THE TREMATODE FAUNA

OF A BRACKISH COASTAL LAGOON

IN TASMANIA

by

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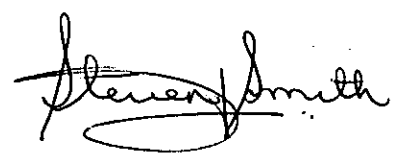

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ABSTRACT

Calvert's Lagoon, a land-locked, brackish lagoon on the south-east coast of Tasmania, Australia, serves as a focus for the life-cycles of a wide variety of digenetic trematodes. A gastropod, *Coxiella badgerensis* (Johnston), is the only molluscan inhabitant, and it is heavily infected by trematodes throughout the year. The incidence of primary trematode infections was found to vary seasonally, with a peak in autumn. Developmental stages of 17 species from 7 trematode families were recorded in snails collected at the lagoon from April 1976 to September 1978. In order of abundance of primary infections, the families were: Microphallidae (3spp.), Schistosomatidae (1sp.), Notocotylidae (4spp.), Renicolidae (2spp.), Psilostomidae (3spp.), Heterophyidae (3spp.) and Strigeidae (1sp.). Gravid adults of 9 of these species were found infecting a wide range of birds at the lagoon, including swans, ducks, grebes, dotterels and coots. Two of the flukes, *Apatemon gracilis* (Rudolphi) and *Psilochasmus oxyurus* (Creplin) are cosmopolitan; however the geographical distributions of the others are unknown.

About 80% of primary trematode infections at the lagoon were caused by the 3 microphallid species: *Maritrema calvertensis* Smith, *Levinseniella tasmaniae* (Smith) and *Atriophallophorus coxiellae* Smith. *M. calvertensis* and *L. tasmaniae* have typical 3 host life-cycles, however, *A. coxiellae* is one of 10 known microphallids that exhibit a reduced life-cycle, with metacercarial cysts being formed in the molluscan host. The daughter sporocysts and xiphidiocercariae of *M. calvertensis* and *L. tasmaniae* are distinguished by several morphological characteristics. The behaviour and ecology of the cercariae were also found to differ in many respects. There is a distinct periodicity in the patterns of emergence of both cercariae from their snail host, however the cercaria of L. tasmaniae emerges during the day, whereas that of *M. calvertensis*

emerges at night. Although both species encyst in the amphipod *Austrochiltonia australis* (Sayce) only *M. calvertensis* is infective to the ostracod, *Mytilocypris tasmanica* McKenzie. Metacercarial cysts of *L. tasmaniae* induce a colour change, from green to bright orange, in the amphipod host. The hoary-headed grebe, the most abundant bird at Calvert's Lagoon, harbours the minute adults of each of the microphallids: *L. tasmaniae* inhabits the intestinal caeca and *Maritrema calvertensis* and *Atriophallophorus coxiellae* have overlapping, but different distributions in the lower small intestine.

The life-cycles of the three microphallids, the psilostomes *Psilochasmus oxyurus* and *Psilostomum* spp.A and B, and the notocotylids *Paramonostomum bursae* n.sp. and *P. caecai* n.sp., were demonstrated experimentally. Growth and development in laboratory ducklings were studied. At Calvert's Lagoon, gravid adults of *Apatemon gracilis* were found in a black duck, and intramolluscan stages were found in *C. badgerensis*, however no second intermediate host was found. Metacercarial cysts of *A. gracilis* were found infecting the freshwater native fish, *Galaxias auratus* Johnston, at Lake Crescent, about 100 km NW of Calvert's Lagoon, (life-history notes on *A. gracilis* and another strigeoid, *Diplostomum galaxiae* n.sp., infecting *G. auratus* at Lake Crescent, are presented in Appendix 2).

A comparison was made of the *in vitro* development of metacercariae of the 3 microphallids developing in *C. badgerensis* at Calvert's Lagoon, and 4 microphallids, *Gynaecotyla hickmani* n.sp., *G. macrocotylata* n.sp., *Maritrema eroliae* Yamaguti and *Microphallus paragrapsi* n.sp., that encyst in an estuarine crab, *Paragrapsus gaimardii* (M. Edw.), at Bruny Island, about 20 km SW of Calvert's Lagoon (descriptions of the microphallids infecting *P. gaimardii* are presented in Appendix 3). All of the microphallids produced eggs *in vitro*; however, the rates of egg production in different culture media varied between species. A direct relationship is suggested between the longevity of each species in its

definitive host and its nutritional requirements *in vitro*, such that very short-lived flukes, like *L. tasmaniae*, relying on endogenous food reserves, can produce eggs at normal rates in balanced salt solution, whereas relatively long-lived flukes require more complex culture media for normal development.

Preliminary investigations of the ecology of other water bodies in Tasmania revealed that the trematodes found at Calvert's Lagoon are widely distributed on the E and NE coasts of Tasmania in enclosed, brackish lagoons inhabited by *C. badgerensis*. The distributions of these trematodes probably extend to similar water bodies that occur on the Bass Strait Islands and in the SE of the Australian mainland.

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PART I

GENERAL INTRODUCTION

Although there have been numerous investigations of the trematodes infecting particular snail species, there have been few studies of the entire trematode fauna of a particular habitat. Different workers have shown the life-cycles of a number of avian trematodes to be established in Australian coastal lagoons, (e.g. Bearup, 1955; Howell and Bearup, 1967; and Smith, 1974), however no previous attempts have been made to characterize the entire trematode fauna of such a lagoon. A study of the life-cycles, and inter-relationships, of the trematode fauna of a brackish coastal lagoon in Tasmania forms the subject of this thesis.

Investigations have been conducted into trematodes infecting snails in a wide range of aquatic environments, from marine (e.g. Lysaght, 1941; Wikgren, 1956; Holliman, 1961; Cable, 1963; and Rebecq, 1964), to freshwater (Dubois, 1929; Rees, 1932; Cort, 1941; Llewellyn, 1957; and Probert, 1966). Typically, trematodes exhibit a high degree of specificity towards their primary intermediate host, hence one of the most important factors determining the trematode fauna of a particular habitat is the identity and availability of the aquatic molluscs. During a 9 year study in Wales, James (1969), found the cercariae of 12 species, from 6 trematode families, developing in the marine snail, *Littorina saxatilis*. He found that most of the trematodes were restricted to a single subspecies of *L. saxatilis*. Some parasite species were restricted to zones within the distribution of the host, related to its vertical position, and degree of exposure to wave action. In Australia, Johnston and his associates studied the developmental stages and life-histories of trematodes infecting freshwater snails in ponds, swamps, and river banks, along the Murray River, over a period of many years, e.g. Johnston and Cleland (1938); Johnston and Angel (1941); Johnston and Beckwith (1946);

and Johnston and Muirhead (1949). They found that although some trematodes occur in more than one snail host, each snail species in a particular habitat is infected by a different assemblage of trematodes.

Various studies have indicated that the trematode fauna of an aquatic biocoenosis is largely determined by the ecological nature of the environment (i.e. physico-chemical characteristics of the water, fauna, flora, and seasonal changes). Wisniewski (1955, 1958a) and his associates, investigated the parasite faunas of an eutrophic and a mesotrophic water body in Poland, and found that although the same kinds of trematode life-cycles occured in both lakes, some were restricted in the mesotrophic lake to the littoral zone. Chubb (1963, 1964), showed that the incidence of trematode infections of fish in oligotrophic lakes in Wales, was markedly less than that in the eutrophic lake studied by Wisniewski. The difference was probably related to the greater size and diversity of the fauna, particularly molluscan and avian, of the latter habitat (Erasmus, 1972). Wikgren (1956), found that on islands of the Finnish Archipelago, the incidence of trematode infections of molluscs in coastal pools was higher than in molluscs of the nearby shore. He suggested that the incidence of infection on the shore was dependent on the density of hatched miracidia, the density of snails, and the action of waves and water currents. The incidence of infection varied from 0 to 100% between different pools, depending largely on the number of birds visiting the pool. Other studies of enclosed aquatic habitats have indicated that there is an inverse relationship between the size of the water body and the incidence of infection of molluscs with trematodes, and that the diversity of the trematode fauna is directly related to the size of the water body. For example, in a 3 year study of a small pond near Warszawa, Styczynska-Jurewicz (1966), found that 52% of the molluscs examined were infected by trematodes, and only 5 species of trematodes were recorded; however in his study of an eutrophic lake, Wisniewski (1958a),

found that about 16% of the molluscs examined were infected by trematodes, a wide variety of which were recorded (54 species of cercariae, and 10 species of metacercariae). Styczynska-Jurewicz (1966), suggested that the high rates of infection observed in small, shallow water bodies were the result of:

(1) a great density of hosts, with mass occurrence of some species due to the uniformity of the environment;

(2) frequent contacts between hosts in a relatively small area; and

(3) relatively high and variable water temperatures.

The life-cycles of many digenetic trematodes are associated with shallow, lentic, water bodies, such as coastal lagoons. These lagoons, whether permanent or temporary, are frequently highly productive habitats, attracting many water birds. The salinity of coastal lagoons varies considerably, mainly in relation to their distance from the ocean, and the balance between evaporation and precipitation in the region. As the salinity of an enclosed lagoon rises, the diversity of species present decreases (Bayly and Williams, 1973). This phenomenon, together with a typically low degree of habitat heterogeneity, and a discrete aquatic community, generates in brackish coastal lagoons, relatively simple ecosystems that are particularly suitable for ecological studies.

The life-cycle of a digenetic trematode typically involves a molluscan host, in which asexual multiplication occurs; an intermediate host, harbouring an encysted metacercaria; and a vertebrate host in which trematode eggs are produced by sexually reproducing adult flukes. However, many variations of life-cycle pattern are known. Pearson (1972), reviewed the literature on the evolution of these cycles, and postulated a phylogeny of life-cycle patterns of digenetic trematodes in which the 3 host life-cycle described above is derived from a 2 host mollusc-vertebrate cycle without a metacercarial stage. The 3 host life-cycle has apparently arisen independently in a number of groups, and has been secondarily reduced several times, by loss of the definitive or second intermediate hosts,

(e.g. in the Microphallidae). The ontogeny of digeneans is complex, with alternation of generations, and up to 7 morphologically distinct stages. In some species the only non-parasitic stage is the egg; however in many, the miracidium, cercaria, and metacercaria are also non-parasitic. Each life-cycle stage is an expression of the genotype of the trematode species, and is adapted to its own particular ecological niche. Any account of the ecology of a trematode should consider the sum total of its life-cycle stages as an ecological unit (Croll, 1966). The successful establishment of a trematode life-cycle depends on the availability of a suitable host, or 'microenvironment', for each parasitic stage, and a suitable aquatic habitat, or 'macroenvironment', for the non-parasitic stages (Dogiel, 1962). Trematodes have adapted to a wide range of aquatic habitats and hosts, from marine to freshwater, and although not conspicuous, they are characteristic and integral components of the biocoenoses of aquatic ecosystems the world over. Each life-cycle is influenced by a complex of ecological factors, such as food chains, migratory and dispersal behaviour of the hosts, and physico-chemical characteristics of the habitat. To understand the selective pressures operating on a trematode species, it is necessary to view the life-cycle in the context of its aquatic environment. The study of trematode life-cycles in relation to their environment has, in the past, been grossly neglected, and there is considerable need for an ecological approach to life-cycle studies of selected habitats (Erasmus, 1972).

In 1967, Dr. J.L. Hickman discovered hundreds of cysts of a trematode, (*Atriophallophorus coxiellae* Smith, 1974), in a snail from Calvert's Lagoon, a brackish water body on the south east coast of Tasmania. Subsequent investigations at that location revealed that the snail, *Coxiella badgerensis* (Johnston, 1878), served as the primary intermediate host for at least 6 trematode species from 4 families (Smith, 1971). It was also discovered that amphipods and ostracods in the lagoon were heavily infected by cysts of microphallid trematodes, and that birds inhabiting

the lagoon served as hosts for a variety of flukes. The present study was undertaken to identify and describe the trematodes of Calvert's Lagoon, elucidate their life-cycles, and investigate the ecological relationships between the parasites and their environment. Calvert's Lagoon was particularly suitable as a study site, because of its proximity to the University of Tasmania; permanence; relatively simple ecosystem ; and most importantly, because it is inhabited by only one molluscan species. With the rare exception of marine polychaete annelids (Linton, 1915; Stunkard, 1929), all known primary intermediate hosts of trematodes are molluscs. As no polychaetes have been recorded at Calvert's Lagoon, all trematodes developing at the lagoon must use *C. badgerensis* as their primary intermediate host.

The circulation of trematodes in the biocoenoses of shallow water bodies may be typified as either localized or expansive (Styczynska-Jurewicz, 1966). In a localized type of circulation, exemplified by the life-cycle of *Astiotrema trituri* (Grabda, 1959), all trematode developmental stages are confined to the same aquatic habitat. In an expansive type of circulation, different developmental stages may be transferred to other ecosystems by natural migration of infected hosts. The life-cycles of avian trematodes occurring at coastal lagoons are examples of expansive circulations, with migration of the adult flukes and eggs in their bird hosts. Birds may also disseminate the trematode developmental stages in snails, and other invertebrate intermediate hosts, when these hosts are transported to other ecosystems, adhering to birds' legs or feathers. A preliminary investigation of coastal lagoons around the eastern seaboard of Tasmania was conducted to determine the likely distribution of the trematodes occurring at Calvert's Lagoon, and the factors influencing the composition of the trematode fauna of a coastal lagoon in general.

In the course of this study, the attention of the author was drawn to trematode infections of a marine crab, *Paragrapsus gaimardii*, in south east Tasmania, and a freshwater fish, *Galaxias auratus*, on the Central

Plateau. These infections were studied to determine whether any of the trematodes utilizing the crab and fish as intermediate hosts, were related to those under investigation at Calvert's Lagoon. White-faced herons that had been feeding on the fish, were examined for adult trematodes. Accounts of these investigations are given in Appendices 1, 2 and 3. The materials and methods used in these 'peripheral' studies are included in Part II.

Descriptions of the new species presented in this thesis have been submitted for publication.

PART II

MATERIALS AND METHODS

Throughout most of the study period, April 1976 to January 1979, various ecological data, (physico-chemical, faunal and floral), were collected during monthly field trips to Calvert's Lagoon. No such data was collected from January 11th to April 16th, 1977, when the author was studying overseas in England and France. Ecological data was also collected at some other coastal lagoons, using similar methods. The incidence of trematode infections in *Coxiella badgerensis* at Calvert's Lagoon was monitored by monthly sampling of adult snails at Site 1, over a 2 year period (Figure 1.2). The spatial, as well as the temporal, distribution of primary trematode infections in the lagoon was investigated. Samples of adult snails were collected on the same day from 4 widely separated locations around the lagoon (Sites 1, 2, 3 and 4), at 3-monthly intervals over a period of one year. Emergence of cercariae from the snail host, their swimming behaviour, and invasion of the second intermediate host, were investigated. The term "post-cercaria" is used here for the developmental phase following invasion of the second intermediate host, prior to encystment. In this thesis, the terminology proposed by Dixon (1965), for the encysted stage in the trematode life-cycle is adopted: the term "metacercaria" referring to the animal which is enclosed in a "cyst wall", the composite structure being a "metacercarial cyst" or simply a "cyst". Snails, amphipods, and ostracods from Calvert's Lagoon were maintained and bred in the laboratory, and served as a source of experimental second intermediate hosts. Ducklings raised under controlled conditions, served as experimental definitive hosts. Growth and development of trematodes within these experimental hosts were investigated. The nutritional and physical habitat requirements of the adults of 3 microphallid species infecting *C. badgerensis* were studied by culturing excysted metacercariae *in vitro*. Comparisons were made with 4 microphallid

species infecting the crab *Paragrapsus gaimardii*, at Bruny Island. *In vitro* culture was a convenient tool in life-history studies, particularly with microphallid species that commence egg production soon after excystment. It enabled ovigerous adults to be obtained quickly, without necessitating the maintenance and slaughter of ducklings. *In vitro* culture also provided a unique opportunity of observing the appearance and behaviour of adult flukes, at the body temperature of their bird hosts.

ECOLOGY

Physico-chemistry

The temperature and level of Calvert's Lagoon were measured at Site 1 (Figure 1.2). Temperature was recorded about 10 cm below the water surface, and the water level was measured relative to a partly submerged, vertical metal stake, located about 25 m from the shore. PH and salinity determinations were made on water samples in the laboratory, on the day of collection. Water was collected in 250 ml, black, screw-cap, glass bottles. PH measurements were made with a glass electrode pH-meter (E 350A, Metrohm A.G., Switzerland). Salinity was determined approximately by volumetric titration against $AgNO_3$ solution (Harvey, 1955, p.125). One ml aliquots were titrated against 2.725 gm/L $AgNO_3$ solution, using dilute K_2CrO_4 solution as the end-point indicator. The titre, (mls of $AgNO_3$ solution), approximated the salinity of the sample (‰). The salinity was taken as the mean of at least 3 replicate titrations, that were in close agreement.

Monthly water samples were collected, for detailed chemical analysis, over a period of one year. Each sample was collected in 2, one litre screw-cap, plastic bottles. The plastic bottles were thoroughly washed in distilled water, cleaned with a few drops of concentrated HNO_3 acid, and rinsed in lagoon water, before each sample was collected. The samples

were analysed by the Government Analyst (Government Analyst Laboratory, Hobart), for the Inland Fisheries Commission, using standard A.P.H.A. methods (Rand *et al.*, 1975). Total dissolved solids (TDS), were determined as the filterable residue, and suspended solids, as the total non-filterable residue, using Method 208D. 'Total hardness', and Ca^{++}, were measured using EDTA titration (Methods 309B and 306C); and Mg^{++} was derived by subtraction of Ca^{++} from 'total hardness'. K^{+} and Na^{+} were measured by flame photometry (Methods 317A and 320A). Cl^{-} was measured using Method 408A; and CO_3^{-} and $HCO_3^{=}$ alkalinities using Method 403. $SO_4^{=}$ was determined gravimetrically as $BaSO_4$, according to Method 427A; and Method 425F was used to determine $PO_4^{=}$. Soluble, 'molybdate-reactive', silica was measured using the 'heteropoly blue' method, Method 426C.

Fauna

Aquatic invertebrates were regularly collected from Calvert's Lagoon, using a long-handled, standard, F.B.A. net. Sweeps of the net were made at varying depths, while wading along a 25 m transect from the shore towards the middle of the lagoon. The animals were transferred to 1L plastic containers for transport to the laboratory. A sample of 30 adult snails (shell length >5 mm), was randomly selected from these containers, for a systematic survey of trematode infections. Snails were dissected individually in a crystal dish, (4 × 4 × 1.5 cm clear glass block, with a 1 cm deep concavity in the upper surface), under a dissecting microscope (Russian Stereoscopic Dissecting Microscope, MBC-1). The shell length, aperture length, number of whorls, and sex of each snail were recorded. Measurements were made with a finely calibrated, ocular graticule. Removal of the snail shell was achieved by holding the body whorl firmly with one pair of forceps, while gently chipping shell fragments away with a sharp metal probe. The soft body of the mollusc was then dissected from the dorsal surface, using 2 pairs of fine watchmaker's forceps. The mantle cavity was opened along the right side, all organs were dissected,

from the pallial gonoduct to the tip of the 'visceral hump', and the location and identity of trematode infections were recorded. All lagoon water used in laboratory procedures, including snail dissection, was decanted from large jerry cans in which it had been kept for at least 2 weeks, to ensure that any cercariae or trematode eggs would have settled to the bottom.

Seasonal variation in the incidence of primary trematode infections at Site 1, in the 12 months from July 1977 to June 1978, was investigated using analysis of variance. The 3 monthly samples collected in each season were treated as replicates, thus June, July and August samples were replicates for winter; September, October and November samples were replicates for spring, etc.. The percentages of snails infected by each trematode family, in each sample of 30 snails, were converted to angles using angular transformation (Fisher and Yates, 1974, Table X), with the aim of transforming the data to a normal distribution (Sokal and Rohlf, 1969). Variation in the incidence of primary infections at Sites 1, 2, 3 and 4, was also investigated by analysis of variance. Quarterly samples of 30 snails were collected at each site, in July (winter) and October (spring) 1977, and in January (summer) and April (autumn) 1978. Replicates were obtained by sub-dividing each sample of 30 snails into 3 groups of 10, using random number charts (Fisher and Yates, 1974, Table XXXIII). The percentages of snails infected by each trematode family, in each group of 10 snails, were converted to angles using angular transformation (as above).

Snails were maintained and bred in the laboratory at room temperature (15 - 25°C), under a normal light regime. Very young snails (average aperture length 1.06 mm; 4 whorls), were collected at Calvert's Lagoon in October 1977; and their growth in the laboratory was monitored for a year. They were kept in lagoon water, about 5 - 7 cm deep, in plastic troughs measuring 40 × 30 × 12 cm. Several small sandstone rocks from Calvert's Lagoon were sterilized by boiling, and added to each aquarium. Snails were fed *ad lib*, on small pieces of dried lettuce.

The leaves were first boiled in tapwater for about 2 mins., and then spread to dry on a sunny window. When dry, the leaves could be stored indefinitely. One teaspoonful of $CaCO_3$ powder was added to the water about every 2 months. The aquaria were aerated, and partially covered to reduce water loss by evaporation. Water that was lost, was periodically replaced with tapwater. Snail growth, under these conditions, was regularly monitored by measuring the aperture length of the 15 largest snails in the laboratory population.

The release of cercariae of various trematode species from *Coxiella badgerensis* was studied by isolating 'wild' snails for a few days in the laboratory. From 50 to 100 large adult snails were selected from snails periodically collected at Site 2, Calvert's Lagoon, and each snail was placed in lagoon water in a separate crystal dish, on a bright window ledge. After 1, 2 and 3 days, the water in each dish was examined under a dissecting microscope for free cercariae. Cercariae were identified by pipetting onto microscope slides for examination at higher magnification under a standard microscope (Carl Zeiss GFL 654-633). Micropipettes, made by drawing out standard pipettes over a bunsen burner, were used for transferring the minute trematodes.

Snails releasing cercariae were maintained in isolation in the laboratory for 1 or 2 days before being used in experiments on cercarial emergence. They were fed lettuce *ad lib*, and their water was changed daily. In the experiments, snails were transferred, at intervals during the day and night, into clean crystal dishes filled with lagoon water. Several drops of aqueous 0.1% brilliant cresyl blue solution were added to the vacated water, to stain and immobilize cercariae to facilitate counting, which was conducted under a dissecting microscope.

Laboratory-bred snails were individually exposed to infection by newly emerged, free swimming, psilostome cercariae. The behaviour of the cercariae was observed under a dissecting microscope, from the time that they were pipetted into each crystal dish containing a snail. The

course of development of metacercarial cysts of the psilostomes was followed by dissecting snails at various intervals, after cercariae had disappeared into their mantle cavities.

Amphipods and ostracods from Calvert's Lagoon were measured and dissected in crystal dishes under a dissecting microscope, and the location, number and identity of trematode infections were recorded. The length of amphipods was measured from the front of the head to the base of the telson, and the length of ostracods was measured as the maximum dimension of the valve. Examination of the third uropoda of amphipods was facilitated by staining with acid fuchsin (Wagstaff and Fidler, 1955). The exoskeleton was punctured in several places, and then the whole animal was boiled in 10% NaOH for 10 - 15 mins. to remove muscle tissue, before being rinsed in glacial acetic acid, and stained in 0.2% acid fuchsin in glacial acetic acid for 3 mins. Surplus stain was removed in glacial acetic acid, and then the third uropoda were dissected with fine needles and transferred and mounted in 'euparal'.

Amphipods and ostracods were maintained in separate aquaria, for nearly 3 years, under the same conditions as snails. Both species bred in the laboratory, and laboratory-bred animals were experimentally infected by cercariae of the microphallids *Maritrema calvertensis* and *Levinseniella tasmaniae*. Snails from which cercariae were emerging, were checked for several days, and if cercariae of only one microphallid species were emerging, then the snail was used as a source for infecting amphipods and ostracods. Infection of crustaceans by cercariae of each microphallid species was investigated experimentally by exposing individuals to infection by a known number of cercariae of the same species. Fifty cercariae were pipetted, within 1 hour of emergence from the same snail, into a crystal dish containing a single laboratory-bred adult ostracod or amphipod, and a laboratory-bred snail that served as a convenient substrate for the crustacean. An upturned crystal dish was used as a cover, and the crustacean was left exposed to cercariae overnight (about 12 hours),

at room temperature. The experimental host was then transferred to a 1L aerated plastic container, containing small pieces of lettuce in lagoon water, kept in a constant temperature room at 15°C, and dissected 6 days later. In other experiments, to investigate the development of the two microphallids within their crustacean hosts, 2 amphipods or 2 ostracods were placed in a crystal dish for about 12 hours, with a snail known to be releasing cercariae of only one of the microphallids. After being exposed to infection, the crustaceans were transferred to 1L aerated plastic containers, as above, and kept in constant temperature rooms. They were dissected and examined for trematodes, from 4 to 58 days later.

The numbers of metacercarial cysts in naturally infected, and experimentally infected amphipods and ostracods, were distributed according to the negative binomial pattern. An attempt was therefore made to transform the data to a normal distribution, using a logarithmic transformation of the $\log_{10}(x + 1)$ type, so that Student's "t" test could be applied to the data (Elliott, 1977). The means of the transformed data were converted by antilogging and subtracting 1 to give geometric, or derived means. The infection of amphipods and ostracods with trematode cysts was tabulated showing the arithmetic mean ($\bar{X}a$), and variance (V_{Xa}); transformed mean ($\bar{X}t$), and transformed variance (V_{Xt}), both being logarithmic terms; and the geometric mean ($\bar{X}g$), and 95% confidence limits. Student's "t" test was only performed on transformed data.

The estuarine crab *Paragrapsus gaimardii*, was collected at low tide, from under rocks at Great Bay, Bruny Island, and maintained in running seawater in a marine aquarium for up to 2 weeks prior to dissection. Crabs were examined for trematodes in seawater, in plastic petri dishes. They were prepared for dissection by pithing, and then removal of limbs at the basi-ischium/merus joint, using strong scissors. The carapace was cut around the margins of the dorsal surface, and removed, and then each of the soft, internal tissues were examined under a dissecting

microscope.

Fish and birds were the only vertebrates collected from Calvert's Lagoon. Five adult brown trout were caught between midday and dusk, on June 19 1978, using grab-all nets set perpendicular to the shore, off the rocky northern point. The fish were taken to the University, where measurements of length and weight were recorded, and scales were removed for age determinations. The fish were kept overnight at 5°C and the following day, the viscera of each one were transferred to a dish of Hank's balanced salt solution (Hank's BSS). The stomach, alimentary tract (cut into 6 cm lengths), eyes, brain, gonads, liver, kidneys and samples of muscle tissue, were examined in Hank's BSS in separate disposable petri dishes, under a dissecting microscope. Fine watchmaker's forceps were used to tease the tissues apart.

Inch long brown trout and adult *Galaxias truttaceus*, supplied by the Inland Fisheries Commission, were exposed to infection by cercariae of *Heterophyid* sp.A. They were maintained in water from Calvert's Lagoon for several hours and then anaesthetized by MS222. The dazed, slow-moving fish were placed in lagoon water in glass dishes, 10 × 6 × 6 cms, under a dissecting microscope. Cercariae were pipetted into each dish, and their reactions observed. After 30 mins., the fish were transferred to aerated 1L plastic containers, with floating duckweed for refuge, fed on dried fish food, and maintained at 15°C prior to dissection and examination for trematodes.

Golden galaxiids, *Galaxias auratus*, were collected from the River Clyde, where it enters Lake Crescent. They were transported to the University in river water, then pithed and examined for metacercarial cysts. The eyes, brain, gonads, liver, kidneys, muscle tissue and connective tissue were dissected under a dissecting microscope, and cysts were removed with fine watchmaker's forceps, and transferred to Hank's BSS.

A variety of birds, floating or standing on the shore, were shot from the cover of bushes on the southern side of Calvert's Lagoon. Birds

were all shot in the morning and taken to the University, where dissection usually began within 4 hours of death. The period from death of the bird until isolation of different regions of the viscera, was made as short as possible, to minimize the possibility of translocation of parasites after the death of their host. When 2 birds were shot on the same day, one was stored at 5°C, and dissected that evening, or the following morning. The viscera of each bird were transferred to a large dish, and the mesenteric blood vessels were examined for schistosomes by stretching the mesentery over an upturned glass petri dish under a dissecting microscope. The gizzard, gall bladder and bile duct, intestinal caeca and bursa fabricius, were removed and placed in Hank's BSS in separate disposable plastic petri dishes. The remainder of the alimentary tract was cut into 6 cm lengths, each of which were also placed in Hank's BSS in separate petri dishes. Fine watchmaker's forceps were used to tease each length of intestine open. The piece of intestine was then flattened, lightly scraped and gently shaken in Hank's BSS to free any trematodes. The number and identity of flukes in each petri dish were recorded, and the worms transferred to fresh Hank's BSS in crystal dishes, and either fixed immediately in boiling 10% phosphate buffered formol saline (B10BFS), or maintained at 5°C for later examination. The distribution of flukes in the small intestine was expressed in terms of 10 zones of equal length (SI 1 to SI 10), with SI 1 commencing at the gizzard, and SI 10 terminating at the opening of the intestinal caeca.

White-faced herons were shot at Lake Crescent, and on the banks of the River Clyde, where it enters Lake Crescent. They were transported to the University where they were dissected, 5 and 26 hours after death, using the techniques outlined above.

Domestic ducklings, *Anas platyrhynchos platyrhynchos*, were experimentally infected with various trematodes, and maintained for 2 months in the laboratory. Duck eggs from local farms were hatched in a laboratory incubator, however this source of bird hosts proved unsatisfactory,

due to the time delay involved, and the variable rate of hatching success. A more reliable and convenient source was found to be a Victorian duck farm. Ducklings were air-freighted, when 1 day old, from Rutherglen Duck Farm, Rutherglen, Victoria. They were kept for several weeks in a large wooden box lined with straw. A shallow plastic bowl was included to permit swimming, and light globes provided heat. The birds were then transferred to a concrete-floored, internal enclosure, surrounding a grate-covered drain. The enclosure was provided with a food container, and a continuous small stream of water, from a hose. The ducklings were fed on 'duck starter crumble' for the first 4 weeks, and then gradually changed to 'duck grower pellets' (Tasmanian Department of Agriculture). When about 1 week old, ducklings were fed gelatin capsules containing metacercarial cysts (Empty Gelatin Capsules No. 4; Parke, Davis and Co., Sydney). They were killed by chloroform, after periods of up to 5 weeks and immediately dissected and examined for adult trematodes, using the techniques described above for wild birds.

The period of infection (PI) of trematodes in experimental hosts has been abbreviated in a standard way e.g. 18 days and 23 hours becomes 18,23 days; and 1 day and 8 hours becomes 1,8 days.

Flora

The plants in and around Calvert's Lagoon, and other coastal lagoons, were identified and recorded in a survey of Tasmanian "wetlands", conducted for the Tasmanian Conservation Trust, by Harwood and Kirkpatrick (report in preparation).

TREMATODE MORPHOLOGY

Live Material

Observations of live trematodes were made under a standard light microscope (Carl Zeiss, GFL 654-633); a phase-contrast light microscope (Wild Heerbrugg, M20); and an inverted microscope (Olympus Inverted

Microscope, CK). Various degrees of flattening of specimens were obtained by supporting the coverslip with a few specks of vaseline, and gradually absorbing the liquid around the specimen with pieces of filter paper. Observations of flame cells and excretory ducts were facilitated by mounting specimens in a small drop of tapwater under an unsupported coverslip, and allowing the water to dry slowly. Aqueous vital stain solutions, 0.001% brilliant cresyl blue and 0.001% neutral red, were useful for studies of live cercariae, as they differentially stained glands and their products.

Fixed Material

The bodies of most trematodes are extremely flexible and contractile. Consequently, unless otherwise stated, measurements of adults and all developmental stages, except metacercarial cysts, were based on specimens fixed by the following standard procedure. The specimen was placed in Hank's BSS, or lagoon water, at room temperature (15 - 20°C), in a crystal dish. Saline, or lagoon water, was carefully removed with a micropipette, leaving the specimen in the least possible amount of liquid. Boiling 10% phosphate buffered formol saline (B10BFS) was immediately poured over the specimen. All measurements were taken, without flattening the specimen, under a standard light microscope, (Carl Zeiss, GFL 654-633), using a finely calibrated ocular graticule. Unless otherwise indicated, all measurements are given in microns: the mean first, followed by the size range in brackets. Other specimens were flattened, for morphological studies, and fixed in alcohol. These worms were slightly compressed under coverslip pressure, relaxed by gentle heating over a slide-warmer, and fixed by adding 70% ethanol at one side of the coverslip, while absorbing it at the other side with filter paper.

After fixation, worms were generally stained in alum carmine (Bronte Gatenby and Beams, 1950, p.465), or Gower's carmine (Bronte

Gatenby and Beams, 1950, pp.136-137). Other stains used for routine examination were Horen's Trichrome (Horen, 1957), Mallory's Heidenhain (Cason, 1950), and toluidine blue (Pearse, 1968). Metacercariae and adults, flattened and fixed in 70% ethanol, were stained by Fast Red Salt B (Johri and Smyth, 1956), and usually counterstained in Gower's carmine. Fast Red Salt B stains phenolic egg-shell precursors in the vitellaria and vitelline ducts. Another method used to determine the stage of development of vitellaria was the catechol reaction for detecting polyphenol oxidase (Bell and Smyth, 1958). Organ primordia, and the various stages of spermatogenesis in metacercariae and juvenile flukes, were easily detected in aceto-orcein squashes, or whole mounts (Bell and Smyth, 1958). For this technique, live specimens were washed 3 times in non-sterile Hank's BSS, and then the saline was withdrawn, and replaced by aceto-orcein (Culling, 1963, p.397). Aceto-orcein stains nuclei clearly, and causes cell volumes to increase, so that cell divisions can easily be seen. Permanent whole mounts of stained specimens were prepared by clearing in clove oil, and mounting in Canada balsam, or D.P.X., via standard procedures (Pantin, 1946). Drawings were made with the aid of a camera lucida.

Specimens were prepared for sectioning by being fixed in B10BFS, and embedded in Paraplast wax using standard techniques, with methyl salicylate replacing the xylene-benzene steps (Pantin, 1946). Specimens were embedded in crystal dishes, orientated with respect to black paper markers. Serial sections were cut on a Cambridge 'rocker' microtome, fitted with a Wigglesworth microtome blade. The material was either block-stained in alum carmine prior to embedding, or stained after sectioning, according to the Periodic Acid Schiff technique, and counterstained with Ehrlich's Haematoxylin (Pearse, 1968).

Scanning electron microscopy

Adults and excysted metacercariae were washed several times in

Hank's BSS, and cercariae and sporocysts were washed in lagoon water, at room temperature, prior to fixation. Some were fixed in 3% glutaraldehyde in Sorenson's 0.1M phosphate buffer (pH 7.3), containing 3% sucrose and $CaCl_2$, for at least 2 - 3 hours at 4°C. They were then washed in 3 changes of chilled buffer containing 5% sucrose and $CaCl_2$ for at least 20 mins. each, followed by 3 rinses in distilled water at room temperature. Other specimens were fixed in B10BFS and then washed in several changes of distilled water at room temperature. Both methods produced satisfactory results, however worms fixed in B10BFS were more relaxed. Fixed and washed worms were dehydrated through a graded series of alcohol solutions, from 30 to 95%, spending 10 mins. at each stage. They were given 3 × 10 minute changes in 100% ethanol, and then transferred to 50% ethanol:amyl acetate, followed by 2 × 15 minute changes in amyl acetate. The worms were placed in a brass specimen-carrier, based on that of Rostgaard and Christensen (1975), and dried in a Critical Point Drying Apparatus (Polaron). The carrier enabled minute adult trematodes, cercariae and metacercariae to be dried. It consisted of 3 hollow cylindrical, brass parts, clamped together by a wire loop attached to the bottom part. The external diameter of each part was 19.15 mm, and the internal diameter 2.25 mm. The top and bottom parts were 4.50 mm long, and the middle part was 7.35 mm long. Round, copper, electron microscope grids (square pores, 50μ side), were placed between the bottom and the middle parts, and between the middle and top parts. Specimens were dropped with a micropipette into the internal chamber, before the upper copper grid and top part were emplaced. After drying, specimens were tipped onto brass stubs, with double-sided sticky tape, and coated with gold in a Polaron SE Coating Unit (at Imperial College, London), or in a JEOL JEE-4B Vacuum Evaporator (at Central Science Laboratory, University of Tasmania). Cysts and excysted metacercariae of *Atriophallophorus coxiellae* were examined at the Zoology Department, Imperial College, London, on a Cambridge Stereoscan Scanning Electron Microscope, and

photographs were taken with a 35 mm camera. Other specimens were examined at the Central Science Laboratory, University of Tasmania, on a JEOL JXA-50A Electron Probe Microanalyser, and photographs were taken with a Polaroid camera.

IN VITRO CULTURE

Excystment of metacercariae

Excystment of metacercariae was conducted under non-sterile conditions. Cysts were dissected free of host tissue under a dissecting microscope, washed twice in isotonic saline and then transferred, using micropipettes, to test-tubes containing digestive enzyme solutions at 40 - 41°C. The test-tubes were shaken for 30 secs. every 15 mins. in an intermittently-shaking water-bath, set at that temperature range. The optimum excystment procedure varies for different trematode species. Some species, such as *Levinseniella tasmaniae*, excyst readily after exposure to one enzyme solution; however others, such as *Apatemon gracilis*, require treatment with a sequence of different solutions. Metacercariae of each species can be stimulated to excyst under a variety of conditions. The treatments shown in Table M.1 , were the most effective of the treatments tested during the present study. Care was taken to minimize temperature fluctuations after metacercariae had been exposed to elevated temperature. All solutions and glassware were kept at about 41°C (which approximates the body temperature of avian hosts), and changes of solution were made promptly. Between successive treatments, cysts were transferred to crystal dishes under a dissecting microscope, and washed twice in Hank's BSS, before being returned to test-tubes in the water-bath. Excystment procedures for the cysts of 4 microphallids infecting *Paragrapsus gaimardii* at Bruny Island, and 2 strigeoids infecting *Galaxias auratus* at Lake Crescent, are included in Table M.1.

TABLE M.1 *In vitro* excystment of trematode metacercariae

Parasite species	Host species	Treatment*
Microphallidae		
M. calvertensis	amphipod, *A. australis*	0.5PAN + 0.2NAT, 60 min → HAN, 60 min.
L. tasmaniae	amphipod, *A. australis*	0.5PAN, 60 min → HAN, 60 min.
A. coxiellae	snail, *C. badgerensis*	0.5PAN, 60 min → HAN, 60 min.
M. eroliae	crab, *P. gaimardii*	0.5PAN + 0.2NAT, 60 min → HAN, 60 min.
M. paragrapsi n.sp.	crab, *P. gaimardii*	0.5PAN, 60 min → HAN, 60 min.
G. hickmani n.sp.	crab, *P. gaimardii*	0.5PAN, 60 min → HAN, 60 min.
G. macrocotylata n.sp.	crab, *P. gaimardii*	0.5PAN, 60 min → HAN, 60 min.
Psilostomatidae		
P. oxyurus	snail, *C. badgerensis*	0.5PAN, 60 min → HAN, 60 min.
Psilostomum sp.A.	snail, *C. badgerensis*	0.5PAN, 60 min → HAN, 60 min.
Psilostomum sp.B.	snail, *C. badgerensis*	0.5PAN, 60 min → HAN, 60 min.
Renicolidae		
Renicolid sp.B.	snail, *C. badgerensis*	0.5PAN, 60 min → HAN, 60 min.
Strigeidae		
A. gracilis	fish, *G. auratus*	2.0PEP, 10 min → HAN → 0.02NAD, 15 min → HAN → 0.5PAN + 0.2NAT, 120 min → HAN, 30 min.
Diplostomatidae		
D. galaxiae n.sp.	fish, *G. auratus*	2.0PEP, 10 min → HAN → 0.02NAD, 15 min → HAN → 0.5PAN + 0.2NAT, 60 min → HAN, 60 min.

(* explanation of treatment code presented opposite)

Hank's BSS, gassed with CO_2 to pH 7.4, was used in each of the excystment solutions:

Excystment solution	Code used in Table M.1
0.5% pancreatin (Viokase) in Hank's BSS	0.5 PAN
2.0% pepsin (B.D.H.) in Hank's BSS	2.0 PEP
0.02M sodium dithionite in Hank's BSS	0.02 NAD
0.2% sodium taurocholate (B.D.H.) in Hank's BSS	0.2 NAT
0.5% trypsin (B.D.H.) in Hank's BSS	0.5 TRY
Hank's BSS	HAN

Metacercariae excysted at different stages during the excystment treatment. Excysted metacercariae were removed, washed in Hank's BSS, and transferred to a sterile Universal container at 41°C, prior to sterilization by washing, and *in vitro* culture.

General sterile techniques

All routine procedures of culture media preparation, establishment of cultures, and periodic gassing of media, were conducted under sterile conditions inside a laminar flow cabinet (Laminar/Flow Bio-Clean Work Station No. CF 33S, Gelman Clemco Pty. Ltd., N.S.W.). The cabinet, 73 × 59 × 52 cm, was swabbed with 90% ethanol before use. All re-usable apparata were thoroughly cleaned in tapwater, rinsed in distilled water, and then dried at 160°C for 90 mins. The wide ends of glass pipettes were plugged with cotton wool after drying, and the pipettes were then placed in paper sterilization bags (Weckink Sterilizing Bag, Edward Weck and Co. Inc., New York). Open ends of bags were folded twice and stapled shut. The cap and neck of each screw-cap media bottle were covered with aluminium foil, which was stuck down with sterilizing tape. The caps of all screw-cap vessels were loosened during sterilization, and tightened immediately afterwards. All apparata were sterilized by autoclaving at 15 lbs per sq. in. for 20 mins. They were then left to dry in an oven at 60°C for several hours.

Physical culture conditions

Leighton tubes (Bellco), were usually used as culture vessels. These are 15 ml screw-cap glass tubes, with one flat side, that facilitates observation of live flukes on an inverted microscope. An Olympus Inverted Microscope CK, was mounted in a thermostatically-controlled, perspex cabinet, and maintained at about 40°C. The cabinet had a floor area of 104 × 51 cm, back height 51 cm, inclined top, and a front face 23 cm high, with two arm-holes sealed by foam rubber. Disposable Universal containers (Sterilin), were used for sterile washing of excysted metacercariae, and also sometimes as culture vessels. These are 30 ml, sterile, screw-cap, plastic vials. Internally, they have an inverted conical end, but externally they have a flat base, and are free-standing.

Gas (5% CO_2 in air), was bubbled through stock bottles of the various media before use. All media were gassed to a pH of 7.4, using phenol red as an indicator. When cultures were established, the air space within the culture vessel was gassed slowly for about 20 secs. before the cap was tightly sealed. Increases in pH of cultures during experiments were corrected by periodic gassing, using sterile precautions. All cultures were maintained in an intermittently-shaking water-bath, at 40 - 42°C. Gentle mechanical agitation automatically occurred for 30 secs. every 15 mins. Due to the small size of the trematodes under investigation, it was not necessary to replace culture media during the course of experiments.

Culture media

NCTC 135 and Medium 858 were used at Imperial College, London, and their preparation is described by Davies (1976). The compositions of both media are given by Paul (1970). At the University of Tasmania, sterile culture media were supplied by the Commonwealth Serum Laboratories, (45 Poplar Road, Parkville, Victoria). The composition of Hank's BSS

and Eagle's MEM are given in Paul (1970). Each of these media were stored at 4°C until used. Sterile, activated foetal calf serum was also supplied by the Commonwealth Serum Laboratories, and stored frozen. The serum was inactivated before use, by heating it for 1 hour in a water-bath at 56°C.

Preparation of cultures

Non-sterile, excysted metacercariae were sterilized by repeated washing with sterile Hank's BSS. They were added with a minimum of non-sterile Hank's BSS, to 10 mls of sterile Hank's BSS in a Universal container at 41°C, and then agitated for 10 mins., in a water-bath. They were allowed to sediment, and were then transferred, again with a minimum amount of liquid (less than 1 ml), to 10 mls of sterile Hank's BSS in a second Universal container. Four sterile washes of 10 mins. each, were usually performed, and then the sterile excysted metacercariae were transferred to 10 mls of culture medium in a Leighton tube, where they remained for the duration of the experiment, maintained in an intermittently shaking water-bath. Cultured worms were periodically observed and photographed on an inverted microscope, in a heated cabinet.

PART III

THE LAGOON

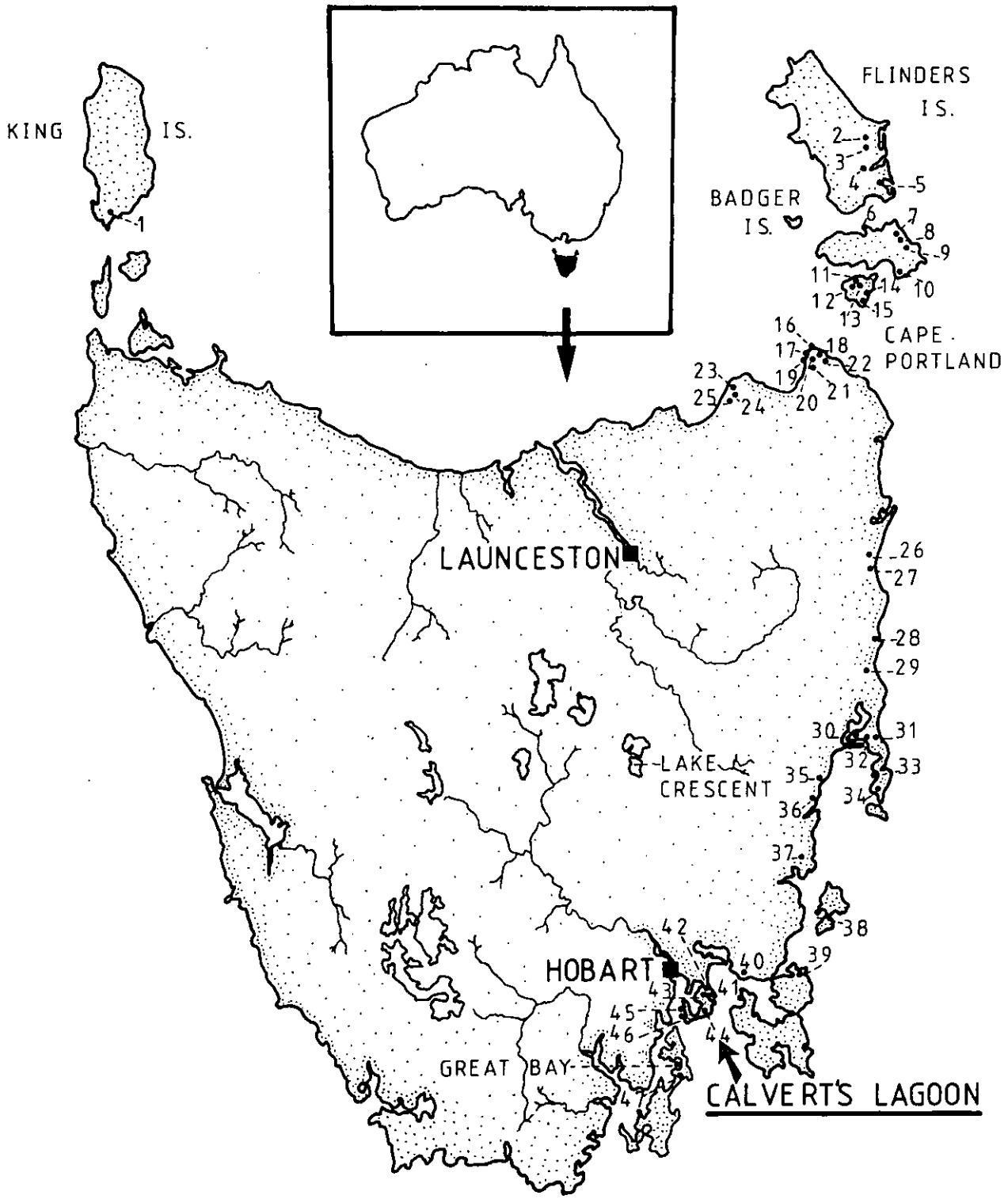
Chapter 1 THE ECOLOGY OF COASTAL LAGOONS

1.1 Introduction

In contrast to the Australian mainland, Tasmania is well-watered, and has a large number of rivers, inland lakes and lagoons (Figure 1.1). This most southerly Australian State, 68,300 square kilometres in area, has a temperate, maritime climate, with relatively mild winters and cool summers. It is generally mountainous, with plateaux in the central and north-eastern regions, and extensive mountain ranges in the western half of the island. Rainfall is distributed unevenly, with areas in the west receiving over 250 cm per year, while a few lowland areas in the east receive less than 50 cm per year (Davies, 1965). Evaporation is relatively low compared to the Australian mainland. The highest amounts occur in the north, east and south-east (Williams, 1974). The great majority of Tasmanian inland waters are very dilute (<300 ppm TDS). All of the more concentrated waters occur in the midlands, north-east coast, east coast, south-east coast and Bass Strait Islands, where annual evaporation is greater than or equal to annual precipitation (Tyler, 1974; Harwood and Kirkpatrick, in preparation).

Coastal lagoons, varying in size, composition and biota, are widely distributed around Tasmania and its offshore islands. Some of these open intermittently to the sea, and are quite saline, their salinities approaching, and sometimes exceeding, that of seawater. Lagoons that never open to the sea vary from fresh to brackish. Following Williams (1967), and Bayly and Williams (1973), the arbitrary boundary between brackish and freshwaters is accepted as a salinity of 3,000 ppm (3‰). A study of the ecology of Calvert's Lagoon, one of the largest of the brackish lagoons, was conducted as an essential background to investigations of trematode life-cycles in that habitat. A preliminary survey

FIG. 1.1 TASMANIA



The locations of coastal lagoons mentioned in this thesis are indicated by numbers. A key to the lagoon numbers is given in Table 1.1.

of other coastal lagoons was made to determine the availability of similar habitats, that appear suitable for establishment of the trematode life-cycles found at Calvert's Lagoon.

TABLE 1.1 The names and map coordinates of coastal lagoons shown in Figure 1.1

Region	Lagoon number	Lagoon name	Map coordinates
King Island	1	Big Lake	8000; 400 545
Flinders Island	2	Singletons	8517; 020 684
	3	Sticks	8517; 020 677
	4	Second Saltpan	8517; 035 580
	5	Logan	8517; 100 520
Cape Barren Island	6	Apple Orchard Point	8517; 032 348
	7	nr. Little Creek	8517; 152 350
	8	nr. Little Creek	8517; 156 343
	9	nr. Little Creek	8517; 156 332
	10	Crystal	8517; 144 182
Clarke Island	11	Kangaroo Bay	8516; 994 151
	12	Sandy	8516; 000 145
	13	E of Sandy	8516; 003 143
	14	nr. Black Point	8516; 037 117
	15	Blue Hills	8516; 030 111
Cape Portland	16	nr. Cape Portland	8416; 816 887
	17	nr. Cape Portland	8416; 811 879
	18	NE of Tregaron	8416; 841 885
	19	N of Tregaron	8416; 825 866
	20	NW of Tregaron	8416; 812 870
	21	S of Tregaron	8416; 816 852
	22	nr. Little Musselroe	8516; 858 850
Waterhouse	23	Little Waterhouse	8416; 520 750
	24	Big Waterhouse	8416, 510 728
	25	Blackmans	8416; 552 710
East Coast	26	Wrinklers	8515; 060 115
	27	Henderson	8515; 045 073
	28	Templestowe	8514; 065 795
	29	Old Mines	8514; 045 675
	30	Big Punchbowl	8513; 976 435
	31	Freshwater	8513; 073 422
	32	Shepherds Hut	8513; 021 398
	33	Hazards	8513; 067 295
	34	Bryans	8513; 057 214
	35	Troyheleener	8513; 864 276
	36	Lisdillon	8513; 826 186
	37	Rostrevor	8413; 768 952
	38	Guards	8512; 842 757

TABLE 1.1 (continued)

Region	Lagoon number	Lagoon name	Map coordinates
South East coast	39	Swan Salt	8412; 760 520
	40	Primrose Sands	8412; 541 515
	41	Sloping	8412; 560 436
	42	Clear	8412; 410 465
	43	Rushy	8412; 415 450
	44	Calvert's	8311; 402 370
	45	Opossum Bay	8311; 333 382
	46	Half Moon Bay	8311; 332 379
	47	Gibbs	8311; 295 106

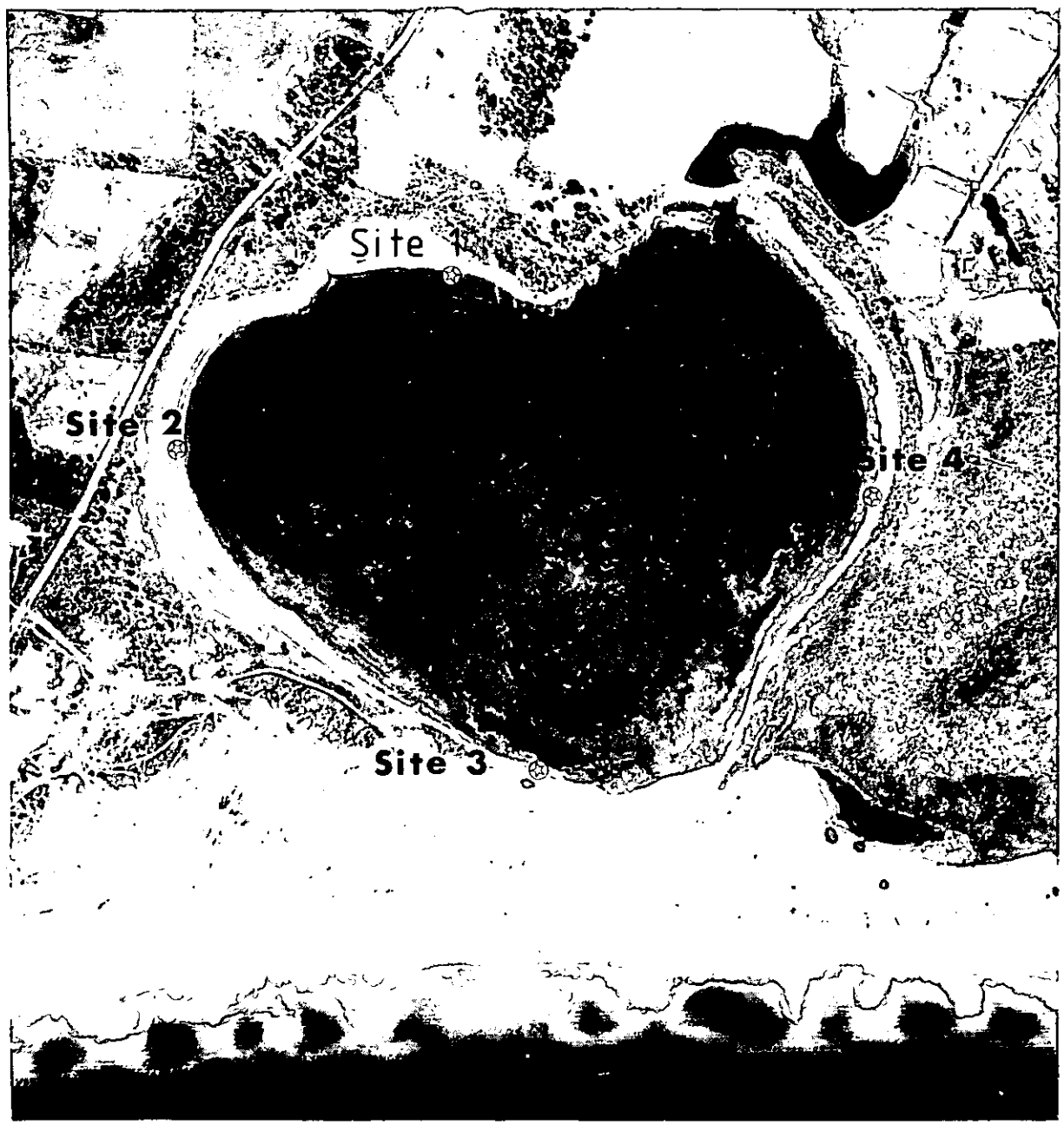
1.2 Ecology of Calvert's Lagoon

1.2.1 Physico-chemistry

Calvert's Lagoon, or Collin's Spring, is a brackish, heart-shaped lagoon or pond, 30 km by road, south-east of Hobart (Figure 1.2). It is separated from the sea by about 200 m of high sand dunes. Management of the lagoon, which is included in the 'South Arm State Recreation Area', is the responsibility of the Lands Department of Tasmania. Seasonal and long-term fluctuations of the water level cause the area of the lagoon to vary, and during the present study, the maximum area observed was about 50 ha. The level of the lagoon reaches a peak each year in spring (Figure 1.3). The depth of the lagoon, which is located in a flat basin, is fairly even, and never exceeds about 2 metres. The clear, pale straw coloured water is surrounded by a sandy shore, except at the mid-northern point, where Permian sandstone outcrops. To the west and north-west, the shore consists of a coarse lithic sand, derived from the sandstone; however, to the south and east, it consists of fine quartz grains from the coastal sand dunes.

Surface water temperatures recorded in the lagoon ranged from 7.2 to 21.5°C (Figure 1.4), however higher temperatures occur in the shallows in summer. In winter, there is little temperature variation with depth; however in summer, rapid growth of slender hydrophytes results

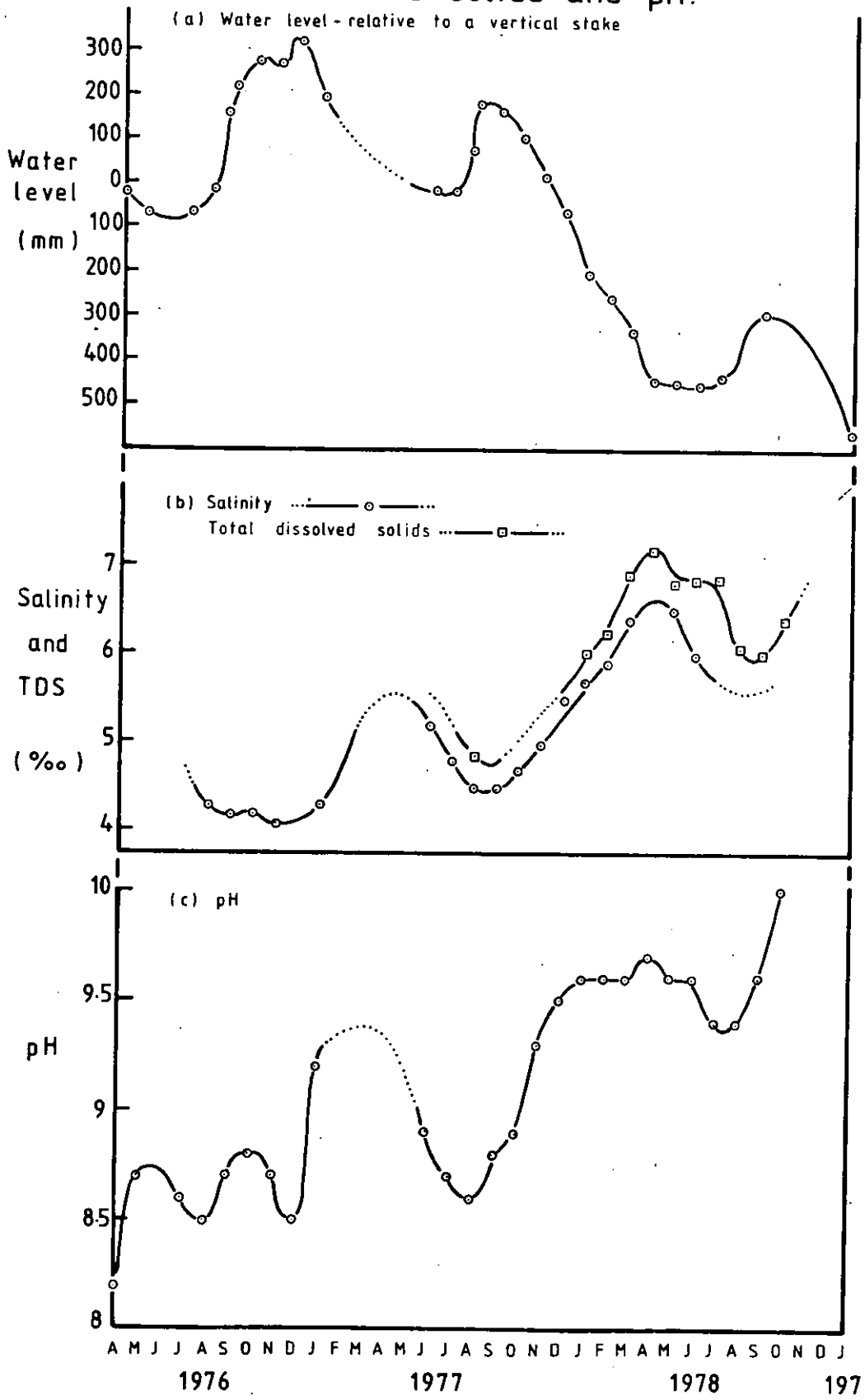
FIG. 1.2 CALVERT'S LAGOON



500 metres

N
W
E
S

FIG. 1.3 CALVERT'S LAGOON: water level, salinity, total dissolved solids and pH.



in the formation of a dense floating weed mat over most of the lagoon, which separates warm surface water from relatively cool, shaded water below. Air temperatures recorded above the lagoon ranged from 7.8 to 23°C. Mean daily temperatures recorded at Hobart Airport (20 km north-east of the lagoon), are shown in Figure 1.4.

Total monthly rainfall recorded at Hobart Airport is shown in Figure 1.4. The annual rainfall at that meteorological station was 620 mm in 1976, 484 mm in 1977, 511 mm in 1978 and 351 mm in 1979.

The salinity of Calvert's Lagoon varied from 4.1 to 6.5‰, between August 1976 and June 1978 (Figure 1.3). This compares with the range of 6.4 to 10.6‰ recorded in 1970 (Smith, 1971), when the level of the lagoon was much lower. Total dissolved solids (TDS), varied from 4.9 to 7.2‰, between August 1977 and October 1978 (Figure 1.3). This compares with previous TDS records of 3.5 to 4.3‰ (Williams, personal communication in Bayly 1964, p.236); 6.8‰ in 1965 (McKenzie, 1966), and 12.5‰ in 1967 (Yasin, 1967). The concentration of suspended solids varied from only <0.5 to 16 mg/L. The water was always alkaline, with pH values varying from 8.2 to 9.7 during the present study, and from 8.4 to 10.2 in 1970 (Smith, 1971). The cationic proportions and dominance order of the lagoon water were very similar to seawater, however the anionic composition differed from seawater, with HCO_3^- and $CO_3^=$ being dominant over $SO_4^=$ (Table 1.3). The composition of water samples collected during the study are shown in Table 1.2. Small amounts of $PO_4^=$ (never more than 0.02 mg/L), were present, and the amount of dissolved silica varied from 0.6 to 9.1 mg/L.

1.2.2 Fauna

A variety of aquatic invertebrates have been recorded in Calvert's Lagoon. The ostracod *Mytilocypris tasmanica* is very abundant, and single specimens of 2 other ostracods, *Gomphothycere* sp. and *Ilyocypris* sp. were identified in a sample collected by McKenzie (1966). The copepods

FIG. 1.4 CALVERT'S LAGOON: temperature and rainfall

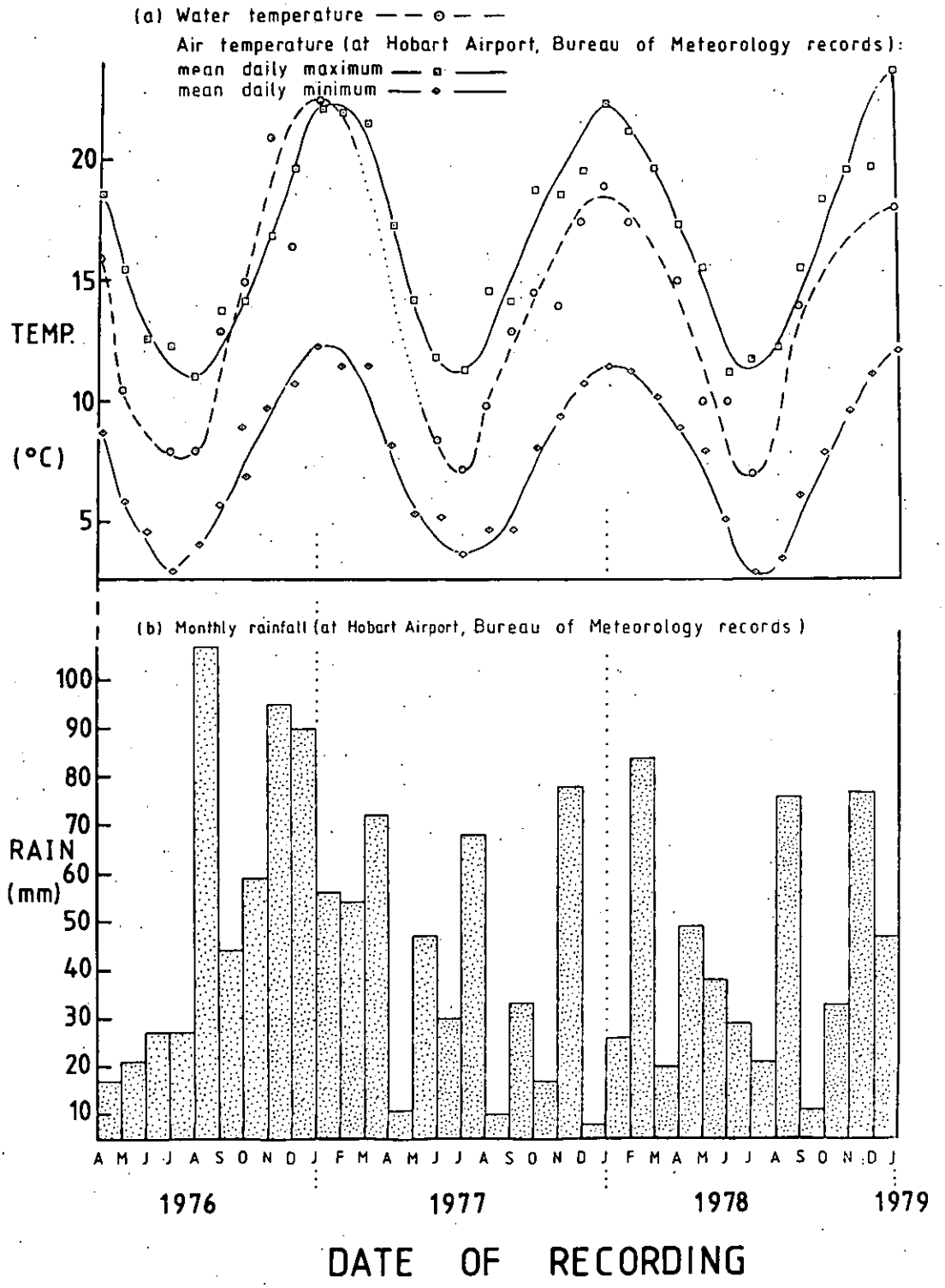


TABLE 1.2 The ionic composition of water in Calvert's Lagoon, (mg/L), from October 1977 to October 1978.

Month	Year	Cations				Anions			
		Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	$SO_4^=$	$CO_3^=$	HCO_3^-
October	1977	1520	52	160	68	2620	157	80	-
November	1977	1620	56	172	66	2800	159	146	-
December	1977	-	-	-	-	-	-	-	-
January	1978	1840	65	183	46	3240	190	192	114
February	1978	1700	70	168	88	3350	190	215	69
March	1978	2080	70	204	60	3630	220	190	-
April	1978	2040	70	197	76	3750	226	164	-
May	1978	1760	58	193	70	3530	212	190	-
June	1978	1880	62	186	92	3690	230	148	162
July	1978	1900	65	206	31	3590	220	117	-
August	1978	1520	52	181	54	3110	192	128	-
September	1978	1640	56	-	-	3260	205	184	-
October	1978	1680	56	199	56	3430	215	224	-
	n	12	12	11	11	12	12	12	3
	average	1765	61	186	64	3333	201	165	115

TABLE 1.3 The average ionic composition and ionic proportions of water in Calvert's Lagoon from October 1977 to October 1978, compared with 'World Average Fresh Water' and 'Average Ocean Water' (Bayly and Williams, 1973).

	Fresh Water		Calvert's Lagoon		Sea Water	
	mg/L	%	mg/L	%	mg/L	%
Cations						
Na^+	8	4.5	1765	30.0	10810	30.7
K^+	3	1.7	61	1.0	390	1.1
Mg^{++}	5	2.8	186	3.2	1300	3.7
Ca^{++}	30	16.9	64	1.1	410	1.2
Anions						
Cl^-	8	4.5	3333	56.6	19440	55.2
$SO_{4=}$	18	10.2	201	3.4	2710	7.7
CO_3^-	-	-	165	2.8	-	-
HCO_3^-	105	59.3	115	2.0	140	0.4
Salinity	177		5890		35200	

Boeckella triarticulata and *Microcyclops arnaudi* were recorded by McKenzie (1966), and Yasin (1967), and were found during the present study. Periodic blooms occur in the population of cladocerans, *Daphnia* sp.. The amphipod *Austrochiltonia australis* is very abundant. A bright red water mite, *Hydrachna* sp., is the only aquatic arachnid present.

Adults and juveniles of several insect orders are represented in the aquatic community. Large numbers of collembolans sometimes gather on the water surface. Case-bearing caddis fly larvae (Trichoptera), of various leptocerid species occur in large numbers on the lagoon bottom. 'Water boatmen', corixids and notonectids, are common, as are the nymphs of damsel flies, including *Austrolestes annulosus* (Lestidae). Dipteran larvae, including red chironomids, occur in the lagoon benthos.

The snail, *Coxiella badgerensis*, is found in great numbers throughout the year. Empty shells of the gastropods *Roblinella* sp. and *Physastra gibbosa* occasionally occur on the lagoon shore, however the only live mollusc found in the lagoon during the present study, other than *C. badgerensis*, was a single specimen of *P. gibbosa*. This snail was collected at Site 4, not far from a freshwater dam that adjoins the lagoon. *P. gibbosa* is only known from freshwater habitats, and this specimen had probably been recently transferred from the dam by birds, or washed into the lagoon by rain.

As well as trematodes, *C. badgerensis* also occasionally harbours nematodes, and a dipteran larva (possibly a chironomid). The latter occurs in the mantle cavity. Amphipods harbour cysts of 2, and ostracods one, trematode species. Amphipods are also rarely infected by the cysticercoid of a cestode, and ostracods are infected by the cysticercoid of a different species (Figure 1.5). Two out of 50 adult ostracods dissected in May 1978 were infected, one with 3 and one with 8 cysticercoids. The adults of the cestodes infecting amphipods and ostracods are believed to infect water birds at the lagoon.

FIG. 1.5 Larval tapeworms

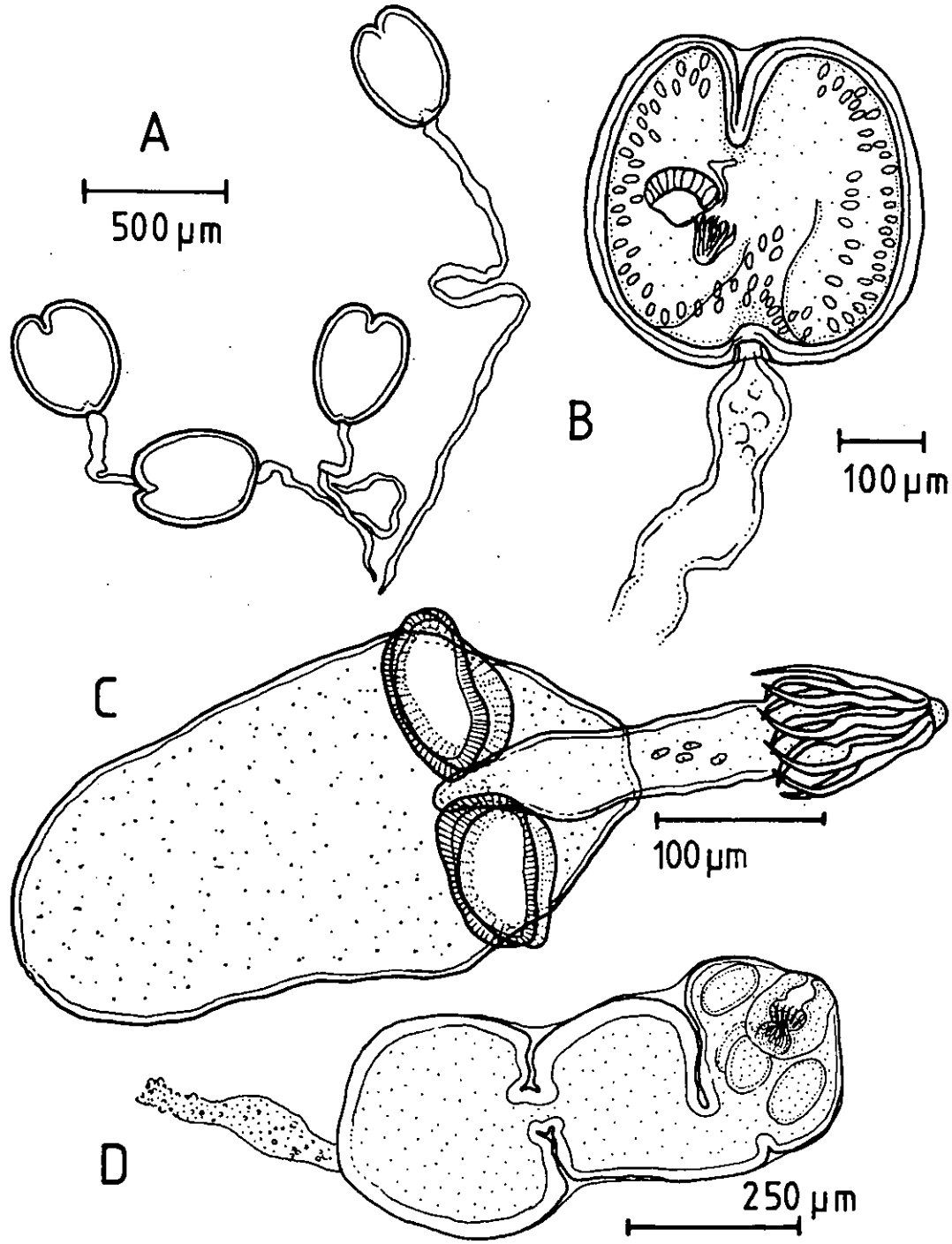


FIGURE 1.5 A, four cysticercoids infecting the ostracod *Mytilocypris tasmanica;* B, detail of one cysticercoid, from A; C, scolex of cestode infecting *M. tasmanica* - after incubation of the cysticercoid *in vitro* in 0.3% pancreatin and 0.1% sodium taurocholate in Hank's saline at 41°C for 1 hour; D, cysticercoid infecting the amphipod *Austrochiltonia australis*.

TABLE 1.4 Birds observed at Calvert's Lagoon

Family	Species		*Status
Accipitridae	Marsh harrier	*Circus aeruginosus*	B,M
Anatidae	Australasian shoveller	*Anas rhynchotis*	B,N
	Black duck (Bd)	*Anas superciliosa*	B,N
	Chestnut teal (Ct)	*Anas castanea*	B,N
	Musk duck (Md)	*Biziura lobata*	B
	Black swan (Bs)	*Cygnus atratus*	B,N
Ardeidae	White-faced heron (Wfh)	*Ardea novaehollandiae*	B
Charadriidae	Black-fronted dotterel	*Charadrius melanops*	B,N
	Hooded dotterel	*Charadrius rubricollis*	B
	Red-capped dotterel (Rcd)	*Charadrius ruficapillus*	B
	Masked lapwing (Ml)	*Vanellus miles novaehollandiae*	B
Corvidae	Forest raven	*Corvus tasmanicus*	B,N
Ephthianuridae	White-fronted chat	*Ephthianura albifrons*	B,N
Falconidae	Brown falcon (Bf)	*Falco berigora*	B
Haematopodidae	Pied oystercatcher (Po)	*Haematopus longirostris*	B
Hirundinidae	Tree martin	*Cecropis nigricans*	B,M
Laridae	Silver gull	*Larus novaehollandiae*	B
Phalacrocoracidae	Great cormorant (Gc)	*Phalacrocorax carbo*	B
Podicipedidae	Hoary-headed grebe (Hhg)	*Poliocephalus poliocephalus*	B,N
Rallidae	Eurasian coot (C)	*Fulica atra*	B,M(?),N
	Tasmanian native hen	*Gallinula mortierii*	B

(* from Thomas (1979): B = breeds in Tasmania; M = migrant wintering on the Australian mainland; N = nomadic within Tasmania.)

The only vertebrates at the lagoon, apart from occasional visits by humans, (viz. anglers and surfers), were fish and birds. Brown trout, *Salmo trutta*, that were reintroduced in 1975, are the only fish recorded in Calvert's Lagoon. The survival of these fish, since a drastic drop in the water level in 1979, is considered unlikely. A variety of birds feed and breed at the lagoon during the year, with hoary-headed grebes being the most common long-term residents. Twelve bird species were recorded from June 1977 to June 1978 (Figure 1.6). At other times, shovellers, hooded dotterels, black-fronted dotterels, white-fronted chats and silver gulls, have also been seen, floating on the water, or walking on the shore of the lagoon. In summer, dotterels are able to walk on the surface of the floating weed mat. Native hens, ravens, marsh harriers and tree martins occur in the environs of the lagoon. The full list of bird species recorded at Calvert's Lagoon is shown in Table 1.4.

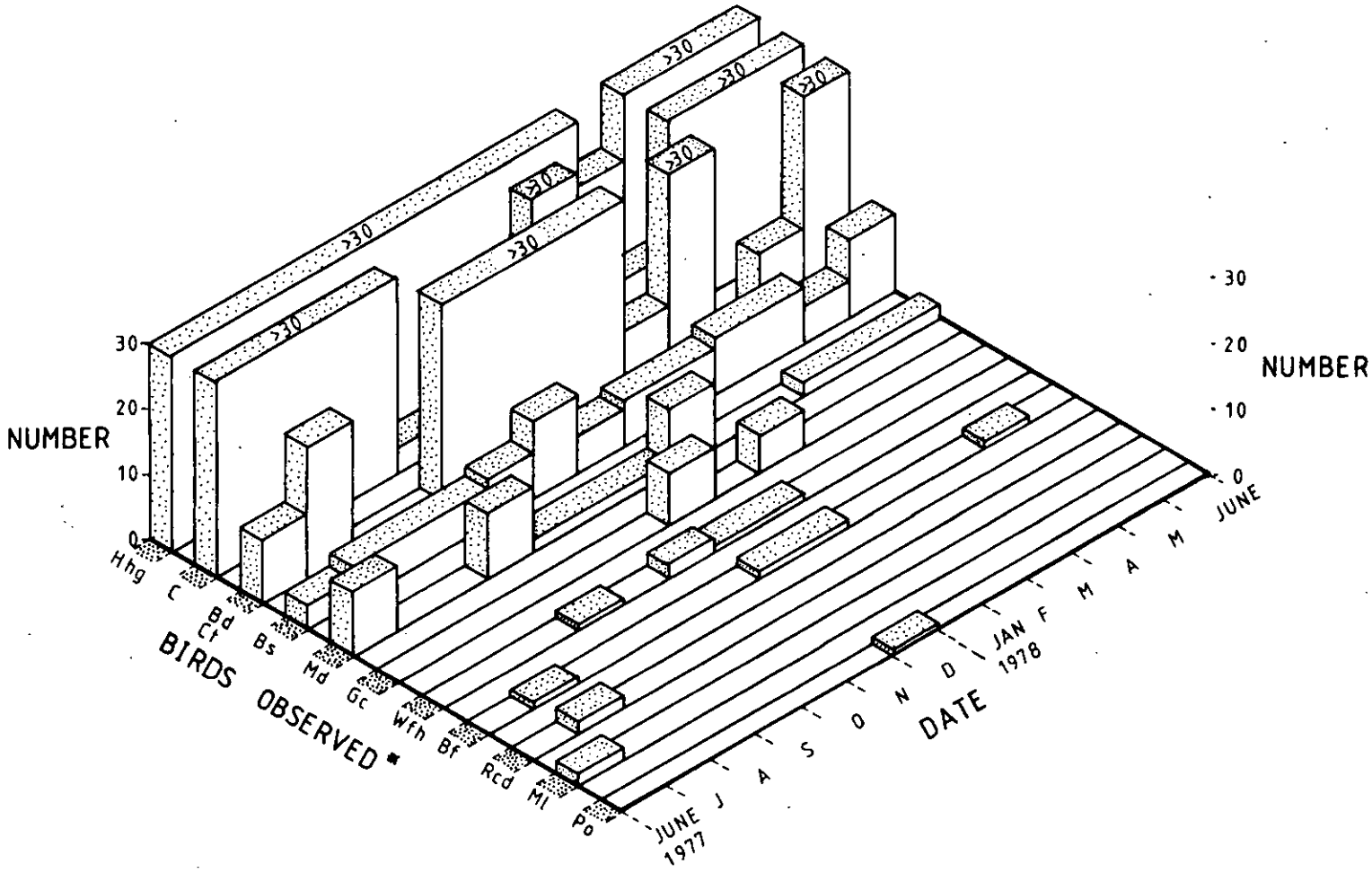
1.2.3 Flora

The lagoon is surrounded by cleared pastures to the north and west, and coastal scrub on sand dunes to the south and east. The aquatic community is 'closed' (i.e. about 80% of the sandy-silty bed supports plant growth). The angiosperms *Ruppia maritima* and *Lepilaena* spp., and alga *Lamprothamnium* sp., are the dominant hydrophytes. *Myriophyllum elatinoides* and *Triglochin procera* occur in freshwater ponds around the lagoon. Sedges, *Gahnia trifida* and *Schoenus intens*, backed by *Acacia verticellata* scrub, occur on the western and northern side, with *Stipa stipoides* and *Scirpus nodosus* on dune sedgeland on the southern and eastern sides.

1.2.4 Discussion

Seasonal changes in the level of Calvert's Lagoon are accompanied by changes in the physico-chemical properties of the lagoon water. As

FIG. 1.6 ABUNDANCE OF BIRDS OBSERVED AT CALVERT'S LAGOON, (JUNE 1977 — JUNE 1978)



(* initials of bird species shown in Table 1.4)

the level increases, the pH, salinity and TDS, decrease, and vice versa. Although the salinity of the lagoon varies, the percentage change in salinity, compared with the minimum concentration during the period of study, was only 59. The mean percentage change for 6 saline, inland waters studied in Victoria, was 330 (Bayly and Williams, 1966). The ionic composition of the lagoon indicates that, like most Tasmanian athalassic waters, ions are mainly derived from precipitation, with HCO_3^- + $CO_3^=$ enrichment from geochemical processes (Tyler, 1974). There is an inverse relationship between the amount of chloride in rain and distance from the ocean (Hutton and Leslie, 1958). Calvert's Lagoon is close to the ocean, and is probably often subject to direct sea spray.

Death of brown trout in the lagoon in 1963 was attributed to high pH (Inland Fisheries Commission Annual Report, 1967; 1969). However, fish grew well in the lagoon from November 1975, at least until June 1978, when pH was generally high (i.e. about 9), reaching a maximum of 9.7 in April 1978. High alkalinity may result from other changes in the lagoon, that are more directly harmful to the fish population. PH values of 9.0 to 11.0 may be caused in inland water bodies by temporary displacement of the normal equilibrium because of intensive photosynthetic activity (Bayly and Williams, 1973). High pH in Calvert's Lagoon occurs when the level is low, and the water becomes clogged by rapid plant growth and decaying organic matter. At such times it seems likely that pH is just one of several factors, such as low oxygen tension and rapid and large temperature fluctuations, that would be deleterious to fish. These inevitable changes in a shallow lagoon mean that in the long-term, Calvert's Lagoon will only be intermittently suitable as a trout fishery.

In contrast to trout, the natural biota is well adapted to the seasonal and long-term physico-chemical fluctuations of a shallow brackish lagoon. Many of the animals are known to be extremely euryhaline. The salinity tolerances of some are shown in Table 1.5. *Coxiella striata*, which may be conspecific with *C. badgerensis* (Smith and Kershaw, 1979),

is included in the table.

TABLE 1.5 Salinity tolerances in saline athalassic waters of south-east Australia, of some invertebrates inhabiting Calvert's Lagoon (from Bayly and Williams, 1973, Tables 10.2 and 10.3).

	Species	Salinity Range (‰)
Ostracoda		
	Mytilocypris	fresh - 36
Copepoda		
	Boeckella triarticulata	fresh - 22
	Microcyclops arnaudi	6 - 93
Amphipoda		
	Austrochiltonia	fresh - 29
Insecta		
	Leptoceridae spp. (Trichoptera)	21 - 62
Mollusca		
	Coxiella striata (Gastropoda)	25 - 112

The dominant plants *Ruppia maritima* and *Lepilaena* spp. are characteristic of moderately saline waters. *Ruppia maritima* can live at a salinity of about 56‰ (Bayly and Williams, 1973).

The distribution of amphipods within the lagoon varied depending on environmental conditions. When water level was high, particularly in winter, amphipods were widely dispersed, right into the shallow margins. During hot weather, however, when the level was low, amphipods were absent from the margins, and could only be found in the middle of the lagoon, near the bottom. Yasin (1967) sampled aquatic invertebrates from the edge of the lagoon, during a period when the level was low, and found no amphipods. The salinity at the time was well within the tolerance limits of the species. Hence, 'migration' of amphipods within the lagoon appears to be a response to unfavourably high temperatures, and/or low oxygen tension in the shallows during periods of hot weather.

1.3 Ecological Comparison of Tasmanian Coastal Lagoons

The geographical range of a trematode is limited by the distribution of its hosts. The life-cycle can only be completed at locations where

conditions exist that are suitable for all of the hosts. Specificity of trematodes is generally greatest at the primary intermediate host level, and progressively less at the second intermediate and definitive host levels. Consequently the geographical distribution of trematodes developing in *Coxiella badgerensis* at Calvert's Lagoon is primarily determined by the distribution of this snail species. A preliminary survey of coastal lagoons along the east and north-east coasts of Tasmania has shown that a number of them are inhabited by *C. badgerensis*.

Most of the lagoons that never open to the sea were found to be fresh (salinity <2‰), and inhabited by one or 2 snail species (Table 1.6). All were inhabited by *Physastra gibbosa*, and most were also inhabited by *Rivisessor gunni*. The most concentrated of these waters, Rushy Lagoon, (salinity 1.8‰), was inhabited by *P. gibbosa* and *Potomopyrgus niger*. The flora of these freshwater lagoons was quite distinct from that of Calvert's Lagoon. They characteristically supported the growth of *Triglochin procera*, and other common dominants were *Myriophyllum elatinoides*, *Eleocharis sphacelata* and *Baumea arthrophylla*.

A small percentage of lagoons that never open to the sea were brackish, ranging in salinity from 3.1 to 15.6‰. All were inhabited by *Coxiella badgerensis* (Table 1.6). These lagoons were ecologically similar to Calvert's Lagoon, being separated from the sea by sand dunes, and sharing a similar biota. They were all dominated by one or more of the salt-tolerant hydrophytes *Ruppia maritima*, *Lepilaena* spp. and *Lamprothamnium* sp.

The salinity of lagoons that open periodically to the sea ranged from 16.2 to 37.8‰, their salinity being a function of the interval between marine incursions, and the balance between rainfall and evaporation in the area. The biota of these waters included many typically estuarine species (Table 1.6). Those that frequently open to the sea, such as Henderson's Lagoon (salinity 37.8‰), and Lisdillon Lagoon (salinity 31.6‰), were inhabited by the marine snail *Salinator fragilis*.

TABLE 1.6 The salinity, snails and dominant plants of some coastal lagoons (a) that never open to the sea, and (b) that periodically open to the sea.

Lagoon No.	Name	Salinity (‰)	pH	Snails	Dominant plants	Area (ha)
(a)	Freshwater					
43	Rushy	1.8	7.8	Pg,Pn	Ba,Tp	500
24	Big Waterhouse	1.4	9.1	Pg,Rg	Es,Me,Tp	130
30	Big Punchbowl	1.4	8.2	Pg,Rg	Ba,Ll	200
23	Little Waterhouse	1.0	8.3	Pg	Lc,Me	10
38	Guards	1.0	-	Pg,Rg	-	5
47	Gibbs	0.6	6.2	Pg	Tp	10
33	Hazards	0.5	6.5	Pg,Rg	Tp	30
37	Rostrevor	0.5	-	Pg	Es,Tp	50
25	Blackmans	0.3	7.6	Pg,Rg	Me,Tp	28
	Brackish					
19	N of Tregaron	15.6	8.5	Cb	Rm	11
44	Calvert's	5.9	9.1	Cb	La,Le,Rm	44
41	Sloping	5.0	9.3	Cb	Le,Rm	40
18	NE of Tregaron	3.1	8.5	Cb,Pg	Rm	0.5
22	Little Musselroe	-	-	Cb	Rm	2
21	S of Tregaron	-	-	Cb	Rm	10
40	Primrose Sands	-	-	Cb	Lc,Rm	10
46	Half Moon Bay	-	-	Cb	La,Rm	2
(b)	Saline					
27	Henderson	37.8	7.7	Sf	Zo	150
36	Lisdillon	31.6	-	Sf	Zo	40
26	Wrinklers	25.6	7.3	Pn	Le,Rm,Zo	16
35	Troyheleener	16.2	7.0	Cb,M,Pn Sf,Tr	Rm	1

Key to snails

Cb = *Coxiella badgerensis*; M = *Melanella* sp.; Pg = *Physastra gibbosa*; Pn = *Potomopyrgus niger*; Rg = *Rivisessor gunni*; Sf = *Salinator frgailis*; Tr = *Tatea rufilaris*

Key to plants

Ba = *Baumea arthrophylla*; Es = *Eleocharis sphacelata*; La = *Lamprothamnium* sp.; Lc = *Lepilaena cylindrocarpa*; Le = *Lepilaena* spp.; Ll = *Lepidosperma longitudinale*; Me = *Myriophyllum elatinoides*; Rm = *Ruppia maritima*; Tp = *Triglochin procera*; Zo = *Zostera* sp.

Troyheleener Lagoon (salinity 16.2‰), was inhabited by *Coxiella badgerensis*, as well as an assemblage of marine and estuarine snails; however *C. badgerensis* was not found in Wrinklers Lagoon (salinity 26‰), a medium sized lagoon inhabited by *Ruppia maritima* and *Lepilaena* spp..

The absence of *C. badgerensis* from Wrinklers Lagoon may reflect a lower salinity tolerance for this species than for *C. striata*, which inhabits hypersaline waters on the Australian mainland (Bayly and Williams, 1973). The absence, however, may be due to other factors, such as the ionic proportions of the lagoon water, the physical effect of marine incursions, or simply chance lack of colonization.

From this limited survey, the typical coastal habitat of *C. badgerensis* appears to be brackish lagoons, that never open to the sea, and that are separated from an exposed surf-beach by high sand dunes. They have a characteristic salt-tolerant flora, dominated by *Ruppia maritima*, *Lepilaena* spp., and *Lamprothamnium* sp.; and a euryhaline fauna, frequently including *Austrochiltonia australis* and *Mytilocypris tasmanica*.

A list of lagoons with the same dominant plants as Calvert's Lagoon was compiled from data collected for the 'Tasmanian Wetlands Survey' (Harwood and Kirkpatrick, in preparation). Some are known to be inhabited by *C. badgerensis*, viz. lagoons no. 18,19,21,22,40,41 and 46; however, the molluscan faunas of the others (Table 1.7) are unknown. All of the lagoons are located on the east and north-east coasts of Tasmania and the Bass Strait Islands (Figure 1.1). Those with conductivities close to the known range of *C. badgerensis*, are more likely to be inhabited by this snail, and therefore to be potential habitats for trematodes found at Calvert's Lagoon. This list of lagoons may be useful for further investigations of the distribution of *C. badgerensis* and its trematode parasites.

TABLE 1.7 Brackish and saline coastal lagoons that have a similar flora to Calvert's Lagoon, and which may be habitats for *Coxiella badgerensis* (from data collected for the 'Tasmanian Wetlands Survey' by Harwood and Kirkpatrick).

Lagoon No.	Name	Dominant plants	Conductivity (µmhos/cm)	Area (ha)
1	Big Lake	Rm, Le	-	80
2	Singletons	Rm, La	-	22
3	Sticks	Rm, La	'high'	22
4	Second Saltpan	Rm, Lc	'high'	6
5	Logan	Lc, La	-	700
6	Apple Orchard Pt.	Lc	-	0.5
7	nr. Little Creek	Lc	-	1
8	nr. Little Creek	Lc	3,000	15
9	nr. Little Creek	Lc	-	23
10	Crystal	Lc, La	-	50
11	Kangaroo Bay	Lc, La	'brackish'	1
12	Sandy	Lc	-	15
13	E of Sandy	Lc	5,000	2
14	nr. Black Point	Lc	7,200	0.5
15	Blue Hills	Lc, La	8,000	2
16	Cape Portland	Rm, La	-	5
17	Cape Portland	Rm	-	1
20	NW of Tregaron	Rm, Le	44,000	5
28	Templestowe	Rm, Le	24,000	60
29	Old Mines	Lc	55,000	22
31	Freshwater	Rm	17,000	12
32	Shepherds Hut	Rm, La	50,000	0.5
34	Bryans	Rm, Le	5,500	25
39	Swan Salt	Rm, La	16,000	2
42	Clear	Rm	36,000	14
45	Opossum Bay	Rm	6,400	1

Key to plants

La = *Lamprothamnium* sp.; Lc = *Lepilaena cylindrocarpa;*

Le = *Lepilaena* spp.; Rm = *Ruppia maritima*

Conductivity (µmhos/cm)

Calvert's Lagoon (no.44) = 15,000; range of known *Coxiella badgerensis* habitats = 9,000 (lagoon no.46) ↔ 27,000 (lagoon no.19).

PART IV

THE PARASITES

Chapter 2 THE MICROPHALLIDAE TRAVASSOS, 1920

2.1 General Introduction

The family Microphallidae consists of about 180 species, and the life-cycles of about 40 of these have been elucidated. The adults are mainly intestinal parasites of birds, however they are more rarely parasitic in fish, amphibia, reptiles and mammals. Anseriform and charadriiform birds commonly serve as the vertebrate host, the migratory behaviour of many of these birds being an important factor in the cosmopolitan distribution of the family. In fact, the Microphallidae have been described as "migration parasites", because birds often become infested, and transmit them to new habitats, in the course of their migrations (Dogiel, 1962).

The typical microphallid life-cycle involves the development of asexual generations in a prosobranch mollusc (viz. hydrobiids and littorinids), and the liberation of free-swimming cercariae, which penetrate, and encyst in crustaceans (viz. amphipods, isopods and decapods). Most microphallids infect marine invertebrates, however some brackishwater, and freshwater hosts are known. The abundance and widespread distribution of these intermediate hosts has facilitated the establishment of microphallids along the migratory routes of their bird hosts. The geographic distribution of the genera of this family is reviewed by Deblock (1971). As this distribution reflects the degree of attention of naturalists in each region, the microphallid faunas of some regions of the world, such as Australia, appear to be relatively impoverished. The results of the present study indicate that the microphallid fauna of Tasmania is, in fact, diverse and abundant.

The intermediate invertebrate hosts of microphallids play a greater role in their biology than the definitive host (Deblock, 1971).

Adults survive for only a few days or a few weeks in the definitive host, and show low host specificity. For example, *Microphallus opacus* can reach maturity in freshwater fish, snakes, turtles, the opossum and the raccoon (Caveny and Etges, 1971); and *Atriophallophorus minutus* can produce eggs in ducks, mice and hamsters (Stunkard, 1958). By contrast, microphallids are relatively long-lived and specific with regard to their intermediate hosts. Intramolluscan development may take about 5 months (Bridgman, 1969), and intracrustacean development about 1 or 2 months (Etges, 1953; Bridgman, 1969). Hence, information on the biology of developmental stages, as well as adults, is essential to understand the ecological pressures on these parasites. Such information is also helpful in elucidating relationships within the family, particularly between species with morphologically similar adults.

The earliest record of a microphallid species in Australia was that of *Levinseniella howensis* (Johnston, 1916) in the Eastern golden plover, *Pluvialis dominica(= Charadrius dominicus)*, from Lord Howe Island. Since then 18 other species have been recorded from Australian birds and mammals (Johnston, 1948; Hickman, 1955; Deblock and Pearson, 1968a, 1968b, 1969 and 1970; Smith, 1974). Two microphallids were recorded in wild New Zealand ducks by Rind (1974).

The species recorded from the Australasian region are:

Super sub-family Maritrematidi

Endocotyle

E. incana (Deblock and Pearson, 1968b)

Maritrema

M. calvertensis (Smith, 1974, 1979)

M. eroliae (Deblock and Pearson, 1968b)

M. oocysta (Deblock and Pearson, 1968b)

M. ornithorhynchi (Hickman, 1955)

Pseudolevinseniella

P. anenteron (Deblock and Pearson, 1968b)

Super sub-family Gynaecotylidi

Basantisia

B. queenslandensis	(Deblock and Pearson, 1968a)

Gynaecotyla

G. brisbanensis	(Deblock and Pearson, 1968a)

Microphalloides

M. australiensis	(Deblock and Pearson, 1968a)

Super sub-family Microphallidi

Atriophallophorus

A. coxiellae	(Smith, 1974, 1979)

Levinseniella

L. howensis	(Johnston, 1916; Pearson and Deblock, 1979)
L. microovata	(Deblock and Pearson, 1970)
L. monodactyla	(Deblock and Pearson, 1970)
L. tasmaniae	(Smith, 1974, 1979)
?Levinseniella sp.	(Rind, 1974)

Microphallus

M. minus	(Deblock and Pearson, 1969)
M. minutus	(Deblock and Pearson, 1969)
M. papillornatus	(Deblock and Pearson, 1969)
M. vaginosus	(Deblock and Pearson, 1969)
Microphallus sp.	(Deblock and Pearson, 1969)
M. (Spelotrema) sp.	(Rind, 1974)

Although clues have been given to the possible life-cycles of some of these species, no previous study of a microphallid life-cycle in the Australasian region has been reported. The present study elucidates the life histories of three microphallids parasitic in Tasmanian fauna, and adds to the existing fund of knowledge on the biology of this distinctive family.

* * * * *

Super sub-family Maritrematidi (Nicoll, 1907)

Sub-family Maritrematinae Nicoll, 1907

Tribe Maritrematini (Nicoll, 1907)

Genus Maritrema Nicoll, 1907

2.2 *Maritrema calvertensis* Smith, 1974

2.2.1 Life-cycle

In 1970, during a study of developmental stages of trematodes infecting *Coxiella badgerensis* at Calvert's Lagoon, over 1,000 snails were dissected, and about 4% of these were found to be infected by sporocysts producing microphallid cercariae (Smith, 1971). During the same study, 3 new species of microphallid trematodes, *Maritrema calvertensis*, *Levinseniella tasmaniae* and *Atriophallophorus coxiellae*, were discovered in birds feeding at the lagoon (Smith, 1974). *M. calvertensis* infected the black-fronted dotterel, hooded dotterel and chestnut teal.

During the present study, investigations of other invertebrates in the lagoon has revealed that nearly all adults of the amphipod *Austrochiltonia australia*, and the ostracod, *Mytilocypris tasmanica*, are infected by trematode cysts. The amphipod harbours a 'large' and 'small' kind of metacercarial cyst, whereas the ostracod harbours metacercarial cysts of only one kind. The cysts in the ostracod are smaller than the 'small' kind of cyst in the amphipod; however, *in vitro* excystment and experimental infection of laboratory ducklings, has shown that both belong to *Maritrema calvertensis*. *In vitro* excystment and experimental infection of ducklings, has shown that the 'large' cyst in the amphipod belongs to *Levinseniella tasmaniae*.

The cercariae of *M. calvertensis* and *L. tasmaniae* develop in *C. badgerensis*, and then emerge and invade crustaceans in the lagoon. To distinguish these cercariae, naturally infected snails releasing microphallid cercariae were isolated in the laboratory in individual containers.

FIG. 2.1 *Maritrema calvertensis*

Life-cycle

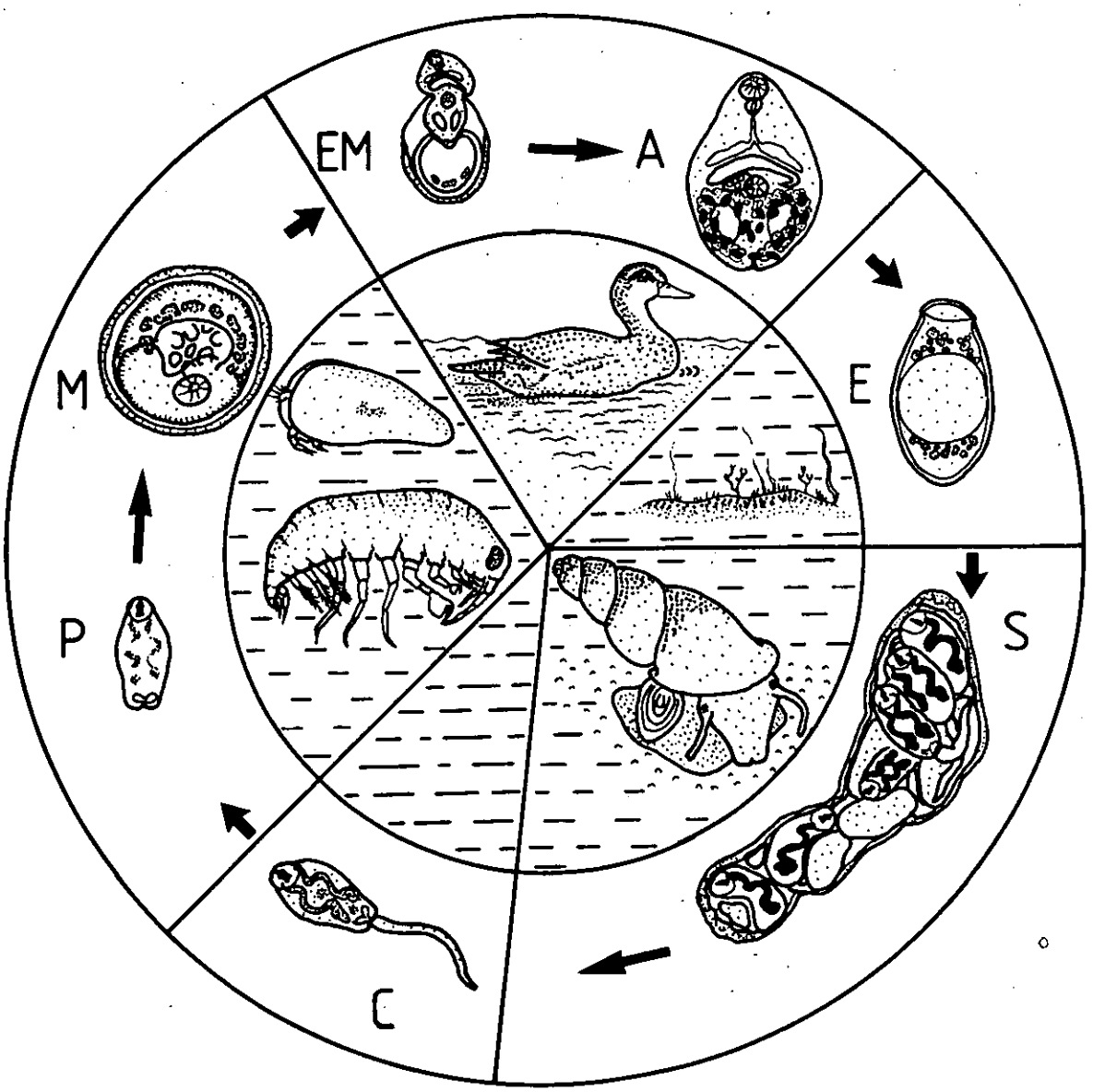


FIGURE 2.1 A, adult; E, egg; S, daughter sporocyst; C, cercaria; P, post-cercaria; M, metacercarial cyst; EM, excysting metacercaria.

Examination of cercariae shed from the same snail, and comparison with those from different snails, revealed many morphological, behavioural and ecological differences between the 2 trematode species. Both types of cercariae experimentally infect laboratory-bred amphipods, giving rise to easily distinguished metacercarial cysts. Intramolluscan developmental stages were identified by dissection of many snails found to be releasing cercariae of only one of the microphallid species.

Examination of various water birds from Calvert's Lagoon during the present study has extended the known vertebrate host range of *M. calvertensis* to include the hoary-headed grebe and black duck. Other birds feeding at the lagoon may also serve as hosts.

The life-cycle of *Maritrema calvertensis* is summarized in Figure 2.1. The primary intermediate host is the brackishwater hydrobiid, *Coxiella badgerensis*, which sheds cercariae capable of forming metacercarial cysts in the amphipod *Austrochiltonia australis*, and the ostracod *Mytilocypris tasmanica*. Birds become infected by adult flukes after ingesting these crustaceans at Calvert's Lagoon.

2.2.2 Adult (Figure 2.2)

The original description is based on ovigerous worms fixed under "slight coverslip pressure", taken from naturally infected dotterels and a chestnut teal (Smith, 1974). The species is named after the lagoon habitat of the invertebrate and bird hosts. A more comprehensive description is possible now that the life-cycle has been experimentally demonstrated, and live adults have been cultured *in vitro*. Dimensions of adults fixed in boiling 10% formol saline, without coverslip pressure, are given in Tables 2.1 and 2.3. The dimensions of the holotype of this species are also given, as these were not included in the original description.

Description:

Body dorsoventrally flattened, slightly concave ventrally.

FIG. 2.2 Maritrema calvertensis - adult

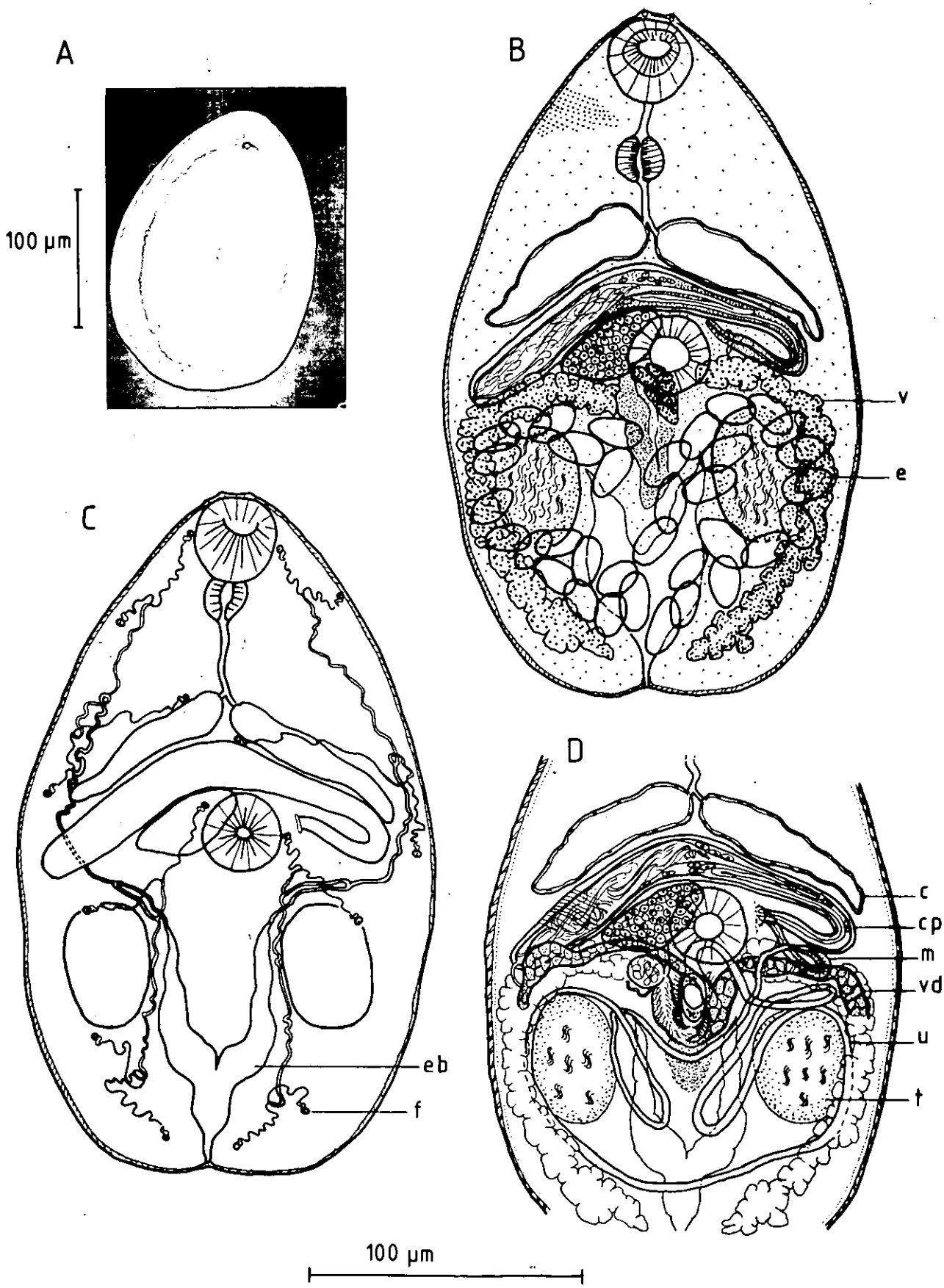


FIGURE 2.2 A, S.E.M. photograph of juvenile adult; B, gravid adult after 1,22 days in laboratory duckling, ventral view; C, excretory system, showing distribution of flame-cells; D, reproductive system. (c: caecum; cp: cirrus pouch; e: egg; eb: excretory bladder; f: flame-cell; m: metraterm; t: testis; u: uterus; v: vitellaria; vd: vitelline duct.)

TABLE 2.1 *Maritrema calvertensis*. Dimensions of excysted metacercariae from naturally infected amphipods (a), after 4 hours at 41°C; and dimensions of adults from experimentally infected ducklings: (b) after 1,22 days, and (c) after 5,19 days. The dimensions of the holotype of the species (an ovigerous adult) are also presented (d).

	(a)	(b)	(c)	(d)*
Sample size	20	10	20	1
Body length (BL)	180 (141 - 217)	200 (182 - 228)	206 (175 - 239)	194
Body width (BW)	118 (99 - 137)	135 (118 - 148)	149 (133 - 175)	141
Oral sucker length	22 (19 - 23)	23 (21 - 27)	23 (23 - 27)	21
Oral sucker width	23 (23 - 27)	26 (25 - 27)	27 (23 - 29)	25
Ventral sucker length	24 (23 - 27)	26 (23 - 27)	28 (23 - 30)	25
Ventral sucker width	23 (19 - 27)	26 (23 - 27)	29 (27 - 30)	27
Prepharynx length	9 (8 - 11)	6 (0 - 15)	4 (0 - 11)	6
Pharynx length	15	17 (15 - 19)	19 (17 - 21)	13
Pharynx width	11	17 (15 - 19)	16 (15 - 19)	15
Oesophagus length	8	8 (4 - 11)	5 (0 - 8)	11
L. caecum length	54 (49 - 57)	64 (53 - 80)	63 (57 - 68)	61
R. caecum length	55 (49 - 61)	59 (49 - 68)	63 (57 - 68)	66
Cirrus pouch length (CPL)	124 (103 - 137)	143 (103-156)	154 (137 - 186)	152
Cirrus pouch width	15 (13 - 17)	18 (17 - 19)	20 (17 - 23)	16
Cirrus pouch thickness	-	3.1 (1.9 - 3.8)	3.9 (2.9 - 4.8)	1.9
Seminal vesicle length	-	63 (49 - 84)	70 (61 - 76)	49
Seminal vesicle width	-	12 (10 - 13)	13 (11 - 15)	11
Ovary length	27 (27 - 30)	36 (29 - 38)	35 (30 - 40)	34
Ovary width	13 (11 - 15)	22 (17 - 27)	21 (15 - 25)	19
L. testis length	35 (23 - 42)	35 (27 - 42)	34 (27 - 38)	38
L. testis width	27 (19 - 30)	29 (27 - 30)	28 (23 - 34)	30
R. testis length	37 (27 - 42)	36 (30 - 38)	35 (30 - 38)	38
R. testis width	27 (19 - 30)	27 (27 - 30)	26 (23 - 30)	30
Roundness (BW/BL)	0.66	0.68	0.72	0.73
OS(l+w)/VS(l+w)	0.96	0.94	0.88	0.88
CPL/BL	0.69	0.72	0.75	0.78

(* fixed under coverslip pressure)

Shape varies from oval to pyriform, maximum width near middle of body. Small, comb-shaped spines with at least 4 teeth, embedded in sockets in outer tegument; arranged quincuncially, sometimes overlapping; over whole body. Round oral sucker subterminal-ventral; round ventral sucker equatorial, slightly sinistral. O.S.:V.S. ratio = 0.93 (0.88 - 0.96). Prepharynx short, length approximately equal to oesophagus. Pharynx barrel-shaped. Oesophageal bifurcation midway between suckers. Caeca diverge obtusely, extending 4/5ths distance to lateral body wall, contiguous to anterior border of cirrus pouch; sometimes overlapping; never extending posterior to cirrus pouch. Small genital pore near left border of ventral sucker. Genital atrium thin-walled, barely discernible. Symmetrical oval testes, posterolateral to ventral sucker; separated by arms of excretory bladder and "oogenotop" (Ebrahimzadeh, 1966). Sperm ducts unite dextrally near base of cirrus pouch. Cirrus pouch arcuate, forming wide upturned V between intestinal caeca and ventral sucker; median flexure of pouch anterior to ovary; distal half of pouch narrows posteriorly, recurves sharply to join genital atrium dorsally, from left side. Oblique muscle fibres discernible in thin pouch wall, which varies from 2 to 4 μ thick in proximal half. Common sperm duct enters elongate seminal vesicle, which occupies proximal half of cirrus pouch when fully distended. Seminal vesicle gives rise to narrow, thin-walled, convoluted ejaculatory duct. Evaginated cirrus filiform, glabrous, about 54μ long; sometimes bent sharply to enter metraterm of same worm. Prostate gland cell nuclei concentrated in distal half of cirrus pouch, around ejaculatory duct and seminal vesicle. Cirrus pouch length: body length = 0.68 (0.62 - 0.75). Dextral to median ovary partly overlies ventral sucker, contiguous with cirrus pouch; irregular in form, varying from oval to triangular. Oviduct descends from

middle of posterior ovary wall. Round seminal receptacle, Laurer's canal, branch off oviduct, which widens into ciliated J-shaped tract, "fertilization chamber", leading to ootype. Vitelline secretions discharged from vitelline reservoir at entrance to ootype. Mehlis' gland cell ducts open through lateral walls of ootype, which ascends towards ventral sucker. Eggs formed in ootype enter ascending loop of proximal uterus, which passes sinistrally to anterior of left testis, then forms large posteromedial loop before passing anteriorly around left testis, then crossing body posteriorly. Uterus passes around outside of right testis, forms posteromedial loop, then crosses body to join genital atrium sinistrally, ventral to cirrus pouch. Distal part of uterus slightly thickened to form weakly developed metraterm. Uterus contains many eggs (182 eggs in fluke after 17 days in laboratory duckling). Vitelline glands form 2 semi-circular brackets in posterior half of body; composed of numerous follicles; arise near stem of excretory bladder, extend anteriorly around testes. Vitelline ducts pass medially from anterior of testes, uniting to form longitudinal vitelline reservoir, posterior to ventral sucker. Arms of Y-shaped excretory bladder extend to anterior margins of testes. Flame-cell formula typical of Microphallidae, $2[(2+2) + (2+2)] = 16$.

Vertebrate hosts: *Anas castanea* (Eyton), *A. platyrhynchos* L. (experimental host), *A. superciliosa* Gmelin; *Charadrius melanops* Vieillot, *C. rubricollis* Gmelin; and *Poliocephalus poliocephalus* (Jardine and Selby).

Habitat: Mainly lower small intestine, also caeca and rectum.

Geographical location: Calvert's Lagoon, Tasmania.

Type material: Tasmanian Museum - holotype K250 (ringed and arrowed); paratypes, K250 (ringed but not arrowed), K249 (ringed), K251 (ringed).

Relationships:

Maritrema calvertensis is morphologically similar to *M. oocysta* (Lebour, 1907), and *M. sobolevi* Kurotsckin, 1962. *M. humile* Nicoll, 1907 has experimentally been shown to be a synonym of *M. oocysta* (Rothschild, 1942; Rothschild and Clay, 1952, p.204), and *M. innae* Leonov, 1958 is also considered to be a synonym of *M. oocysta*, (Deblock and Combes, 1965). *M. calvertensis* differs from these species in having a smaller, rounder body, a relatively large, thin-walled, cirrus pouch and larger eggs. The cirrus pouch of *M. calvertensis*, although variable in size and shape, is relatively longer and narrower than those of the other species, with conspicuously thinner walls. It is shaped like a wide, inverted V, with more or less equal limbs, whereas those of the other species are J-shaped.

Following the key to species of *Maritrema* constructed by Deblock (1971), *M. calvertensis* keys out with *M. oocysta* and *M. sobolevi*. The following modification would enable *M. calvertensis* to be distinguished from these species:

22. - Ratio of the length of cirrus pouch to body length greater than 3/5 .....22A

Ratio of the length of cirrus pouch to body length less than 3/5 .....22B

22A.- Body length, 129-239µ. O.S., 19 - 29µ diameter. V.S., 23 - 30µ diameter. O.S./V.S. = 0.88 - 0.96. Pharynx, 15 - 21 × 11 - 19µ. Oesophagus short. Caeca short, "présacculaires". Cirrus pouch, 91 - 186 × 11 - 23µ. Cirrus pouch/body length about 2/3. Evaginated filiform cirrus, about 54µ long. Median to dextral ovary. Uterus surrounding the testes. Weakly developed metraterm, about 40µ . Eggs, 18 - 23 × 10 - 13µ.

Cercariae develop in the brackishwater hydrobiid, *Coxiella badgerensis*. Metacercariae encyst in amphipods and

ostracods. Intestinal flukes in water birds (Anseriformes, Charadriiformes and Podicipediformes), in Australia, (Tasmania) ..... *M. calvertensis* Smith, 1974

22B.- Body length, 250 - 290µ. O.S., 33µ diameter. V.S., 40µ diameter. O.S./V.S. about 0.83. Pharynx, 19µ diameter. Oesophagus short. Caeca short, "présacculaires". Cirrus pouch, 165 × 30µ. Cirrus pouch/body length = 0.5. Evaginated filiform cirrus, 48 × 9µ. Ovary submedian dextral. Uterus surrounding the testes. Metraterm not described. Eggs, 17 - 18µ.

Metacercariae in amphipods (Pontogammarus). Adults parasitic in the digestive tube of marine mammals (Pinnipedes), in the Caspian Sea.....*M. sobolevi* Kurotsckin, 1962

Body length, 190 - 420µ, (average 250 - 300µ). O.S., 23 - 28µ. V.S., 23 - 28µ. O.S./V.S. = 1.00. Pharynx, 15 - 17µ. Oesophagus short. Caeca short, "présacculaires", 50 - 70µ. Cirrus pouch, 100µ. Cirrus pouch/body length = 0.50 - 0.33. Filiform, glabrous cirrus, 25 - 35µ long. Median ovary. Uterus surrounding the testes. Unobtrusive metraterm, 40µ. Eggs, 16 - 18µ.

Metacercariae encyst in hydrobiid molluscs. Adults parasitic in the digestive tube of birds, Charadriiformes of Eastern Europe (Black Sea) and Western Europe, Pelicaniformes and Podicipediformes of Australia; and of mammals (Rodentia) of Australia.....*M. oocysta* (Lebour, 1907)

syn.: *M. humile* Nicoll, 1907

M. innae Leonov, 1958

Biology:

To investigate the *in vivo* biology of the adult worms under controlled conditions, domestic ducklings were fed metacercariae, encysted in naturally infected amphipods, and, on one occasion, ostracods. The

birds were then sacrificed after different periods, ranging from 4 hours (0,4 days), to 24 days 21 hours (24,21 days), post infection.

Sixty-one percent (11/18) of exposed ducklings became infected, with from 4 to 130 flukes. The duckling exposed to cysts from *Mytilocypris tasmanica* was infected by 40 worms. The proportion of encysted metacercariae that were recovered from infected birds varied from 0 to about 60%. The longest recorded period of infection was 19,4 days. This is relatively long for microphallids, which are believed to be generally very short-lived in vertebrate hosts (Dogiel, 1962).

The adult flukes showed marked site specificity. The preferred habitat was from SI6 to SI8. After 4 and 46 hours, the worms were concentrated in SI6. After 5,19 and 17,5 days, they were concentrated in SI7, and after 19,4 days, they were in SI8. No flukes were recovered from a duck dissected 24,21 days post infection. The apparent posterior displacement of worms after 19,4 days, may reflect a loss of vigour, due to aging. The distribution of *M. calvertensis* in the alimentary tract, at different intervals after infection, is shown in Figure 2.3.

The size and sexual maturity of newly excysted metacercariae are quite variable, however about 20% of flukes had produced eggs after 4 hours in a laboratory duckling. After this period, all other flukes had phenolic egg-shell precursors present in the vitelline cells, however the vitelline ducts and reservoir were empty, and no mature sperm were present. After 24 hours in ducklings, all flukes were producing eggs. The number of eggs contained in the uterus varied with the age of the fluke, as shown in Table 2.2, increasing gradually up to 17 days. The decreased number of eggs in flukes after 19,4 days may reflect a decreased rate of egg production in adults of this age. This observation is consistent with other evidence indicating that the longevity of *M. calvertensis* is little more than 19 days.

FIG. 2.3 Maritrema calvertensis. Distribution of adults in the gut of laboratory ducklings, after different periods of infection, (n = number of adults)

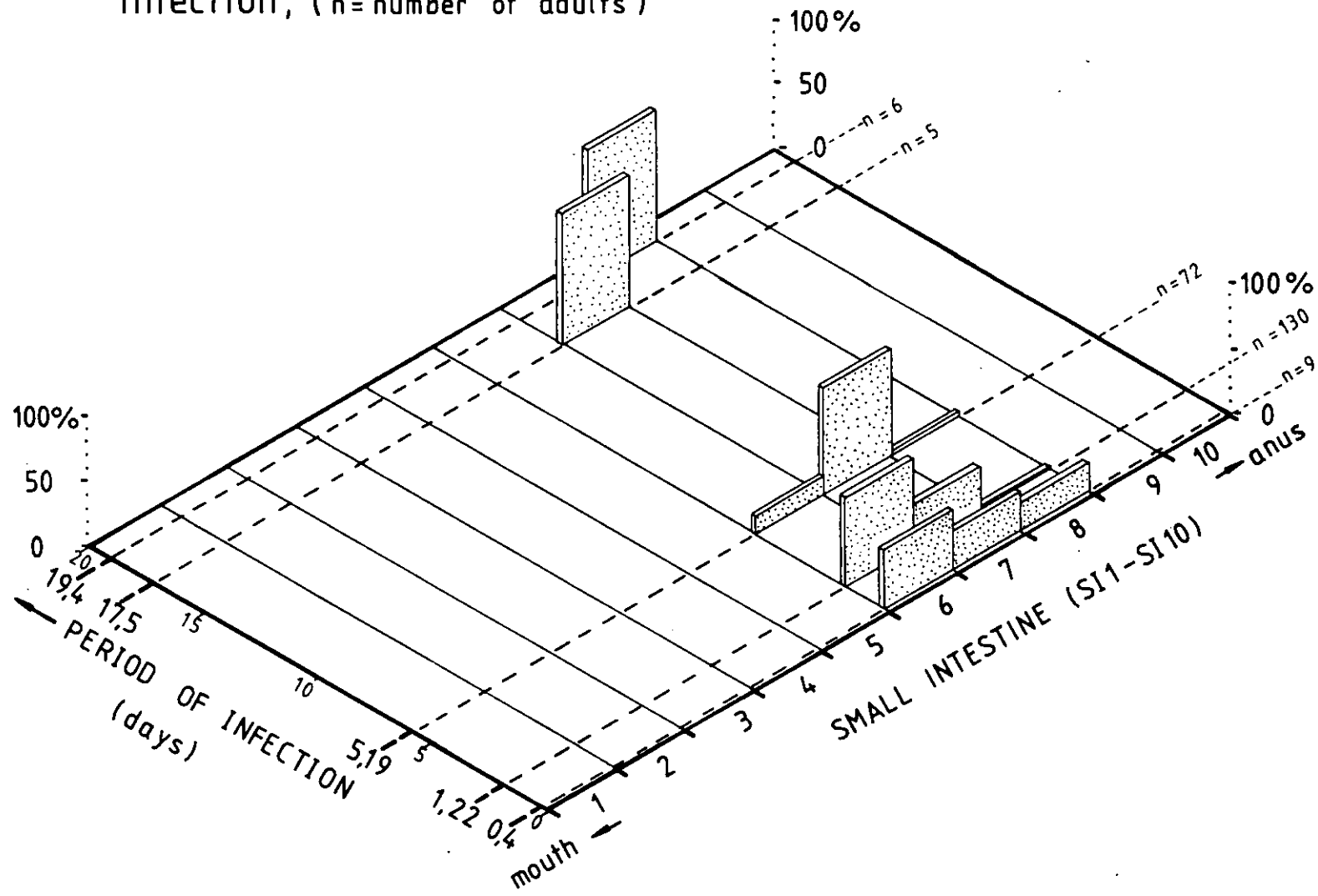


TABLE 2.2 *Maritrema calvertensis*. The number of uterine eggs in adults recovered from experimentally infected ducklings, after different periods of infection.

Period of Infection (days)	Sample size (no. of flukes)	Number of intra-uterine eggs Mean	S.D.	Range
1, 0	10	18.3	7.1	2 - 27
1, 22	10	49.8	7.4	36 - 61
5, 19	10	62.8	10.6	51 - 79
8, 0	2	115.5	14.9	105 - 126
11, 0	5	132.6	23.8	100 - 156
17, 5	4	169.3	19.7	140 - 182
19, 4	4	72.3	13.0	58 - 89

In Table 2.1, morphometric data of flukes taken from experimentally infected ducklings are compared with metacercariae excysted *in vitro*. The flukes, recovered after 1,22 and 5,19 days, showed very little body growth, if any, after excystment in the laboratory host; however, there were increases in the size of the ovary and suckers, and the OS:VS ratio changed gradually from 0.96 to 0.88. The length of the cirrus pouch increased, and the ratio of cirrus pouch length to body length increased from 0.69 to 0.75.

M. calvertensis naturally infects a variety of water birds, however measurements of fixed, unflattened specimens are only available from the hoary-headed grebe, and these are shown in Table 2.3. The slight difference in size between ovigerous specimens and those without eggs, indicates that very little growth occurs in this host. These flukes are significantly smaller than those taken from laboratory ducklings, and also smaller than metacercariae excysted *in vitro* (Table 2.1). Many factors, such as the parasite population density, the host's diet, the physico-chemical characteristics of the host's gut, and the time between death of the host and fixation of the parasites, could contribute to the observed size differences. Although body size differs, the relative dimensions of the organs, the ratio of suckers, relative size of the cirrus pouch and the size of the eggs, do not differ significantly in flukes from the

laboratory ducklings and wild grebes.

TABLE 2.3 *Maritrema calvertensis*. Dimensions of (a) ovigerous, (b) non-ovigerous adults recovered from naturally infected hoary-headed grebes.

	(a)	(b)
Sample size	20	20
Body length (BL)	157 (129 - 175)	156 (129 - 175)
Body width (BW)	122 (99 - 144)	122 (95 - 133)
Oral sucker length	22 (19 - 25)	
Oral sucker width	24 (23 - 27)	
Ventral sucker length	24 (23 - 27)	
Ventral sucker width	25 (23 - 27)	
Prepharynx length	2 (0 - 4)	
Pharynx length	16 (15 - 19)	
Pharynx width	13 (11 - 15)	
Oesophagus length	13 (10 - 19)	
L. caecum length	52 (42 - 61)	
R. caecum length	51 (46 - 61)	
Cirrus pouch length (CPL)	98 (91 - 103)	
Cirrus pouch width	15 (11 - 17)	
Cirrus pouch thickness	2.3 (1.9 - 2.9)	
Seminal vesicle length	43 (38 - 46)	
Seminal vesicle width	10 (8 - 11)	
Ovary length	33 (27 - 38)	
Ovary width	16 (13 - 19)	
L. testis length	27 (23 - 30)	
L. testis width	24 (23 - 30)	
R. testis length	29 (23 - 28)	
R. testis width	24 (23 - 27)	
Roundness (BW/BL)	0.78	0.72
OS (l+w)/VS(l+w)	0.94	
CPL/BL	0.62	

The numbers of eggs in flukes from some naturally infected hosts are shown in Table 2.4. If the rate of egg-production by *M. calvertensis* is not significantly different in these natural hosts and laboratory ducklings, then the results indicate that nearly all flukes resident in the wild birds were only 1 or 2 days old, with very few older flukes. As hoary-headed grebes are long-term residents at Calvert's Lagoon, it appears that although *M. calvertensis* is capable of living for 19 days in laboratory ducklings maintained under controlled conditions, it lives for much shorter periods in its natural bird hosts.

TABLE 2.4 *Maritrema calvertensis*. The number of uterine eggs in adults recovered from some naturally infected water birds, from Calvert's Lagoon.

Host	Sample size (no. of flukes)	Number of intra-uterine eggs Mean	S.D.	Range
Hoary-headed grebe, No.1.	15	36.2	22.6	2 - 88
Hoary-headed grebe, No.2.	10	27.5	9.2	13 - 44
Chestnut teal	10	5.9	6.2	1 - 20

2.2.3 Egg

The egg is operculate, oval to urn-shaped (Figure 2.1). The egg-shell is of uniform thickness, except where it thins around the rim of the operculum. Transparent and colourless when first formed, it becomes tanned, as the egg passes through the uterus. There is a gradation in colouring from the eggs on the left side of the body, which are generally clear, to those on the right, which are yellow.

A large ovum, 8μ diameter, can be seen in the middle of newly formed eggs, with scattered vitelline granules at either end. The ovum divides to form the developing miracidial embryo while in the uterus, however, the miracidium does not complete development *in utero*. Eggs of microphallids apparently spend a period free in the lagoon before the miracidium is mature and ready to hatch. Bridgman (1969), found that the eggs of *Microphallus choanophallus* were not fully developed until incubated in tapwater and saline for 10 days. The eggs of microphallids do not hatch until ingested by a suitable snail host (Stunkard, 1957; Deblock, 1971).

Preliminary attempts to infect laboratory-bred *Coxiella badgerensis* with *Maritrema calvertensis* failed. Adult flukes, from experimentally infected ducklings were crudely dissected to release their eggs, in small petri dishes, each containing 3 snails. Snails dissected up to 1 month after exposure were uninfected. Bridgman (1969), successfully infected snails with *Microphallus choanophallus* by adding large numbers of ovigerous

flukes to aquaria containing 100 to 200 uninfected snails. After 5 months, 50% of the surviving snails were infected with sporocysts containing cercariae.

The dimensions of eggs in flukes from experimentally infected ducklings, and a naturally infected hoary-headed grebe, are shown in Table 2.5. There is little variation in egg size between the different hosts.

TABLE 2.5 *Maritrema calvertensis*. The dimensions of eggs in flukes from laboratory ducklings, and from a wild hoary-headed grebe.

Host	P.I. (days)	No. eggs	Length	Width
Duckling, No. 1.	1, 22	20	20 (19 - 23)	11 (10 - 13)
Duckling, No. 2.	5, 19	20	21 (19 - 21)	11 (10 - 11)
Hoary-headed grebe	-	20	19 (18 - 22)	11 (10 - 11)

2.2.4 Sporocyst (Figure 2.4)

There are at least two generations of sporocysts. No mother sporocysts have been found, but when daughter sporocysts are present in wild snails they are always in large numbers, up to about 550, distributed throughout the viscera. Two generations of sporocysts have been experimentally demonstrated in *Microphallus similis* (Stunkard, 1957); however, according to James (1969), daughter sporocysts of *M. similis* produce further generations of sporocysts, until the snail host's visceral haemocoel is packed with up to 3,000 sporocysts producing cercariae. Similar multiplication of daughter sporocysts may occur in all microphallids. Daughter sporocysts of *Maritrema calvertensis* are concentrated in the gonad, and around the hepatopancreatic tubules, but extend anteriorly to the kidney region. In female snails they are often massed in the pallial oviduct, adjacent to the mantle cavity. The sporocysts detach readily from snail tissue, and tumble free when infected snails are dissected. Young sporocysts are almost spherical, and contain

only germ balls, whereas older ones are much larger and very elongate, and contain cercariae in all stages of development. Some sporocysts have 1 or 2 fully developed active cercariae surrounded by inactive cercarial embryos and germ balls. The largest sporocysts, which lack a birth pore, have up to 25 very active, mature cercariae, and few germ balls. Some large sporocysts appear to have collapsed along part of their length, and contain a few mature cercariae and some cercarial embryos. These are believed to be old sporocysts, from which many cercariae have escaped.

Dimensions of live, and fixed, daughter sporocysts are given in Table 2.6. Computations are based on 20 of the larger sporocysts, and 20 taken at random, from snails that were releasing only cercariae of *M. calvertensis*.

TABLE 2.6 *Maritrema calvertensis*. Dimensions of live and fixed sporocysts. The 5 largest, and 5 selected at random, were taken from 4 different snails.

		No.	Length	Width
Largest	Live	20	415 (304 - 551)	139 (99 - 175)
	Fixed	20	336 (266 - 403)	138 (72 - 281)
Random	Live	20	306 (194 - 410)	140 (91 - 334)
	Fixed	20	303 (190 - 376)	128 (76 - 247)

2.2.5 Cercaria

Morphology and anatomy:

It is a small, monostome, leptocercous, xiphidiocercaria, typical of the family Microphallidae (Figure 2.4). The body and tail are very contractile. Dimensions of cercariae, measured shortly after emerging from naturally infected snails, are shown in Table 2.7. The oral sucker is subterminal ventral and well differentiated from the parenchyma. Fine, needle-like, tegumental spines, obvious under light microscope, cover the body, except in the region of the oral sucker. A sharp stylet is embedded in the dorsal side of the oral sucker. Its tip

FIG. 2.4 Maritrema calvertensis sporocyst and cercaria

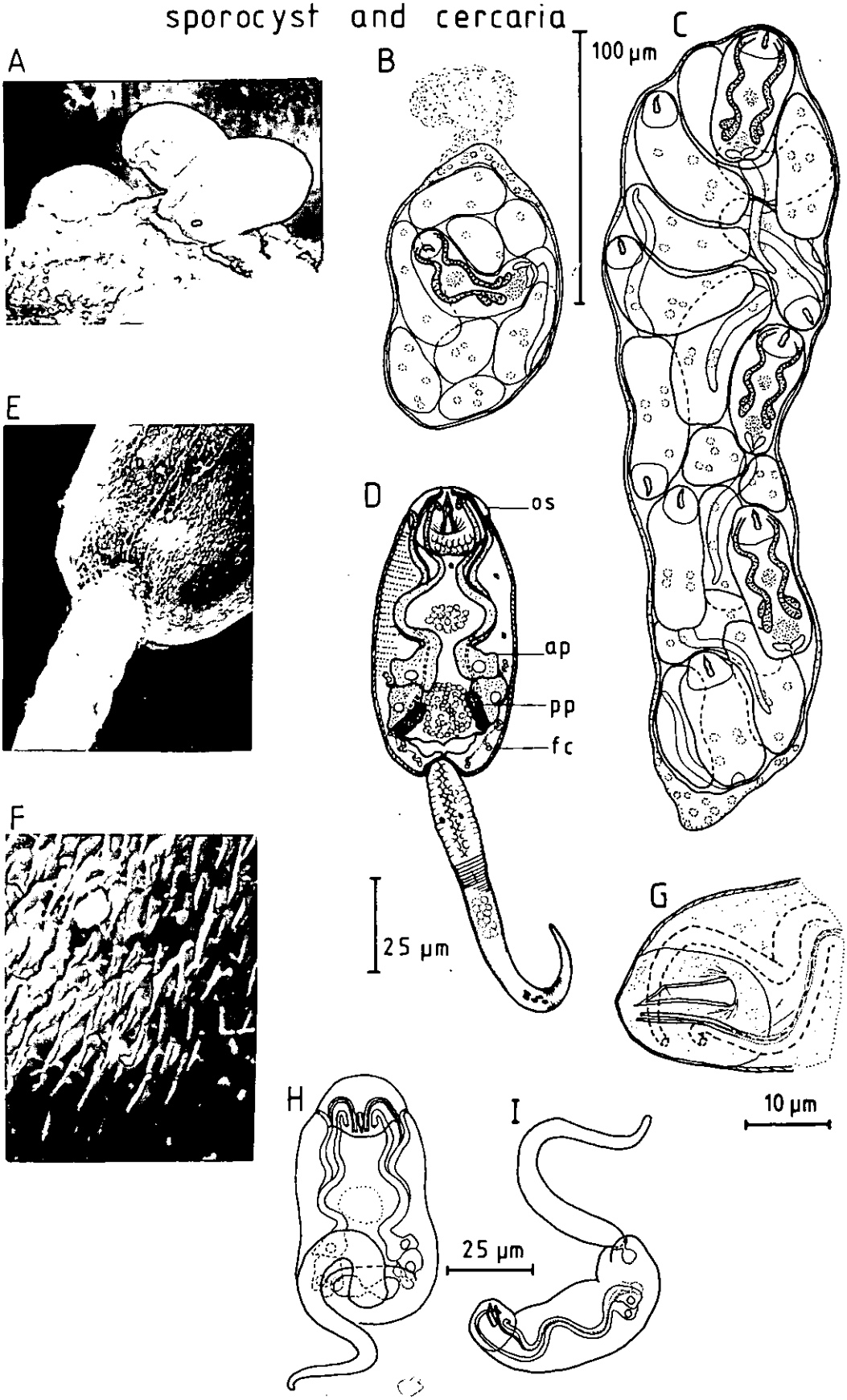


FIGURE 2.4 A, sporocysts protruding from snail hepatopancreatic tissue, S.E.M. × 260; B, daughter sporocyst containing germ balls and one mature cercaria; C, mature daughter sporocyst containing many cercariae; D, whole mature cercaria, ventral view; E, ventral view of junction of cercarial body and tail, S.E.M. × 2850; F, sensory papilla protruding through overlapping tegumental spines of cercarial body, S.E.M. × 20,000; G, cercaria, lateral view of stylet and terminal portions of penetration gland ducts; H and I, swimming position of cercaria, plan and lateral views respectively. (ap: anterior penetration gland; fc: flame-cell; os: oral sucker; pp: posterior penetration gland.)

can be protruded through a terminal aperture, and moved in an arc, by muscles attached to its base. The stylet is asymmetrical in lateral aspect, with the anterior 3/8ths tapering and inclined ventrally. It is uniformly thickened except at its base, which is quite thin. The aspinose tail joins the body in a postero-ventral socket, and narrows gradually from there to its tip. Tegumental annulations, or folds, occur along its length, and a small ventral keel is visible under S.E.M.

The cercaria has four pairs of unicellular penetration glands, with long sigmoidal ducts opening anteriorly. The position of the sinuosities is consistent and characteristic. The two anterior pairs are large, with wide ducts, whereas the two posterior pairs are small and have fine ducts. The glands and ducts are stained by neutral red and brilliant cresyl blue; the posterior pair staining more intensely. The outer large pair of ducts wind forwards and open laterally, at the level of the oral sucker. The inner large pair of ducts follow a similar course, but enter the oral sucker and recurve ventro-medially, on either side of the stylet, opening about halfway along its length. The two pairs of fine ducts from the posterior glands follow the large outer ducts along most of their length, and then traverse the oral sucker, opening near the stylet aperture.

The term 'penetration glands' seems appropriate for all 4 pairs of glands, as all discharge part of their products during invasion of the crustacean host. The coarse-grained contents of the 2 large anterior pairs appear indistinguishable and show identical staining properties in neutral red and brilliant cresyl blue. These glands are almost completely emptied within minutes of contact with the host. The anterior section of the fine ducts of the small posterior pairs of glands are filled with secretions for many days after invasion. These secretions, as well as aiding penetration of the host's exoskeleton, may also facilitate invasion of the host's soft tissues. Similar fine ducts from

the homologous glands in the cercaria of *Microphallus claviformis* also persist after invasion, and Deblock and Rosé (1965) suggested that they may be cystogenous glands. In the case of *Maritrema calvertensis* this function seems unlikely as the glands and ducts disappear in post-cercariae long before encystment commences.

The bilobed excretory bladder is conspicuous when dilated. Flame-cells and sinuous excretory ducts are most visible in cercariae, immediately after invasion of the crustacean host. The flame-cell formula appears to be 2[(2 + 2)] = 8. A cluster of small cells near the middle of the body may be the anlagen of the ventral sucker. Another larger area of small cells, located between the excretory vesicle and penetration glands, is believed to be the genital anlagen.

TABLE 2.7 *Maritrema calvertensis*. Dimensions of cercariae, measured shortly after emerging from the snail host: (a) fixed, (b) live.

(a) Sample size	20
Body length	72 (68 - 84)
Body width	34 (30 - 38)
Body depth	29 (24 - 34)
Tail length	73 (57 - 103)
Tail width	8 (8 - 10)
(b)* Sample size	**10**
Oral sucker length	15 (11 - 17.5)
Oral sucker width	21.5 (17.5 - 25.5)
Length of posterior penetration gland and duct, (P.G.L.)	73 (59 - 94)
Body length, (B.L.)	87 (73 - 105)
P.G.L./B.L.	0.84
Stylet length (S.L.)	12.5 (11 - 13.5)
Stylet width	2.5 (1.5 - 3)
Stylet point (S.P.)	5 (4 - 5.5)
S.P./S.L.	0.4

(*compressed under coverslip pressure)

Emergence from the molluscan host:

In the laboratory, emergence of cercariae from snails exposed to

normal light and temperature conditions is periodic, with a peak occurring at night. Different numbers of cercariae, up to about 1200 per day, emerge from each snail. There is great variation in the numbers of cercariae emerging daily from the same snail, and from different snails. The longest recorded period of 'continuous' emergence of cercariae of *M. calvertensis* from one snail is 37 days.

The pattern of emergence of cercariae in September, from 7 isolated naturally-infected snails is shown in Figure 2.5. The results, expressed as the average number of cercariae emerging per hour, indicate that most cercariae emerge from the host during the evening and night. In November, under hotter conditions, the peak of emergence was still at night, but more cercariae emerged during the day than did in September (Figure 2.6). To take account of the variation in the numbers of cercariae leaving different snails, the November results are also expressed as a percentage of the daily output from each snail. About 80% of cercariae emerged between 4 p.m. and 4 a.m.

To investigate the factors determining the periodicity of emergence of cercariae, 15 snails were kept at a constant temperature, 15°C, and exposed alternately to 12 hours of light, 43 lumens/sq.ft., coinciding with day, and 12 hours of dark, coinciding with night, over a 2 day period. These snails were conditioned at 15°C, in continuous light at 43 lumens/sq.ft., for 12 hours before being exposed to the first experimental dark period. The resultant emergence of cercariae under this 'normal', controlled light regime, shown in Figure 2.6, was investigated by analysis of variance (Table 2.8).

TABLE 2.8 Analysis of variance table showing the influence of light and dark periods on the emergence of *M. calvertensis* from its snail host, in a 'normal' controlled light regime.

Source of variation	d.f.	M.S.	F	P	Sig.
light:dark periods (P)	1	107357	36.8	P<0.001	***
Day (D)	1	15746	5.4	0.01<P<0.05	*
Interaction (P - D)	1	13862	4.8	0.01<P<0.05	*
Residual	56	2914			

FIG. 2.5 Maritrema calvertensis. Emergence of cercariae from the snail host.

(mean ± 1s.d.)

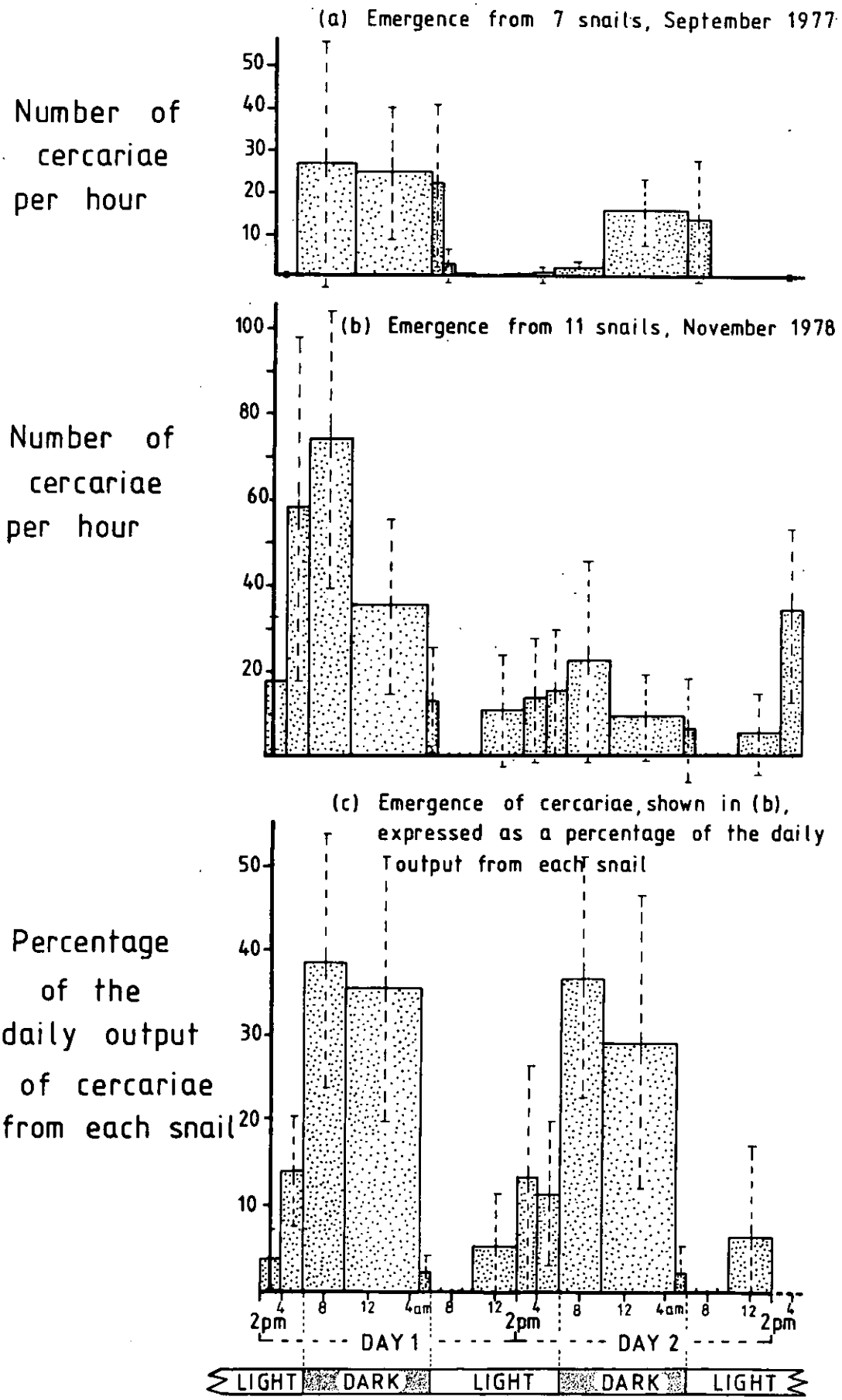
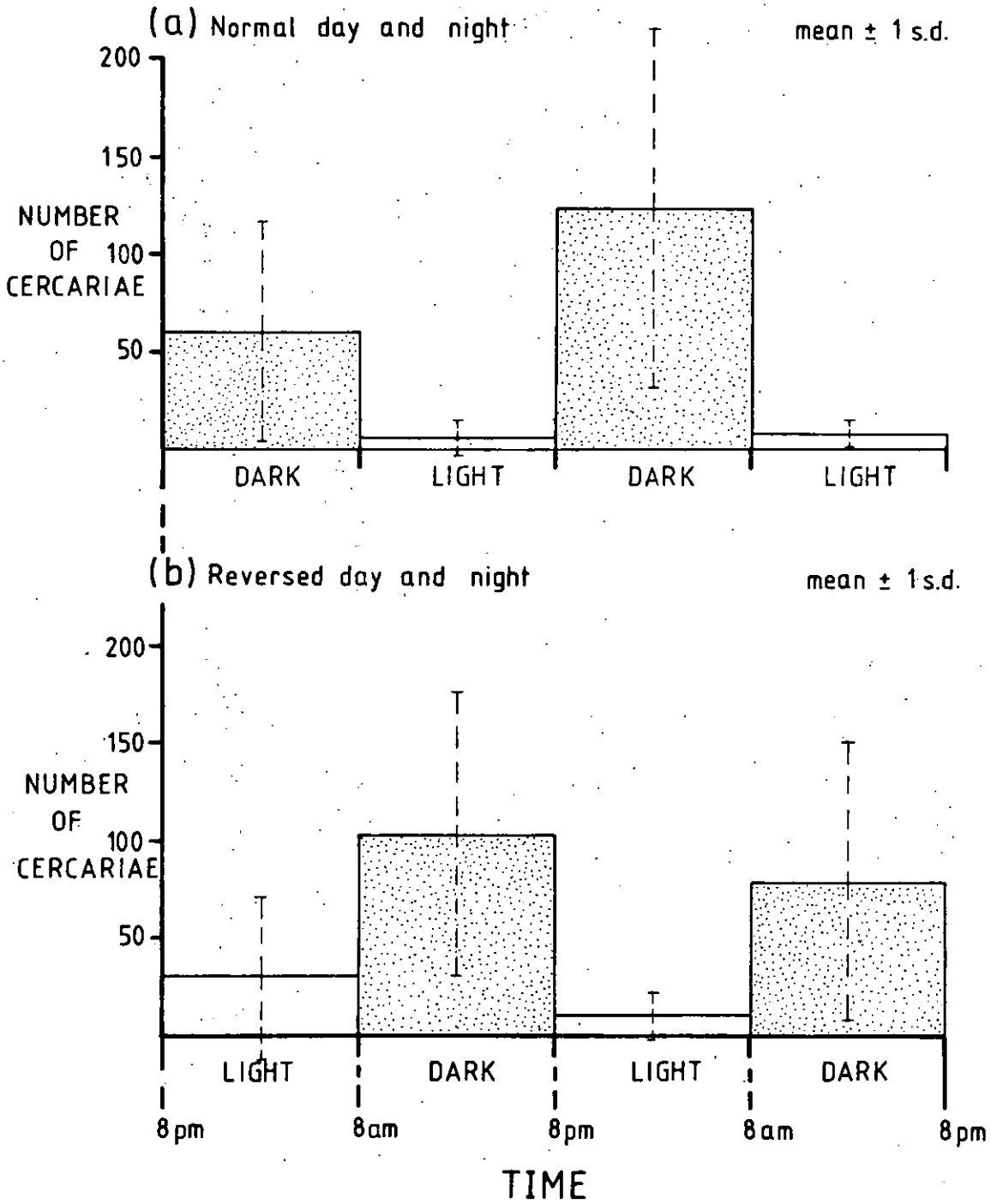


FIG. 2.6 Maritrema calvertensis. Emergence of cercariae under controlled light conditions, at 15°C, (number of host snails = 15).



There was a highly significant difference in the levels of emergence between the light and dark periods, and a slightly significant difference between the levels of emergence on Day 1 and Day 2. The mean level of emergence in the dark periods was 91.5 cercariae per snail, and in the light periods was 6.9 cercariae per snail. The mean level of emergence was 33.0 cercariae per snail on Day 1, compared with 65.4 on Day 2.

A parallel experiment was simultaneously conducted with 15 different snails, except that the light periods coincided with night, and the dark periods with day. These snails were conditioned at 15°C in continuous dark for 12 hours before being exposed to the first experimental light period. Emergence of cercariae under this 'reversed' controlled light regime, shown in Figure 2.6, was also investigated by analysis of variance, (Table 2.9).

TABLE 2.9 Analysis of variance table showing the influence of light and dark periods on the emergence of *M. calvertensis* from its snail host, in a 'reversed' controlled light regime.

Source of variation	d.f.	M.S.	F	P	Sig.
Light:dark periods (L)	1	72176	23.9	P<0.001	***
Day (D)	1	7684	2.5	0.05<P<0.2	N.S.
Interaction (L - D)	1	94	0	0.2<P	N.S.
Residual	56	3022			

There was a highly significant difference in the levels of emergence between the light and dark periods, and, as under 'normal' light conditions, there was significantly more emergence during the dark periods than during the light periods. The mean level of emergence in the dark periods was 91.1 cercariae per snail, and in the light periods was 21.7 cercariae per snail. In this experiment there was a slight decrease in the average levels of emergence from Day 1, (67.7 cercariae per snail), to Day 2, (45.1 cercariae per snail), however the difference was not significant.

The effect of temperature on emergence was investigated by simultaneously maintaining groups of snails in constant dark, for 2 days, under controlled temperature conditions: 5, 10, 15 and 25°C. Each group consisted of 8 snails. The number of cercariae that emerged from each snail was counted daily, and the results, shown in Figure 2.7, were subjected to analysis of variance (Table 2.10).

FIG. 2.7 Maritrema calvertensis. Emergence of cercariae under controlled temperature conditions, in the dark, (number of host snails in each group = 15).

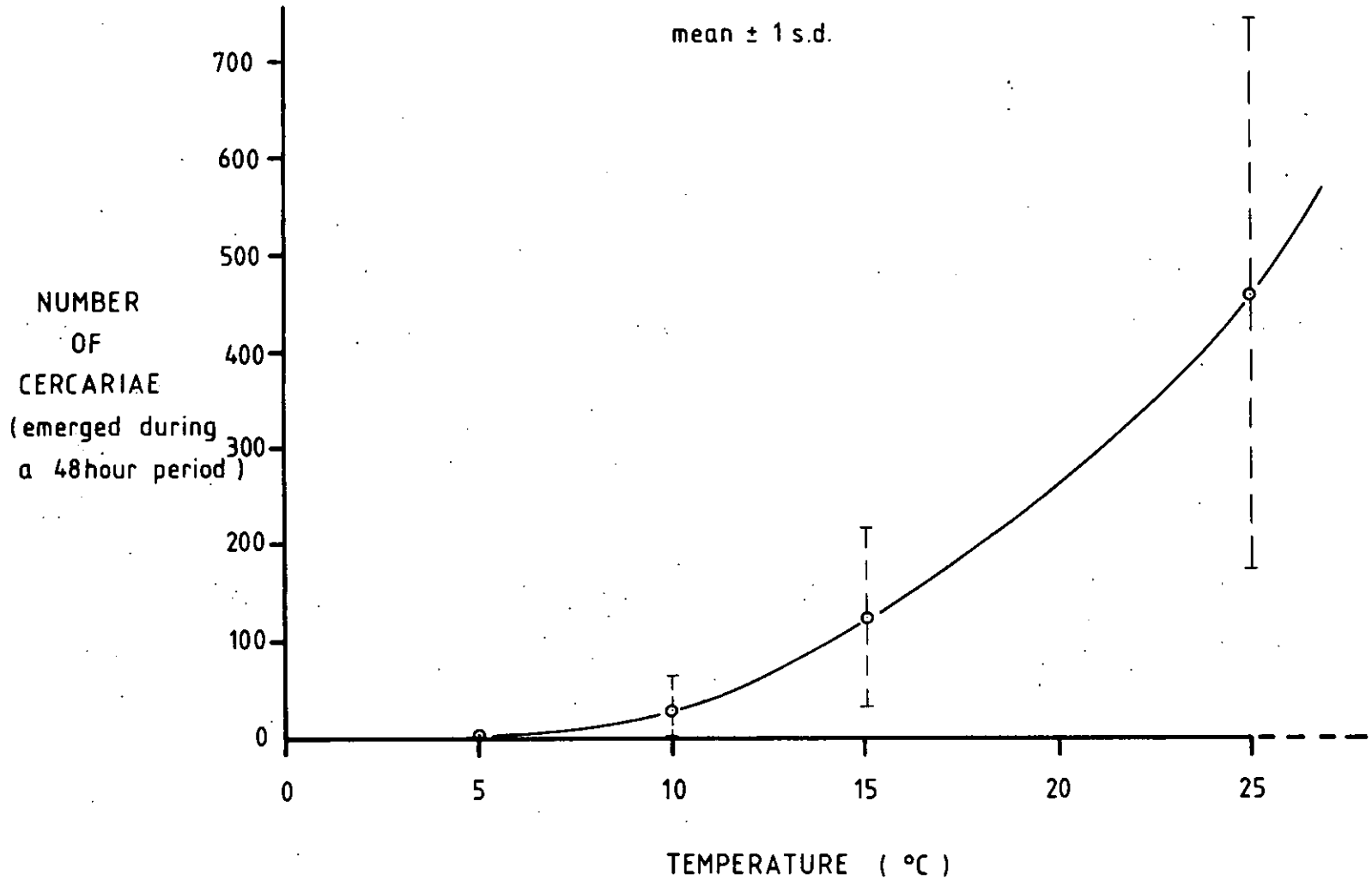


TABLE 2.10 Analysis of variance table showing the effect of temperature on emergence of *M. calvertensis* from its snail host.

Source of variation	d.f.	M.S.	F	P	Sig.
Temperature (T)	3	178097	29.0	P<0.001	***
Day (D)	1	1630	0.3	0.2<P	N.S.
Interaction (T - D)	3	2168	0.4	0.2<P	N.S.
Residual	56	6137			

The effect of temperature was highly significant. The mean rate of emergence was directly related to temperature: 0.3 cercariae per snail, per day, at 5°C; 14.6 at 10°C; 62.7 at 15°C; and 230.0 at 25°C. In this experiment there was no significant difference in the average levels of emergence on Day 1, (71.8 cercariae per snail) and Day 2,(81.9) cercariae per snail.

The results of these investigations indicate that environmental temperature and light conditions are important factors influencing emergence of the cercaria of *M. calvertensis* from its snail host. The effect of temperature is probably a general one, with activity and metabolic rate of the parasite, and its poikilothermic host, being related to environmental temperature. Under experimental conditions, the periodicity of emergence of the cercaria of *M. calvertensis* was reversed when host snails were placed in a 'reversed' day-night regime. Significantly more cercariae emerged during the dark periods than the light periods, whether under natural or artificial light conditions, and whether under 'normal' or 'reversed' day-night regimes. The results indicate that emergence of the cercaria of *M. calvertensis* is stimulated by dark conditions, or inhibited by light, or both; and that the periodicity observed under natural light conditions is due to the normal diurnal changes in environmental light intensity, i.e. night and day. As microphallid cercariae have no eyespots, and show no light-sensitive responses in isolation of the snail, the mechanism of this control of emergence is obscure. However, the association between developmental stages of trematodes and molluscs is very intimate, and variations in the physiology

of one are known to have a pronounced effect on the other (Wright, 1971; Erasmus, 1972). It is possible that the cercaria of this trematode species responds to changes in environmental light conditions through their effects on the behaviour and/or physiology of the snail host.

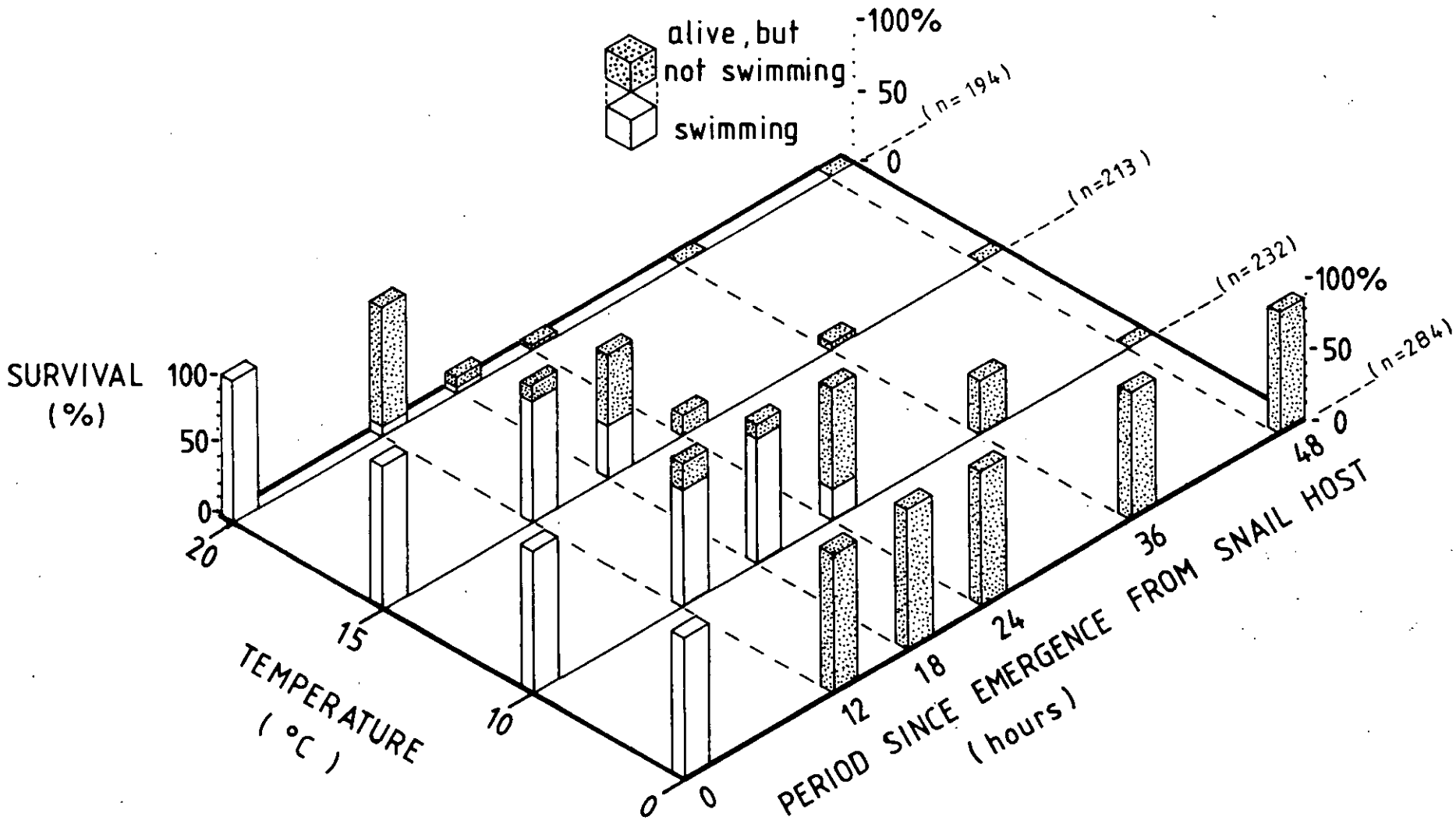
Swimming:

Following emergence, the cercaria swims continuously, until exhausted, or until contacting a suitable crustacean host. The cercaria swims with the body curled ventrally, and the ventral side up; the body appearing rectangular in plan view (Figure 2.4). Rapid, whip-like, tail strokes cause the anterior to jerk from side to side, and the cercaria to rise in the water, with the anterior usually leading. The cercaria swims an erratic course, sometimes describing a spiral. When tiring, it may creep briefly on the substrate, before resuming swimming. In lagoon water, the longevity of the cercaria was found to be inversely related to water temperature in the range from 5°C to 20°C (Figure 2.8). At 5°C swimming ceased almost immediately, however cercariae continued wriggling and creeping for at least 48 hours. Fifty percent of cercariae continued swimming at 10°C for about 21 hours, at 15°C for about 14 hours, and at 20°C for about 6 hours.

Invasion of the crustacean host: (Figure 2.9)

The cercaria invades the amphipod, *Austrochiltonia australis*, and ostracod, *Mytilocypris tasmanica*. It approaches amphipods indirectly, and is drawn to the postero-ventral region by posterior flowing currents caused by the beating pleopods. There it rapidly attaches, and invades through intersegmental membranes, principally of the pleopods and other limbs. After landing on a limb, the cercaria creeps quickly towards the body, tail sweeping from side to side, until reaching an intersegmental membrane. It attaches itself at the anterior end, discharging the contents of the large pairs of penetration glands over the zone of contact.

FIG. 2.8 Maritrema calvertensis. Longevity of cercariae at different temperatures, (n = no. of cercariae at each temperature).



Within 2 minutes it penetrates the membrane, leaving its tail wriggling on the surface. Invasion sometimes occur when a cercaria is presented with a paralysed amphipod, or severed amphipod limb; however, in such situations the cercaria usually lands on the exoskeleton, briefly prods with the anterior end, and then resumes swimming.

The infectivity of cercariae was investigated by exposing a number of laboratory-bred amphipods, in separate crystal dishes, to 50 newly emerged cercariae, for about 12 hours at room temperature. The amphipods were then transferred to 1L containers and maintained at 15°C for 6 days. Nineteen out of 23 amphipods (82.6%), were found to be infected, and the incidence of post-cercariae is shown in Table 2.11.

When placed in a crystal dish with a live ostracod, the cercaria appears to swim randomly, and does not react when contacting the ostracod valves. When drawn inside the valves by ventilating currents, however, the cercaria invades and later encysts, forming clusters of metacercarial cysts at the base of the swimming appendages. In a similar experiment to that described above, 7 out of 16 ostracods (43.7%), were found to be infected, and the incidence of post-cercariae is shown in Table 2.11.

TABLE 2.11 The incidence of post-cercariae in laboratory-bred amphipods and ostracods after each individual was exposed to 50 cercariae of *Maritrema calvertensis*.

Host Species	No.	Number of post-cercariae $\bar{X}a$	$\bar{X}t$	V_{Xt}	$\bar{X}g$	95% conf. limits
amphipod	23	11.5	0.797	0.307	5.3	2.6 - 9.9
ostracod	16	1.2	0.229	0.090	0.7	0.2 - 1.4

($t = 4.13$, d.f. = 37, $P<0.001$, ***)

The results indicate that *A. australis* is more susceptible to infection with *Maritrema calvertensis* than is *Mytilocypris tasmanica*. Amphipods were infected by significantly more post-cercariae of *Maritrema calvertensis*, than were ostracods.

FIG. 2.9 *Maritrema calvertensis*

Invasion of *Austrochiltonia australis*

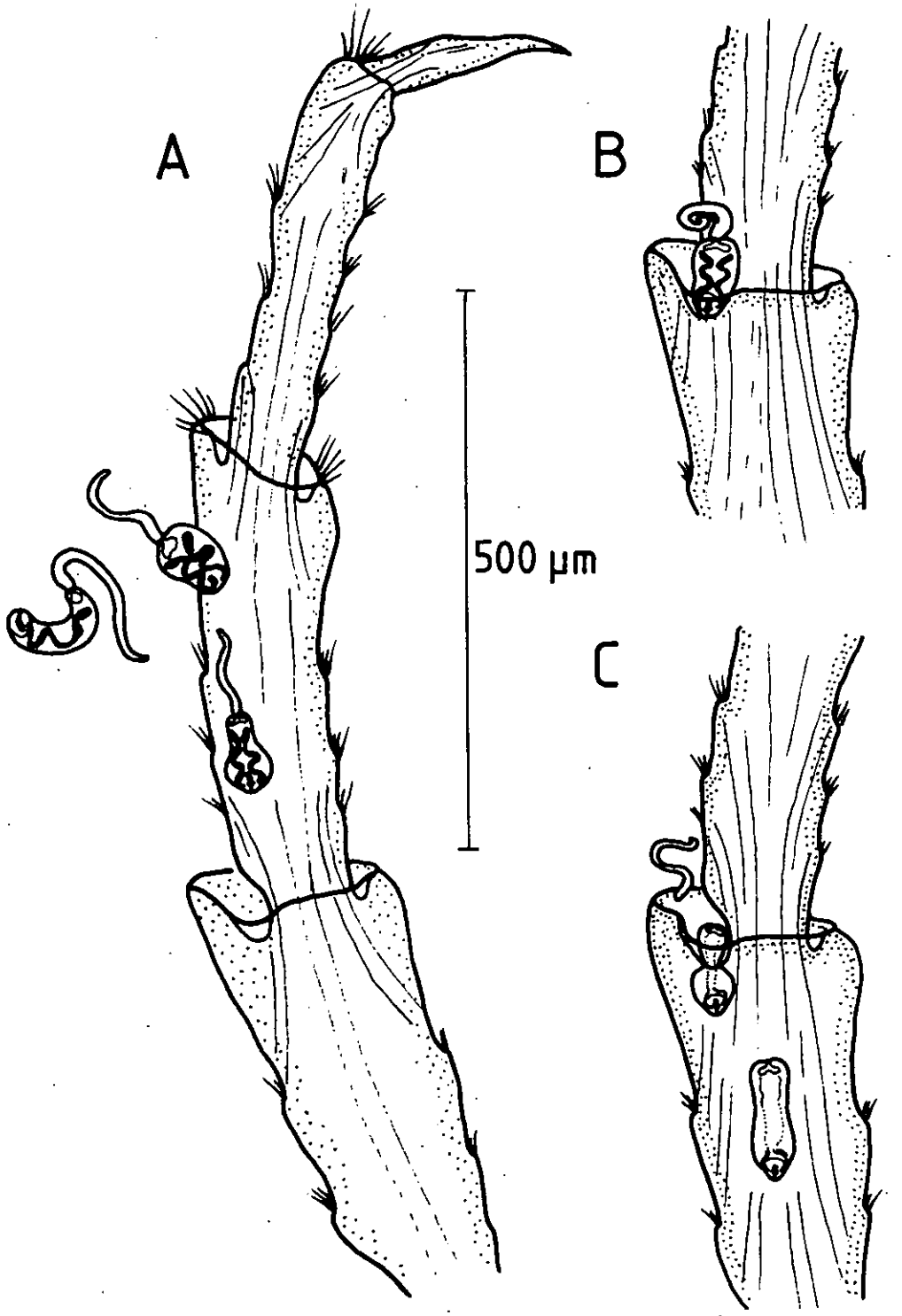


FIGURE 2.9 A, cercaria landing on peraeopod of amphipod in cavity slide under microscope, and creeping towards the body; B, cercaria attached to intersegmental membrane, tail strongly contracted; C, tail is shed, and cercaria invades the amphipod limb.

2.2.6 Growth and development in the crustacean host

Introduction:

Relatively little attention has been directed to the development of trematodes in their second intermediate hosts. This period of development is an important phase in the life-cycles of the parasites. The typical development of a microphallid within the crustacean host proceeds from the invading cercaria to an encysted, sexually advanced metacercaria. During invasion, the tail of the cercaria is shed, and the tail-less cercarial body, or post-cercaria, grows, develops and encysts within the tissues of its host. Development continues after encystment, and in most species is arrested after the reproductive organs are well developed, and vitellaria are producing egg-shell precursors. Further development and egg production are usually inhibited until the metacercaria experiences the body temperature of the vertebrate host.

The influence of temperature on the biology of digenetic trematodes has been well documented (Smyth, 1966, 1976; Erasmus, 1972). Environmental temperatures influence the rate of development and longevity, of the free-living trematode stages, and also the developmental stages parasitic within poikilothermic intermediate hosts. Apart from its general effect on trematode metabolism, and rates of development, temperature changes also act as trigger stimuli, inducing important physiological and behavioural changes in the life-cycle, such as hatching of eggs, emergence of cercariae from snails and excystment of metacercariae (Smyth, 1966, 1976; Erasmus, 1972). Threshold and optimum temperatures exist for each of these processes, related either to the external environment (e.g. hatching of eggs and emergence of cercariae), or to the host's internal environment (e.g. excystment of metacercariae).

In the present study, the effect of temperature on the development of *Maritrema calvertensis* was examined. Development in the amphipod,

TABLE 2.12 *Maritrema calvertensis*. The dimensions of post-cercariae (P) and metacercarial cysts (M), recovered from experimentally infected amphipods and ostracods.

(i) Growth in *Austrochiltonia australis*, at 5°C

P.I. (days)	P/M	No.	Length Mean	Length S.D.	Width Mean	Width S.D.
6, 0	P	30	76	3	34	1
7, 0	P	30	75	3	34	2
15, 0	P	30	78	3	35	2
22, 0	P	30	79	3	36	2
28, 0	P	30	79	3	36	2

(ii) Growth in *Austrochiltonia australis*, at 15°C

P.I. (days)	P/M	No.	Length Mean	Length S.D.	Width Mean	Width S.D.	
(0, 17	P	30	76	5	33	2	...at room temperature)
6, 0	P	30	84	4	39	2	
7, 0	P	30	84	5	40	3	
13, 0	P	30	99	5	55	5	
15, 0	P	30	118	10	62	5	
19, 0	P	20	141	17	74	7	
19, 0	M	30	124	13	109	12	
23, 0	P	4	116	15	55	10	
23, 0	M	30	127	10	112	6	
30, 0	M	30	126	13	99	9	
42, 0	M	30	155	7	128	5	
49, 0	M	30	157	8	144	8	
58, 0	M	30	156	8	142	5	

(iii) Growth in *Austrochiltonia australis*, at 25°C

P.I. (days)	P/M	No.	Length Mean	Length S.D.	Width Mean	Width S.D.
4, 0	P	30	111	6	55	3
6, 0	P	30	133	7	71	5
7, 0	M	30	134	19	119	16
10, 0	M	30	149	8	131	4
15, 0	M	30	156	11	133	9
21, 0	M	30	160	11	135	10
30, 0	M	30	163	5	148	3

(iv) Growth in *Mytilocypris tasmanica*, at 15°C

P.I. (days)	P/M	No.	Length Mean	Length S.D.	Width Mean	Width S.D.
6, 0	P	3	78	7	35	1
7, 0	P	6	83	4	41	2
14, 0	P	3	100	8	50	8
23, 0	P	5	138	6	76	4

Austrochiltonia australis, is briefly compared with that in the ostracod, *Mytilocypris tasmanica*.

Results:

The dimensions of *Maritrema calvertensis* in its crustacean hosts, at different temperatures, and at different intervals after invasion, are shown in Table 2.12. Where possible, the mean length and width of 30 post-cercariae, or metacercarial cysts, are given. Encystment did not occur simultaneously in post-cercariae of the same age, so for a period after invasion, hosts were infected by both free and encysted stages.

Growth and development in the amphipod at 15°C is described below (average dimensions given in microns), and illustrated in Figure 2.10 -

After penetrating in the postero-ventral body region and limbs, post-cercariae normally migrated via the haemolymph before encysting in haemolymphatic sinuses, throughout the body. When migration was blocked (e.g. in the propod of gnathopods, and the carpus, merus and basis of peraeopods), viable, though sometimes mis-shapen cysts were formed at the site of penetration.

Seventeen hours after invasion the post-cercaria was relatively unchanged, except for the condition of penetration glands and ducts. The 2 large anterior pairs were almost empty, and disappeared within 7 days. The fine ducts of the 2 posterior pairs were filled with secretions in the oral sucker region, and remained so for about 15 days.

After 7 days, the post-cercaria measured 84 × 40. The excretory vesicle, oral sucker and stylet were unchanged from the cercaria. Small, round, cystogenous glands were scattered through the body. When compressed, their secretions oozed out through the spinous tegument around the body, looking like unwinding scrolls. After 15 days, the post-cercaria measured 118 × 62. The body appeared darker, due to the increased accumulation of coarse, cystogenous granules. The excretory bladder

FIG. 2.10

Growth and development of *Maritrema calvertensis* in *Austrochiltonia australis*.

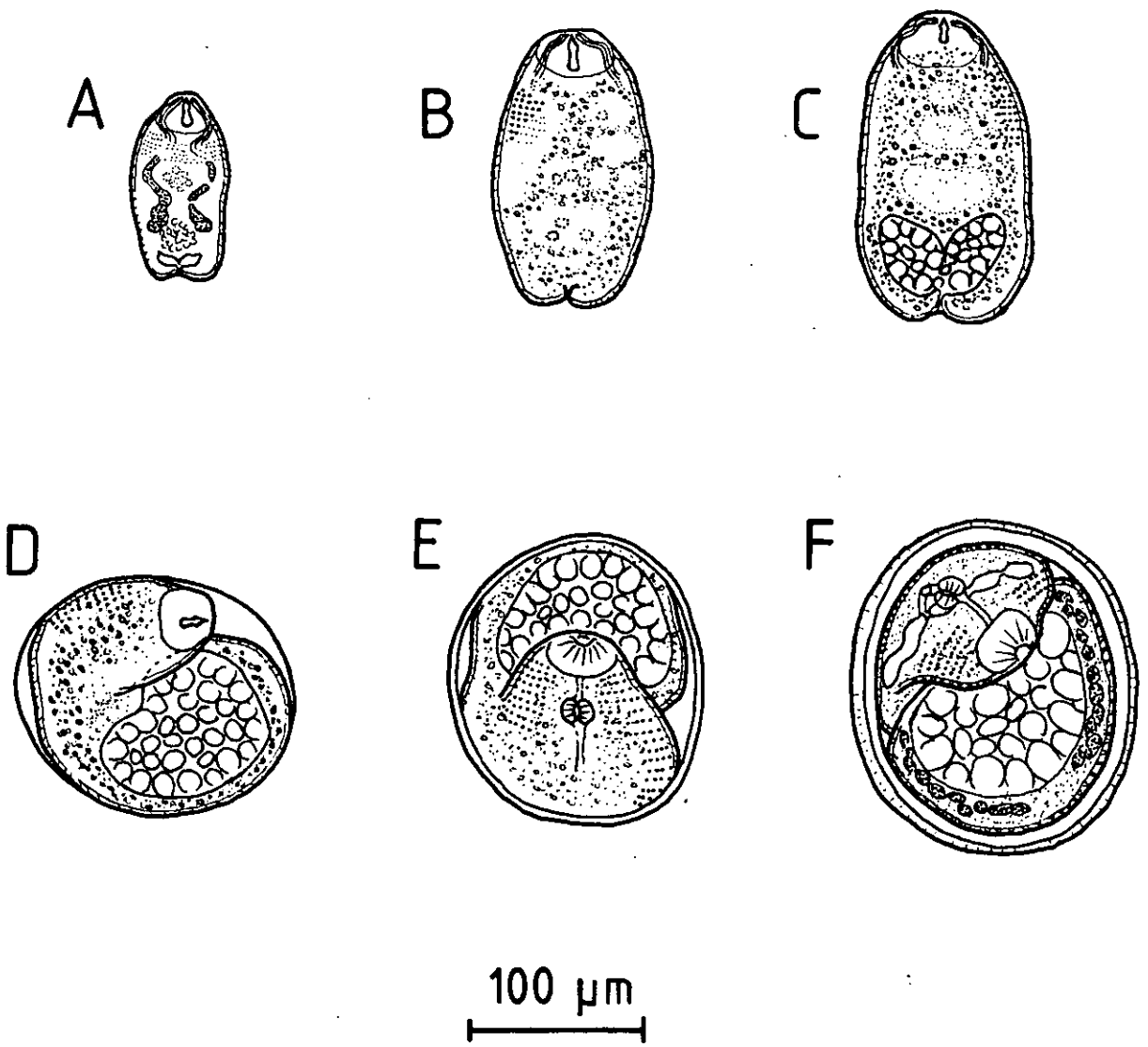


FIGURE 2.10 A, after 17 hours at room temperature; B, after 4 days at 25°C, or 15 days at 15°C; C, after 6 days at 25°C; D, after 7 days at 25°C, or 23 days at 15°C; E, after 10 days at 25°C; F, after 21 days at 25°C, or 58 days at 15°C.

was not visible and the stylet was thinner, and less distinct. No encystment had occurred. Nineteen days after invasion, 75% of post-cercariae had encysted. The free post-cercaria measured 141 × 74. The excretory bladder was very enlarged and loosely packed with large granular cells. All trace of penetration glands and ducts had disappeared. The stylet and oral sucker were visible, but the rest of the body was obscured by cystogenous granules. The encysted metacercaria was enclosed by a single, thin, pliable, transparent membrane, about 1.5μ thick. The cyst measured 124 × 109. After 23 days, 77% of post-cercariae had encysted, forming thin-walled, pliable cysts. The cyst measured 127 × 112, and was about 1.5μ thick. The body of the metacercaria, still obscured by masses of granules, appeared little changed from a post-cercaria. The stylet, oral sucker and large excretory bladder were distinct. No excystment occurred after 2 hours in 0.3% pancreatin in Hank's saline. The worms that had not encysted by this stage were small, 116 × 55, and moribund. After 30 days, all surviving specimens were encysted. The cyst was still about the same size and thickness, and the stylet was still present in the metacercaria. By the 39th day after invasion, the cyst was about 10μ thick, composed of 2 layers, each about 5μ thick. The inner layer was light and hyaline, and the outer layer was darker, apparently laminated, sometimes with radiating fissures. The metacercaria had undergone great development, having both oral and ventral suckers, a digestive system and complete but immature reproductive system. There were no developing spermatids in the testes, and although vitellaria were formed, they contained no phenolic egg-shell precursors. After 42 days, the cyst measured 155 × 128, and a very thin stylet was still present in some metacercariae. No excystment occurred when cysts were incubated in 0.3% pancreatin in Hank's saline for 3 hours. No spermatids or phenolic egg-shell precursors were present in the testes or vitellaria respectively. Fifty eight days after invasion, the cyst measured 156 × 142, and the wall was

uniformly 11μ thick, still composed of 2 more or less equal layers. A membranous outer layer, of host origin, sometimes connected cysts into groups of 2 or more. After 2 hours in 0.3% pancreatin in Hank's saline, 23% of metacercariae had excysted. Phenolic egg-shell precursors were present in the vitellaria, but no developing spermatids were in the testes.

Growth and development in the amphipod at 5°C -

Six days after invasion the post-cercaria measured 76 × 34, and by 28 days it measured 79 × 36. After 28 days, the post-cercaria was active, but almost unchanged since penetration of the host. The stylet and excretory bladder were unaltered, the large ducts of the anterior penetration glands were partly filled, and the fine ducts of the posterior penetration glands were filled in the oral sucker region.

The 28 day old post-cercariae were capable of developing normally at elevated temperatures. Some were transferred from 5 to 25°C, and after 8 days measured 106 × 49. After 15 days, there was 100% encystment, cysts measuring 148 × 131, with walls about 8μ thick.

Growth and development in the amphipod at 25°C - (Figure 2.10)

After 4 days the post-cercaria measured 111 × 55. The large penetration glands had disappeared, but the fine ducts of the small glands were distinct in the oral sucker region. The stylet and oral sucker were unchanged. Small cystogenous granules had been produced, and were distributed unevenly through the body. After 6 days the body of the post-cercaria appeared darker, due to masses of accumulated cystogenous granules. It measured 133 × 71. The stylet was very thin and indistinct, but the oral sucker was fairly well defined. The fine ducts of the posterior penetration glands were still evident anteriorly, and the excretory bladder was very enlarged. Three pale regions of small cells, along the mid-line of the body, were possibly the anlagen

of the digestive system, ventral sucker and the reproductive system. Seven days after invasion, 94% of post-cercariae had encysted, forming cysts 134 × 119, bounded by a thin, pliable membrane about 2μ thick. A very thin stylet was still visible within the oral sucker. After 10 days, 100% of post-cercariae had encysted. The cyst measured 149 × 131, and was bounded by a single-layered wall, about 4μ thick. The stylet had disappeared, but little other change had occurred, and no adult organs were discernible. No excystment occurred after 2 hours in 0.3% pancreatin in Hank's saline. Rapid development occurred in the next few days, and after 15 days the metacercaria was a fully developed pre-adult, able to excyst *in vitro*. Phenolic egg-shell precursors were in the vitellaria, and spermatogenesis had reached the "comma" stage. The ovary contained only uniformly small cells, and no apparently mature ova. The cyst measured 156 × 133, and the cyst wall, which was composed of 2 distinct layers, was about 10μ thick. The cyst continued to grow, and after 21 days measured 160 × 135, and was about 15μ thick. The outer layer was pitted with radial cracks that extended inwards as far as the boundary with the inner layer. After 30 days, the cyst measured 163 × 148, and was about 16μ thick, still composed of 2 distinct layers. More spermatids were developing in the testes, however no mature sperm were formed. Metacercariae excysted readily *in vitro*, but no mature sperm or eggs were present in excysted worms after 4 hours in Medium 199, at 40°C.

Growth and development in the ostracod at 15°C -

After 7 days the post-cercaria measured 83 × 41. The fine ducts of the small posterior penetration glands were clearly visible in the anterior region. The stylet and oral sucker were well-defined and unchanged from the free swimming cercaria. Fourteen days after invasion, the post-cercaria measured 100 × 50. The stylet was thinner, and the oral sucker less distinct. The penetration glands and their ducts were

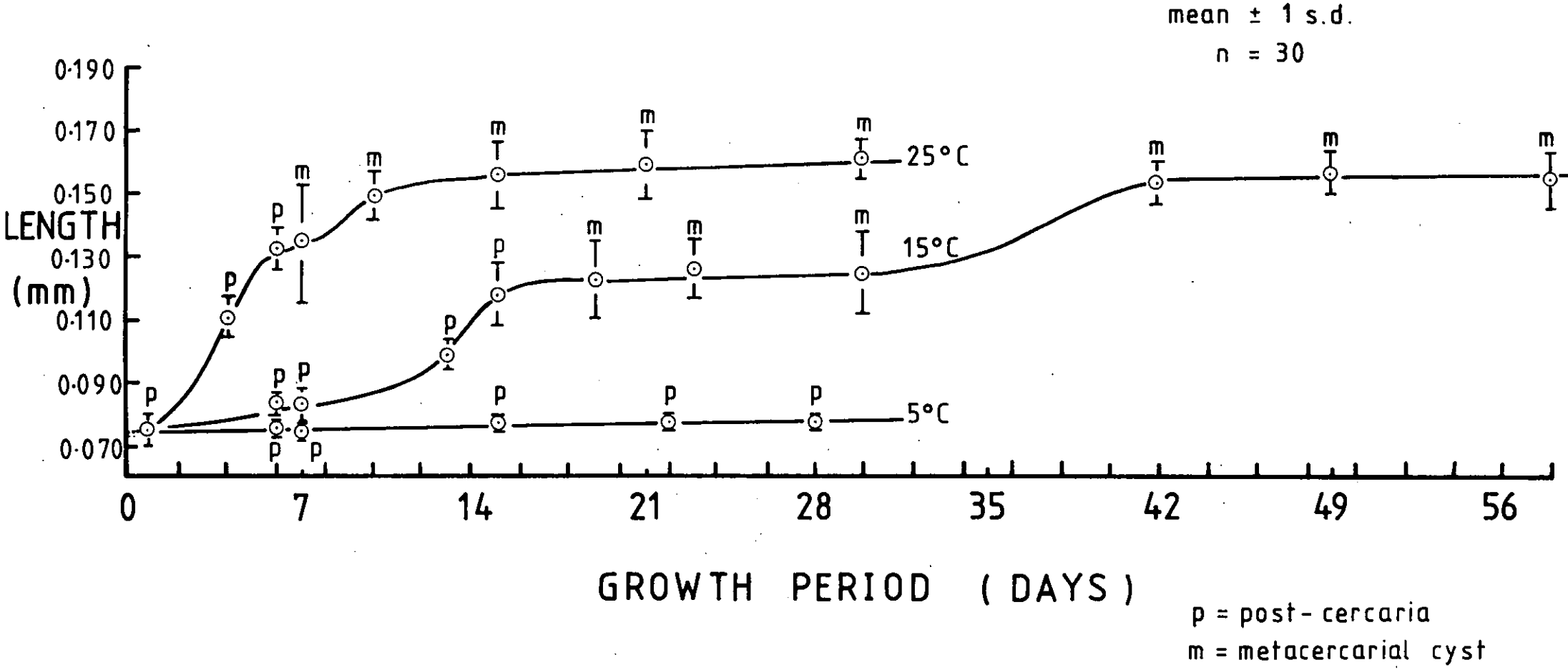
no longer visible. After 23 days, the post-cercaria was 138 × 76. It appeared quite dark due to the accumulation, all through the body, of masses of cystogenous granules, varying greatly in size. The stylet was very thin and indistinct.

Discussion:

At 15°C, the growth of *Maritrema calvertensis* in *Austrochiltonia australis* followed 2 continuous, sigma-shaped curves. The first curve representing growth of the post-cercaria prior to, and including, encystment, and the second, the growth of the metacercarial cyst (Figure 2.11). At 25°C, the same growth pattern was telescoped into a much shorter period of time. Little growth, and no development, occurred at 5°C. The initial size of the post-cercaria was 76 × 33, and after 1 month at 5°C its size (length + width), had increased by only 6%. At 15°C, the size of the post-cercaria increased by 65% before encystment, and at 25°C, the size increased by 87% before encystment. Encystment first occurred after 19 days at 15°C, and after 7 days at 25°C. After encystment, a period of slow growth was followed by a rapid acceleration of growth of the metacercarial cyst. Rapid development of the metacercaria coincided with this acceleration of growth of the cyst, and it was during this period that the metacercaria developed into a pre-adult, with all of the adult organs. This period of maturation occurred between the 30th and 39th day after invasion at 15°C, and between the 10th and 15th day at 25°C. Phenolic egg-shell precursors were present in the vitellaria of encysted metacercariae after 58 days at 15°C, and after only 15 days at 25°C.

The results of this experiment show that development of *M. calvertensis* in *A. australis* is inhibited by cold conditions (5°C), and that in warmer conditions (i.e. 15 - 25°C), it occurs at a rate directly related to environmental temperature. A minimum threshold temperature for development lies somewhere between 5 and 15°C. Although

FIG. 2.11 Growth and development of *Maritrema calvertensis* in *Austrochiltonia australis*, at different temperatures



no development occurs at 5°C, the post-cercaria is able to survive for at least 28 days, and then develop normally when the temperature is raised.

Bridgman (1969), examined the development of another microphallid, *Microphallus choanophallus*, in the shrimps, *Macrobrachium ohione* and *Palaemonetes pugio*, at 27°C. Encystment of this trematode occurred after 24 hours in the crustacean host, however metacercariae were not infective to definitive hosts until the 30th day after invasion. Bridgman suggested that metacercariae would not survive in the definitive hosts until the vitellaria were developed. It is not known when metacercariae of *Maritrema calvertensis* were infective to birds, however vitellaria had formed after 39 days at 15°C, and after 15 days at 25°C. The extremes of water temperature recorded at Calvert's Lagoon are 5.1°C and 22.5°C, and water temperature is below 15°C for most of the year (Figure 1.4). Thus, if *M. calvertensis*, like *Microphallus choanophallus*, is infective when vitellaria are formed, the development of infective metacercariae would usually take longer than 39 days. During the summer, when water temperatures exceed 20°C, infective cysts may develop in about 3 weeks. As well as varying seasonally, the natural rate of development of metacercariae is probably also a function of the temperature tolerance, and behaviour of the crustacean host. Observations of amphipods at the lagoon, indicate that they move into deeper, cooler water during hot, dry periods, behaviour that would slow the rate of development of their trematode parasites.

Although the number of post-cercariae of *M. calvertensis* recovered from ostracods in this experiment was small, the results indicate that growth and development may be slightly slower in the ostracod, than in the amphipod. At 15°C, 77% of post-cercariae had encysted after 23 days in the amphipod, however no encystment had occurred after this period in the ostracod.

2.2.7 *Metacercaria*

Metacercarial cyst: (Figure 2.10)

The mature metacercarial cyst is more or less round, with a thick, resilient wall. Dimensions of cysts from naturally infected amphipods and ostracods are shown in Table 2.13. Cysts in the ostracods were significantly smaller than those in the amphipods (t, difference between mean lengths = 4.96; d.f. = 28; $P<0.001$).

TABLE 2.13 *Maritrema calvertensis*. Dimensions of thick-walled metacercarial cysts taken from naturally infected amphipods and ostracods.

		Length		Width		Thickness	
	No.	Mean	Range	Mean	Range	Mean	Range
Austrochiltonia australis	15	179	(160-194)	156	(141-167)	15.8	(7.6-20.9)
Mytilocypris tasmanica	15	163	(148-171)	150	(144-163)	14.3	(11.4-17.1)

The cyst wall is composed of 2 layers of approximately equal thickness, surrounded by a very thin outer membrane. The inner layer is proteinaceous, and the outer layer is composed of acid and neutral mucopolysaccharides (Smith, 1971). The outer membrane, about 70 nm thick, is of host origin, and can be seen extending between cysts, often binding them in clusters. The inner layer seems to be relatively hard, and resilient to damage. In older cysts the wide outer layer is often traversed by fissures. These may be due to internal pressure caused by the growing metacercaria, or perhaps,breakdown of mucopolysaccharide due to decay or aging.

Excystment:

Mature metacercariae will excyst readily at about 41°C, after exposure to digestive enzymes, or bile. Incubation for 1 or 2 hours in 0.5% pancreatin in Hank's saline, or bile (sheep or chicken); or 0.5%

pancreatin with 0.2% sodium taurocholate in Hank's saline, followed by transfer to Hank's saline, will successfully induce excystment. Pretreatment in 2% pepsin at pH 1.5, for varying periods, before transfer to pancreatin solution, was found to be lethal.

The process of excystment *in vitro* has been described previously (Smith, 1971). The metacercaria is activated by elevation of temperature to about 41°C, and begins revolving within the cyst. Excystment does not occur unless the cyst wall is weakened externally by digestive enzymes or bile. This external 'digestion' of the cyst, stimulates the metacercaria to escape actively. It makes vigorous stretching movements, pushing against the thin remnant cyst wall until it bursts through. Enzymes of parasite origin, with localized action, may be involved in excystment, as the metacercaria sometimes emerges through a small hole when the cyst wall is still relatively thick.

Excysted metacercariae:

Mature excysted metacercariae are of adult size, and have apparently mature ova in the ovary, active vitellaria and testes containing clusters of comma-shaped spermatids. Metacercariae can excyst *in vitro* before they are fully grown, after their reproductive organs have developed, but before the vitellaria are producing phenolic egg-shell precursors. A group of 20 excysted metacercariae, taken from naturally infected amphipods, varied in size and sexual maturity (Table 2.1). On average they were smaller than adult worms from laboratory ducklings; however, the largest excysted metacercaria was larger than the average adult.

2.2.8 Discussion

Life-cycle studies of *Maritrema calvertensis* provide more information with which to assess its relationship to other microphallids. The distinction between *M. calvertensis* and the closely related *M. oocysta* (syn. *M. humile* and *M. innae*) is supported by comparison of their life

histories. *M. oocysta* develops in the brackish hydrobiid snail, *Hydrobia ulvae*, on the north coast of France (Deblock, 1975), and in the same host on the east coast of England (Lebour, 1907). It has a xiphidiocercaria, that is aspinous, incapable of active swimming, and encysts within the daughter sporocyst. The cercarial penetration glands are difficult to see, however all 4 pairs of penetration gland ducts open near the tip of the stylet (Deblock, 1975). As described previously, *M. calvertensis* develops in the brackish hydrobiid *Coxiella badgerensis* in Tasmania. It also has a typical microphallid cercaria, which, however, is spinous, and which emerges from its snail host and swims until contacting and penetrating a suitable crustacean intermediate host. The 4 pairs of penetration glands are well developed and very conspicuous. Two pairs of ducts open near the tip of the stylet, 1 pair opens ventrally, and another pair opens laterally.

The metacercarial cyst of *M. oocysta*, after which the species is named, is characteristically oval in shape, 125-145 × 70-85μ, and only 4μ thick. It is relatively weak and easily ruptured mechanically. The cyst of *M. calvertensis* is more or less round, and in naturally infected amphipods measures 160-194 × 141-167μ, and is about 16μ thick. The cyst wall is tough and resilient.

Maritrema sobolevi, whose adult is morphologically similar to that of *M. oocysta*, and to a lesser extent *M. calvertensis*, has a 3 host life-cycle. Its intramolluscan developmental stages have not been described, however it encysts in marine amphipods, and the adult lives in seals in the Caspian Sea. The specific distinction between *M. sobolevi* and *M. calvertensis* is retained on the basis of the slight morphological and anatomical differences between the adults and eggs, and differences between their hosts and aquatic habitats. Hopefully, future life-history studies of the former species will provide more information with which to assess its relationship to *M. calvertensis*.

Reimer (1963), having fed young gulls exclusively on shrimps caught in the Baltic Sea, later recovered from their intestines, flukes that he called *M. humile*. Deblock (1975), proposed 2 hypotheses to account for these findings: (i) the species was not *M. humile(=M.oocysta)* but *M. sobolevi;* and (ii) the species was *M. oocysta* i.e. snails infected with metacercarial cysts of *M. oocysta* were in the digestive tracts of shrimps, when they were fed to the gulls. A third hypothesis had been proposed previously (Deblock, 1965): (iii) *M. oocysta* has alternative life-cycles, sometimes involving 2 hosts, and sometimes involving 3 hosts, and *M. sobolevi* is a synonym of *M. oocysta*. There is, however, no direct evidence that a microphallid species can have such drastically different life-cycles. On the contrary, elucidation of the life-history of *M. calvertensis* has demonstrated that different species may have very similar adults, and quite different life-cycles i.e. the life-histories of *M. calvertensis* and *M. oocysta* are very different, but their adults are very similar. Hence, it seems most likely that hypothesis (i) is correct, and that *M. sobolevi* and *M. oocysta* are in fact different species.

Minute flukes, conforming to the description of *Maritrema oocysta*, have been found in the white-faced heron from Lake Crescent in Tasmania (Appendix 1), and in the water rat, darter, and little grebe in Queensland (Deblock and Pearson, 1968b). The life-cycles of neither the Tasmanian nor the Queensland specimens are known. It is believed, however, that the specimens which commonly infect the water rat in Queensland encyst in a freshwater atyid prawn (Pearson, pers. comm., 1978). Thus, the Queensland specimens may represent a *Maritrema* species which, like *M. sobolevi*, has a 3 host life-cycle, but which utilizes freshwater snails and decapods as intermediate hosts. It is evident that life-cycle studies are required in Tasmania, and in Queensland, to elucidate the natural relationships of these minute trematodes which are provisionally identified as *M. oocysta*.

Super sub-family MICROPHALLIDI (Ward, 1901)

Sub-family MICROPHALLINAE (Ward, 1901)

Tribe LEVINSENIELLINI (Stiles and Hassall, 1901)

Sub-tribe LEVINSENIELLINA (Stiles and Hassall, 1901)

Genus LEVINSENIELLA Stiles and Hassall, 1901)

Sub-genus LEVINSENIELLA Deblock and Pearson, 1970

2.3 *Levinseniella tasmaniae* (Smith, 1974)

2.3.1 Life-cycle

The life-cycle of *L. tasmaniae*, shown in Figure 2.12, is similar to that of *Maritrema calvertensis*. The primary intermediate host is *Coxiella badgerensis*. Cercariae emerge from the snail and infect the secondary intermediate host, *Austrochiltonia australis*. Water birds feeding on infected amphipods ingest metacercarial cysts, and become hosts to the adult fluke. During a study at Calvert's Lagoon in 1970, adults of *L. tasmaniae* were recorded from the chestnut teal, black-fronted dotterel, hooded dotterel and red-capped dotterel (Smith, 1974), and during the present study, the black duck and hoary-headed grebe have also been found to serve as definitive hosts.

2.3.2 Adult (Figures 2.13 and 2.14)

The adult, originally described as *Microphallus tasmaniae* Smith, 1974, was transferred recently to the genus *Levinseniella*, (Smith, 1979). The original description, which was based on adults that were fixed under "slight coverslip pressure", does not mention diverticula from the genital atrium of this species, which are characteristic of the genus *Levinseniella*.

The adult of the species is redescribed here, based on specimens from naturally infected water birds, and laboratory ducklings infected with cysts from naturally infected amphipods. Dimensions of ovigerous and non-ovigerous worms fixed in boiling 10% formal saline, without coverslip

FIG. 2.12 Levinseniella tasmaniae

Life-cycle

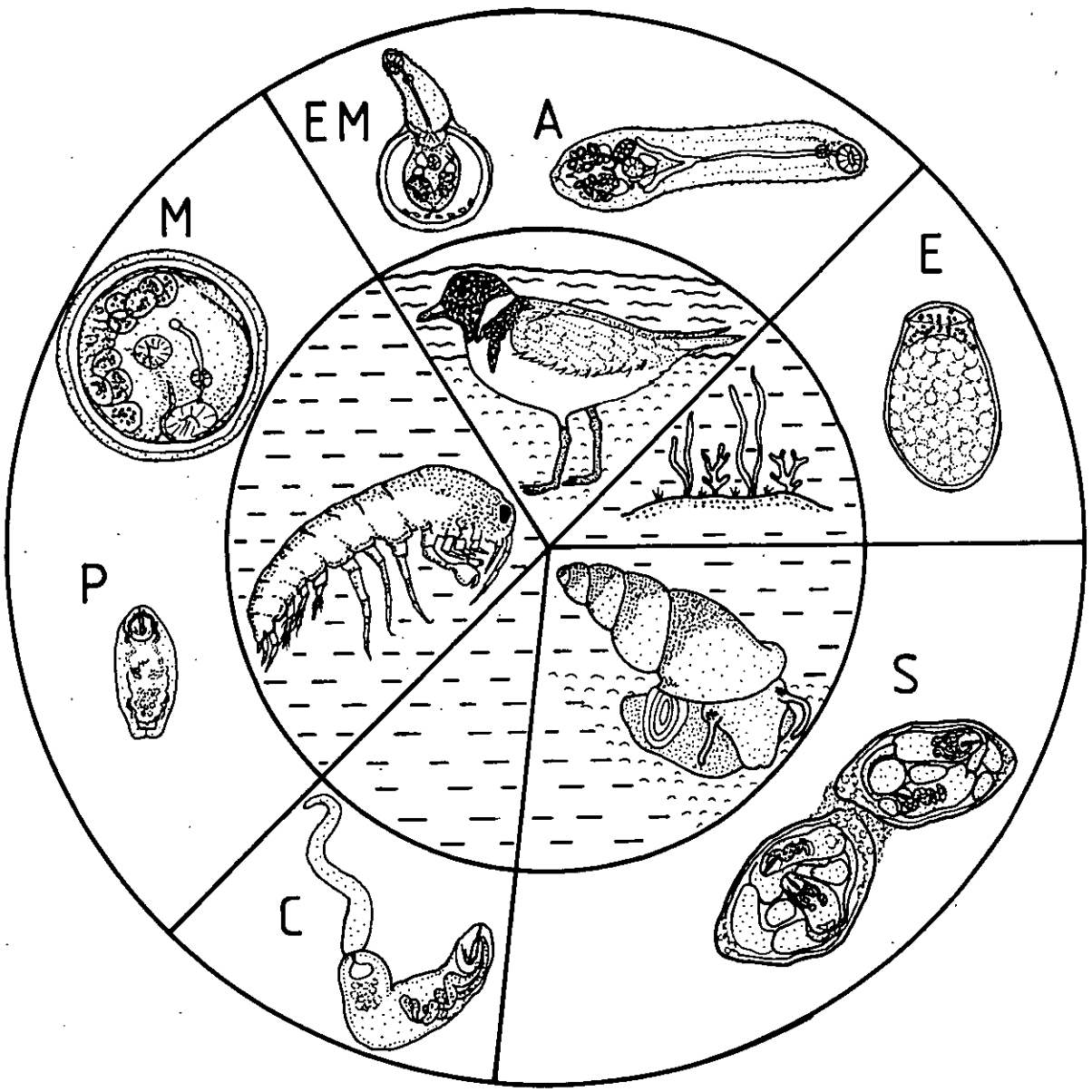


FIGURE 2.12 A, gravid adult; E, egg; S, two daughter sporocysts; C, swimming cercaria; P, post-cercaria; M, metacercarial cyst; EM, excysting metacercaria.

pressure, are given in Table 2.14. The dimensions of the holotype are also given, as these were not included in the original description.

TABLE 2.14 *Levinseniella tasmaniae*. Dimensions of adults recovered from an experimentally infected duckling 4 hours post infection: (a) ovigerous specimens, (b) non-ovigerous specimens. The dimensions of the holotype of the species (an ovigerous adult) are also presented, (c).

	(a)	(b)	(c)*
Sample size	5	5	1
Body length (BL)	711 (680 - 726)	668 (590 - 816)	892
Body width (BW)	195 (182 - 201)	190 (171 - 209)	287
Oral sucker length	54 (53 - 57)	53 (49 - 57)	57
Oral sucker width	52 (49 - 53)	49 (46 - 53)	53
Ventral sucker length	49 (46 - 53)	49 (46 - 53)	53
Ventral sucker width	45 (42 - 49)	43 (38 - 49)	53
Prepharynx length	106 (84 - 144)	67 (30 - 91)	125
Pharynx length	27 (25 - 30)	27 (-)	30
Pharynx width	23 (21 - 25)	23 (-)	25
Oesophagus length	185 (171 - 201)	181 (156 - 194)	258
L. caecum length	149 (144 - 179)	141 (137 - 144)	182
R. caecum length	162 (152 - 175)	135 (125 - 148)	190
Seminal vesicle length	49 (38 - 61)	44 (34 - 61)	95
Seminal vesicle width	34 (27 - 38)	30 (23 - 46)	49
Ovary length	76 (68 - 84)	72 (61 - 84)	80
Ovary width	50 (42 - 53)	48 (42 - 57)	49
L. testis length	73 (68 - 76)	72 (68 - 76)	91
L. testis width	64 (61 - 72)	60 (57 - 61)	72
R. testis length	76 (72 - 80)	77 (76 - 80)	91
R. testis width	64 (61 - 68)	60 (57 - 65)	76
BW/BL	0.27	0.28	0.32
OS(1+w)/VS(1+w)	1.13	1.11	1.04

(* Fixed under coverslip pressure)

Description:

Body dorsoventrally flattened, slightly concave ventrally, especially anteriorly; elongate pyriform, maximum width at level of testes. Spines embedded in outer tegument, quincuncially arranged, diminish in size posteriorly; anteriorly comb-like, with up to 9 teeth, number of teeth diminish posteriorly, peg-like spines on postero-ventral surface. Round, well-developed oral sucker sub-terminal, mouth ventral. Round, medial ventral sucker, protrusive in live worm, encircled by

FIG. 2.13 Levinseniella tasmaniae

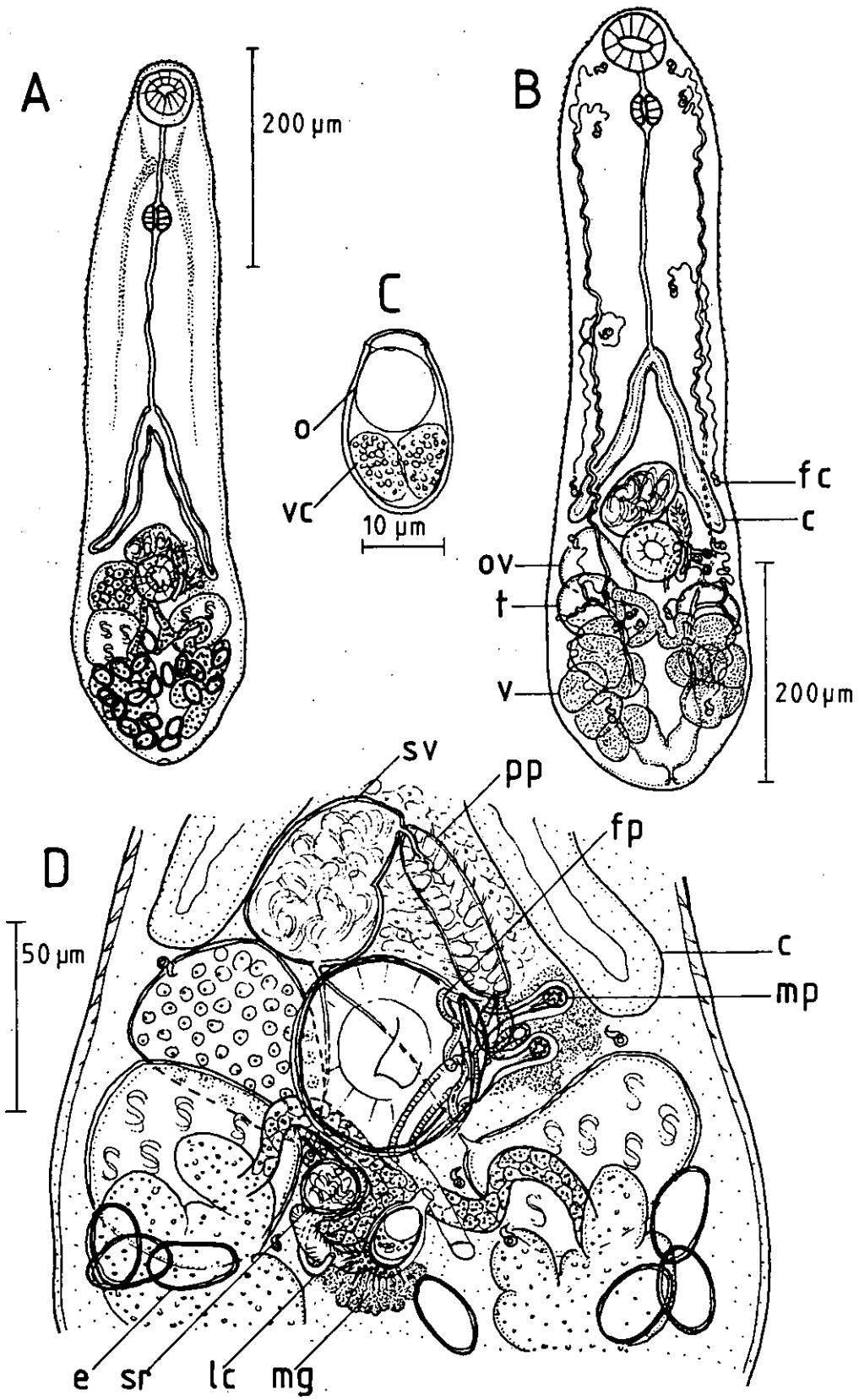


FIGURE 2.13 A, gravid adult, ventral view, from caecum of hoary-headed grebe; B, mature metacercaria, ventral view, excysted *in vitro*, showing distribution of flame-cells; C, egg in proximal part of uterus; D, detail of reproductive system of gravid adult, ventral view. (c: caecum; e: egg; fc: flame-cell; fp: female pouch; l: Laurer's canal; mg: Mehlis' gland; mp: male pouch; o: ovum; ov: ovary; pp: pars prostatica; sr: seminal receptacle; sv: seminal vesicle; t: testis; v: vitellaria; vc: vitelline cell.)

acetabulo-atrial muscle fibres; about 2/3 body length from anterior end. O.S.:V.S. ratio = 1.12 (1.11 - 1.13). Prepharynx long, pharynx barrel-shaped. Oesophageal bifurcation near middle of body, caeca diverge acutely, extend to level of anterior border of ventral sucker, dextral caecum contiguous with ovary, sinistral caecum contiguous with left testis. Unicellular tegumental glands scattered anterior to ventral sucker. Sinistral genital pore, longitudinal cleft, adjacent to ventral sucker, opening into genital atrium, occupied by small, male papilla. Two sub-parallel or divergent, pendulous diverticula, "male pouches", arise separately, in same plane, from sinistral, ventral wall of atrium, curve sinistrally, dorsally, terminate in slightly expanded bulbs. Internal surfaces of diverticula have sclerotized, longitudinal folds or ribs, forming 'rosette' of hooks or loops in each terminal bulb. Anterior male pouch about 19 (15 - 27)μ long, posterior one about 17 (15 - 19)μ long. Pouches surrounded by apparently glandular tissue. Large diverticulum, "female pouch", arises dorsally from dextral wall of genital atrium, near base of male papilla; thick walls convoluted, not sclerotized. Female pouch about 36 (30 - 42) × 14 (8 - 19)μ long. Metraterm joins genital atrium dorsally, in middle of sinistral wall, between male pouches. Oval testes posterolateral to ventral sucker, sinistral testis just anterior to dextral testis. Sperm duct arises from antero-medial wall of each testis; ducts unite dorsally, dextral to ventral sucker, at base of seminal vesicle. No cirrus pouch. Oval to reniform seminal vesicle lies between caecal arch and ventral sucker; attitude varies from transverse to nearly longitudinal. Numerous prostate gland cells scattered in parenchyma around distal ½ of seminal vesicle, and pars prostatica; ducts discharge through small papillae into lumen of thick-walled pars prostatica. Pars prostatica, 35 (23 - 49) × 15 (11 - 19)μ long, joins short ejaculatory duct at base of male papilla.

FIG. 2.14 Levinseniella tasmaniae

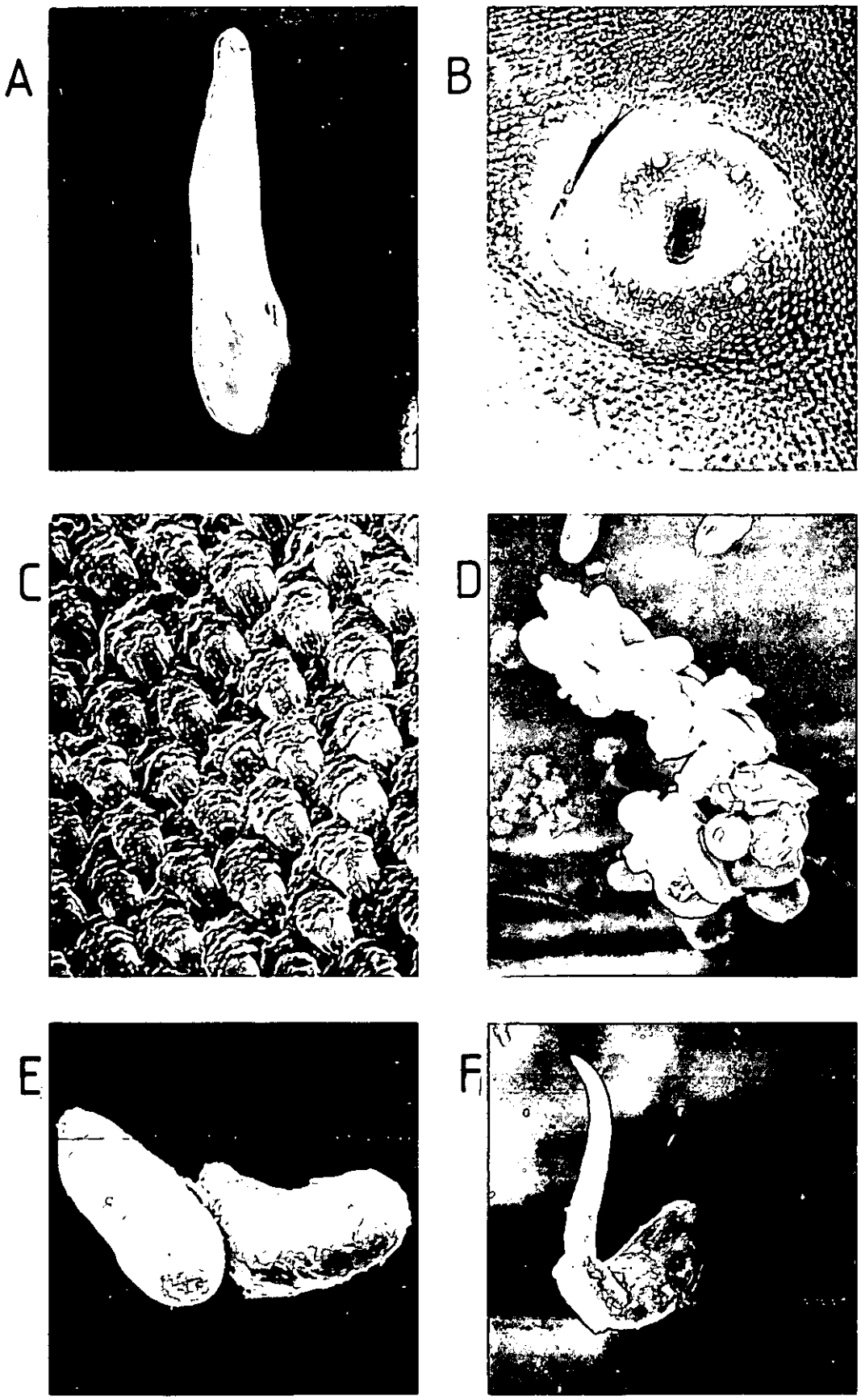


FIGURE 2.14 S.E.M. photographs - A, adult, ventrolateral view, ×130; B, ventral sucker and genital pore of adult, ×1,000; C, tegumental spines on anterodorsal surface of adult, ×4,000; D, daughter sporocysts clustered along portion of posterior vas deferens of snail host, ×60; E, two daughter sporocysts ×190; F, cercaria in swimming position, ventral view, ×600.

Stumpy male papilla, when everted through genital pore, measures 23 (15 - 27) × 30 (27 - 34)μ. Oval to triangular, dextral ovary overlies dextral border of ventral sucker, abuts dextral caecum and testis. 'Oogenotop' typical of family: short, narrow oviduct leads from sinistral border of ovary to large globular seminal receptacle, 27 (23 - 30) × 22 (19 - 23)μ; oviduct becomes wider, lined with cilia, leads sinuously to ootype; Laurer's canal arises from oviduct near seminal receptacle, opens dorsally; vitelline reservoir discharges contents into oviduct near ootype; radiating Mehlis' gland ducts enter proximal wall of ootype. Uterus passes posteriorly, dextrally, from ootype, forming series of loops posterior to, overlapping, dextral testis; crosses body posteriorly, forming loops posterior to, overlapping, sinistral testis; passes dorsal to left side of ventral sucker; joins genital atrium between male pouches. Many uterine eggs, (186 in fluke after 24 hours in laboratory duckling). Vitelline glands clustered in 2 groups of 6 to 9 large, irregular follicles; posterior, overlapping, testes. Excretory bladder V shaped, flame-cell formula 2[(2+2) + (2+2)] = 16.

Vertebrate hosts: *Anas castanea* (Eyton), *A. platyrhynchos* L. (experimental host), *A. superciliosa* Gmelin; *Charadrius melanops* Vieillot, *C. rubricollis* Gmelin, *C. ruficapillus* Temminck; and *Poliocephalus poliocephalus* (Jardine and Selby).

Habitat: Mainly caeca, also small intestine and rectum

Geographical location: Calvert's Lagoon, Tasmania

Type material: Tasmanian Museum - holotype K254 (ringed); paratypes K254 (not ringed), K253, K255, K256, K257 (metacercaria), K258 (metacercariae).

Relationships:

The elongate shape, acetabulo-atrial muscle fibres, acute angle between the intestinal caeca, and diverticula from the genital atrium (male and female pouches), are diagnostic of the genus *Levinseniella*. *L. tasmaniae* is characterised by its relatively small size, a genital atrium with two separate male pouches and one female pouch, a larger oral sucker than ventral sucker, and eggs lacking any ornamentation. The presence of a female pouch is a characteristic of the sub-genus *Levinseniella*. The only other species of this sub-genus having 2 male pouches are *L.* (L.) *heardi*, from East African reptiles; *L.* (L.) *cipangi*, from charadriiform birds of Eastern Siberia; and *L.* (L.) *howensis*, from charadriiform birds of Lord Howe Island, Australia. The latter species has only recently been redescribed (Pearson and Deblock, 1979).

L. tasmaniae can be distinguished from *L. heardi* and *L. cipangi* by its shape, smaller body, smaller suckers and pharynx, and by having 2 male pouches that arise separately from the atrial wall, rather than being at the extremities of the branches of a Y, as in the other 2 species (Figure 2.13). *L. tasmaniae* most closely resembles *L. howensis*. Both species have male pouches that arise separately from the atrial wall, and both infect charadriiform birds of Eastern Australia. The former species, however, has a smaller body, suckers and pharynx, and its eggs lack the characteristic and unusual ornamentation that is a feature of the eggs of *L. howensis*. Neither of the second anterior pair of flame-cells of *L. tasmaniae* are intercaecal, unlike those of *L. howensis*, one of which consistently lies between the caeca.

Use of the key to the sub-genus *Levinseniella*, presented by Deblock (1971), leads by the following steps to *L.* (L.) *heardi* and *L.* (L.) *cipangi*: 1 - 3 - 4. The following modification of the key would distinguish these species from *L.* (L.) *howensis* and *L.* (L.) *tasmaniae* (all measurements given in microns):

4 - Eggs ornamented by 2 transverse bands of thickening, like hoops around a barrel 4A

Eggs without such ornamentation 4B

4A - Body length, 720 - 1100. O.S., 60 - 80 diameter. V.S., 65 - 80 × 65 - 75. O.S./V.S. = 1.0. Pharynx, 50 - 63 × 42 - 53. Caeca long, 340 - 440, extending to the level of the equatorial plane of the ventral sucker. 2 semi-parallel male pouches arise separately from the genital atrium, in the same plane, the first 20 - 30 long, and the second 15 - 25 long. Female pouch thick-walled, 50 × 34, partly overlying the left side of the ventral sucker. Club-shaped male papilla, 20 - 25 × 10 - 15. Seminal vesicle, 90 × 50. Pars prostatica, 75 × 25. Eggs 16 - 22 × 10 - 13, with 2 transverse bands of thickening.

Intestinal parasite of birds (Charadriiformes) of Australia (Pacific Coast).....*L.* (L.) *howensis* Johnston, 1916

4B - 2 semi-parallel male pouches arise separately from the genital atrium.....4C

2 male pouches situated at the extremities of the 2 branches of a Y.....4D

4C - Body length, 423 - 816. O.S., 50 - 57 × 46 - 57. V.S., 45 - 51 × 41 - 49. O.S./V.S. = 1.12. Pharynx, 25 - 32 × 19 - 23. Caeca long, 123 - 162, extending to the level of the anterior border of the ventral sucker. 2 semi-parallel male pouches arise separately, the anterior, 15 - 27 long and the posterior, 15 - 19 long. Female pouch thick-walled, 36 × 14, overlying the left side of the ventral sucker. Stumpy male papilla, 15 - 27 × 27 - 34. Seminal vesicle, 58 - 39. Pars prostatica, 23 - 49 × 11 - 19. Eggs, 19 - 25 × 11 - 15, without ornamentation.

Intestinal parasite of birds (Anseriformes, Charadriiformes, and Podicipediformes) of Australia (Tasmania).....

L. (L.) *tasmaniae* (Smith, 1974)

4D - Body length, 1200 - 1700. O.S., 80-108 × 100-116. V.S., 84-96 × 68-96. O.S./V.S. = 1.20. Pharynx, 77 × 55. Caeca relatively short, 290, reaching the anterior border of the ventral sucker. Male genital sinus, 95 × 60, provided with 2 male pouches, 17 long, situated at the extremity of the 2 branches of a Y. Small, club-shaped, male papilla, 25 diameter. Female pouch well developed, 160-67. Metraterm, 120. Well-defined atrial musculature. Eggs, 17.5 × 10.

Intestinal parasite of reptiles (Scincoides) in East Africa (Kenya).....*L.* (L.) *heardi* Canaris, 1971

Body length, 900-1200. O.S., 85-95. V.S., 65-75. Pharynx, 55-60 × 43-48. Prepharyngial sphincter absent. Caeca long, 400-500. Male genital sinus, 80 × 80, provided with 2 small, equal male pouches, (12 × 12), situated at the extremities of the 2 branches of a transverse Y, 20-30 long. Small, club-shaped male papilla, 30 × 10-15. Small, membranous, female pouch, 50-55. Eggs, 20-22 long. Well-defined, acetabulo-atrial musculature.

Intestinal parasite of birds (Charadriiformes) of East Siberia (Sea of Japan)...*L.* (L.) *cipangi* Deblock and Pearson, 1970

syn.: *L. bucephalae* sensu Belopolskaia, 1954

Biology:

Domestic ducklings, raised under controlled conditions were fed metacercarial cysts from naturally infected amphipods from Calvert's Lagoon. The ducklings were then sacrificed at periods ranging from 0,4 to 19,4 days.

Nineteen percent (3/16) of laboratory ducklings were infected with from 1 to 29 worms. The percentage of flukes recovered compared to the number of cysts fed to the birds, varied from 0 to 36. No flukes were recovered from 12 ducklings killed more than 2 days after ingesting cysts, whereas flukes were recovered from 75% (3/4) of ducklings killed less than 2 days after ingesting cysts. This indicates that the longevity

of *L. tasmaniae* in domestic ducklings is only about 2 days.

The adult fluke shows marked site specificity within the alimentary tract of its experimental host - the intestinal caeca being the preferred habitat. After 4 hours, about 50% of excysted flukes were established in the caeca; and after 1,0 day, and 1,18 days, all flukes were living in the caeca. Metacercariae vary greatly in maturity at the time of excystment. The most advanced specimens are similar in size to adult flukes, and undergo very little development in the definitive host: the testes contain sperm, oogenesis is advanced, and phenolic egg-shell precursors are present in the vitellaria. After 4 hours *in vivo*, 75% (18/24) of excysted flukes had commenced egg production, and after 24 hours *in vivo*, all flukes were producing eggs. Eggs were produced at a very rapid rate during the one or two days that flukes lived in the definitive host, the maximum number of uterine eggs being found in one adult 24 hours post infection.

TABLE 2.15 *Levinseniella tasmaniae*. The number of eggs contained within the uterus of adults recovered from experimentally infected ducklings, after different periods of infection.

Period of Infection (days)	Sample size (no. of flukes)	Number of intra-uterine eggs Mean	S.D.	Range
0, 4	20	11.9	10.0	1 - 29
1, 0	1	186	-	-
1, 18	4	125.3	45.1	59 - 160

In naturally infected dotterels, ducks and grebes, flukes were located mainly, or exclusively, in the intestinal caeca (Figure 7.16). Dimensions of unflattened, fixed adults are only available for specimens taken from the hoary-headed grebe. These are shown in Table 2.16.

The sizes of adults from different individual grebes vary slightly; however, they do not differ significantly from metacercariae that produced eggs *in vitro* at 41°C, within 4 hours of excystment (Table 2.26). The mean number of uterine eggs in flukes taken from 2 hoary-headed grebes,

one black duck, one chestnut teal and 2 hooded dotterels, are shown in Table 2.17. If the rate of egg production is similar in experimentally infected ducklings and wild birds, then the results indicate that adults of *L. tasmaniae* live for only about 2 days in their natural hosts.

TABLE 2.16 *Levinseniella tasmaniae*. Dimensions of adults recovered from naturally infected hoary-headed grebes: (a) ovigerous flukes from Grebe No. 1; (b) ovigerous flukes from Grebe No. 2; and (c) non-ovigerous flukes from Grebe No. 2.

	(a)	(b)	(c)
Sample size	15	20	8
Body length (BL)	569 (484 - 673)	647 (507 - 741)	667 (575 - 801)
Body width (BW)	156 (129 - 171)	198 (167 - 217)	186 (167 - 198)
Body depth	92 (80 - 110)	112 (99 - 133)	118 (106 - 133)
Oral sucker length	50 (46 - 53)	57 (49 - 65)	54 (53 - 55)
Oral sucker width	46 (42 - 49)	52 (49 - 57)	52 (49 - 53)
Oral sucker depth	41 (36 - 46)	49 (46 - 53)	49 (42 - 53)
Ventral sucker length	45 (42 - 49)	50 (46 - 53)	50 (42 - 53)
Ventral sucker width	41 (38 - 46)	48 (46 - 49)	43 (38 - 49)
Ventral sucker depth	39 (38 - 40)	42 (-)	42 (-)
Prepharynx length	51 (19 - 95)	83 (46 - 125)	62 (30 - 91)
Pharynx length	25 (23 - 29)	27 (25 - 30)	25 (23 - 27)
Pharynx width	22 (19 - 23)	21 (21 - 23)	21 (-)
Oesophagus length	157 (129 - 198)	181 (152 - 228)	206 (175 - 243)
L. caecum length	123 (103 - 141)	141 (118 - 160)	159 (144 - 179)
R. caecum length	125 (103 - 137)	144 (125 - 156)	153 (137 - 175)
Seminal vesicle length	35 (27 - 42)	57 (46 - 65)	35 (30 - 38)
Seminal vesicle width	22 (19 - 27)	35 (27 - 38)	23 (19 - 30)
Ovary length	49 (38 - 61)	58 (46 - 65)	63 (53 - 72)
Ovary width	32 (27 - 42)	33 (29 - 34)	45 (30 - 53)
L. testis length	52 (46 - 61)	49 (42 - 57)	74 (65 - 76)
L. testis width	44 (40 - 46)	40 (38 - 42)	62 (57 - 76)
R. testis length	53 (42 - 65)	56 (46 - 61)	78 (65 - 91)
R. testis width	42 (34 - 53)	46 (34 - 57)	69 (57 - 80)
BW/BL	0.27	0.31	0.28
OS(1+w)/VS(1+w)	1.12	1.11	1.14

2.3.3 Egg

The egg is operculate, oval to urn-shaped and of uniform thickness, except for the edge of the operculum, which is thinner (Figure 2.13). Newly-formed egg-shells are colourless; however, they become golden-yellow when the shell protein becomes tanned, as they pass through the uterus. Ova in the proximal oviduct and in newly-formed eggs in the

TABLE 2. 17 *Levinseniella tasmaniae*. The number of eggs contained within the uterus of adults recovered from naturally infected water birds from Calvert's Lagoon: (a) *Anas castanea*, (b) *A. superciliosa*, (c) *Charadrius rubricollis* No. 1, (d) *C. rubricollis* No. 2, (e) *Poliocephalus poliocephalus* No. 1, (f) *P. poliocephalus* No. 2.

Bird host	Sample size (no. of flukes)	Number of intra-uterine eggs Mean	S.D.	Range
(a)	10	59.7	35.9	2 - 98
(b)	1	135	-	-
(c)	4	20.0	13.8	4 - 32
(d)	11	25.6	45.3	1 - 155
(e)	10	85.3	27.5	27 - 128
(f)	8	128.8	14.7	102 - 147

ootype and uterus, measure 10μ diameter. Vitelline granules are either scattered around the ovum in the egg, or occasionally contained in 2 membrane-bound, vitelline cells at the abopercular end. The ovum undergoes cell division in the uterus, however the miracidium does not complete development until after the egg is voided. Dimensions of fixed, unflattened uterine eggs, in flukes taken from experimentally infected domestic ducklings and naturally infected hoary-headed grebes, and in excysted metacercariae after 4 hours *in vitro* at 41°C, are shown in Table 2.18. There is little variation in size between the different groups of eggs.

TABLE 2.18 *Levinseniella tasmaniae*. The dimensions of eggs in flukes taken from a laboratory duckling, a naturally infected hoary-headed grebe and *in vitro* culture.

Bird host	P.I. (hours)	No. Eggs	Length	Width
Duckling	4	10	20 (19 - 23)	12 (11 - 14)
Hoary-headed Grebe	-	20	22 (19 - 25)	13 (11 - 15)
In vitro culture	4	10	23 (21 - 25)	13 (11 - 15)

Eggs deposited by adults in saline at room temperature were transferred to lagoon water. They were then exposed to laboratory-bred snails in petri-dishes at room temperature. The snails were dissected at intervals of 6 days, 2 weeks and 3 weeks; however none

were infected.

2.3.4 Sporocyst (Figures 2.14 and 2.15)

Sporocysts producing the cercaria of *L. tasmaniae* always occur in large numbers in *Coxiella badgerensis*, which indicates that, as in other microphallids, there must be at least 2 generations of sporocysts. The daughter sporocysts are concentrated in the gonad, and clustered linearly along the posterior portion of the oviduct in females, and the posterior vas deferens in males. They occur throughout other visceral tissues, particularly the digestive gland, pallial oviduct and prostate gland. These germinal sacs develop in tight clusters, adhering firmly to snail tissue. Young, daughter sporocysts are small, round, and contain only germ balls, whereas older ones are oval to pyriform and contain cercariae in all stages of development. Mature cercariae become very active within the sporocyst, and leave by pushing through the sporocyst tegument. After the departure of a mature cercaria, the sporocyst appears intact, and no escape pore can be seen. Sporocysts may contain from 1 to 10 fully developed cercariae.

Dimensions of unflattened, live and fixed daughter sporocysts are given in Table 2.19. They are based on the 5 largest sporocysts and 5 taken at random from 4 different snails that were releasing only cercariae of *L. tasmaniae*.

TABLE 2.19 *Levinseniella tasmaniae*. Dimensions of live and fixed sporocysts. The 5 largest and 5 selected at random, were taken from 4 different snails.

		No.	Length	Width
Largest	Live	20	190 (122 - 270)	107 (80 - 141)
	Fixed	20	187 (152 - 236)	104 (65 - 137)
Random	Live	20	147 (68 - 217)	97 (68 - 141)
	Fixed	20	156 (84 - 217)	103 (68 - 129)

FIG. 2.15 Levinseniella tasmaniae

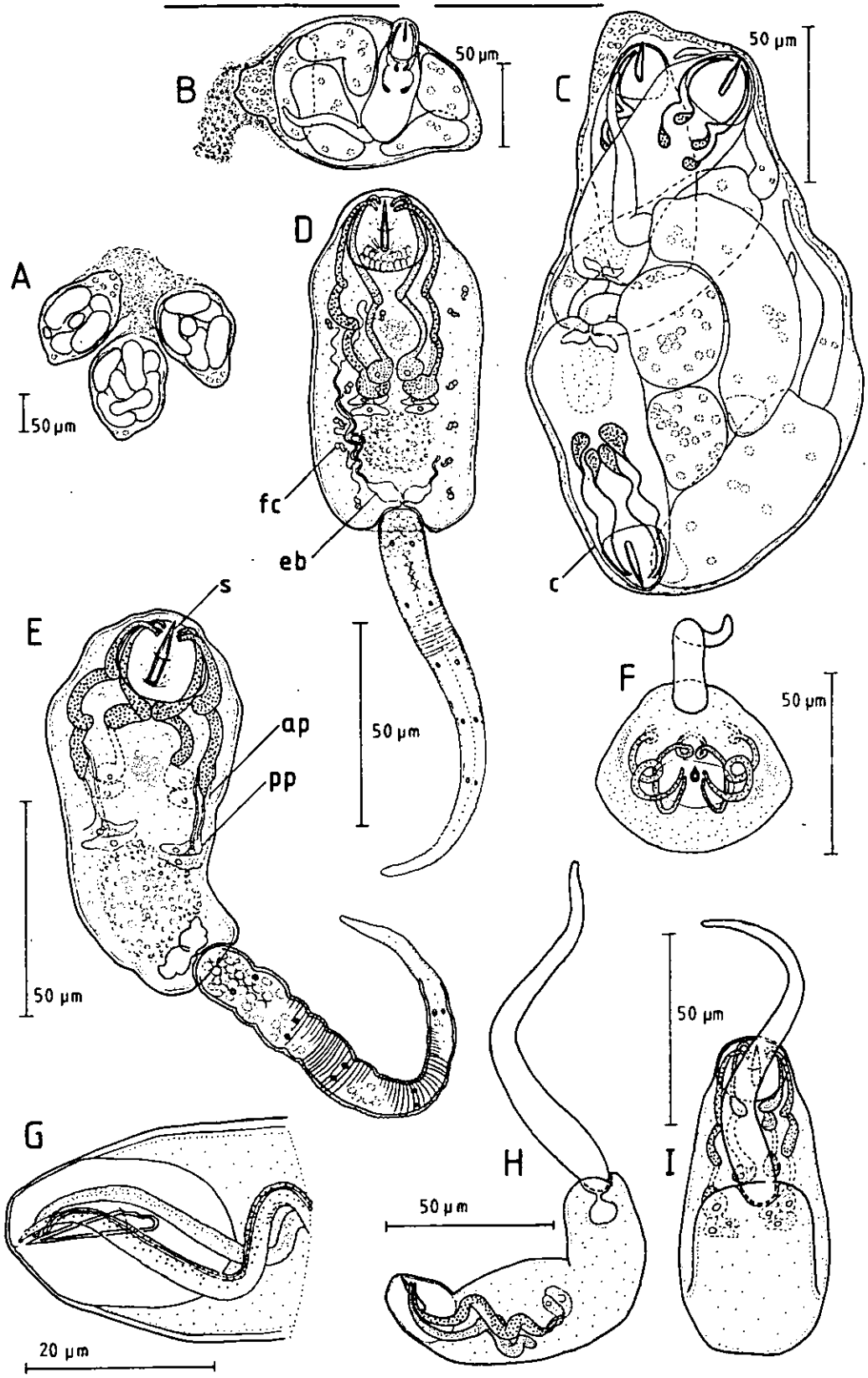


FIGURE 2.15 A, characteristic cluster of oval daughter sporocysts, embedded in snail tissue; B, cercaria emerging from daughter sporocyst; C, daughter sporocyst containing cercariae at varying stages of development and germ balls; D, mature cercaria, showing distribution of flame-cells, ventral view; E, mature cercaria, ventral view; F, anterior view of swimming cercaria, showing helical penetration gland ducts; G, cercaria, lateral view of stylet and terminal portion of penetration gland ducts; H and I, swimming position of cercaria, plan and lateral views respectively. (ap: anterior penetration gland; c: cercaria; eb: excretory bladder; fc: flame-cell; pp: posterior penetration gland; s: stylet.)

2.3.5 Cercaria

Morphology and anatomy:

The cercaria is a small xiphidiocercaria, typical of the family Microphallidae (Figures2.14 and 15). The body and tail are very contractile. Dimensions of specimens, measured shortly after emerging from naturally infected snails, are shown in Table 2.20. The oral sucker is subterminal, ventral, and well differentiated from the parenchyma. Prone, needle-like spines, not visible under light microscopy but seen under S.E.M., are embedded in the tegument of the body, except in the region of the oral sucker.

A sharp stylet is embedded in a muscular sheath in the dorsal side of the oral sucker. Its tip can be protruded terminally and moved in an arc. The stylet is asymmetrical in lateral aspect, with a long tapering point, curving slightly ventrally. It is uniformly thickened on all sides, including the base. A cluster of small cells near the middle of the body may be the rudimentary ventral sucker, and a larger group of such cells between the excretory bladder and penetration glands, may be the genital anlagen.

This cercaria has 4 pairs of so-called penetration glands: 2 large anterior pairs and 2 small posterior pairs that are difficult to discern. The ducts from the large glands spiral helically forward, and open near the stylet tip, the outer pair opening ventral to the inner pair. The fine ducts from the 2 pairs of small posterior glands follow the course of the ducts from the outer large glands, and open with them near the stylet tip. The coarse granular contents of the large pairsof glands are stained intensely by the vital stains neutral red and brilliant cresyl blue, whereas the contents of the smaller glands are very fine-grained, and pale staining.

The term "penetration glands" is appropriate, as the contents of the glands are discharged during invasion of the amphipod second inter-

mediate host. The ducts of the small posterior glands retain some of their contents after invasion, and are visible in the anterior region of post-cercariae for 6 days at 15°C. The body of the cercaria is stained blue by brilliant cresyl blue, but develops a pink outer coat after 1 or 2 hours. The pink coat is not formed around the cercaria of *Maritrema calvertensis* after staining with brilliant cresyl blue, and may be used to distinguish these microphallid cercariae at low magnification. This staining phenomenon may be related to the mucoid secretion, or capsule, that is formed by the cercaria of *L. tasmaniae* on the surface of the amphipod host, during invasion (Figure 2.21).

The tail joins the body in a postero-ventral socket, and narrows gradually from there to its tip. It has tegumental annulations and a small ventral keel. At the posterior end of the body, a V-shaped, bilobed excretory bladder is conspicuous when dilated. The distribution of flame-cells is shown in Figure 2.15. Not always visible in free-swimming cercariae, flame-cells are best seen in post-cercariae immediately after invasion of the second intermediate host. The flame-cell formula is $2[(2+2) + 2] = 12$.

Emergence from the molluscan host:

Cercariae emerge periodically from snails maintained in the laboratory under ambient light and temperature conditions. The peak of emergence occurs during the day (Figure 2.16). The number of cercariae emerging daily varies greatly for each snail, up to about 850. The average rate of emergence of cercariae of *L. tasmaniae* from their snail host appears to be markedly greater than that of *L. amnicolae* from *Amnicola pilsbryi*. Etges (1953), found that the cercariae of *L. amnicolae* emerged "at all times of the day, at a rate of 1 to 15 per day". Cercariae continue to develop in, and emerge from, isolated infected snails over long periods, the longest recorded period being 56 days.

The emergence from naturally infected snails, kept in the laboratory

FIG. 2.16 Levinseniella tasmaniae. Emergence of cercariae from the snail host. (mean ± 1 s.d)

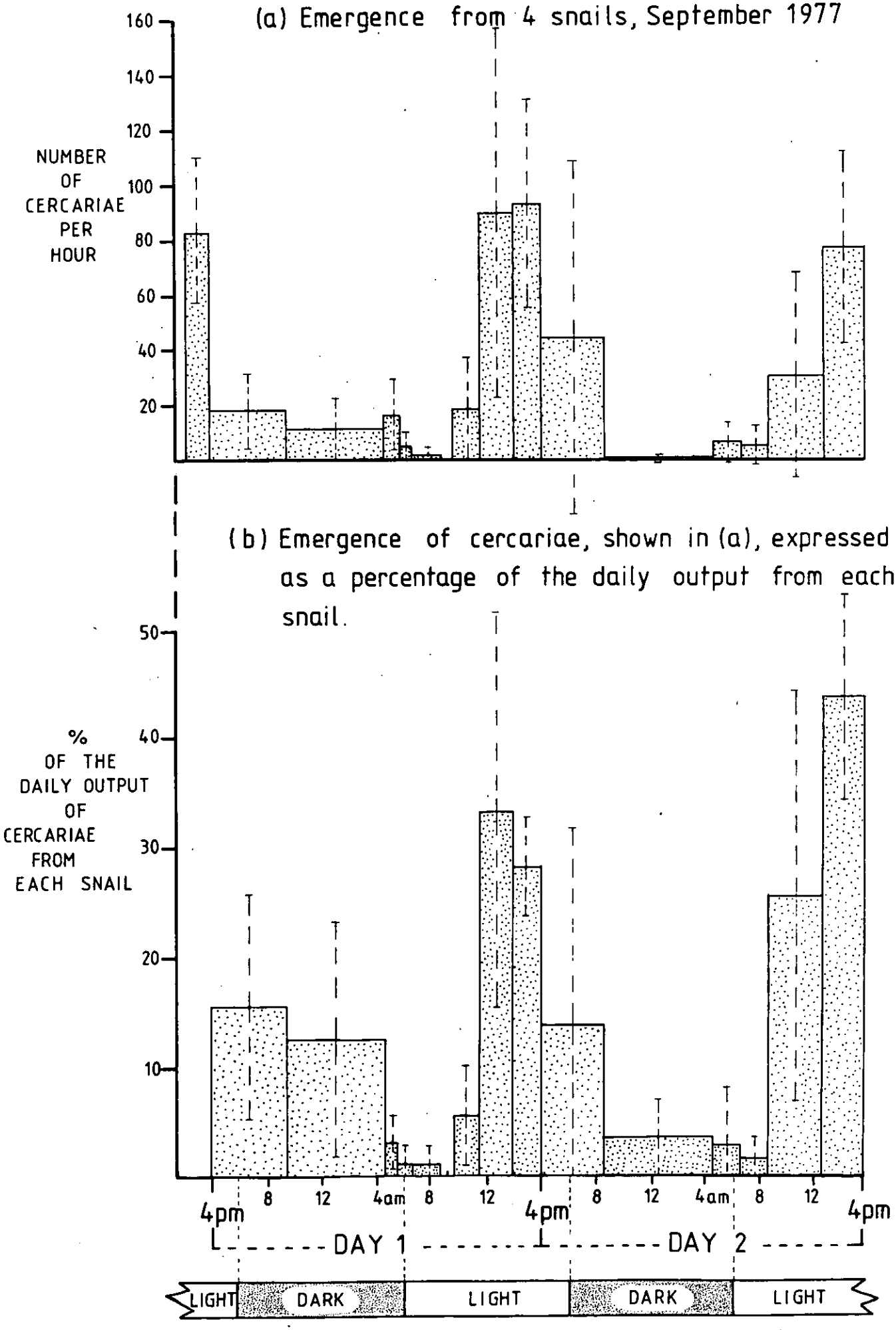


TABLE 2.20 *Levinseniella tasmaniae*. Dimensions of cercariae, measured, shortly after emerging from the snail host: (a) fixed, (b) live.

(a) Sample size	20
Body length	83 (76 - 89)
Body width	48 (46 - 52)
Body depth	29 (27 - 30)
Tail length	121 (118 - 125)
Tail width	11 (11 - 12)

(b)* Sample size	10
Oral sucker length	24 (20.5 - 27)
Oral sucker width	18 (14.5 - 25.5)
Length of posterior penetration gland and duct (P.G.L.)	51 (40 - 55.5)
Body length (B.L.)	92 (79.5 - 103.5)
P.G.L./B.L.	0.55
Stylet length (S.L.)	17 (16 - 17.5)
Stylet width	3 (-)
Stylet point (S.P.)	8 (7 - 8.5)
S.P./S.L.	0.5

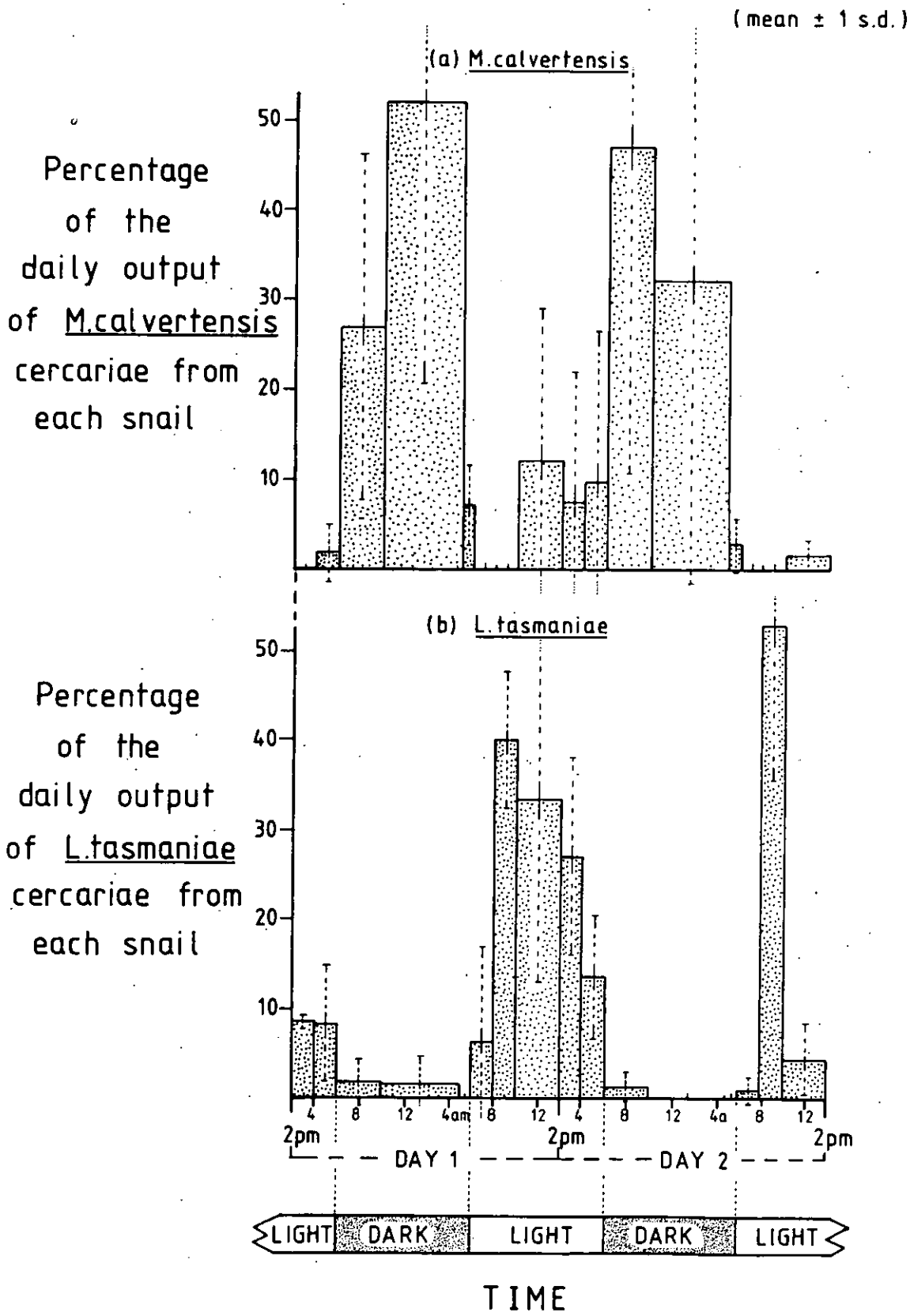
(*compressed under coverslip pressure)

in September, is shown in Figure 2.16. The results, expressed as the average number of cercariae emerging from 4 snails per hour, show that most cercariae emerged during the afternoon. To take account of the variation in the numbers of cercariae leaving different snails, the same results are also expressed as a percentage of the daily output from each snail.

Many wild snails are concurrently infected by both *Levinseniella tasmaniae* and *Maritrema calvertensis*. In such cases the cercariae of each species behave as in single infections, i.e. the peak of emergence of cercariae of *L. tasmaniae* occurs during the day, about 12 hours out of phase with that of *M. calvertensis*, which occurs at night (Figure 2.17).

The peak of emergence of cercariae of *L. tasmaniae* in November (Figure 2.17), was markedly earlier in the day than in September (Figure 2.16), when the climate was cooler.

FIG. 2.17 Maritrema calvertensis and Levinseniella tasmaniae. Cercarial emergence from 4 snails concurrently releasing cercariae of both microphallid species, in November 1978.



To investigate the factors determining the periodicity of emergence of cercariae, 10 snails were kept at a constant temperature, 15°C, and exposed alternately to 12 hours of light, 43 lumens/sq.ft., coinciding with day, and 12 hours of dark coinciding with night, over a 2 day period. These snails were conditioned at 15°C, in continuous light at 43 lumens/sq.ft., for 12 hours before being exposed to the first experimental dark period. The resultant emergence of cercariae under these 'normal' light conditions, shown in Figure 2.18, was investigated by analysis of variance.

TABLE 2.21 Analysis of variance table showing the influence of light and dark periods on the emergence of *L. tasmaniae* from its snail host, in a 'normal' controlled light regime.

Source of variation	d.f.	M.S.	F	P	Sig.
Light: dark periods (L)	1	278389	41.2	P<0.001	***
Day (D)	1	2706	0.4	P>0.1	N.S.
Interaction (L - D)	1	4347	0.6	P>0.1	N.S.
Residual	36	6761			

There was a highly significant difference between the numbers of cercariae that emerged during the light and the dark periods, but no significant difference between the levels of emergence on Day 1 and Day 2. The mean number of cercariae emerging from each snail during the light periods was 176.0, and during the dark periods was 9.1. The mean level of emergence was 84.3 cercariae per snail on Day 1, compared with 100.8 cercariae per snail on Day 2.

A similar experiment was conducted simultaneously, using 10 different snails, and with the light periods coinciding with night, and the dark periods coinciding with day. These snails were conditioned at 15°C in continuous dark for 12 hours before being exposed to the first experimental light period. Emergence of cercariae under this 'reversed' controlled light regime, shown in Figure 2.18, was also investigated by analysis of variance.

FIG. 2.18 Levinseniella tasmaniae. Emergence of cercariae under controlled light conditions, at 15°C, (number of host snails = 10).

mean ± 1 s.d.

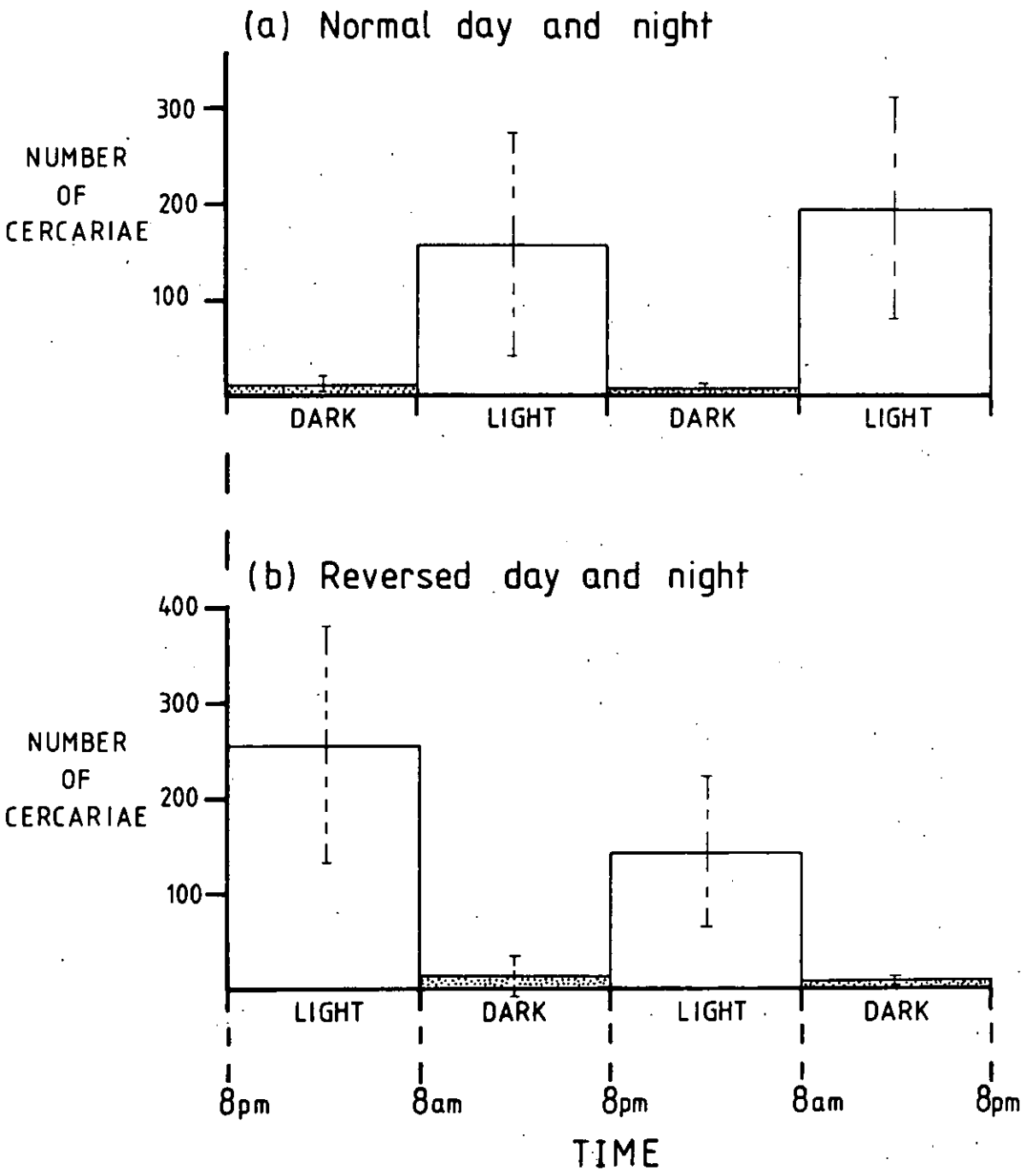


TABLE 2.22 Analysis of variance table showing the influence of light and dark periods on the emergence of *L. tasmaniae* from its snail host, in a 'reversed' controlled light regime.

Source of variation	d.f.	M.S.	F	P	Sig.
Light:dark periods (L)	1	354568	63.5	P<0.001	***
Day (D)	1	33408	6.0	0.01<P<0.05	*
Interaction (L - D)	1	27667	5.0	0.01<P<0.05	*
Residual	36	5577			

The difference between the numbers of cercariae emerging from *Coxiella badgerensis* during the light and dark periods was highly significant. The average number of cercariae emerging from each snail during the light periods was 199.0 and during the dark periods was 10.7. In this experiment, there was a significant decrease in the average number of cercariae emerging from each snail from Day 1 (133.8), to Day 2 (76.0).

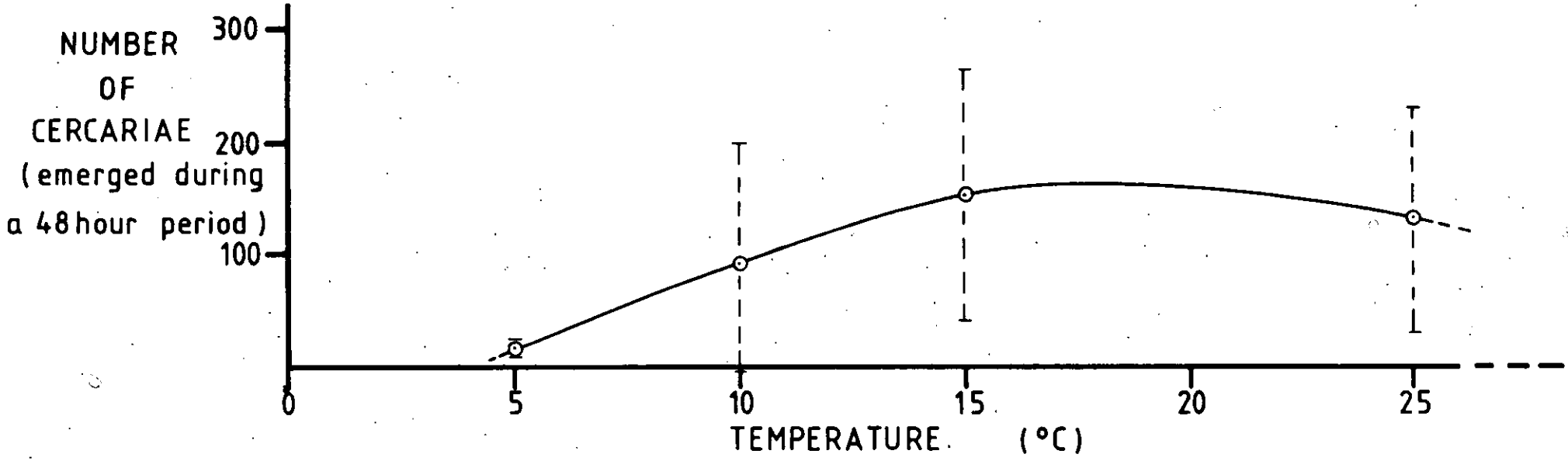
The effect of temperature on emergence was investigated by simultaneously keeping groups of 5 snails in constant dark for 2 days, under controlled temperature conditions: 5, 10, 15 and 25°C. The number of cercariae emerging from each snail was counted daily, and the results, shown in Figure 2.19, were investigated by analysis of variance (Table 2.23).

TABLE 2.23 Analysis of variance table showing the effect of temperature on emergence of *L. tasmaniae* from its snail host.

Source of variation	d.f.	M.S.	F	P	Sig.
Temperature (T)	3	8918	1.9	0.2>P>0.05	N.S.
Day (D)	1	11223	2.3	0.2>P>0.05	N.S.
Interaction (T - D)	3	13694	2.9	0.2>P>0.05	N.S.
Residual	32	4783			

There was no significant difference between the numbers of cercariae that emerged at each temperature, nor between the numbers of cercariae that emerged on Day 1 and Day 2. The average number of cercariae emerging per snail per day was: 8.0 at 5°C, 46.1 at 10°C, 75.9 at 15°C and 65.2 at 25°C. The average number of cercariae emerging per snail on

FIG. 2.19 Levinseniella tasmaniae. Emergence of cercariae under controlled temperature conditions, in the dark, (number of host snails in each group = 5).



Day 1 was 65.6, and on Day 2 was 32.5. Under the conditions of this experiment, emergence of *L. tasmaniae* from its snail host was not directly related to temperature, however this result may be misleading. Two previous experiments (Tables 2.21 and 2.22), have shown that more cercariae of *L. tasmaniae* emerge in the light, than in the dark; hence, the effect of temperature on emergence was obscured in this experiment by keeping the host snails in the dark. A more valid measure of the effect of temperature on emergence of cercariae of *L. tasmaniae*, would be to maintain the host snails at different constant temperatures under constant light conditions.

The results of this investigation of the periodicity of emergence of the cercaria of *L. tasmaniae*, indicate that, as with *Maritrema calvertensis*, emergence is influenced by environmental light conditions. Under controlled conditions, the periodicity of emergence was reversed when host snails were kept in a 'reversed' day-night regime. In direct contrast to *M. calvertensis*, however, emergence occurred mainly during the periods of light. In fact, more cercariae emerged during the light periods than the dark periods, whether under natural or artificial light conditions, and whether under 'normal' or 'reversed' day-night regimes. Emergence of the cercaria of *L. tasmaniae* is either stimulated by light conditions, or inhibited by dark conditions, or both; and the periodicity observed under natural light conditions is due to the normal alternation of a dark period (night), and a light period (day). Under natural conditions, this periodicity of emergence is probably influenced by environmental temperature, and probably varies seasonally with changes in temperature and photoperiod. The emergence of *L. tasmaniae*, like *M. calvertensis*, is probably stimulated by behavioural or physiological responses of the host snail to changes in the external environment, (viz. light). The 2 microphallid species apparently take different 'cues' from their molluscan host.

Swimming:

The cercaria swims continuously until contacting a suitable host, or becoming exhausted. It swims with the ventral surface uppermost, and the anterior of the body often extended. As the tail lashes vigorously from side to side in the vertical plane, the cercaria swims, jerkily, along an erratic course. The longevity of the cercaria at 15°C was found to be much less than that of *M. calvertensis*. All swimming had ceased 12 hours after emergence, and 50% of cercariae had ceased swimming after about 7 hours (Figure 2.20).

Invasion of the crustacean host: (Figure 2.21)

The cercaria is only known to infect the amphipod *Austrochiltonia australis*. When placed in a crystal dish with a live amphipod, the cercaria continues to swim erratically until contacting the host cuticle, whereupon swimming immediately stops. The invading cercaria attaches by the anterior end, and the tail contracts, curls and is shed after about 30 seconds. Slow rhythmic contractions pass anteriorly over the body for several minutes, after which it can move freely within a sticky outer capsule which is firmly attached to the host. This outer capsule, probably formed by exudation of a mucoid secretion, holds the cercaria in position during penetration. A hole is made in the host cuticle, following discharge of the secretions of the large penetration glands over the zone of contact, and vigorous probing and rotating movements by the stylet in the same area. The cercaria squirms through the hole, leaving the 'capsule' on the surface, where it remains long afterwards. The process of invasion takes up to about 10 minutes.

A similar cyst-like capsule attaches the virgulate xiphidiocercaria of *Acanthatrium oregonense* to the gills of its arthropod intermediate host (Burns, 1961a). No previous reports have been found of such a capsule being formed by microphallid xiphidiocercariae.

FIG. 2.20 Levinseniella tasmaniae and Maritrema calvertensis.
Longevity of cercariae at 15°C.

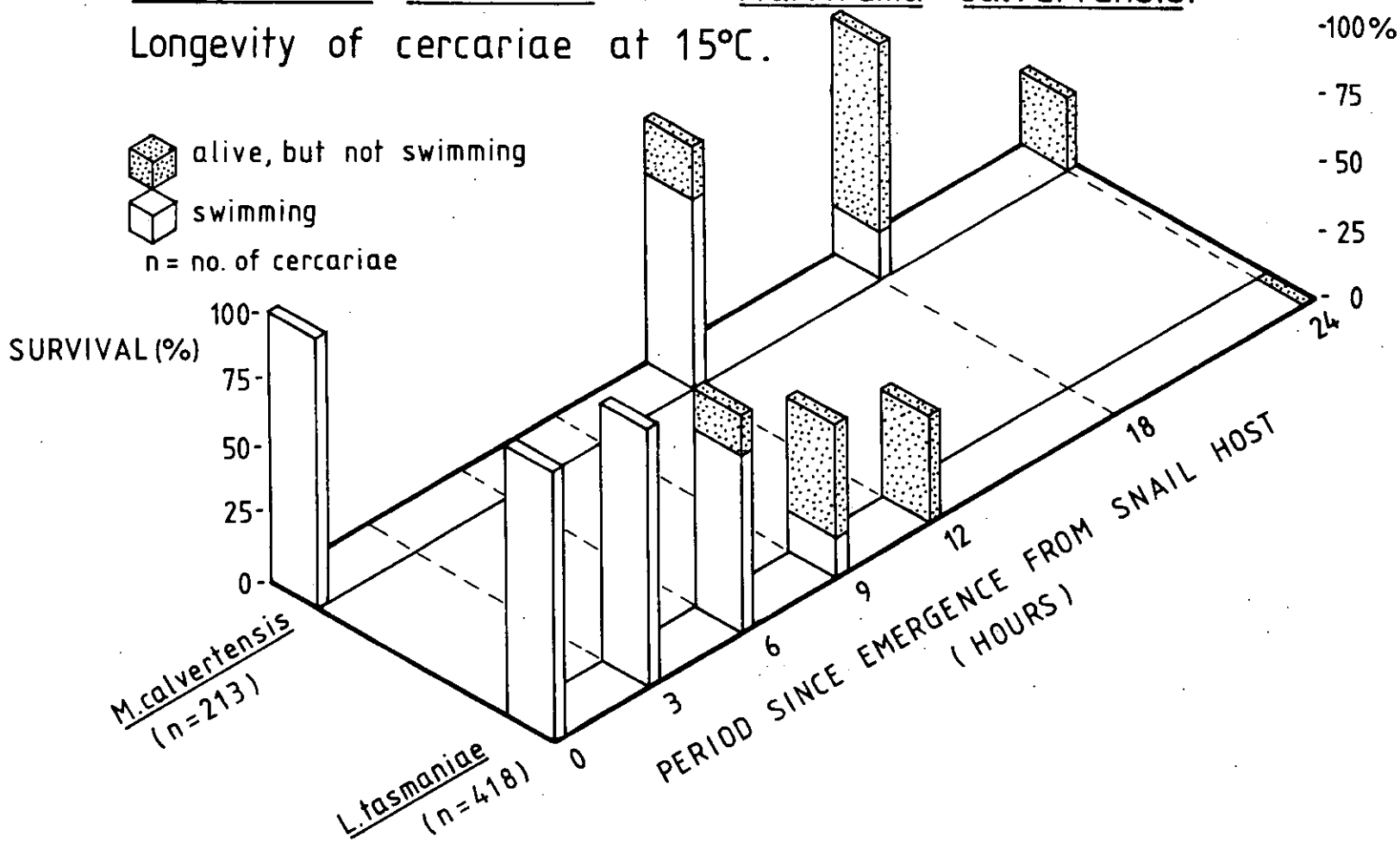


FIG. 2.21 Levinseniella tasmaniae

Invasion of Austrochiltonia australis

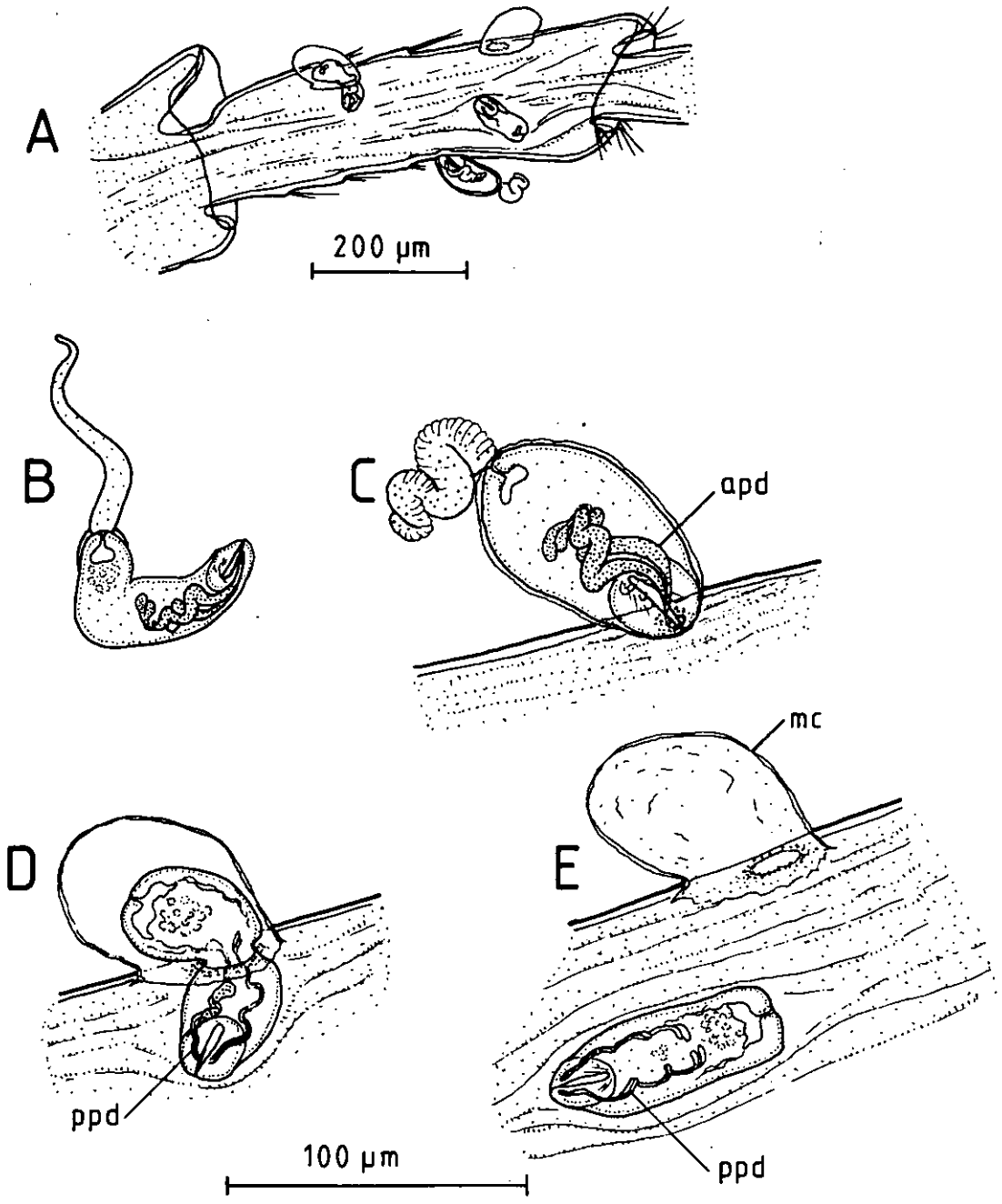


FIGURE 2.21 A, portion of a peraeopod of *A. australis*, showing 3 individuals of *L. tasmania* at different stages of invasion; B, swimming cercaria; C, cercaria attached at anterior end to host cuticle, tail strongly contracted; D, mucoid capsule adhering to host cuticle as cercaria squeezes through a small hole, anterior penetration gland ducts almost completely discharged; E, mucoid capsule remains on surface of amphipod as post-cercaria migrates through host tissues. (apd: anterior penetration gland duct; mc: mucoid capsule; ppd: posterior penetration gland duct.)

Invasion often occurs through the dorsal surface of the amphipod, and rarely through the anterior region or antennae. Ventilating currents caused by beating pleopods, draw cercariae posteriorly and ventrally, and hence most invade through the limbs or at the base of the limbs. A few hours after exposure of an amphipod to infection, post-cercariae were concentrated in the posterior body segments.

In a controlled experiment, groups of 50 newly emerged cercariae were each placed with a laboratory-bred amphipod, in separate crystal dishes, for about 12 hours at room temperature, and then transferred to aerated 2L containers of lagoon water, where they were maintained for 6 days at 15°C. When dissected, 15 out of the 26 amphipods (58%), were infected, and the incidence of post-cercariae is shown in Table 2.24. The results, compared to the results of a similar experiment involving *M. calvertensis*, indicate that *L. tasmaniae* is less infective to amphipods than is *M. calvertensis*. Amphipods were infected by significantly more post-cercariae of *M. calvertensis* than *L. tasmaniae*. A similar experiment was conducted, exposing laboratory-bred ostracods to cercariae of *L. tasmaniae;* however no ostracods became infected.

TABLE 2.24 The incidence of post-cercariae in laboratory-bred amphipods, after each individual was exposed to 50 cercariae of: (a) *L. tasmaniae*, and (b) *M. calvertensis*.

	No. hosts	$\bar{X}a$	$\bar{X}t$	V_{Xt}	$\bar{X}g$	95% conf. limits
(a)	26	3.0	0.387	0.170	1.4	0.7 - 2.6
(b)	23	11.5	0.797	0.307	5.3	2.6 - 9.9

($t = 2.90$, d.f. = 47, $0.01>P>0.001$, **)

2.3.6 Growth and development in the crustacean host

Introduction:

The effect of temperature on the growth and development of *Maritrema calvertensis* in its crustacean intermediate hosts, has been described and discussed (2.2.6). The results of a similar study of

TABLE 2.25 *Levinseniella tasmaniae*. The dimensions of post-cercariae, (P) and metacercarial cysts (M), recovered from experimentally infected amphipods.

(i) Growth in *Austrochiltonia australis*, at 5°C

P.I. (days)	P/M	No.	Length Mean	Length S.D.	Width Mean	Width S.D.
(0, 8	P	30	83	5	35	3...at room temperature)
6, 0	P	30	82	5	36	2
14, 0	P	30	82	3	37	2
22, 0	P	30	81	3	38	2
28, 0	P	30	85	3	38	2

(ii) Growth in *Austrochiltonia australis*, at 15°C

P.I. (days)	P/M	No.	Length Mean	Length S.D.	Width Mean	Width S.D.
6, 0	P	30	96	5	42	2
7, 0	P	30	104	5	45	2
13, 0	P	30	135	12	59	5
20, 0	P	30	219	22	107	9
28, 0	P	30	246	36	124	17
28, 0	M	10	215	13	198	7
35, 0	P	30	278	41	142	25
35, 0	M	23	258	32	241	34
42, 0	P	30	230	21	101	13
49, 0	P	30	257	33	129	26
49, 0	M	30	284	41	261	41
57, 0	M	23	305	11	282	15

(iii) Growth in *Austrochiltonia australis*, at 25°C

P.I. (days)	P/M	No.	Length Mean	Length S.D.	Width Mean	Width S.D.
4, 0	P	30	128	19	59	7
6, 0	P	30	207	19	96	12
10,0	P	30	226	35	98	15
10, 0	M	16	230	33	209	28
13, 0	P	30	200	22	93	11
13, 0	M	30	282	35	266	14
21, 0	P	30	249	29	103	22
21, 0	M	30	306	12	288	13
28, 0	M	30	311	19	290	17

Levinseniella tasmaniae in the amphipod *Austrochiltonia australis*, are presented here. The growth and development of these 2 microphallid species in *A. australis* are briefly compared and contrasted.

Results:

The dimensions of *L. tasmaniae* in its crustacean host, at different temperatures, and at different intervals after invasion, are shown in Table 2.25. Where possible, the mean length and width of 30 post-cercariae, or metacercarial cysts, are given. Encystment did not occur simultaneously for all post-cercariae so that for a period after invasion, hosts were infected by both free and encysted stages.

Growth and development at 15°C is described below (average dimensions are presented, in microns), and illustrated in Figure 2.22-

After penetration of the cuticle of the body and limbs, the post-cercariae migrate in the haemolymph, encysting in haemolymphatic sinuses after 4 to 8 weeks. Metacercarial cysts are mainly distributed in the thoracic and anterior abdominal segments, however, one or 2 cysts have rarely been found in the enlarged propod of the second gnathopod of mature males.

Seven and a half hours after penetration, the post-cercaria measured 83 × 35. Fine penetration gland ducts (containing a non-granular secretion that is stained by brilliant cresyl blue), were visible in the oral sucker region. No other evidence of the penetration glands remained, except for some scattered granular bodies, probably degenerating gland cells. These bodies stained deeply with vital stains viz. neutral red and brilliant cresyl blue. The stylet and V-shaped excretory bladder were unchanged, and the tegument appeared aspinous under light microscopy.

After 6 days, the post-cercaria measured 96 × 42. No trace of penetration glands remained except for the fine anterior ducts still visible in the oral sucker region. The stylet, oral sucker and

FIG. 2.22 Levinseniella tasmaniae. Growth and development in Austrochiltonia australis.

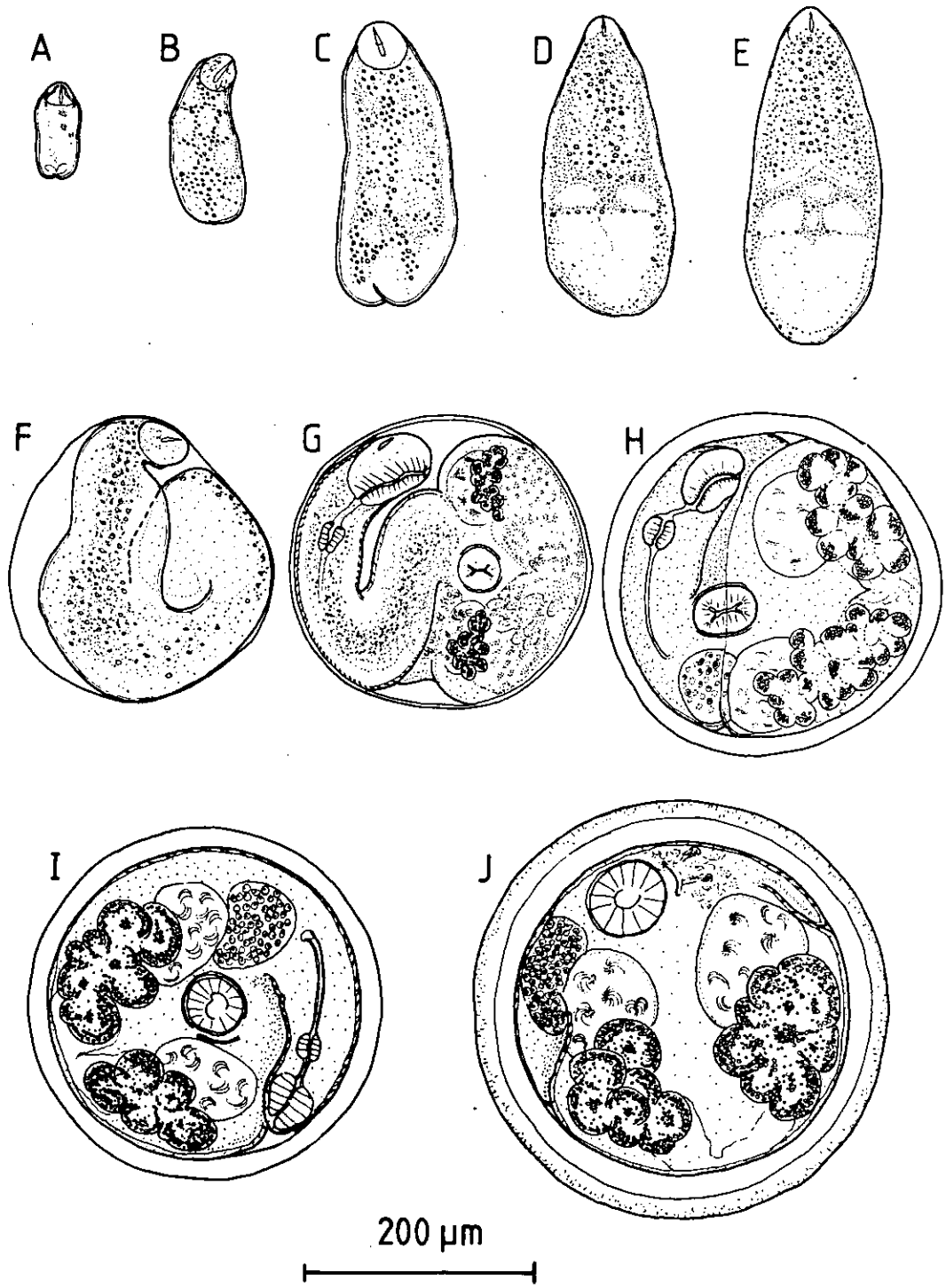


FIGURE 2.22 A, after 8 hours at room temperature; B, after 4 days at 25°C, or 13 days at 15°C; C, after 21 days at 15°C; D, after 28 days at 15°C; E, post-cercaria after 10 days at 25°C, or 35 days at 15°C; F, metacercarial cyst after 10 days at 25°C, or 35 days at 15°C; G, after 13 days at 25°C, or 57 days at 15°C; H, after 21 days at 25°C; I, after 28 days at 25°C; J, after 14 days at 25°C, and a further 165 days at room temperature.

excretory bladder were unchanged. After 13 days the post-cercaria measured 135 × 59. The body appeared darker, due to the accumulation of small cystogenous granules which oozed out through the aspinous tegument under pressure and looked like unwinding scrolls. Fine anterior penetration gland ducts still persisted in some post-cercariae, but were absent in most. After 20 days the body, 219 × 107, was very dark, due to the increased accumulation of cystogenous granules, up to 2μ diameter. Regions in the body where adult organs were developing appeared pale. The stylet was thin-walled and less distinct. No encystment had occurred. By the 28th day, 19% of post-cercariae had encysted, the cyst measured 215 × 198, and was bounded by a single, pliable membrane, about 2μ thick. The remaining post-cercariae measured 246 × 124, the body appearing very dark, except around the periphery and anterior end. The developing testes, thin vestigial stylet and enlarged excretory bladder, were visible among the cystogenous granules. After 35 days 27% of post-cercaria had encysted, forming thin-walled cysts, 258 × 241, and about 4μ thick. One hundred percent excystment occurred in 0.5% pancreatin, after 2 hours at 40°C, however the excysted meta-cercariae were very immature, and indistinguishable from the remaining post-cercariae. The body of the encysted metacercaria was still densely packed with cystogenous granules, and the stylet, although very thin, persisted. The excretory bladder occupied almost the posterior 1/3rd of the body, and the tegument remained aspinous. After 49 days, 53% of post-cercariae had encysted. The cysts, mostly thin-walled about 5μ thick, measured 284 × 261. The bodies of the remaining post-cercariae were obscured by cystogenous granules ranging up to 4μ diameter, but the outlines of the reproductive organs, suckers and excretory bladder, were discernible. The stylet had been resorbed, and small tegumental spines were visible in the anterior region of the body. After 57 days, all post-cercaria had encysted. The cysts measured 305 × 282, with a single-

layered wall, up to 11μ thick. All of the metacercariae excysted after 1 hour in 0.5% pancreatin at 40°C. The reproductive system was well-developed: the testes contained sheafs of spermatids but no mature sperm; the vitellaria, phenolic egg-shell precursors;and the ovary, small immature ova. The voluminous excretory bladder was packed with membrane-bound refractile granules, making the rear of the body appear dark. No large cystogenous granules remained, however the anterior of the body contained large numbers of small irregular cells, packed with very fine grains. These may have been cystogenous glands, responsible for producing the inner proteinaceous cyst layer present in fully developed cysts, but not yet formed in these cysts. Tegumental spines were well-developed, and widespread. Tegumental gland cells were scattered in the anterior of the body, where spines were most prominent.

Growth and development at 5°C -

Although little or no development occurred at this temperature, the post-cercariae survived and kept moving for at least 28 days. After 6 days, the post-cercaria measured 82 × 36, and after 28 days, it measured 85 × 38. The stylet and oral sucker were still distinct, and the fine ducts of the small penetration glands still visible in the anterior region, 4 weeks after invasion. No encystment occurred.

Growth and development at 25°C - (Figure 2.22)

The post-cercaria measured 207 × 96 after 6 days. The stylet and oral sucker were distinct, the tegument appeared aspinous and the body dark, due to the accumulation of cystogenous granules of varying size. After 10 days, 16% of post-cercariae had formed thin-walled, pliable cysts, within which the metacercariae actively squirmed and revolved. The cyst, measuring 230 × 209, and about 2μ thick, was easily ruptured mechanically. The remaining post-cercariae, 226 × 98, were densely packed with cystogenous granules up to 7μ diameter. Organogeny

was advanced and the rudimentary reproductive organs and developing caeca were visible, though indistinct. Thirteen days after invasion, 26% of post-cercariae had encysted, the cyst measuring 282 × 266, with walls varying up to 10μ thick. One hundred percent excystment occurred when these cysts were exposed to 0.5% pancreatin in Hank's saline at 40°C, for 1½ hours. Thin stylets persisted in the least mature metacercariae. The adult organs of these worms were rudimentary, and their bodies still contained masses of cystogenous granules. Tegumental spines had developed in the oral sucker region. The more advanced metacercariae had well-developed vitellaria, producing phenolic egg-shell precursors. The testes contained bundles of spermatids but no mature sperm, and the ovary, only small, undifferentiated cells. After 21 days, 56% encystment had occurred. The cysts measured 306 × 288, with walls composed of a single uniform layer, up to 19μ thick. All of the metacercariae excysted after 3 hours in 0.5% pancreatin in Hank's saline at 41°C, and the ovaries of the most mature worms contained mature ova. These worms had motile sperm in their seminal vesicles, and were producing eggs, within 4 hours of exposure to elevated temperature. After 28 days, all post-cercariae had encysted, the cyst measuring 311 × 290. The cyst wall, up to 23μ thick, was still composed of only one homogeneous layer. All excysted metacercariae were very mature: after 1 hour, at 41°C, they had sheafs of immotile spermatids in the testes, and mature ova in the ovary; and after 4 hours they had motile sperm packed into the seminal vesicle and seminal receptacle, and up to 28 eggs in the uterus.

After 14 days at 25°C, some infected amphipods were transferred in containers, to a sunny window ledge at room temperature. After 165 days, the cysts from these amphipods measured 357 × 358, and were about 37μ thick. The cyst wall was composed of 2 distinct layers: the outer one pitted and of variable thickness, was about 15μ thick, and the inner, resilient, hyaline layer, was about 22μ thick. This inner layer must

be formed well after the metacercaria is sexually mature. *In vitro* excystment was easier to induce when the cyst wall was relatively thin. Only 20% of the 179 day old cysts had excysted after 6½ hours in 0.5% pancreatin in Hank's saline at 41°C, but all had excysted after 21 hours.

Discussion:

The patterns of growth and development of *L. tasmaniae* and *M. calvertensis* in *Austrochiltonia australis* were very similar, however the rates of growth and development were markedly different (Figure 2.23). The cercariae of these species are very similar in size, however, the post-cercaria of *L. tasmaniae* grew at a much faster rate, and, before encysting, reached a much greater size than that of the latter. At 15°C, the post-cercaria of *M. calvertensis* encysted after 19 days, and that of *L. tasmaniae* encysted after 28 days; however, the encysted metacercaria of *L. tasmaniae* matured at a faster rate, so that the metacercariae of both species produced phenolic egg-shell precursors about 8 weeks after invasion of the amphipod.

The growth and development of *L. tasmaniae* in *A. australis*, like that of *M. calvertensis*, is related to environmental temperature (Figure 2.24). The initial size of the post-cercaria of *L. tasmaniae* was 83 × 35, and after one month at 5°C its size, (length+width), had increased by only 4%. At 15°C, the size of the post-cercaria increased by 214% before encystment, and at 25°C, the size increased by 198% before encystment. Encystment occurred much sooner at 25°C (i.e. after 10 days), than at 15°C, and phenolic egg-shell precursors were present in the vitellaria of metacercariae after only 13 days at 25°C. The metacercariae of *L. tasmaniae*, like those of *M. calvertensis*, are probably infective when their vitellaria contain phenolic egg-shell precursors, if not earlier, hence they would be infective to birds after about 8 weeks at 15°C, and about 2 weeks at 25°C.

FIG. 2.23 Growth and development of Maritrema calvertensis (—⊙—) and Levinseniella tasmaniae (—✶—) in Austrochiltonia australis at 15°C

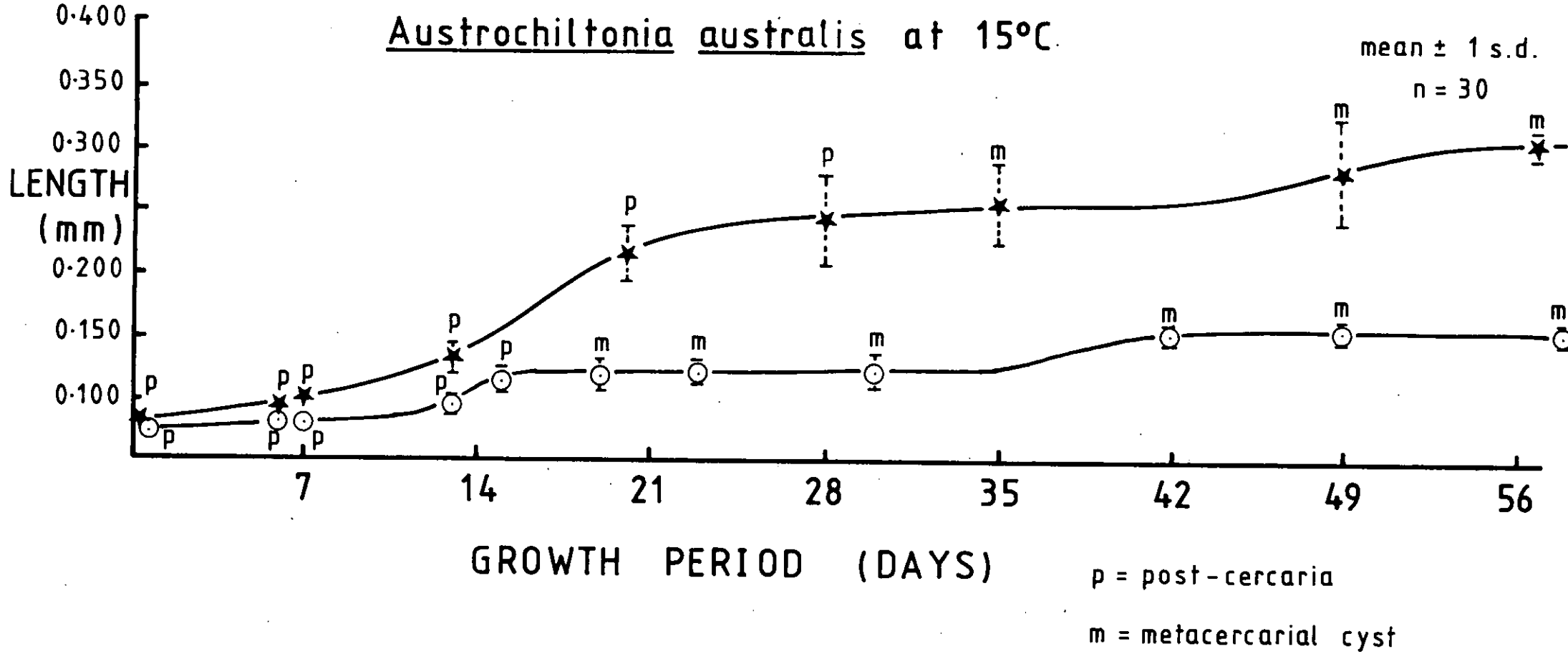
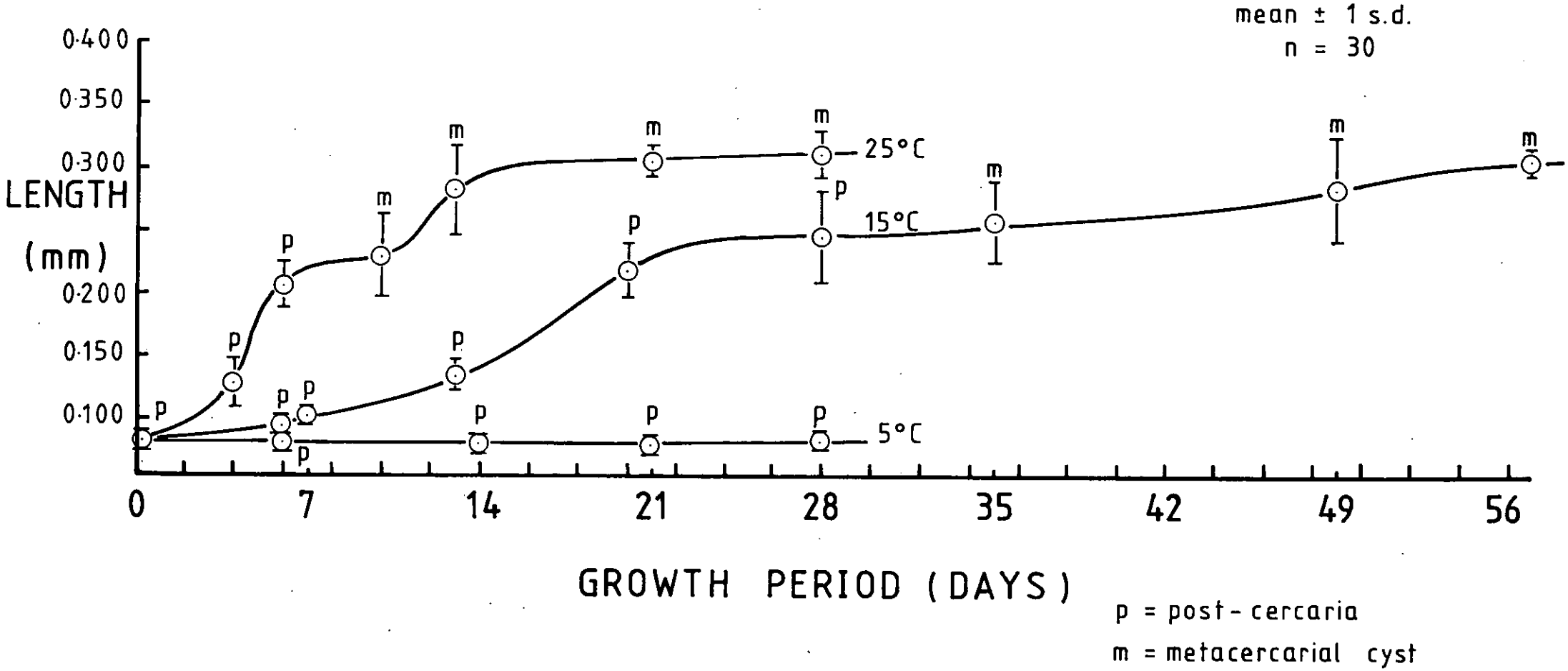


FIG. 2.24 Growth and development of Levinseniella tasmaniae in Austrochiltonia australis at different temperatures



Etges (1953), reported that, in the laboratory, metacercarial cysts of *Levinseniella amnicolae* reached full size in the isopod *Asellus communis* after 5 to 6 weeks. Bridgman (1969), found that, at 27°C, metacercarial cysts of *Microphallus choanophallus* were infective to definitive hosts after 4 to 5 weeks in the shrimp *Macrobrachium ohione*. Although the rates and patterns of growth and development of microphallids in their crustacean hosts differ in detail, they are, in general, quite similar. In all species that have been studied, a post-cercaria migrates and grows in the haemocoel before encysting. The meta-cercaria continues to grow after encystment, and the cyst expands and thickens, for a period of weeks or months, depending on temperature and species. The encysted metacercaria undergoes great development, losing its cercarial features (e.g. stylet), and gradually acquiring the morphology and anatomy of an adult worm. Metacercariae eventually reach an advanced stage of sexual maturity, at which development is arrested, and consequently are able to commence egg production soon after entering the definitive host.

2.3.7 Metacercaria

Metacercarial cyst: (Figure 2.22)

The mature cyst is more or less round, and has a thick, tough wall. Fifteen such cysts, taken from naturally infected amphipods from Calvert's Lagoon, measured 377 (361 - 395) × 355 (319 - 391), and were about 32μ thick. The largest cysts from experimentally infected amphipods main-tained under controlled temperature conditions, were only 311 × 290, and up to 23μ thick, (after 28 days at 25°C). These cysts had a single-layered homogeneous wall. The cysts grew to 357 (350 - 365) × 348 (327 - 365), and were about 37μ thick, after 14 days at 25°C, and 165 days at room temperature. These older cysts were each bounded by a wall composed of 2 distinct layers, the wide inner layer having been formed much later than the outer layer, long after maturation of the metacercaria.

The ultrastructure and composition of the cyst are similar to that of *M. calvertensis*. The translucent inner layer is proteinaceous, about 22μ thick, and the denser, osmiophilic outer layer, is composed of neutral mucopolysaccharide, about 15μ wide (Smith, 1971). In older cysts, the outer layer is traversed by many fissures, that are most numerous at the border with the inner layer. The cyst is enclosed by a fine outermost membrane, about 130 nm thick, composed of neutral mucopolysaccharide. This outer sheath, may, like that around the cyst of *M. calvertensis*, be of host origin.

Excystment:

Metacercariae will excyst *in vitro* at 40 - 42°C, after exposure to digestive enzymes, with or without, bile salts. As previously mentioned, metacercariae excyst after 1 or 2 hours in 0.5% pancreatin in Hank's saline, before the inner proteinaceous layer is formed. After the cyst wall is completed, excystment under the same conditions, takes from 3 to 10 hours. The same result occurs when 0.5% pancreatin and 0.2% sodium taurocholate, in Hank's saline is used.
As the inner protein layer inhibits excystment under these conditions, pretreatment with a protease may be necessary to accelerate the process. Pretreatment in 2% pepsin at pH 1.5 for various periods, from 5 to 30 minutes, proved to be toxic to metacercariae, however, varying the concentration of pH of the pepsin, and the period of exposure, may prove successful in accelerating excystment.

The process of excystment has been described previously (Smith, 1971), and is very similar to that of *Maritrema calvertensis*. The metacercaria is usually activated by elevation of temperature to about 41°C, however excystment rarely occurs at room temperature, after bacterial decay of the cyst wall for several days. When exposed to digestive enzymes *in vitro* at 41°C, the mature metacercaria becomes increasingly active within the cyst, and eventually, usually escapes through a small hole in an

otherwise uniformly thick cyst. The formation of a small escape hole in an apparently uniform, resilient cyst indicates that the cyst wall is weakened locally, internally, by enzymes of parasite origin. Sometimes metacercariae do not escape until the cyst wall has been reduced to a thin membrane. The process of excystment appears to consist of 2 phases: the initial, passive phase, involving activation of the metacercaria by elevation of temperature, and weakening of the cyst wall by digestive enzymes; and the second, active phase, involving internal dissolution of the cyst wall by parasite enzymes, and/or mechanical rupture of the weakened wall by vigorous stretching movements of the metacercaria.

Excysted metacercaria:

The development of the sexually precocious metacercaria of *L. tasmaniae*, is arrested when the worm is fully grown, at the stage of having mature ova in the ovary, sperm in the testes, and vitellaria producing phenolic egg-shell precursors. Within 4 hours of the elevation of temperature to 41°C, motile sperm are packed into the seminal vesicle, and the first eggs have been produced, whether or not the metacercaria has left its cyst. Metacercariae can excyst before the cyst wall is fully formed, when the reproductive organs are discernible, but neither vitellogenesis nor gametogeny have commenced.

The dimensions of metacercariae taken from naturally infected amphipods from Calvert's Lagoon, and excysted in 0.5% pancreatin in Hank's saline after 4 hours, are given in Table 2.26. Ovigerous worms were separated from those without eggs. The gravid worms have slightly larger bodies and organs than the less mature worms, and fall within the size range of gravid adults taken from naturally infected birds (Table 2.16).

TABLE 2.26 *Levinseniella tasmaniae*. Dimensions of excysted metacercariae after 4 hours *in vitro* at 41°C: (a) ovigerous specimens, and (b) non-ovigerous specimens.

	(a)	(b)
Sample size	10	11
Body length (BL)	635 (423 - 756)	538 (469 - 665)
Body width (BW)	174 (152 - 198)	175 (144 - 224)*
Body depth	114 (91 - 141)	94 (84 - 103)
Oral sucker length	54 (42 - 65)	51 (46 - 57)
Oral sucker width	57 (49 - 68)	48 (42 - 57)
Oral sucker depth	46 (42 - 49)	43 (38 - 46)
Ventral sucker length	51 (42 - 55)	45 (42 - 51)
Ventral sucker width	49 (42 - 53)	45 (38 - 55)
Ventral sucker depth	42 (-)	34 (-)
Prepharynx length	40 (10 - 72)	39 (19 - 65)
Pharynx length	29 (27 - 34)	32 (25 - 38)
Pharynx width	21 (19 - 23)	19 (15 - 23)
Oesophagus length	157 (95 - 190)	122 (99 - 156)
L. caecum length	155 (133 - 167)	124 (99 - 152)
R. caecum length	155 (114 - 171)	128 (95 - 148)
Seminal vesicle length	58 (46 - 68)	40 (32 - 46)
Seminal vesicle width	39 (34 - 46)	32 (27 - 34)
Ovary length	68 (53 - 80)	62 (53 - 70)
Ovary width	44 (29 - 53)	43 (36 - 53)
L. testis length	68 (61 - 76)	65 (57 - 76)
L. testis width	57 (53 - 61)	52 (40 - 61)
R. testis length	69 (65 - 76)	71 (65 - 76)
R. testis width	55 (51 - 61)	61 (49 - 72)
BW/BL	0.27	0.33
OS (1+w)/VS (1+w)	1.02	1.02

2.3.8 Discussion

The life-history of only one other species of the genus *Levinseniella*, viz. *L. amnicolae* Etges, 1953, is known. *L. amnicolae* inhabits the caeca of the mallard, *Anas platyrhynchos* in New York. It develops in a freshwater hydrobiid *Amnicola pilsbryi*, and encysts in the isopod *Asellus communis*. Deblock (1974), found a microphallid that he believed may have been a species of *Levinseniella*, encysted in its hydrobiid primary intermediate host on the French Atlantic coast; however, the immature state of the available metacercariae prevented determination of the species.

The closest relative of *L. tasmaniae* seems to be *L. howensis*, one of the 3 other species of this genus recorded in Australian fauna.

The adult of *L. howensis* infects the Eastern golden plover, *Pluvialis dominica, (=Charadrius dominicus)* on Lord Howe Island (Johnston, 1916; Pearson and Deblock, 1979). The adults of *L. monodactyla* and *L. microovata* infect the Mongolian dotterel, *Charadrius mongolus*, at Raby Bay, Queensland, and the adults of the latter species were taken from an Eastern golden plover at the same location (Deblock and Pearson, 1970). Both the Eastern golden plover and the Mongolian dotterel are non-breeding migratory visitors to Australia from the Palaearctic region, and both species visit the coast of Tasmania (Thomas, 1979).

Tribe MICROPHALLINI (Ward, 1901)

Sub-tribe MICROPHALLINA (Ward, 1901)

Genus ATRIOPHALLOPHORUS Deblock and Rosé, 1964a

2.4 Atriophallophorus coxiellae Smith, 1974

2.4.1 Life-cycle

The life-cycle of *A. coxiellae* is shown in Figure 2.25. The primary intermediate host, *Coxiella badgerensis* is probably infected by ingesting eggs in the lagoon. Intramolluscan development results in the formation of hundreds of metacercarial cysts in each infected snail. Water birds serve as the definitive hosts, after they feed on infected snails, and the life-cycle is completed when eggs of *A. coxiellae* drop with the faeces of these birds into the lagoon. In 1970, during a study at Calvert's Lagoon, metacercarial cysts of *A. coxiellae* were found in 17% of the 1035 snails dissected, and immature adults were found in the small intestines of hooded dotterels and a red-capped dotterel feeding on the shore of the lagoon. During the present study, the hoary-headed grebe and coot have also been found to serve as definitive hosts.

2.4.2 Adult (Figures2.26 and 2.27)

The original description of this species was based on sexually mature excysted metacercariae, fixed under "slight coverslip pressure". The gravid adult is described here for the first time, based on specimens recovered from experimentally infected ducklings and naturally infected hoary-headed grebes. The dimensions of adults fixed in boiling 10% formol saline, without coverslip pressure, are given in Table 2.27, together with the dimensions of the holotype of the species, which were not included in the original description.

FIG. 2.25 Atriophallophorus coxiellae

Life-cycle

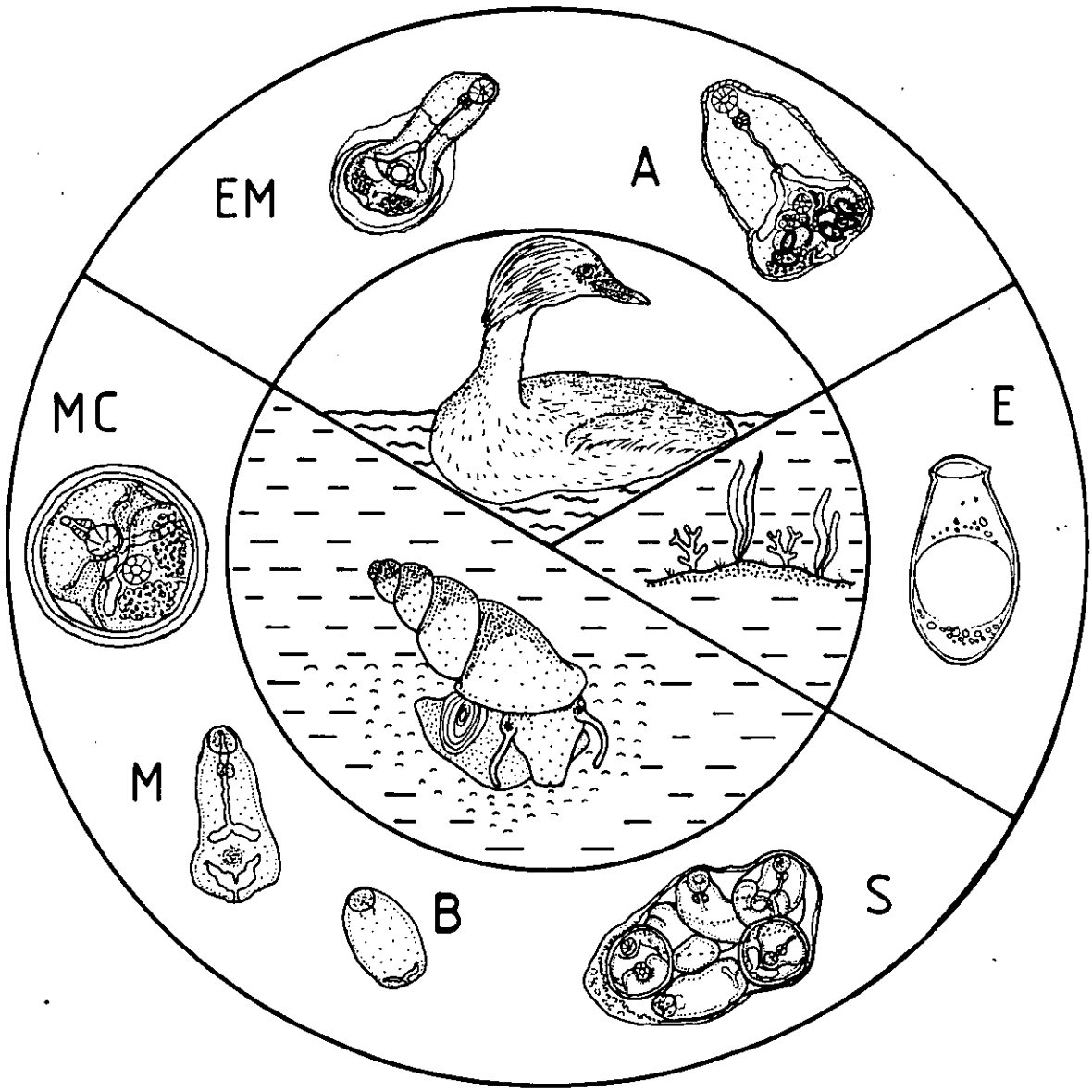


FIGURE 2.25 A, gravid adult; E, egg; S, daughter sporocyst; B, blastocercaria; M, metacercaria; MC, metacercarial cyst; EM, excysting metacercaria.

Description:

Body dorsoventrally flattened, pyriform to triangular; folded concave ventrally, particularly in posterior region, forming 2 postero-lateral stumps, which, with oral sucker, support living worm. Quincuncially arranged spines embedded in outer tegument: large, comb-like with up to 10 teeth, on dorsal, antero-ventral surfaces, diminish posteriorly; peg-like on postero-ventral surface; absent around excretory pore. Spines absent from modified tegument of internal surfaces of round oral, ventral suckers. Oral sucker sub-terminal ventral; ventral sucker protuberant, median, slightly smaller than oral sucker. O.S.:V.S. = 1.07(1.02 - 1.16). Ciliated sensory papillae scattered among tegumental spines, concentrated near suckers. Specialised aspinous regions of convoluted tegument, symmetrical, posterolateral to ventral sucker. Tegumental glands distributed over anterior 2/3 of body. Pre-pharynx short or absent, pharynx spatulate. Oesophagus narrows from pharynx to fine tube extending to middle of body; bifurcates, giving rise to obtusely divergent caeca. Caeca, slender or saccate, extend to level of anterior border of testes. Genital atrium adjacent to sinistral border of ventral sucker. Genital pore longitudinal, about 12μ long, sinistral lip surmounted by arc of 5-6 ciliated sensory papillae. Oval testes symmetrical, posterolateral to ventral sucker. Cirrus pouch absent, oval seminal vesicle antero-dorsal to, sometimes overlapping, ventral sucker. 'Canal déférent' leads from seminal vesicle, to pars prostatica within phallophore. Prostate cells situated in intercaecal arch, mainly anterior to seminal vesicle, extending posteriorly as far as ventral sucker; prostate cell ducts enter phallophore with 'canal déférent', in 2 bundles of 4, one ventral, one dorsal; pars prostatica discharges into prostatic chamber at base of retracted male papilla; sphincter muscle surrounds proximal opening of phallophore. Retracted male

FIG. 2.26 Atriophallophorus coxiellae

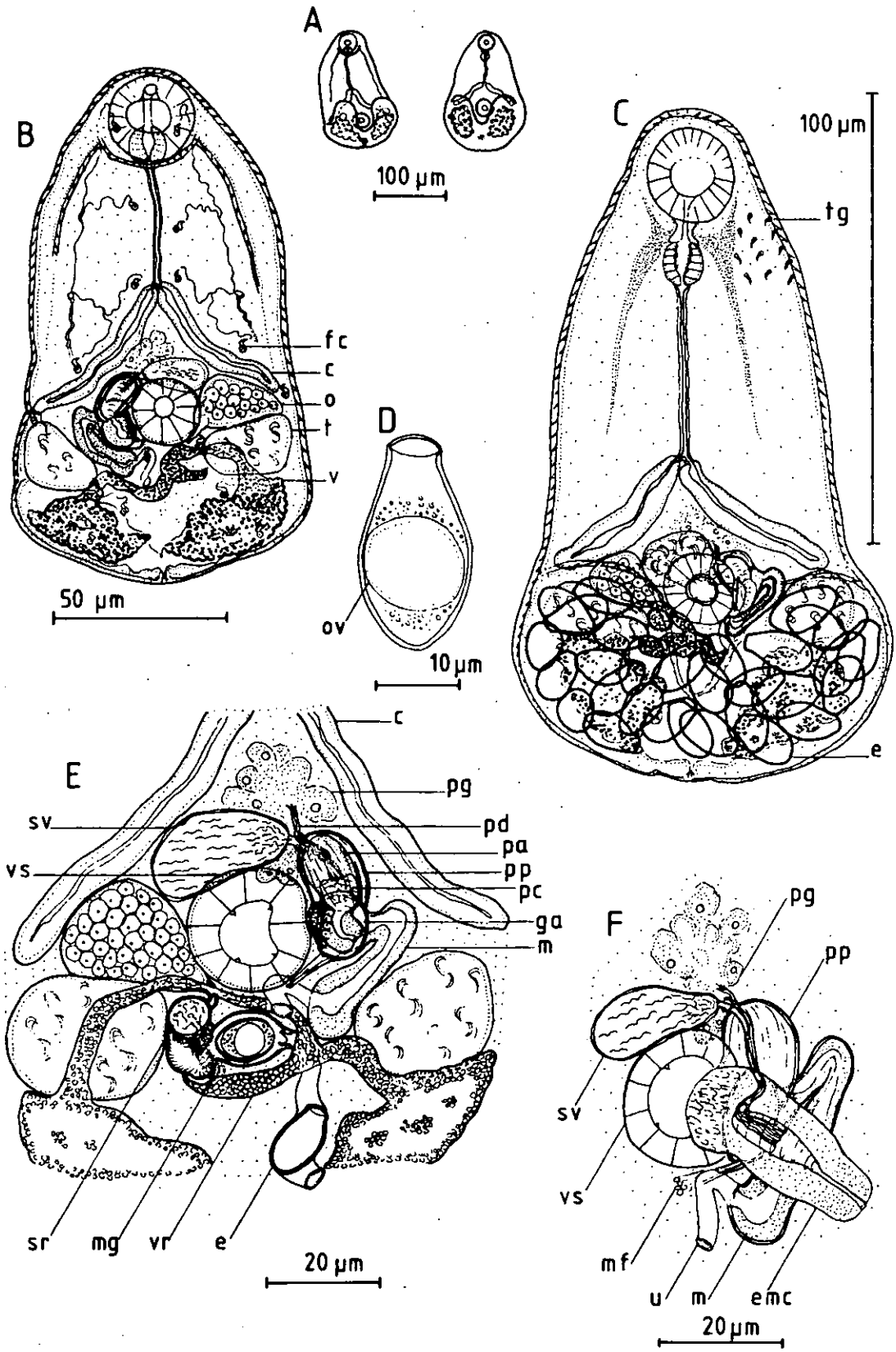


FIGURE 2.26 A, characteristic form of adult; B, mature excysted metacercaria, showing distribution of flame-cells, dorsal view; C, gravid adult from the hoary-headed grebe, ventral view; D, newly formed egg; E, detail of reproductive system, ventral view; F, detail of genital atrium region, showing everted male copulatory organ, ventral view. (c: caecum; e: egg; emc: everted male copulatory organ; fc: flame-cell; ga: genital atrium; m: metraterm; mf: muscle fibres, mg: Mehlis' gland; ov: ovum; pa: pars prostatica; pc: prostatic chamber; pd: prostate gland ducts; pg: prostate gland; pp: phallophore; sr: seminal receptacle; sv: seminal vesicle; t: testis; tg: tegumental gland; u: uterus; v: vitellaria; vr: vitelline reservoir; vs: ventral sucker.)

TABLE 2.27 *Atriophallophorus coxiellae.* Dimensions of ovigerous adults recovered from: (a) a laboratory duckling, 1, 0 day post infection; and (b) a naturally infected hoary-headed grebe. The dimensions of the holotype of the species (a mature excysted metacercaria) are also presented, (c).

	(a)	(b)	(c)*
Sample size	4	20	1
Body length (BL)	169 (133 - 190)	169 (141 - 194)	133
Body width (BW)	99 (91 - 110)	112 (87 - 118)	89
Oral sucker length	24 (19 - 27)	25 (23 - 30)	19
Oral sucker width	26 (25 - 27)	25 (21 - 30)	25
Ventral sucker length	22 (21 - 23)	24 (19 - 27)	21
Ventral sucker width	21 (-)	24 (17 - 27)	21
Prepharynx length	0 (-)	1 (0 - 6)	0
Pharynx length	12 (11 - 13)	16 (13 - 19)	13
Pharynx width	11 (10 - 11)	13 (11 - 14)	14
Oesophagus length	36 (23 - 48)	40 (30 - 53)	27
L. caecum length	62 (53 - 72)	48 (40 - 57)	49
R. caecum length	61 (53 - 68)	48 (40 - 57)	51
Seminal vesicle length	22 (21 - 23)	21 (15 - 27)	17
Seminal vesicle width	14 (13 - 15)	14 (10 - 19)	11
Ovary length	30 (27 - 32)	25 (23 - 29)	27
Ovary width	14 (11 - 17)	16 (13 - 19)	15
L. testis length	26 (23 - 29)	32 (30 - 34)	27
L. testis width	25 (23 - 29)	26 (25 - 27)	21
R. testis length	29 (23 - 34)	29 (27 - 30)	23
R. testis width	25 (23 - 27)	26 (23 - 30)	19
BW/BL	0.59	0.66	0.67
OS (l+w)/VS (l+w)	1.16	1.04	1.05

(* Fixed under cover-slip pressure)

papilla protrudes into genital atrium from anterodorsal atrial wall; accessory lobe, at base of retracted papilla, impinges on sinistral border of ventral sucker. Everted male copulatory organ relatively large, club-shaped, from 16 to 32μ, depending on degree of retraction; when fully everted, base about 16μ diameter, tip about 10μ diameter; 'canal déférent', 2 bundles of prostatic ducts, enter base of everted organ, extending about ½ of its length, to ejaculatory duct which opens terminally. Accessory lobe projects from base of everted copulatory organ, like heel of foot, overlies sinistral side of ventral sucker. Ovary oval to triangular, dextral to ventral sucker, bounded anteriorly by dextral caecum, seminal vesicle, posteriorly by dextral testis. Thin, unciliated oviduct passes posteromedially from posterior, sinistral corner of ovary; widens, becomes ciliated, enters median ootype. Round seminal receptacle, 12μ diameter, between ventral sucker, ovary, branches from oviduct. Laurer's canal not discernible. Transverse vitelline reservoir discharges into ciliated oviduct; Mehlis' gland ducts open through small papillae at proximal opening of ootype. Uterus forms loops posterior to, overlapping, dextral testis, then posterior to, overlapping, sinistral testis, then passes anteriorly to metraterm. Metraterm large, thick-walled, ear-shaped, about 27μ long, 6μ diameter, opens into sinistral wall of genital atrium. Uterus contains many relatively large eggs. Vitelline glands in 2 compact groups posterior to, just overlapping testes. Vitelline ducts pass anteromediad from each group, to level of posterior border of ventral sucker, recurve posteriorly, uniting posterior to ventral sucker to form vitelline reservoir, extends transversely towards dextral testis. Excretory $\overset{\text{bladder}}{\wedge}$ V-shaped, flame-cell formula: $2[(2+2) + (2+2)] = 16$.

FIG. 2.27 Atriophallophorus coxiellae

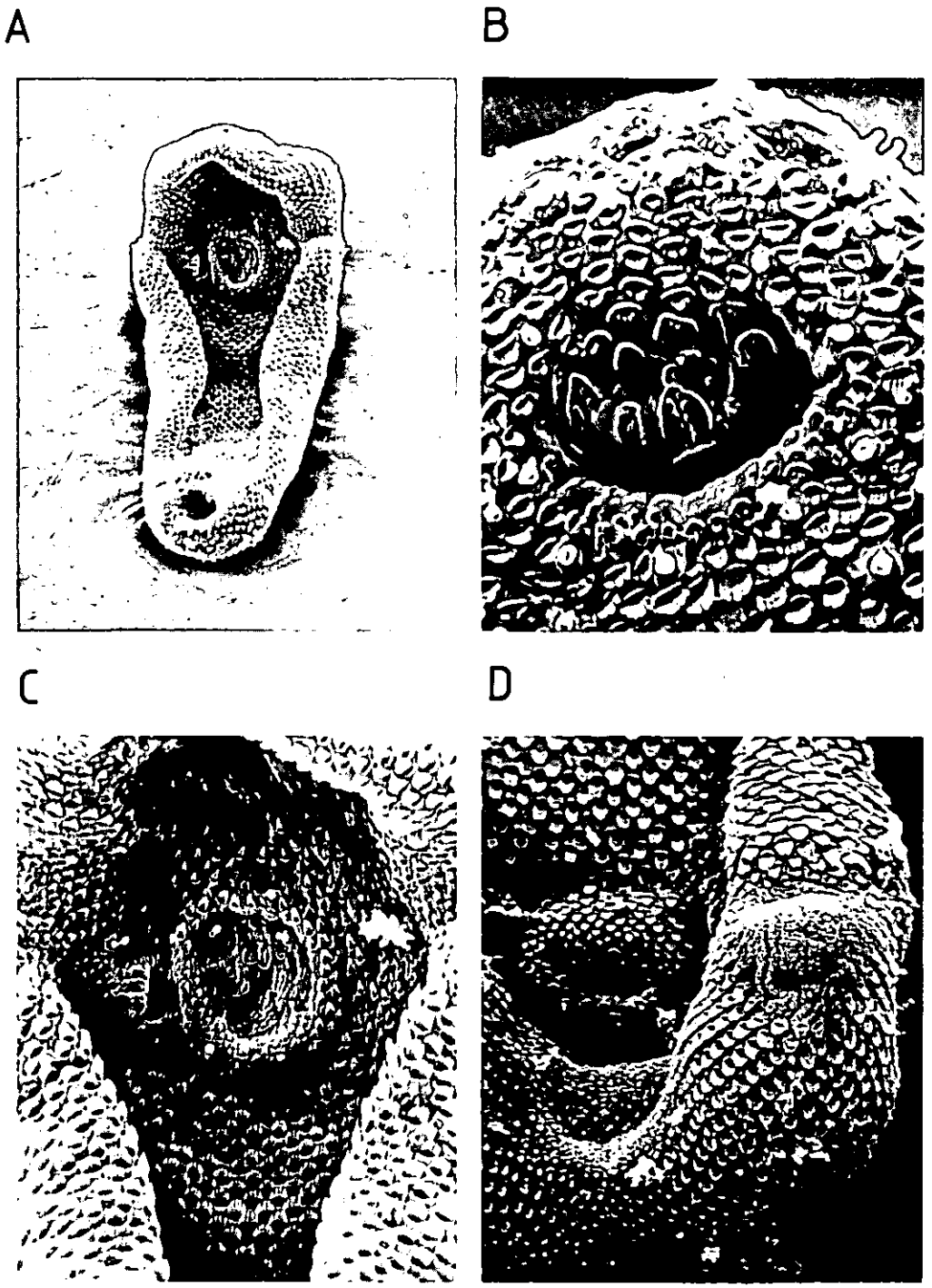


FIGURE 2.27 S.E.M. photographs of mature excysted metacercariae - A, antero-ventral view of whole worm, ×550; B, oral sucker region, showing tegumental spines and ciliated sensory papillae, ×5,300; C, ventral sucker and genital pore, ×2,200; D, aspinous region of convoluted tegument on posterolateral stump, ×2,200.

A feature of the surface of this fluke is the symmetrical pair of aspinous pads behind the ventral sucker (Figure 2.27D). These areas of highly convoluted tegument, in living worms, would be on the bottom of the posterolateral stumps, and *in vivo*, would be directly in contact with host epithelium. Davies (1976), described similar regions on *Microphallus similis*. She also found that unusual tegumental cells, packed with mitochondria, underlie these regions, and suggested that they may be specialised respiratory cells. There is evidence that oxygen tension approaches zero in the lumen of the intestine, but is markedly higher adjacent to the mucosa (Rogers, 1949; Crompton *et al.*, 1965). The convolutions of the aspinous tegument in these posterolateral pads could provide increased surface area for exchange of gasses. Two muscular envelopes associated with the terminal male genital tract are unusual and characteristic of this species. A thick-walled outer envelope, about 25 × 16μ, extends from the anterior wall of the genital atrium, almost to the seminal vesicle, and contains a thin-walled inner envelope. The outer envelope is considered to be the homologue of the 'phallophore' of *Atriophallophorus minutus*. The inner envelope of annular muscle fibres is continuous with the base of the male papilla, and moves within the phallophore like a piston. The vitelline glands of *A. coxiellae* are located unusually posterior for the Microphallidae (Deblock, 1979, pers. comm.).

Vertebrate hosts: *Anas platyrhynchos* L. (experimental host); *Charadrius rubricollis* Gmelin, *C. ruficapillus* Temminck; *Fulica atra* L.; and *Poliocephalus poliocephalus* (Jardine and Selby).

Habitat: Mainly lower small intestine, also caeca and rectum

Geographical location: Calvert's Lagoon

Type material: Tasmanian Museum - holotype K246; paratypes K245, K247 and K248.

Relationships:

This diminutive parasite of Tasmanian birds conforms to the definition of the genus *Atriophallophorus*, and closely resembles the only other species in that genus, *A. minutus* (Price, 1934), (syn. *Levinseniella minuta* Price, 1934 and *A. samarae* Deblock and Rosé, 1964, according to Deblock, 1971). *A. minutus* is a parasite of wild ducks in the U.S.A., the West Indies and France.

The genus *Atriophallophorus* is characterised by, and named after, an unusual periatrial envelope termed a 'phallophore'. The contents of this membrane form a support, or base, for the male papilla. In *A. coxiellae*, the phallophore surrounds an inner membrane, the contents of which, when everted, form the basal part of a relatively large copulatory organ. Describing the "atrial cavity" of *Levinseniella minuta*, Stunkard (1958), stated that "the wall is fibrous, without nuclei, and can be everted, forming a large, curved, club-shaped copulatory organ, about 18μ wide at the base and 40 - 60μ long, with the muscular papilla at its tip". This description corresponds well with observations of the copulatory apparatus of *A. coxiellae* and with those of *A. samarae* (Deblock and Rosé, 1964).

Although *A. coxiellae* and *A. minutus* are very similar in the size, shape and anatomy of the adult and developmental stages, there are significant and consistent differences which are considered to justify the taxonomic distinction between them. Differences in the life-cycles and developmental stages will be discussed later. The most significant difference between the adults is in the shape and position of the phallophore. In *A. minutus* it is concentric around the genital atrium, enclosing a hemispherical portion of parenchyma. In *A. coxiellae* it is not concentric around the genital atrium, but is elongate oval, longitudinal or obliquely angled towards the seminal vesicle. In *A. coxiellae* the "canal déférent" and the ducts of the prostatic cells terminate about 2/3 along the phallophore, forming a prostatic chamber

at the base of the papilla, anterodorsal to the genital atrium. In *A. minutus* the "canal déférent" and the ducts of the prostatic cells terminate within the base of the male papilla, within the genital atrium. Another difference between these species is the muscular scale, which always projects over the left border of the ventral sucker in *A. minutus*. In *A. coxiellae*, a fleshy accessory lobe overlies the sinistral border of the ventral sucker, only when the copulatory organ is everted. The maximum number of uterine eggs recorded in *A. coxiellae* from laboratory ducklings is 62, whereas that of *A. minutus*, from wild ducks in France, is only 25 (Deblock and Rosé, 1964). Dimensions of some of the latter specimens, kindly made available by Prof. Deblock, are shown in Table 2.28.

A key to the species of *Atriophallophorus*, based on the abbreviated description of *A. minutus* by Deblock (1971), is provided below (all measurements given in microns):

1 - Phallophore concentric around the genital atrium.....2

Phallophore not concentric around the genital atrium, but elongated towards the seminal vesicle.....3

2 - Body very small, 150 - 200 long. O.S., 24 diameter. V.S., 22 diameter. Pharynx, 12 - 15. Oesophagus short; caeca short, 58, extending to the ventral sucker. Genital pore sinistral, juxtacetabular. Acetabular scale projects from the left border of the atrium, 8 - 15 × 2. Seminal vesicle and prostate free in the parenchyma. Phallophore, 20 - 25 diameter. Fleshy eversible male papilla, from 13 × 16 to 20 × 50. Pars prostatica intrapapillary. Dextral ovary. Uterus postacetabular and postcaecal. Metraterm 30 - 40. Vitellaria as in *Microphallus*. Eggs few, (about 20 - 25), and large, 21 - 25 × 10 - 13.

Intestinal parasite of birds (Anseriformes), in North America and in Europe. Abridged life-cycle. Metacercariae encysted in the first intermediate host *(Hydrobia, Amnicola,*

Oxytrema).....

Atriophallophorus minutus (Price, 1934) Deblock and Rosé, 1964

syn.: *Levinseniella minuta* Price, 1934

A. samarae Deblock and Rosé, 1964

TABLE 2.28 *Atriophallophorus minutus*. Dimensions of adults taken from naturally infected *Anas platyrhynchos* in NE France: (a) ovigerous specimen, and (b) non-ovigerous specimens. (Material provided by Prof. Deblock, Université de Lille, France.)

	(a)	(b)
Sample size	1	9
Body length (BL)	190	169 (141 - 196)
Body width (BW)	84	95 (80 - 122)
Oral sucker length	25	25 (21 - 27)
Oral sucker width	25	25 (23 - 29)
Ventral sucker length	23	22 (21 - 23)
Ventral sucker width	23	20 (19 - 21)
Prepharynx length	-	0.5 (0 - 2.0)
Pharynx length	-	14 (11 - 15)
Pharynx width	-	12 (11 - 14)
Oesophagus length	-	38 (23 - 53)
L. caecum length	-	59 (49 - 65)
R. caecum length	-	57 (51 - 61)
Seminal vesicle length	-	23 (-)
Seminal vesicle width	-	11 (-)
Ovary length	-	27 (25 - 30)
Ovary width	-	17 (15 - 19)
L. testis length	-	28 (27 - 30)
L. testis width	-	23 (-)
R. testis length	-	32 (30 - 34)
R. testis width	-	25 (23 - 27)
Egg length (n = 5)	22 (19 - 23)	-
Egg width (n = 5)	11 (10 - 11)	-
No. eggs *in utero*	20	-
BW/BL	0.44	0.56
OS (l+w)/VS (l+w)	1.09	1.19

3 - Body very small, 130 - 200 long. O.S., 25 diameter. V.S., 23 diameter. Pharynx, 11 - 19 × 10 - 13. Oesophagus short: caeca short, 42 - 73, extending to the ventral sucker. Genital pore sinistral, juxta-acetabular. No acetabular scale. Seminal vesicle and prostate free in the parenchyma. Phallophore, about 25 × 16. Fleshy eversible male papilla, 16 - 32 long. Pars prostatica outside the retracted male papilla. Dextral ovary.

Uterus postacetabular and postcaecal. Metraterm about 27 × 6. Vitellaria as in *Microphallus*. Eggs large, 21 - 27 × 11 - 15; up to about 60 per fluke.

Intestinal parasite of birds (Charadriiformes, Gruiformes and Podicipediformes), in Australia. Abridged life-cycle. Metacercariae encysted in the first intermediate host *(Coxiella)*

.....*Atriophallophorus coxiellae* Smith, 1974

Biology:

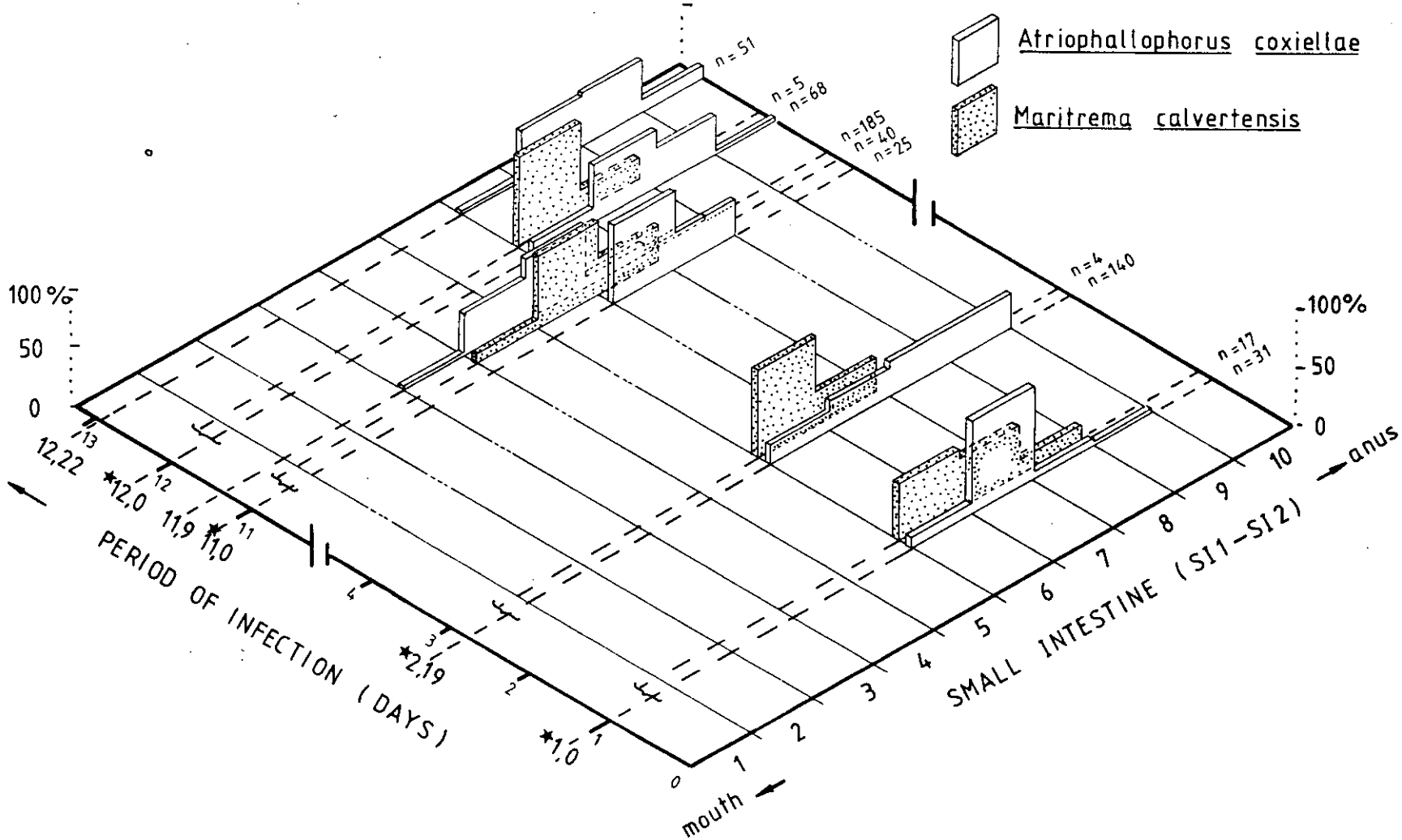
Domestic ducklings, raised under controlled conditions, were fed mature metacercarial cysts from naturally infected snails and dissected at intervals ranging from 1,0 day to 23,23 days.

Fifty percent (7/14), of the ducklings were found to be infected at the time of dissection, with from 1 to 185 flukes. The percentage of flukes recovered, compared to the number of metacercarial cysts used, varied from 0 to 84. The oldest flukes found in laboratory infections, were recovered after 12,22 days. Ducks dissected after 17,0 and 23,23 days, were not infected.

The preferred habitat of this fluke is the lower small intestine. The distribution of *A. coxiellae* in experimentally infected ducklings is shown in Figure 2.28. In a duckling that had been infected only by *A. coxiellae* for 11,9 days, adults were distributed from SI4 to SI6, with over 50% in SI6. In a duckling infected only by *A. coxiellae*, for 12,22 days, the range of flukes was from SI7 to SI10, with the greatest concentration in SI9. The apparent posterior shift in distribution, may be due to aging of the flukes, as these were the oldest specimens found in laboratory ducklings.

Birds infected with *A. coxiellae* at Calvert's Lagoon are also likely to be infected with *Maritrema calvertensis*, which lives in the same region of the small intestine (viz. SI6 to SI8). In fact, 71% (5/7), of wild birds infected with *A. coxiella*, were also infected by

FIG. 2.28 Atriophallophorus coxiellae. Distribution of adults in the gut of laboratory ducklings, after different periods of infection, (n = no. of adult flukes).



(*duckling also infected with Maritrema calvertensis).

Maritrema calvertensis. In ducklings experimentally infected with both species, their distributions overlapped, but the greatest concentration of *A. coxiellae* was always more posterior in the small intestine than that of *M. calvertensis*. *A. coxiellae* always extended further posteriorly in the intestine than *M. calvertensis* and *M. calvertensis* always extended further anteriorly than *A. coxiellae*. The same relative distribution of these species was found in wild birds, simultaneously infected with both microphallids. The distribution of *A. coxiellae* in a laboratory duckling, infected only with that species (infection period 11,9 days), was markedly more anterior than its distribution in mixed infections with *M. calvertensis* after 11,0 days and 12,0 days. This suggests that *A. coxiellae* was displaced posteriorly in the host's intestine when in competition with *M. calvertensis*.

The size and maturity of excysted metacercariae are variable. *In vitro*, the most advanced metacercariae begin egg production within a few hours of the temperature being raised to about 40°C, whether or not excystment has occurred. The number of uterine eggs in adults from experimentally infected ducklings is shown in Table 2.29. The number of uterine eggs increased greatly from 1,0 day to 11,9 days. Low numbers of eggs after 2,19 and 6,9 days were probably due to differences in the average maturity of the groups of cysts fed to the ducklings. They indicate that metacercariae require different periods to commence egg production in ducklings, from a few hours to a few days; and that metacercariae are infective at different stages of maturity.

In naturally infected birds, the range of *A. coxiellae* was from SI6 to SI10, and the rectum and caeca, with the greatest concentration in the lower small intestine (about SI9). Dimensions of unflattened fixed adults are only available from the hoary-headed grebe (Table 2.27). There was no marked difference between the sizes of adults from a grebe and from a laboratory duckling. Further, there was no marked difference between the sizes of these adults and mature excysted metacercariae after

2 hours *in vitro* at 41°C (Table 2.32). The average number of uterine eggs in 20 flukes from the hoary-headed grebe was 36(20 - 48). Assuming that rates of egg production are comparable in different bird hosts, this observation indicates that the longevity of *A. coxiellae* in natural hosts is similar to that in laboratory ducklings, i.e. up to about 13 days.

TABLE 2. 29 *Atriophallophorus coxiellae*. The number of uterine eggs in adults recovered from experimentally infected ducklings, after different periods of infection.

Period of Infection (days)	Sample size (no. of flukes)	Number of intra-uterine eggs Mean	S.D.	Range
1, 0	10	12.1	1.8	(10 - 16)
2, 19	10	10.0	2.2	(7 - 14)
6, 9	1	10	-	-
11, 9	20	46.9	9.0	(33 - 62)

2.4.3 Egg

The egg is operculate, oval to urn-shaped and very large relative to the size of adult worms (Figure 2.26). Colourless when formed, it becomes tanned yellow as it passes through the uterus. The ovum, about 12μ diameter, undergoes division within the uterus, but development of the miracidium is incomplete when the egg is shed. Adult worms begin shedding eggs after 2 days *in vitro* at 41°C, even when still contained within a cyst. Dimensions of eggs in flukes taken from a wild hoary-headed grebe and from a laboratory duckling, are shown in Table 2.30. There was little variation in the size of eggs of *A. coxiellae* in different bird hosts.

Attempts to infect laboratory-bred snails, *Coxiella badgerensis* with *A. coxiellae*, were unsuccessful. Isolated snails were fed whole, ovigerous adults of *A. coxiellae*, taken from experimentally infected ducklings. The snails were kept in lagoon water at room temperature and dissected at intervals up to 3 weeks; however no infections were

found.

TABLE 2.30 *Atriophallophorus coxiellae.* Dimensions of eggs in flukes taken from a laboratory duckling and a wild hoary-headed grebe.

Host	P.I. (days)	No. of eggs	Length	Width
Duckling	1,0	15	25 (23 - 27)	14 (12 - 15)
Hoary-headed grebe	-	20	23 (21 - 27)	13 (11 - 15)

2.4.4 Sporocyst (Figure 2.29)

Sporocysts of *A. coxiellae* always occur in large numbers, from 20 to 60, in each snail host. Within the same snail, sporocysts are usually at about the same stage of development. This indicates that there must be at least 2 generations of sporocysts. Daughter sporocysts occur throughout the viscera of *C. badgerensis*, but are concentrated in the gonad, around the tubules of the digestive gland and in the pallial oviduct of female snails. They adhere firmly to snail tissue and are difficult to separate intact. Each daughter sporocyst produces germ balls, which develop relatively synchronously, forming metacercarial cysts. As the cysts expand and thicken, the sporocyst is stretched and eventually ruptures, liberating mature cysts among the snail tissues. Free cysts can pass from the digestive gland to the style sac, where they are compacted with indigestible food residues and eliminated. Faecal pellets of infected snails contain up to about 25 cysts of *A. coxiellae*. These cysts contain active metacercariae, and vary in maturity from having thin, single layered walls, to thick, double layered walls.

Daughter sporocysts containing 5 to 9 offspring at different stages of development, from blastocercariae to immature cysts, are oval and measure about 228 × 156µ. One sporocyst contained 9 immature cysts and measured 304 × 213µ.

FIG. 2.29 Atriophallophorus coxiellae

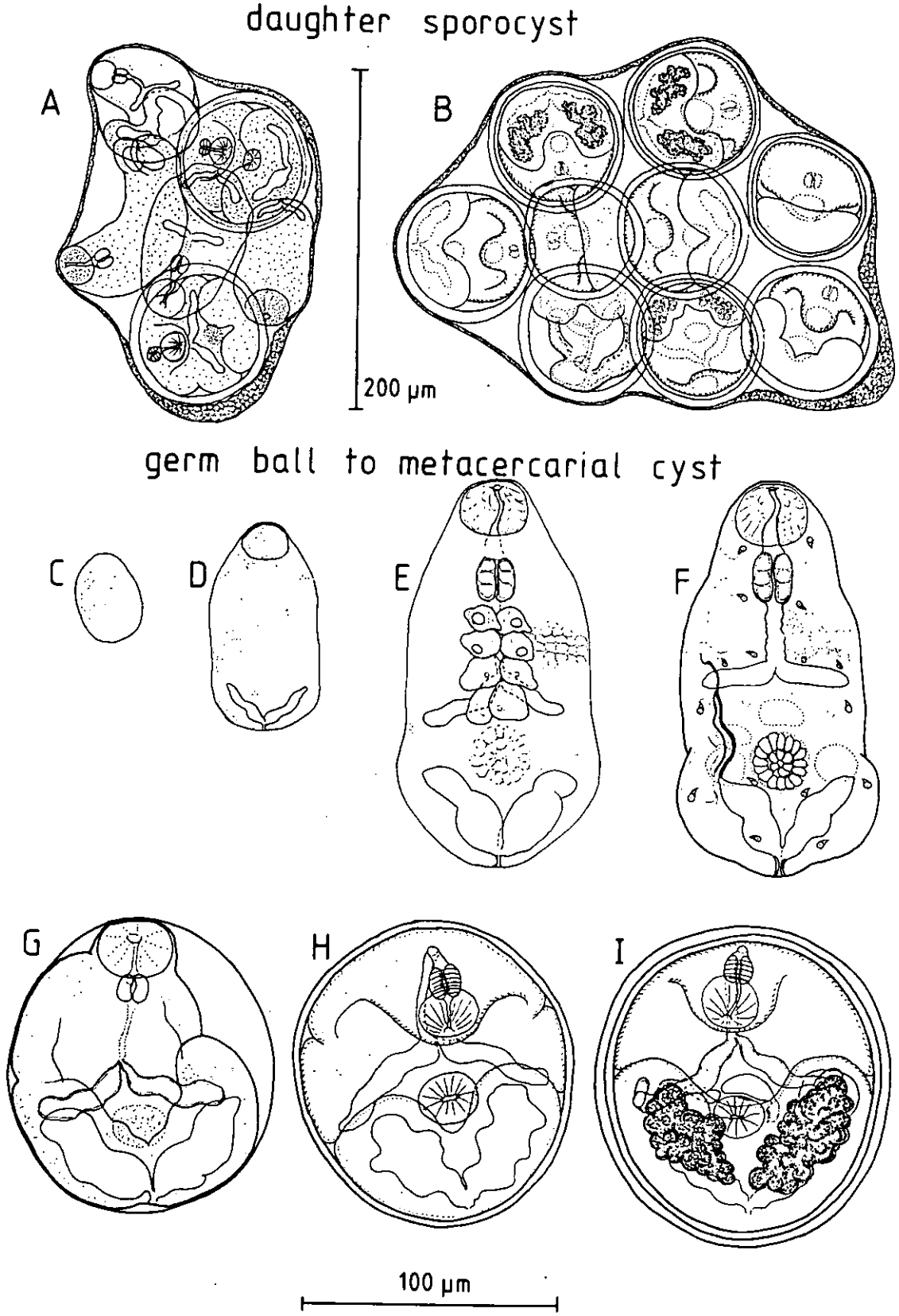


FIGURE 2.29 A, daughter sporocyst containing a blastocercaria, metacercariae, and metacercarial cysts; B, daughter sporocyst containing metacercarial cysts of varying maturity; C, germ ball; D, blastocercaria; E, early metacercaria, showing 4 axial pairs of apparently glandular cells; F, metacercaria, showing distribution of flame-cells; G, initial metacercarial cyst; H, immature metacercarial cyst, with single-layered cyst wall; I, mature metacercarial cyst.

2.4.5 Germ ball to early metacercaria (Figure 2.29)

Spherical germ balls, 38μ diameter, grow and differentiate within daughter sporocysts. Early in development, a discrete oral sucker (19μ diameter), and bilobed, V-shaped excretory bladder appear. This stage, the so-called "blastocercaria" (Deblock, 1971), has a thin aspinous tegument and measures about 80 × 49μ. The digestive tract, and 4 axial pairs of large cells (differentially stained by brilliant cresyl blue), soon develop. The 8 cells, possibly vestigial penetration gland cells, do not persist for long. A barrel-shaped, muscular pharynx is separated from the oral sucker by a short pre-pharynx, and the oesophagus extends to the middle of the body, where it bifurcates, giving rise to stumpy, widely divergent caeca. Following Deblock (1977), the developmental stage within the daughter sporocyst is called a blastocercaria after the appearance of an oral sucker, and before the development of a pharynx, because the latter is not found in typical microphallid cercariae. In *A. coxiellae*, the blastocercaria is a brief stage in the continuous development towards a metacercaria, within the daughter sporocyst. The metacercaria initially measures about 110 × 57μ. Flame-cells develop symmetrically and are connected on each side of the body by branching excretory canals, which join together, and empty into each lobe of the excretory bladder. The 4 anterior pairs of flame-cells develop simultaneously and the posterior pairs develop sequentially. The observed flame-cell formulae were: 2[(2+2) + (1)] = 10; 2[(2+2) + (1+1)] = 12 and 2[(2+2) + (2+1)] = 14; a few free metacercariae were observed that had the full adult complement of 16 flame-cells. The aspinous metacercaria grows to about 133 × 65μ before encysting. It curls ventrally into a ball, within the flimsy initial cyst, which measures 87 × 68μ and is 1 to 2μ thick.

2.4.6 Metacercaria

Metacercarial cyst: (Figure 2.29)

The initial cyst wall is single layered, but gradually thickens until it is 4.5μ wide, and appears to be composed of 2 layers under light microscopy. At this stage, the round "immature" cyst measures about 94μ diameter and the metacercaria has large tegumental spines over the body. Tegumental glands are well-developed in the anterior of the body, and rudimentary gonads are discernible. The cyst continues to expand and thicken, and is considered "mature" when the vitellaria of the encysted metacercaria are actively producing phenolic egg-shell precursors. Dimensions of some "mature" and "immature" cysts are shown in Table 2.31.

TABLE 2.31 *Atriophallophorus coxiellae*. Dimensions of metacercarial cysts from naturally infected snails: (a) mature, and (b) immature.

	No. Cysts	Length Mean	S.D.	Range	Width Mean	S.D.	Range	Cyst thickness Mean	Range
(a)	20	122	5	(114 - 137)	116	7	(103 - 125)	9	(8 - 11)
(b)	20	94	4	(87 - 99)	-	-	-	4.5	(3 - 6)

The ultrastructure and composition of the cyst of *A. coxiellae* are rather different from those of the cysts of *Maritrema calvertensis* and *Levinseniella tasmaniae*, which encyst in crustacean hosts. The fully developed cyst wall is composed of 4 layers (Smith, 1971). The thin innermost membrane, which closely invests the encysted metacercaria, is proteinaceous and 120 nm wide. The next layer, about 5μ thick, is composed of mucoprotein; outside this layer is a relatively ductile, slightly more electron dense layer, about 4μ thick, that is also composed of mucoprotein. An external membrane, 200 nm wide, is the most electron dense of the cyst layers, and is composed of acid mucopolysaccharide and mucoprotein.

Excystment:

Mature metacercariae usually excyst *in vitro* within about an hour, when exposed to digestive enzymes at 41°C. The optimum excystment conditions were found to be 0.5% pancreatin in Hank's saline, for 60 mins., followed by incubation in Hank's saline. Metacercariae were sometimes found to excyst at room temperature, when left for several days in lagoon water. In such cases, excystment seems to have been stimulated by weakening of the cyst wall by bacterial decay. A large number of immature metacercariae, with tegumental spines and partially developed reproductive systems, were once found to have excysted within their primary host. Bacterial action within the snail is assumed to have induced this premature excystment.

The process of excystment is very similar to that of *Maritrema calvertensis* and *Levinseniella tasmaniae*. Like *M. calvertensis*, the metacercaria of *A. coxiellae* was usually found to escape only after the cyst wall had been reduced to a thin membrane. This remnant membrane was eventually ruptured by vigorous stretching movements of the metacercaria.

Excysted metacercaria:

The metacercaria of *A. coxiellae* grows to adult size within the cyst, and development progresses to the stage of having mature ova and sperm in the gonads, and vitellaria producing phenolic egg-shell precursors. When the temperature of a mature metacercaria is raised to about 40°C, motile sperm move into the seminal vesicle and vitelline secretions fill the vitelline ducts and vitelline reservoir after only 1 or 2 hours; and the first eggs are produced within 4 hours. Metacercariae can excyst *in vitro* at earlier stages of development. In the more immature metacercariae that excysted *in vitro*, rudimentary reproductive organs were discernible, but gametogeny and vitellogenesis had not commenced. The different rates of egg production observed in

adults recovered from experimentally infected ducklings are evidence that metacercariae are infective to birds at different stages of maturity. The dimensions of excysted metacercariae from thick-walled "immature" cysts and "mature" cysts, are shown in Table 2.32. They were excysted *in vitro*, and measured after 2 hours at 41°C. The "mature" metacercariae fell within the size range of ovigerous adults taken from bird hosts (Table 2.27); however the "immature" specimens were significantly smaller than ovigerous adults. The "immature" metacercariae were also more elongate and had a greater oral sucker to ventral sucker ratio than gravid adults.

TABLE 2.32 *Atriophallophorus coxiellae*. Dimensions of excysted metacercariae after 2 hours *in vitro* at 41°C: (a) "mature" specimens, with active vitellaria (i.e. Fast Red Salt B positive), but no eggs; and (b) "immature" specimens, with inactive vitellaria.

	(a)	(b)
Sample size	20	20
Body length (BL)	165 (122 - 217)	122 (99 - 144)
Body width (BW)	93 (87 - 105)	59 (40 - 65)
Oral sucker length	22 (19 - 27)	21 (15 - 23)
Oral sucker width	23 (19 - 27)	21 (13 - 23)
Ventral sucker length	23 (19 - 27)	14 (10 - 17)
Ventral sucker width	21 (17 - 23)	14 (10 - 17)
Prepharynx length	0 (-)	5 (0 - 11)
Pharynx length	13 (11 - 15)	12 (10 - 15)
Pharynx width	10 (8 - 13)	11 (8 - 11)
Oesophagus length	44 (27 - 57)	25 (19 - 34)
L. caecum length	61 (46 - 72)	29 (19 - 38)
R. caecum length	55 (42 - 72)	28 (19 - 34)
Ovary length	27 (25 - 30)	14 (10 - 17)
Ovary width	17 (13 - 23)	10 (8 - 11)
L. testis length	27 (23 - 34)	19 (15 - 23)
L. testis width	22 (15 - 27)	16 (13 - 17)
R. testis length	27 (23 - 30)	17 (15 - 19)
R. testis width	21 (15 - 25)	14 (10 - 19)
BW/BL	0.56	0.48
OS (1+w)/VS (1+w)	1.02	1.50

2.4.7 Discussion

The life-cycle of *A. coxiellae* is interesting, because it is reduced by having metacercarial cysts develop within the first intermediate

host. Although only 9 other microphallid species are known to have such a 2 host life-cycle, one of them is *A. minutus*, the only other species in the genus *Atriophallophorus*.

The earliest record of this genus was that of the adults of *A. minutus* in the small intestine of the scaup duck, *Nyroca affinis*, taken at Flamingo Lake, Culebra Island, in the West Indies (Price, 1934). Many years later, the same fluke was rediscovered in freshwater hydrobiid snails in the U.S.A.: *Hydrobia minuta* from Durham, New Hampshire, and *Amnicola limosa* from Cape Cod, Massachusetts (Stunkard, 1958). The metacercarial cysts were later found in the freshwater snail, *Oxytrema silicula*, and adults were found in naturally infected domestic ducks, from Woods Creek, Oregon (Burns, 1963). The following year, adults from wild ducks, *Anas platyrhynchos*, in the marshes of the Somme, in NE France, were described in detail and named *A. samarae* (Deblock and Rosé, 1964a). Comparison of the specimens from Europe with those from America revealed that they were of the same species, *A. minutus*, (Deblock and Rosé, 1964b).

A. minutus adults have been experimentally obtained from white mice and hamsters, and excysted metacercariae were even found in the intestine of a fish, the guppy, 30 minutes after ingestion of snail tissue containing cysts (Stunkard, 1958; Burns, 1963). The longevity of these adults was found to be between 3 and 8 days in white mice, and 4 and 7 days in hamsters.

Comparison of the life-histories of *A. coxiellae* and *A. minutus*, shows that although they are very similar, there are differences. The intermediate hosts of *A. minutus* are freshwater snails of the family Hydrobiidae. The snail host of *A. coxiellae* is also an hydrobiid, however it lives in brackish water habitats. The daughter sporocysts of *A. minutus* each produce about 3 blastocercariae which emerge from the sporocyst before encysting. The daughter sporocysts of *A. coxiellae* each produce 5 to 9 blastocercariae which do not emerge, but encyst

within the sporocyst of origin. The cysts of each species are about the same thickness, however those of *A. minutus* are about 100μ diameter, whereas those of *A. coxiellae* are about 120μ diameter. The metacercariae of each species develop precociously to an advanced stage of maturity, and begin egg production soon after ingestion into the definitive host. The longevity of *A. minutus* in white mice and hamsters is about 1 week, whereas that of *A. coxiellae* in domestic ducklings is about 2 weeks. It is not known how much this difference in longevity reflects host differences and how much it is due to differences between the parasites. The adults of both species infect water birds of inland and coastal water bodies.

2.5 General Discussion

2.5.1 Comparison of the developmental stages of microphallids

There is little variation in the gross morphology of sporocysts, cercariae and metacercarial cysts, in the family Microphallidae. The problem of distinguishing the developmental stages of 2 microphallid species concurrently infecting the same snail, has been encountered many times previously, but this appears to be the first record of individual snails simultaneously being parasitized by 3 microphallid species. Some examples of double infections are: from the U.S.A., *Levinseniella amnicolae* and *Maritrema obstipum* in *Amnicola pilsbryi*, New York (Etges, 1953), *Atriophallophorus minutus* and *Microphallus limuli* in *Hydrobia minuta*, Massachusetts (Stunkard, 1968), and *Microphallus choanophallus* and *M. basodactylophallus* in *Lyrodes parvula*, South Louisiana (Bridgman, 1969); from Canada, *Maritrema laricola* and *Ascorhytis charadriformis* in *Littorina scutulata*, British Columbia (Ching, 1963); from Britain, *Microphallus pygmaeus* and *M. similis*, in *Littorina saxatilis*, Wales (James, 1969); and from France, *Maritrema syntomocyclus* and *Microphallus scolectroma* in *Hydrobia acuta*, Corsica (Deblock and

Tran Van Ky, 1966).

Cercariae have proven most useful in distinguishing the intra-molluscan developmental stages of different microphallid species. Shape and dimensions of the body and tail; the shape and size of the stylet; and the arrangement of penetration glands and ducts; are very uniform in cercariae of the same species. These features of the cercaria of *Microphallis similis* have been shown not to vary significantly in spite of development occurring in different snail species in different parts of the world (Stunkard, 1957; James, 1969). The cercariae of different microphallids may also vary in their swimming behaviour (Ching, 1963). Sporocysts are relatively featureless, but their size, when fully developed, and the number of contained cercariae, have been found to be characteristic (James, 1969). The shape and size of metacercarial cysts are fairly constant for each species, but vary significantly between species.

The 3 microphallid species infecting *Coxiella badgerensis* are not distinguishable until development in the daughter sporocysts has passed the germ ball stage. The blastocercaria of *A. coxiellae* can then be distinguished as it does not develop a tail, stylet, or penetration gland ducts, and soon develops into a metacercaria, which encysts within the daughter sporocyst.

The sporocysts of *Maritrema calvertensis* and *Levinseniella tasmaniae*, although superficially similar, have been found to differ in many respects. These differences are summarised in Table 2.33.

TABLE 2.33 Comparison of the fully-developed daughter sporocysts of *Maritrema calvertensis* and *Levinseniella tasmaniae* in *Coxiella badgerensis*.

	Maritrema calvertensis	*Levinseniella tasmaniae*
Tenacity to snail tissue.	Separate readily	Adhere firmly
Distribution	Not clustered in bunches	Clustered in bunches
Shape	Elongate sausage shaped	Oval to tear-drop shaped
Size (microns)	336(266-403) × 138(72-281)	187(152-236) × 104(65-137)
Contents	Up to about 25 cercariae	Up to about 10 cercariae

The morphological characters used to distinguish other microphallid cercariae, as well as some behavioural and ecological characters, have proved useful in distinguishing the cercariae of *M. calvertensis* and *L. tasmaniae*. Morphological differences between the cercariae are summarised in Table 2.34.

TABLE 2.34 Comparison of the fully-developed cercariae of *Maritrema calvertensis* and *Levinseniella tasmaniae*.

	Maritrema calvertensis	*Levinseniella tasmaniae*
Body size (μ)	72(68 - 84) × 34(30 - 38)	83(76 - 89) × 48(46 - 52)
Tail size (μ)	73(57 - 103) × 8(8 - 10)	121(118-125) × 11(11-12)
Tegument	Spinous, (light microscopy)	Non-spinous (light microscopy)
Stylet size (μ)	12.5(11-13.5) × 2.5(1.5-3)	17(16-17.5) × 3
Stylet point: Stylet length ratio	0.4	0.5
Penetration glands	4 pairs in posterior half the body	2 pairs in anterior half of the body and 2 pairs at or about the middle.
Penetration gland ducts	- follow sinuous path - 2 small pairs open beside the stylet aperture; 1 large pair opens ventrally and 1 large pair open laterally.	- follow helical path - all 4 pairs open beside the stylet aperture.

The most obvious differences between the cercariae are the size and shape of the stylet and the position of the penetration glands and their ducts. The stylet of *L. tasmaniae* is distinctly larger and has a relatively longer point, than the stylet of *M. calvertensis*. The penetration glands of *L. tasmaniae* are located further anteriorly than those of *M. calvertensis*; and the penetration gland ducts of *L.tasmaniae*, which are helical, all open beside the stylet aperture, whereas those of *M. calvertensis* are sigmoidal, and open at different places in the anterior region.

The patterns of emergence of these cercariae from *Coxiella badgerensis* are both periodic; however, they are distinctly different. The emergence of *M. calvertensis* is at a peak during the night, whereas that of *L. tasmaniae* at a peak during the day, about 12 hours out of phase. Emergence of *M. calvertensis* is stimulated by dark, and/or

inhibited by light, whereas that of *L. tasmaniae* is inhibited by dark and/or stimulated by light. The only previous record of emergence of microphallid cercariae from their snail host is that of Etges (1953), who reported that cercariae of *Levinseniella amnicolae* emerged "at all times of the day".

The swimming behaviour of cercariae of *L. tasmaniae* and *M. calvertensis* are very similar; however, under the same conditions of temperature and salinity, the former swim for only about half as long as the latter. *M. calvertensis* is infective to *Mytilocypris tasmanica*, whereas *L. tasmaniae* is not. Although cercariae of both species can infect *Austrochiltonia australis*, they have different routes of invasion and rates of infection. The cercaria of *L. tasmaniae* penetrates the exoskeleton of the amphipod almost all over the body, leaving a cyst-like, mucoid capsule at the site of invasion. The cercaria of *M. calvertensis* penetrates intersegmental membranes of the limbs or at the base of the limbs, and does not leave such a capsule at the surface. When amphipods were exposed to the same number of cercariae from each microphallid species, under the same experimental conditions, more amphipods were infected by significantly more cercariae of *M. calvertensis* than of *L. tasmaniae*.

The growth and development of each species within the same crustacean intermediate host differ greatly. *L. tasmaniae* grows faster and forms much larger cysts than *M. calvertensis*. *M. calvertensis* encysts sooner; however, at 15°C, both species require about 8 weeks to complete development. After this period, the mature cysts of *M. calvertensis* are about 156µ diameter and those of *L. tasmaniae* are about 305µ diameter.

2.5.2 Reduction of the life-cycle in the Microphallidae

The life-cycles of many digenetic trematodes have been secondarily reduced from the classic 3 host life-cycle by telescoping of various developmental stages. This has occurred in many ways to varying degrees. For example, the encysted metacercaria of *Coitocaecum anaspidis*

(Opecoelidae), develops progenetically and deposits eggs in the syncarid mountain shrimp, *Anaspides tasmanica*, in Tasmania (Hickman, 1934), thus, perhaps, removing the dependence on a vertebrate host in the life-cycle. *Bunocotyle progenetica* and some other hemiurids develop progenetically in the molluscan host with gravid adult flukes developing within rediae (Jamieson, 1966; Deblock, 1974a). Perhaps the ultimate in this regressive trend is the development of active miracidia within sporocysts. This phenomenon has been described in India several times (Sewell, 1922; Premvati, 1955; Mohandas and Nadakal, 1970 and Mohandas, 1975), and similar progenetic sporocysts have recently been discovered by Dr. J.L. Hickman, in a marine snail in Tasmania.

Reduction of the life-cycle is relatively common in the family Microphallidae. Belopolskaia (1962), first noted a sequence of gradual shortening of the life-cycle in this family, from a 3-host to a 2-host cycle. In recent years, many more microphallids have been discovered with 2-host life-cycles, in fact 14 species, other than *Atriophallophorus coxiellae*, are presently known. This is a high proportion of the 40 odd microphallids whose life-cycles have been elucidated.

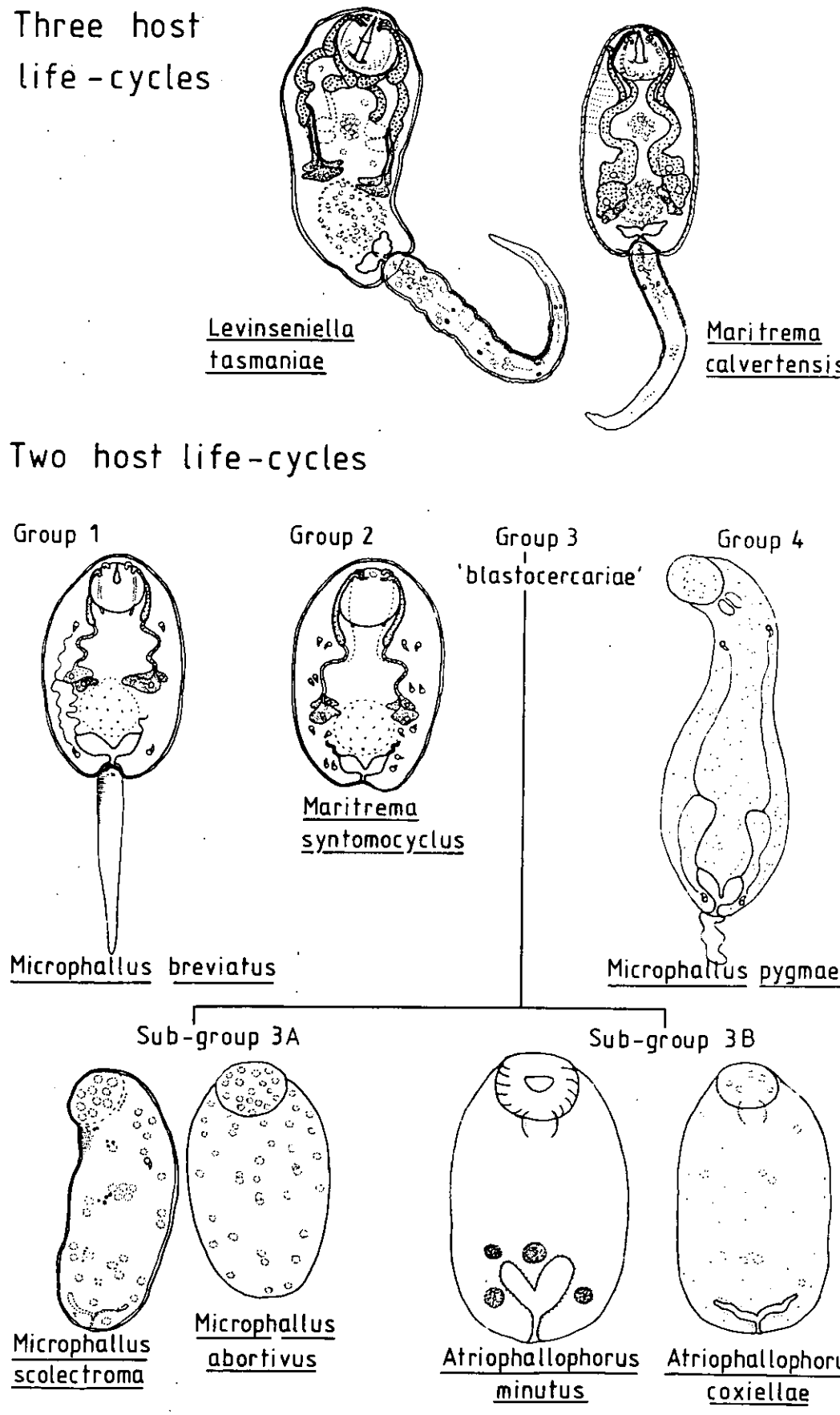
In most microphallids, encysted metacercariae develop precociously and begin egg production soon after entering the vertebrate host (e.g. *Maritrema calvertensis* and *Levinseniella tasmaniae)*, however in 5 species, *Quasimaritrema caridinae*, *Microphallus opacus*, *Sogandaritrema progeneticus*, *Gynaecotyla adunca* and *G. longiintestinata*, progenetic metacercariae commence egg production within the crustacean host (Deblock, 1977). At least some metacercariae of each of the latter 3 species become ovigerous without encysting, thus increasing the chance of eggs being transmitted to the snail host.

In 10 species, the life-cycle has been reduced by elimination of the crustacean host. These species, from 4 genera, develop into sexually advanced metacercariae within the snail host. The cercariae of these species are morphologically atypical and show a gradual sequence of

regression. Deblock (1977), classified them into 4 groups (Figure 2.30): (1) *Maritrema oocysta, Microphallus somateriae* and *M. breviatus* have the least altered monostome, leptocercous xiphidiocercariae; (2) *Maritrema syntomocyclus* has a 'pseudo-cercariaeum', that is without stylet or tail; (3) *Atriophallophorus minutus, Microphallus scolectroma, M. abortivus* and an undescribed *Levinseniella* species, have very vestigial cercariae, i.e. 'blastocercariae'; and (4) *M. pygmaeus* has a unique blastocercaria with a vestigial tail, which develops into a metacercaria and remains unencysted within the daughter sporocyst. *Atriophallophorus coxiellae* has a blastocercaria like those in group (3), however it develops into a metacercaria before encysting within the daughter sporocyst. The unencysted metacercaria has a rudimentary ventral sucker and well-developed pharynx and digestive tract. The development of *A. coxiellae* differs from that of *M. abortivus* and *M. scolectroma*, which encyst as blastocercariae, (sub-group 3A), but is very similar to that of *A. minutus*. Stunkard (1958), describing the cercaria of *A. minutus*, stated that: "there is no acetabulum and the only obvious features of the cercaria are the oral sucker and the excretory vesicle", however in Figure 4, p.230, a pharynx is clearly shown in the "cercaria" (sic). This indicates that *A. minutus*, like *A. coxiellae*, develops into a metacercaria before encysting and hence they constitute a distinct sub-group of the species that have a blastocercaria, sub-group 3B.

The development of sexually advanced metacercariae within the molluscan host results in a high probability that flukes from the same snail (i. e. probably of the same genotype), will fertilize each other within the vertebrate host (Deblock, 1971). This must reduce the amount of variability from genetic recombination within the species and tend to produce more or less genetically isolated populations. Such development, which places greater dependence on mutation as the source of variability, must have great selective value, as it has arisen independently in diverse genera, in the sub-families Maritrematinae and Microphallinae.

FIG. 2.30 Cercariae of the Microphallidae



Considerable energy is conserved by ensuring that every cercaria develops into a metacercaria in the host snail. This telescoping of the life-cycle has the effect of "placing all the eggs (metacercariae) in one basket", rather than dispersing them widely in the environment, and thus provides an alternative parasitic strategy to the 3-host life-cycle. In an open environment like the sea or a river, it would probably result in fewer infected birds, but ensures that every bird host is almost immediately infected by hundreds of adult trematodes. In a closed environment like Calvert's Lagoon, a high proportion of birds become infected by a large number of adults.

Precocious sexual development of microphallid metacercariae ensures that eggs are likely to be dropped with the definitive host's faeces into the aquatic habitat of the primary intermediate host (Deblock, 1971). Furthermore, the development of sexually precocious metacercariae in molluscs (e.g. *Atriophallophorus coxiellae)*, and short-lived adults that produce eggs quickly (e.g. *Levinseniella tasmaniae)*, enables large numbers of eggs, or packets of eggs in the form of gravid flukes, to be dropped into the snail host's habitat within a few hours or days of a bird becoming infected. Dogiel (1962), described microphallids as "migratory parasites" because of their association with migratory birds. The trends that have been noticed in the Microphallidae, towards:

(1) development of metacercariae within the snail host,

(2) sexually advanced metacercariae, and

(3) short-lived adults,

may be adaptations to the itinerant, or migratory, behaviour of the bird hosts.

Chapter 3 THE PSILOSTOMIDAE LOOSS, 1900

3.1 General Introduction

The family Psilostomidae comprises about 60 species from 21 genera (Yamaguti, 1971; 1975). These flukes closely resemble, both morphologically and ontogenetically, the Echinostomatidae, and they are placed together in the superfamily Echinostomatoidae. Psilostomes are mainly intestinal parasites of birds, but are rarely found in reptiles and mammals. One species, *Psilorchis hominis*, infects humans in Japan (Kifune and Takao, 1973). Adult psilostomes are distomate, generally elongate and relatively large. They have a characteristic fine, subcuticular excretory network, which gives the appearance of black lacework over the forebody. *Sphaeridiotrema globulus* has been implicated in the deaths of wild and domestic ducks in North America (McDonald, 1969b), and it seems likely that heavy infections by other large psilostomes would also be deleterious to their hosts.

Hitherto the life histories of only 8 psilostome species have been elucidated (Yamaguti, 1975). In each case rediae develop within a prosobranch snail. The immature cercaria emerges from a redia and grows and develops in the host tissues, before emerging. After a period swimming free in the aquatic habitat of the snail, cercariae encyst quite rapidly on vegetation, or on an animal, or within an animal (usually a snail). The cercaria is gymnocephalous and has a tail which is either simple or has dorso-ventral finfolds. Little or no development occurs within the cyst; however, after excysting in the gut of a suitable vertebrate host, the fluke undergoes rapid growth and development.

There are few records of psilostomes in the Australasian region. *Psilochasmus oxyurus* has been recorded in wild ducks and the black swan in New Zealand (Rind, 1974). Some large *Psilostomum* specimens,

recovered from black swans at Lake Bookar, Queensland, in 1975, were kindly sent to the author by Dieter Palmer (Department of Veterinary Pathology, Zurich University, Switzerland). Two immature psilostomes, *Psilostomum sp.A*, were found in a hooded dotterel killed at Calvert's Lagoon in 1970 (Smith, 1971). During the present study, a high percentage of adult snails in Calvert's Lagoon were found to have metacercarial cysts embedded in the pericardium, and sometimes lodged between the mantle and shell in the region of the pericardium. The mantle of infected snails was often found to be distended by these hard cysts. Excystment *in vitro* and experimental infection of ducklings, revealed that the cysts were of 3 species, all belonging to the family Psilostomidae. One of these species is *Psilochasmus oxyurus* and the other two belong to the genus *Psilostomum*, and are referred to as *Psilostomum sp.A* and *Psilostomum sp.B*. Both *Psilostomum* species are believed to be new; however, specific designation has been deferred until ovigerous adults have been obtained from experimentally infected birds and fixed by standard procedure without coverslip pressure.

Subfamily PSILOSTOMINAE Looss, 1900

Genus PSILOCHASMUS Luhe, 1909

3.2 Psilochasmus oxyurus (Creplin, 1825) Luhe, 1909

3.2.1 Life-cycle (Figure 3.1)

The cercaria of *P. oxyurus* develops in a large orange-pigmented redia in *Coxiella badgerensis*. When fully developed, the cercaria leaves the primary intermediate host and after swimming for a period, it invades and encysts in the same snail, or another of the same species. Definitive hosts become infected by eating snails that harbour the metacercarial cyst. Adult flukes initially established themselves in the lower small intestine of experimentally infected ducklings and after about 1 week, migrated to the upper small intestine, where egg production

FIG. 3.1 Psilochasmus oxyurus

Life-cycle

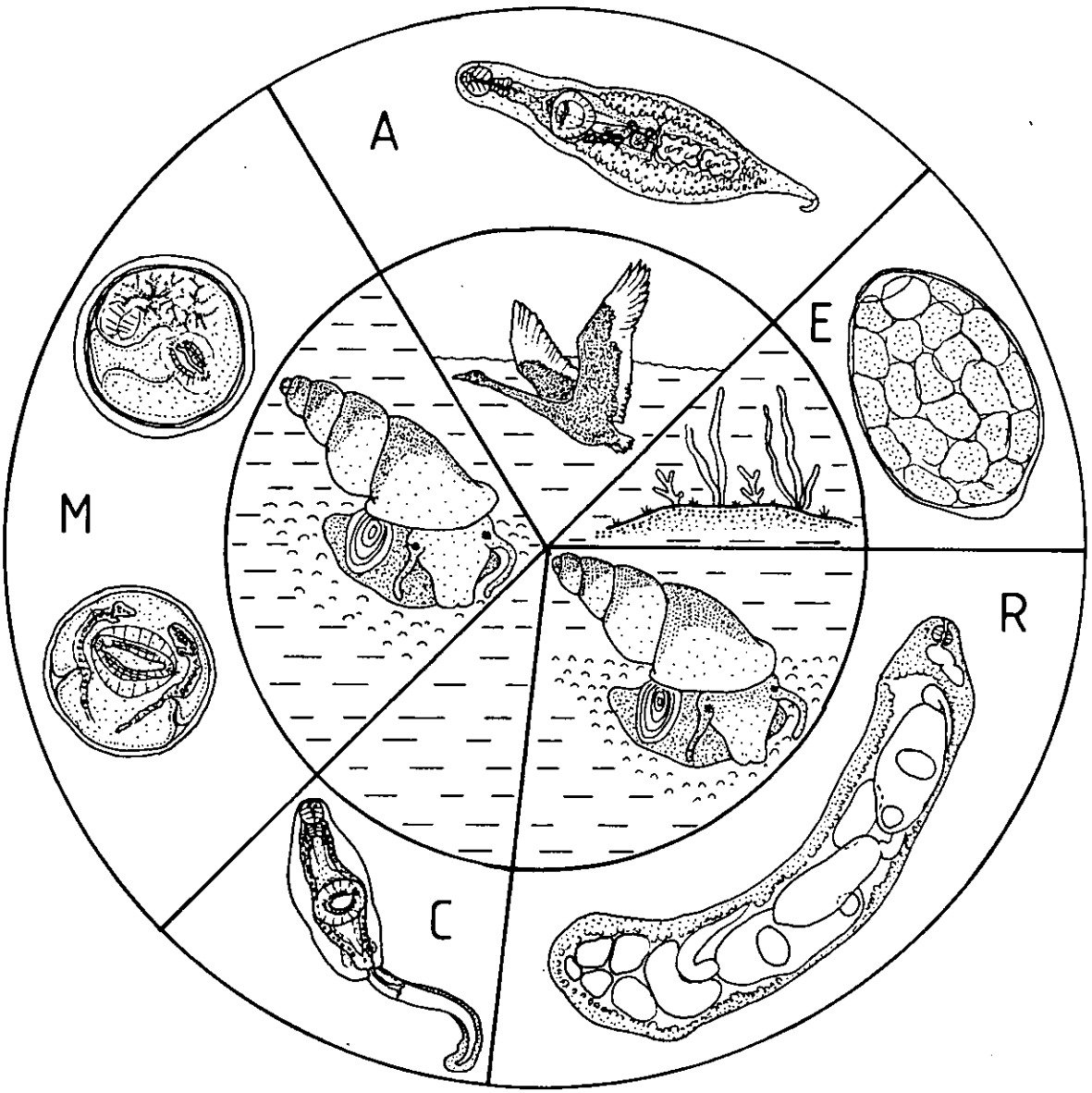


FIGURE 3.1 A, gravid adult; E, egg; R, daughter redia; C, cercaria; M, metacercarial cyst.

commenced. Adults can survive in laboratory ducklings for at least 33 days. At Calvert's Lagoon, mature adults were found in the black swan and black duck, and immature specimens were found in the hoary-headed grebe and coot.

3.2.2 Adult (Figure 3.2)

Psilochasmus oxyurus is mainly an intestinal parasite of anatiform birds. First named *Distomum oxyurum*, (Creplin, 1825), it was later transferred to the genus *Psilochasmus* by Luhe (1909). The species has a cosmopolitan distribution, occurring in anatids in both the northern and southern hemispheres. It has been found in Germany (Creplin, 1825; Braun, 1902); in the U.S.A. (Stunkard and Dunihue, 1931); in Argentina (Szidat, 1957); in Poland (Wisniewski, 1958); in West Siberia, China, India and Egypt (Yamaguti, 1971) and in New Zealand (Rind, 1974).

The description of the adult given below is based on specimens recovered from experimentally infected ducklings. The dimensions of worms of different ages are given in Table 3.1.

Description:

Fore-body more or less cylindrical; hind-body wider, dorsoventrally flattened. Protrusible terminal 'horn', composed of muscle fibres continuous with those of body wall; retracted into terminal socket, or protruded, curving ventrally. Outer tegument aspinous, about 11μ thick, traversed by fine striae. Oral sucker oval, mouth subterminal-ventral. Ventral sucker larger than oral sucker, powerful, sphincter-like; O.S.:V.S. = 0.62 (0.52 - 0.72). Large pharynx contiguous with oral sucker; oesophagus wide with well-developed epithelium, bifurcating anterior to ventral sucker, at level of genital pore. Caeca diverge obtusely around ventral sucker, terminate posterior to posterior testis. Tandem testes post-equatorial, large, more or less rectangular, with distinct irregular indentations

FIG. 3.2 *Psilochasmus oxyurus*

Growth in the domestic duckling

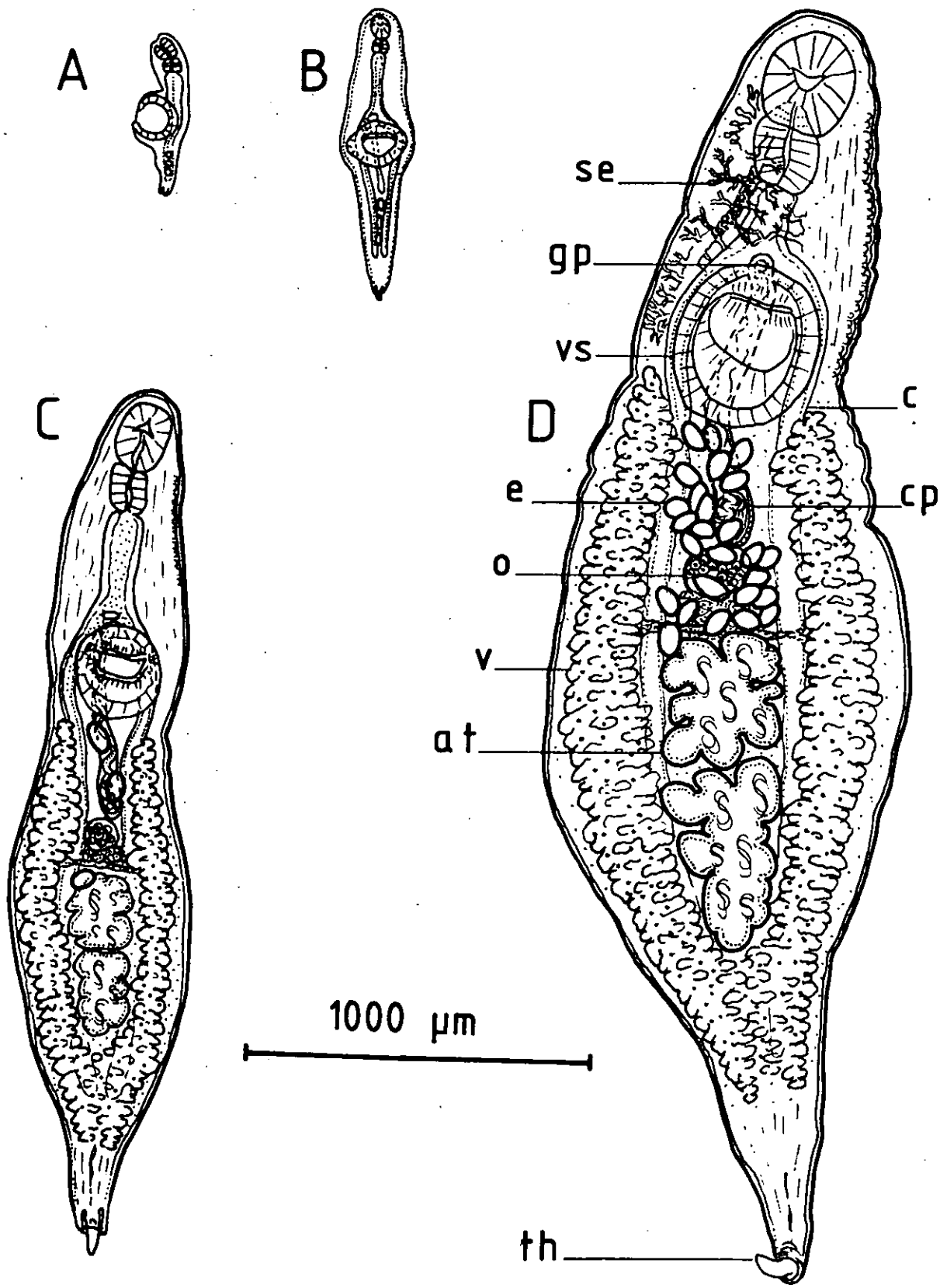


FIGURE 3.2 A, excysted metacercaria, after 4 hours at 41°C; B, juvenile adult, after 1,7 days in duckling; C, gravid adult, after 9,20 days in duckling, ventral view; D, gravid adult, after 23,23 days in duckling, ventral view. (at: anterior testis; c: caecum; cp: cirrus pouch; e: egg; gp: genital pore; o: ovary; se: subcuticular excretory network; th; terminal horn; v: vitellaria; vs: ventral sucker.)

TABLE 3.1 *Psilochasmus oxyurus*. Dimensions of excysted metacercariae after 4 hours at 41°C (a); and dimensions of adults from experimentally infected ducklings: (b) after 1,7 days, (c) after 4,17 days, (d) after 9,20 days, (e) after 18,22 days, (f) after 23,23 days, (g) after 32,23 days. Dimensions of flukes from a naturally infected black swan (killed at Calvert's Lagoon) are shown for comparison, (h).

	(a)	(b)	(c)	(d)
Sample size	8	9	7	9
Body length	569 (469 - 658)	816 (635 - 922)	1681 (1421 - 1914)	2709 (2407 - 2900)
Body width	157 (148 - 163)	168 (133 - 205)	343 (239 - 408)	550 (469 - 635)
Body depth	175 (171 - 179)	133 (122 - 148)	271 (209 - 333)	-
Anterior to V.S. (fore-body)	230 (151 - 295)	334 (287 - 408)	469 (378 - 757)	667 (522 - 783)
V.S. to posterior (hind-body)	225 (166 - 272)	346 (249 - 438)	771 (665 - 907)	1647 (1131 - 1972)
Oral sucker length	74 (67 - 84)	96 (84 - 106)	151 (144 - 163)	209 (186 - 228)
Oral sucker width	62 (61 - 65)	85 (72 - 95)	122 -	166 (144 - 179)
Oral sucker depth	63 (61 - 67)	87 (80 - 91)	122 -	-
Prepharynx length	8 (0 - 27)	4 (0 - 15)	0 -	0 -
Pharynx length	49 (42 - 53)	62 (46 - 76)	117 (106 - 125)	171 (152 - 190)
Pharynx width	36 (34 - 38)	47 (46 - 49)	106 -	115 (99 - 129)
Pharynx depth	48 (42 - 57)	68 (65 - 70)	101 (99 - 103)	-
Oesophagus length	88 (49 - 114)	138 (110 - 190)	95 (38 - 152)	205 (163 - 315)
Ventral sucker length	114 (84 - 141)	140 (96 - 156)	259 (224 - 302)	306 (277 - 338)
Ventral sucker width	116 (114 - 118)	149 (129 - 167)	269 (232 - 302)	282 (247 - 342)
Ventral sucker depth	136 (122 - 141)	185 (175 - 198)	253 (201 - 287)	-
Ovary length	-	32 (23 - 38)	61 (46 - 80)	94 (84 - 103)
Ovary width	-	21 (11 - 34)	46 -	89 (80 - 106)
Ovary depth	-	-	70 (61 - 80)	-
Anterior testis length	-	24 (15 - 30)	98 (87 - 114)	243 (171 - 296)
Anterior testis width	-	19 (11 - 30)	68 -	187 (160 - 228)
Anterior testis depth	-	-	59 (49 - 68)	-
Posterior testis length	-	31 (23 - 38)	104 (80 - 137)	301 (228 - 350)
Posterior testis width	-	19 (11 - 30)	61 -	169 (133 - 198)
Posterior testis depth	-	-	76 (72 - 80)	-
Horn length	32 (29 - 34)	33 (19 - 46)	49 (46 - 57)	87 (72 - 110)
Body length:body width ratio	3.62	4.86	4.90	4.93
O.S.(l+w):V.S.(l+w) ratio	0.59	0.63	0.52	0.64
Fore-body:hind-body ratio	1.02	0.97	0.61	0.40

	(e)	(f)	(g)	(h)
Sample size	20	1	3	5
Body length	3022 (2436 - 3451)	3654	4135 (3886 - 4408)	5614 (4524 - 6438)
Body width	703 (665 - 741)	1058	968 -	907
Body depth	390 (318 - 499)	-	386 (333 - 438)	552 (393 - 696)
Anterior to V.S. (fore-body)	789 (580 - 928)	754	795 (754 - 870)	1305 (1102 - 1508)
V.S. to posterior (hind-body)	1908 (1392 - 2204)	2552	2917 (2494 - 3132)	3776 (2900 - 4176)
Oral sucker length	275 (247 - 304)	277	328 (318 - 333)	431 (393 - 469)
Oral sucker width	228 -	239	287 -	318
Oral sucker depth	194 (171 - 220)	-	280 (272 - 287)	358 (302 - 393)
Prepharynx length	0 -	0	0 0	0
Pharynx length	195 (167 - 266)	251	212 -	257 (227 - 287)
Pharynx width	144 -	137	121 -	302
Pharynx depth	186 (167 - 201)	182	151 -	257
Oesophagus length	184 (156 - 209)	454	464 (408 - 529)	-
Ventral sucker length	339 (257 - 408)	423	529 -	490 (333 - 605)
Ventral sucker width	363 -	-	484 (408 - 559)	578 (529 - 635)
Ventral sucker depth	378 (348 - 454)	-	-	227
Ovary length	103 -	-	-	212
Ovary width	133 -	393	590	-
Ovary depth	-	408	423 -	711
Anterior testis length	304 -	-	-	423
Anterior testis width	247 -	529	665 -	-
Anterior testis depth	-	348	333 -	907
Posterior testis length	350 -	-	-	333
Posterior testis width	209 -	-	129 (121 - 136)	-
Posterior testis depth	-			160 (121 - 212)
Horn length	98 (72 - 110)			
Body length:body width ratio	4.30	3.45	4.27	6.19
O.S.(l+w):V.S.(l+w) ratio	0.72	0.59	0.62	-
Fore-body:hind-body ratio	0.41	0.30	0.27	0.35

around margins; anterior testis shorter, wider than posterior testis. Cirrus pouch about 3μ thick, long, dextral to ovary, terminating at mid-level of ovary; seminal vesicle, about 150 × 90μ, opens into ejaculatory duct, which is coiled 2-3 times, before opening at genital pore; prostate gland cells concentrated near junction of seminal vesicle, ejaculatory duct; everted cirrus about 230 × 90μ. Round, sometimes indented ovary, sub-median sinistral, situated about 1/3 distance from anterior testis to ventral sucker. Oviduct passes posteriorly from posterior border of ovary to ootype, midway between ovary and anterior testis. Ootype surrounded by large, indiscrete Mehlis' gland. Laurer's canal, about 75μ long, extends from ootype to sinistral pore on dorsal surface. Proximal uterus, "receptaculum seminis uterinum", expanded, filled with sperm. Uterus sinuous, passing posteriorly to anterior testis, then anteriad, passing dorsally over ventral sucker, to median genital pore; many uterine eggs, (93 in one fluke from wild black swan). Vitellaria extend from posterior border of ventral sucker to termination of caeca; 2 elongate clusters of glands, mainly extracaecal, meet posterior to posterior testis; longitudinal ducts on each side of body conduct vitelline secretions to 2 large transverse vitelline ducts, arising anterolaterally to anterior testis, unite medially, forming vitelline reservoir. Flame-cell pattern not observed. Paranephridial system well-developed, reticulate arrangement of large paranephridial canals extends throughout hind-body; opague, subtegumental excretory network in fore-body, containing fine droplets, extends from oral sucker to middle of ventral sucker; excretory pore located on dorsal side of terminal 'horn'.

Vertebrate hosts: *Anas platyrhynchos* L. (experimental host), *A. superciliosa* Gmelin; *Cygnus atratus* (Latham); *Fulica atra* L.; *Poliocephalus poliocephalus* (Jardine and Selby).

Habitat: Small intestine

Geographic location: Calvert's Lagoon

Material: Tasmanian Museum - K916, gravid adult (flattened); K917, gravid adults; K918, immature adults; K919, excysted metacercariae.

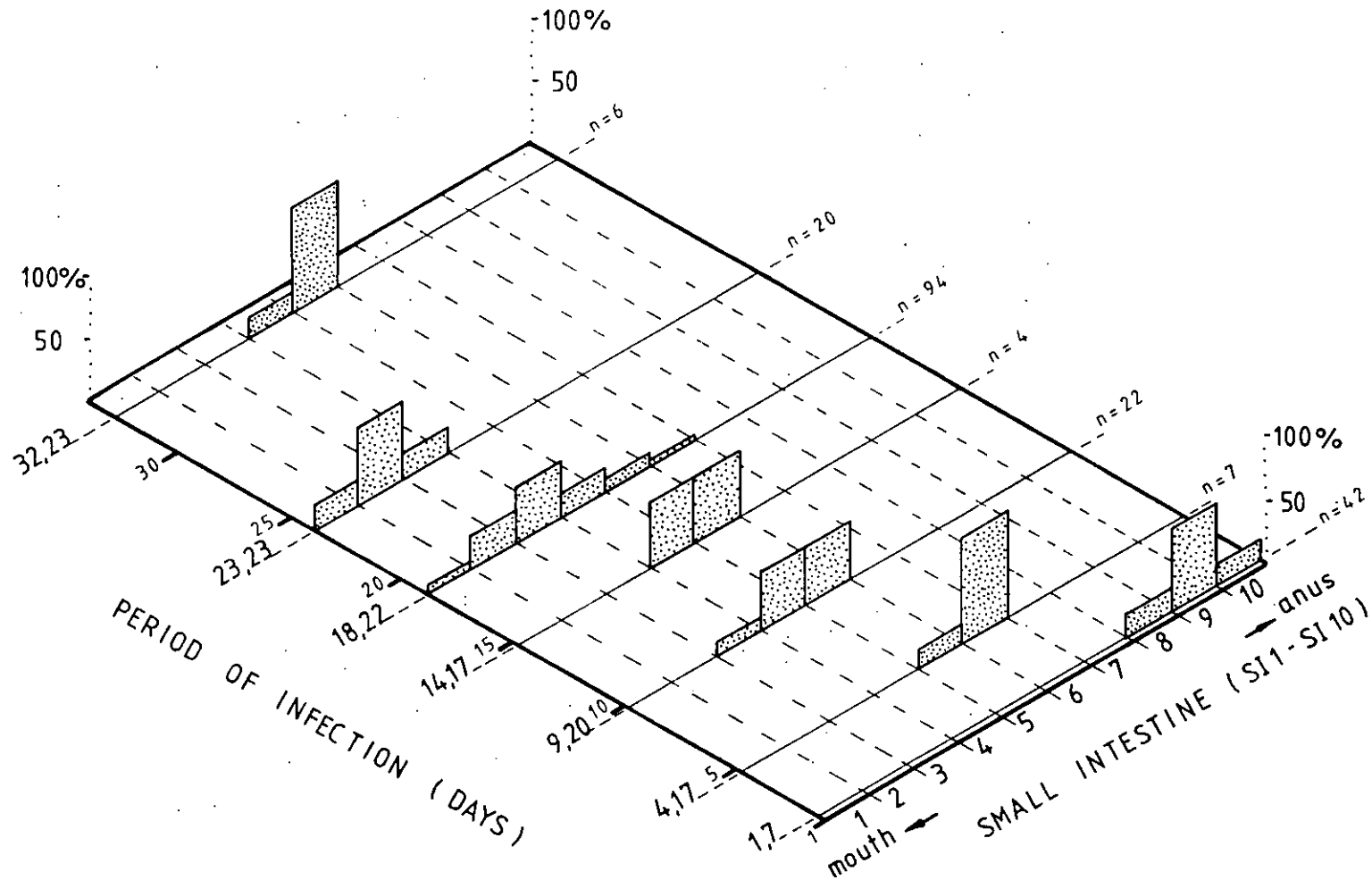
Relationships:

The adults recovered from experimentally infected ducklings and naturally infected birds from Calvert's Lagoon, fall well within the previously recorded range of variation of size and morphology of *Psilochasmus oxyurus*. The discovery of *P. oxyurus* in Australia, extends its known distribution in the southern hemisphere, where it was previously known only from New Zealand and Argentina.

Biology:

Domestic ducklings, raised under controlled conditions, were fed encysted metacercariae from naturally infected and occasionally, experimentally infected, snails. The birds were sacrificed after different periods, ranging from 1,0 to 32,23 days post infection. Sixty-three percent (10/16) of ducklings were infected by from 1 to 94 adults of *P. oxyurus*. The proportion of encysted metacercariae that were recovered, as adults, from the intestines of infected birds, varied from about 25 to 75%. The longest recorded period of infection was 32,23 days. The distribution of *P. oxyurus* in the digestive tract, at different intervals post infection, is shown in Figure 3.3. The adults showed marked site specificity and were more or less normally distributed in the small intestine. The preferred site varied in relation to the age of the worms. They were concentrated in SI9 after 1,7 days, but migrated anteriorly as they grew and matured, being concentrated in SI6 after 4,17 days; SI4 and SI5 after 9,20 days; SI4 and SI5 after 14,17 days; SI3 after 18,22 days and SI2 after 23,23 days. A small number of adults (6), were found in SI4 and SI5 after 32,23 days, possibly indicating a regression due to senescence.

FIG. 3.3 Psilochasmus oxyurus. Distribution of adults in the gut of laboratory ducklings, at different intervals after infection, (n = no. of adult flukes).



Newly excysted metacercariae are very immature. They undergo enormous growth in the bird host, as they become sexually mature. The rate of growth is shown in Figure 3.4. Primordia of the ovary and testes were barely discernible in some worms after 1,0 day *in vivo*, but were present in all worms after 1,19 days. The gonads were large and obvious after 4,17 days. As the reproductive system developed the hind-body became proportionally larger (Figure 3.5). Vitelline cells were visible after 6,21 days, however no phenolic egg-shell precursors were being produced. By this stage, sheafs of spermatids were present in the testes and the paranephridial system was well developed and ramified throughout the body. After 9,20 days, the vitellaria were producing phenolic egg-shell precursors, the seminal vesicle and the proximal part of the uterus were filled with sperm and the first eggs occupied the uterus. Growth continued and eggs were produced over the next 24 days. The average number of intra-uterine eggs increased with the age of the flukes (Table 3.2).

TABLE 3.2 *Psilochasmus oxyurus*. The number of eggs contained within the uterus of adults: (a) recovered from experimentally infected ducklings, after different periods of infection; and (b) from a wild black swan.

Period of Infection (days)	Sample size (No. of flukes)	Number of intra-uterine eggs Mean	S.D.	Range
(a) 9, 20	15	4.9	3.0	0 - 10
11, 0	8	2.9	2.7	0 - 7
14, 17	4	14.5	0.6	14 - 15
18, 22	20	21.4	3.9	16 - 30
23, 23	10	25.3	2.8	21 - 29
32, 23	3	39.7	7.5	32 - 47
(b) -	5	61.2	22.4	38 - 93

The only bird killed at Calvert's Lagoon that harboured ovigerous specimens of *P. oxyurus*, was a black swan. The 8 flukes were evenly distributed in SI4 and SI5. Two specimens of *P. oxyurus*, found infecting a black duck, were relatively mature, with phenolic egg-shell

FIG. 3.4 Psilochasmus oxyurus. Growth of adults in laboratory ducklings.

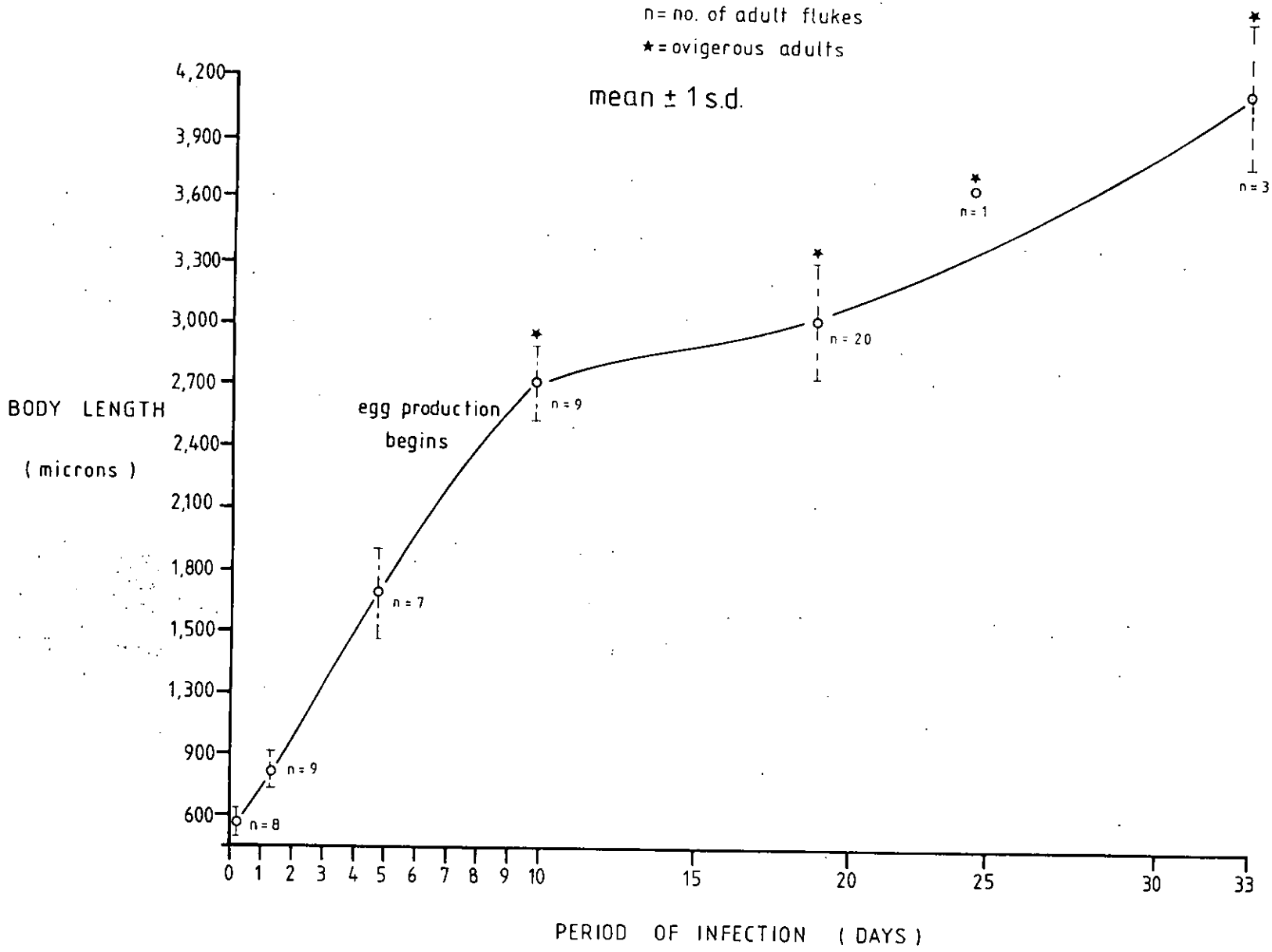
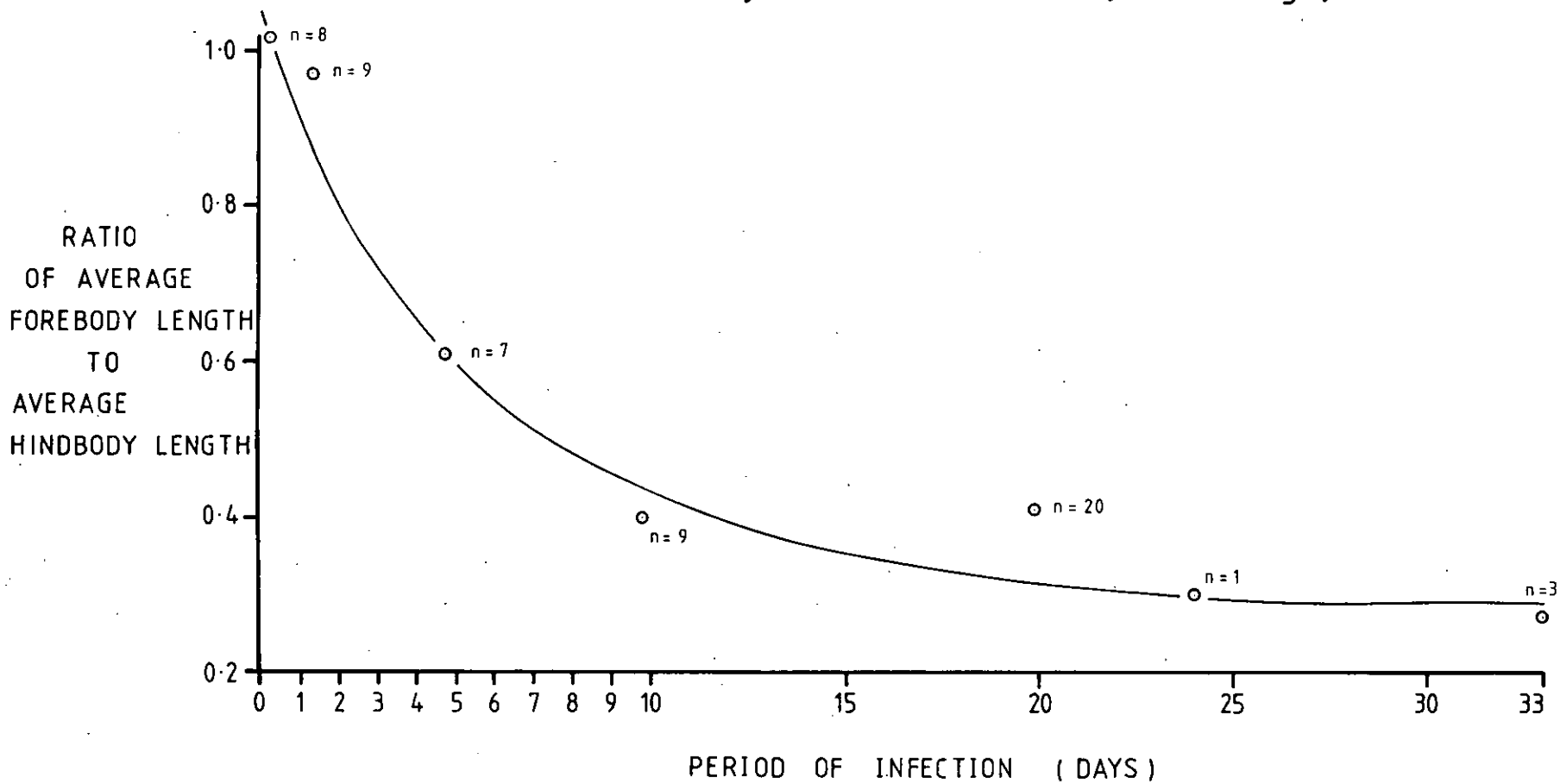


FIG. 3.5 Psilochasmus oxyurus. Changes in the relative size of forebody and hindbody of adult flukes, with age, (n = no. of flukes).



precursors in the vitellaria; however, specimens recovered from a coot and hoary-headed grebes, were very immature. If the rate of egg production does not differ significantly in black swans and domestic ducklings, then the numbers of eggs in flukes found in the swan indicate that the flukes were more than 33 days old (Table 3.2). Such an age is also indicated by the great size of the adults in the black swan.

3.2.3 Egg

The egg is broadly oval with a small round operculum about 20μ diameter (Figure 3.6). The egg-shell is of uniform thickness, except around the rim of the operculum, where it thins and at the abopercular pole, where it is slightly thicker. When first formed, it is thin, transparent and colourless, but soon thickens, and, after oviposition, is tanned yellow. The egg is densely packed with vitelline nutritive cells, each about 24μ diameter with a nucleus and many yolk granules. Eggs are not embryonated within the uterus. A clear round body, 18μ diameter, resembling the 'viscous cushion' in the egg of *Fasciola hepatica* (Wilson, 1968), underlies the operculum. Dimensions of live eggs deposited in the intestine of a duckling after 18,22 days, are shown in Table 3.3, together with those of fixed intra-uterine eggs in flukes of different ages. Fixation appeared to cause some shrinkage, as fixed eggs were slightly smaller than live eggs of the same age. The size of eggs varied markedly within and between eggs from different ducklings, but was not related to the age of the flukes. Eggs found in the intestine of a duckling 18,22 days P.I., were maintained in the dark at room temperature in lagoon water. After 19 days, a moribund miracidium was found in the opening of one egg. The miracidium was covered in small cilia, that were still active. Except for a round apical organ, 14 × 7μ, no other features could be discerned. The flattened miracidium was 116 × 105μ. The operculum was still attached to the egg. At the

FIG. 3.6 Psilochasmus oxyurus

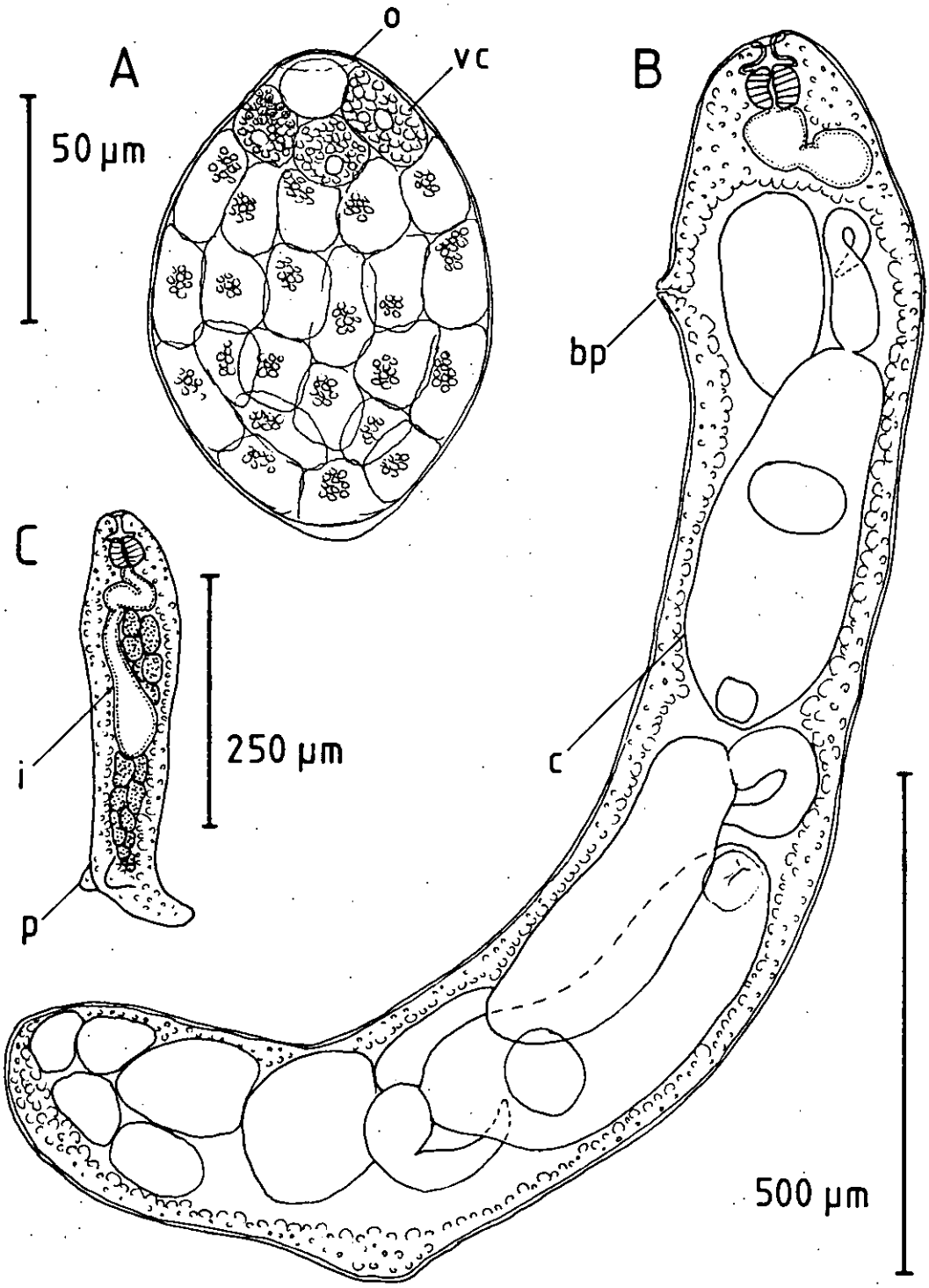


FIGURE 3.6 A, egg, from intestine of duckling 14,17 days after infection with *P. oxyurus;* B, mature pigmented daughter redia; C, immature colourless daughter redia. (bp: birth pore; c: cercaria; i: intestine; o: operculum; p: procrusculum; vc: vitelline cell.)

same time, some other eggs were discovered empty, but no free miracidia were found. Intact eggs were mechanically opened, but none were found to contain a miracidium. Attempts to infect adult laboratory bred snails with eggs from laboratory ducklings were unsuccessful.

TABLE 3.3 *Psilochasmus oxyurus*. Dimensions of live and fixed eggs from adults in a laboratory duckling 18,22 days P.I.: and fixed eggs from adults in other experimentally infected ducklings and a naturally infected black swan.

Host	Live/ fixed	No. eggs	Length	Width
Duckling, 18, 22 days P.I.	Live	10	114 (106 - 118)	81 (76 - 84)
Duckling, 18, 22 days P.I.	Fixed	20	108 (99 - 114)	70 (57 - 80)
Duckling, 9, 20 days P.I.	Fixed	20	96 (87 - 105)	57 (49 - 65)
Duckling, 23, 23 days P.I.	Fixed	5	109 (103 - 114)	72 (65 - 78)
Duckling, 32, 23 days P.I.	Fixed	5	100 (91 - 106)	58 (53 - 61)
Wild black swan	Fixed	10	104 (91 - 121)	59 (53 - 60)

3.2.4 Redia (Figure 3.6)

Rediae, in which cercariae develop, are distributed throughout the viscera of infected snails. They appear to be concentrated in the gonad, but extend among the hepatopancreatic tubules, into the kidney and, in female snails, into the pallial oviduct. There is a gradation in size and development from minute colourless rediae, containing undifferentiated germ balls to large very motile orange pigmented rediae, containing distinct developing cercariae. The dimensions of these 2 classes of rediae are shown in Table 3.4.

TABLE 3.4 *Psilochasmus oxyurus*. Dimensions of colourless, immature rediae (a), and pigmented, mature rediae (b).

	(a)	(b)
Sample size	10	20
Body length	419 (348 - 514)	1119 (484 - 1618)
Body width	88 (65 - 121)	227 (181 - 302)
Pharynx length	26 (23 - 27)	35 (27 - 40)
Pharynx width	30 (23 - 34)	35 (30 - 38)

The small rediae are more or less cylindrical in shape and have a small pharynx, a colourless intestine, and 2 prominent procruscula. No birth pore is evident. The intestine is coiled and relatively long, extending about half the length of the redia. In the largest rediae, cercarial embryos and germ balls are densely packed into the posterior part of the brood chamber and cercariae at different stages of development are loosely packed in the remaining space. Cercariae, when still immature, emerge through a birth pore in the tegument at the anterior of the brood chamber, and complete their development in the viscera of the host snail. Orange pigment is unevenly distributed through the tegument of these rediae. Carotenoid pigments have been reported from sporocysts and rediae of several trematode species (Marshall, 1974; Hoskin and Cheng, 1975 and Zavras and James, 1979). The carotenoids, which are absorbed from the host tissues, have been implicated in the electron transport system of the parasite, and it has been suggested that they aid survival of trematodes under extreme conditions of parasite-induced anoxia (Zavras and James, 1979). In the mature rediae, a terminal mouth opens into a small pharynx and a relatively small, colourless intestine. The procruscula are much less conspicuous than in immature rediae.

3.2.5 Cercaria (Figure 3.7)

Morphology and anatomy:

It is large and gymnocephalous, with dorso-ventral tail finfolds. The opaque body, densely packed with masses of cystogenous granules, has its maximum width at the level of the ventral sucker. Both oral and ventral suckers are large and powerful. The outer tegument is thick and aspinous. Ciliated sensory papillae are symmetrically arranged around the subterminal ventral mouth, which leads via a short prepharynx to a large muscular pharynx. The wide oesophagus, with narrow lumen and well-developed epithelium, bifurcates anterior to the ventral sucker.

FIG. 3.7 Psilochasmus oxyurus

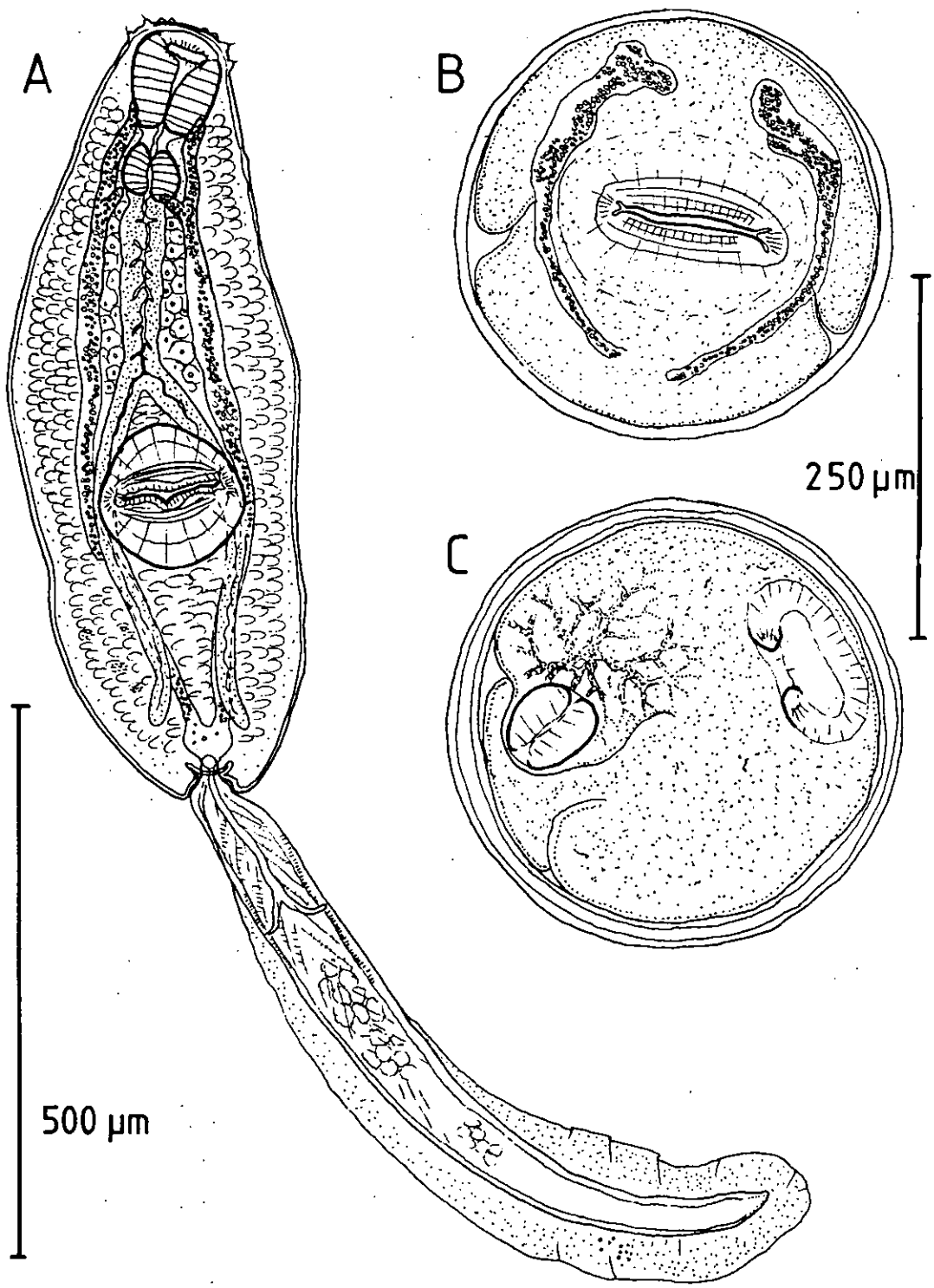


FIGURE 3.7 A, whole mature cercaria, ventral view; B, metacercarial cyst after 2 hours in experimental snail host; C, metacercarial cyst after 7 days in experimental snail host.

The caeca, which contain very fine granules, extend to the posterior of the body. Cystogenous glands are concentrated in the hind-body, but extend anterolaterally as far as the oral sucker. The gland cells, stained differentially by neutral red and brilliant cresyl blue, are packed with grains, about 1μ long. When flattened, each cell measures about 17μ diameter. Large cells with very distinct nuclei lie on either side of the oesophagus, between the oral sucker and gut bifurcation. They are not granular, and although no anteriorly-opening ducts were seen, they may be a type of 'frontal gland'. The excretory pattern is stenostomate; excretory bladder branching about halfway between the excretory pore and ventral sucker, forming 2 wide primary vessels, which extend nearly to the anterior of the oral sucker, each giving rise to a short internal branch at the level of the pharynx. The primary vessels contain irregular-shaped, refractile excretory granules, up to about 2μ diameter, which are more densely packed anterior to the ventral sucker. A caudal canal extends about ½ of the length of the tail, then bifurcates to form 2 short canals which open laterally. The tail has an asymmetrical, dorsoventral finfold: the ventral part extends along the full length of the tail, and the dorsal part extends along the posterior half. Dimensions of the cercaria are shown in Table 3.5.

TABLE 3.5 *Psilochasmus oxyurus*. Dimensions of cercariae, after emerging from the snail host, (n = 20).

Body length	649 (575 - 786)	Oral sucker length	91 (80 - 106)
Body width	227 (186 - 302)	Oral sucker width	79 (70 - 91)
Body depth	174 (151 - 197)	Ventral sucker length	116 (87 - 156)
Tail length	579 (454 - 680)	Ventral sucker width	136 (106 - 171)
Tail width	64 (53 - 76)	Pharynx length	48 (42 - 53)
Dorsal finfold length	340 (272 - 393)	Pharynx width	49 (42 - 57)
Ventral finfold length	591 (514 - 711)		
D.F.L.:V.F.L. ratio	0.56		
Max. finfold width	122 (118 - 125)		

Behaviour and ecology:

The cercaria swims upside down, the tail lashing rapidly in a vertical plane, perpendicular to the body, causing the latter to jerk from

side to side. The cercaria is neither phototactic, nor geotactic, but follows an apparently random course. It swims continuously for many hours, until exhausted, or until encountering a suitable snail host. Mature cercariae periodically emerge from their primary host. Over a 4 day period in March, 6(2-12) cercariae were released daily from one snail. All emerged in the afternoon or evening, with a peak at about 4 p.m. Cercariae continued to emerge for at least 18 weeks, from one infected snail in the laboratory.

When exposed to a laboratory-bred snail in a crystal dish, the cercaria approaches and soon lands. Contact is made with the head or foot of the snail within 2 or 3 minutes. The cercaria then quickly creeps up the head of the snail, using its oral and ventral suckers, and disappears into the mantle cavity. After a few minutes, the detached tail reappears and 'swims' away.

Metacercarial cysts were recovered from experimentally infected snails after periods ranging from 2 hours to 7 days (Table 3.6). In each case the cysts were loosely attached to the internal surface of the shell, near the pericardium. This is surprising, as cysts in naturally infected snails are usually lodged around the heart, in the wall and cavity of the pericardium. Only in very heavy natural infections are cysts found between the shell and mantle.

Snails which serve as the primary host may be reinvaded, and serve as the secondary host. This has been observed experimentally, but is also indicated by the relatively high incidence of these cysts in snails serving as primary intermediate hosts for *P. oxyurus*. During the present study, the average number of cysts in naturally infected snails, also serving as primary hosts for *P. oxyurus*, was 49 (12 - 121). In snails collected at Site 1, from August 1977 to September 1978, that were infected with cysts of *P. oxyurus* and/or *Psilostomum sp.B*, the average number of cysts was only 1.7 (1 - 6). The similar large metacercarial cysts of *P. oxyurus* and *Psilostomum sp.B* were not distinguished until

later in the study.

3.2.6 Metacercaria

Metacercarial cyst: (Figure 3.7)

Two hours after invasion the cyst is only about 8μ thick, however, after 50 hours, it is fully developed. The mature cyst is large and more or less round, up to about 20μ thick. In optical section it appears to have 3 clear, uniform layers. The dimensions of cysts from naturally infected snails and some cysts of different ages, from experimentally infected snails, are shown in Table 3.6.

TABLE 3.6 *Psilochasmus oxyurus*. Dimensions of metacercarial cysts: (a) from experimentally infected snails; and (b) from naturally infected snails.

	Infection Period (days)	No. cysts	External dimensions Length × Width	Cyst thickness (average)
(a)	0, 2	1	293 × 289	7
	0, 4	1	300 × 296	13
	2, 2	4	313 (308 - 319) × 302 (295 - 315)	15
	2, 23	14	309 (293 - 331) × 296 (251 - 315)	15
	7, 0	4	306 (304 - 310) × 304 (300 - 306)	16
(b)	Wild snails	20	308 (293 - 334) × 302 (285 - 334)	19

Excystment:

Only a relatively small percentage of metacercarie, collected from naturally infected snails, excysted *in vitro* when exposed to 0.5% pancreatin in Hank's saline at 41°C: 5% had excysted after 2 hours: 9% after 5 hours: and after 11 hours, 11% had excysted. The process of excystment, *in vitro*, is stimulated by elevation of the temperature to about 40°C and by exposure to pancreatin solution. The metacercaria actively escapes through a small hole that is probably dissolved in the cyst wall by enzymes of parasite origin.

Excysted metacercaria: (Figure 3.2)

The excysted metacercaria is smaller than the body of the cercaria, presumably due to the loss of cystogenous material; very little development occurs within the cyst. The dimensions of the excysted metacercaria are shown in Table 3.1. Genital anlagen are barely visible, however the characteristic terminal horn is clearly developed. Szidat (1957), recorded the horn in excysted metacercarie of *P. oxyurus*, but Wisniewski (1958b), did not. The latter author may have mechanically excysted metacercariae that were not fully developed.

3.2.7 Discussion

The life cycle of *Psilochasmus oxyurus* has been studied previously by Szidat (1957) and Wisniewski (1958b). The former author discovered that the brackishwater snail, *Littoridina australis*, served as both the primary and secondary intermediate host for *P. oxyurus* in Argentina, and that wild ducks served as the definitive host. Wisniewski found the species infecting fauna in the Mazury Lake District in Poland, where the snail *Bithynia tentaculata* served as the primary intermediate host, and the snails *B. tentaculata*, *Radix* sp. and *Spiralina vortex* were found to be secondary intermediate hosts. The adult fluke infected wild ducks, *Anas platyrhynchos* and *Nyroca fuligula*, at the same location.

In each of the studies, the morphology of the redia was very similar. The mature redia in *Coxiella badgerensis* has a slight "collar" at the level of the birth pore. A "collar" was reported by Szidat, but not by Wisniewski. The size of the body and pharynx of rediae from the 3 different snail hosts, varied considerably (Table 3.7). Cercariae in each of the studies were morphologically identical. Variation in their dimensions is shown in Table 3.7. Szidat found that in Argentina, cercarial emergence occurred in the morning, whereas, in the present study, emergence in summer occurred in the afternoon and evening. In each of the studies, cysts were found to be large and round. Variation in the

size of cysts from different second intermediate hosts is shown in Table 3.7. Szidat reported that the cercaria encysts in the snail host's "visceral sac"; however, Wisniewski found that encystment was between the snail's shell and mantle. In naturally infected snails at Calvert's Lagoon, cysts were usually localized in the wall of the pericardium, but occasionally, in heavy infections, some cysts occurred between the shell and mantle. In experimentally infected specimens of *C. badgerensis*, cysts were always formed between the shell and mantle. It is apparent that environmental conditions influence the site of encystment in the second intermediate host. Szidat recovered adult flukes from experimentally infected ducks and chicks up to 21 days after infection. In the present study, *P. oxyurus* was found to be very long-lived in the definitive host. The longest period of infection in laboratory ducklings was nearly 33 days, and it is believed, from their size, and the number of uterine eggs, that flukes recovered from a black swan at Calvert's Lagoon, were more than 33 days old.

TABLE 3.7 *Psilochasmus oxyurus*. Comparison of the dimensions of the redia, cercaria and metacercarial cyst in 3 different snail hosts: (a) *Littoridina australis*, (Szidat, 1957); (b) *Bithynia tentaculata*, (Wisniewski, 1958b) and (c) *Coxiella badgerensis*.

		(a)	(b)	(c)
Redia	Body	950 × 100	1450 × 205	1119 × 227
	Pharynx	30 × 30	70 × 70	35 × 35
Cercaria	Body	580 × 220	870 × 225	649 × 227
	Tail	500 × -	650 × -	579 × 64
	O.S.	70 × 70	105 × 110	91 × 79
	V.S.	150 × 150	180 × 185	116 × 136
Cyst	Outer wall	300 × 300	340 × 340	308 × 302
	Thickness	-	25	19

The results of the present study, support those of Szidat (1957), who found that the adult of *P. oxyurus* continues to grow after commencing egg production, with the result that ovigerous specimens of this species vary greatly in size (Figure 3.4). He showed that the adults of *P. longicirratus* and *P. japonicus* fall within the size range of *P. oxyurus*.

Stunkard and Dunihue (1931), considered that *P. longicirratus* was a synonym of *P. oxyurus*. Yamaguti (1971), however, contended that *P. japonicus* was a synonym of *P. longicirratus*, but that differences of egg measurements justified the specific distinction between *P. oxyurus* and *P. longicirratus*. In the present study, the egg size of *P. oxyurus* was found to vary markedly between flukes of the same age and of different ages, taken from laboratory ducklings (Table 3.3). Consequently, *P. longicirratus* and *P. japonicus*, whose eggs actually fall within the size range of eggs of *P. oxyurus*, are considered by the author to be synonyms of *P. oxyurus*. In view of the size variation that has been demonstrated in the adults and eggs of the type species, a revision of the genus *Psilochasmus* is needed to determine the validity of some other species, such as *P. agilis*.

Genus PSILOSTOMUM Looss, 1899

3.3 Psilostomum sp.A.

3.3.1 *Life-cycle*

A metacercarial cyst, significantly smaller than that of *Psilochasmus oxyurus*, frequently occurs in the pericardium, and between the shell and mantle of ***Coxiella badgerensis***. The cyst belongs to a relatively small species of ***Psilostomum***, to be referred to as ***Psilostomum sp.A***. Its life-cycle is similar to that of *Psilochasmus oxyurus*. The cercaria develops in a redia in *C. badgerensis*, and after emerging and swimming for a period, it invades and encysts in the same snail, or another snail of the same species. Laboratory ducklings became infected by *Psilostomum sp.A.* after being fed with cysts from naturally infected snails. The adults inhabit the middle region of the small intestine and can survive for at least 11 days. Hoary-headed grebes and possibly black swans, serve as natural hosts for this trematode at Calvert's Lagoon.

TABLE 3.8 *Psilostomum sp.A.* Dimensions of metacercariae, excysted *in vitro* after about 4 hours at 41°C (a); and dimensions of adults from experimentally infected ducklings: (b) after 1,19 days, (c) after 4,23 days, and (d) after 9,0 days (*fixed under coverslip pressure).

	(a)	(b)	(c)	*(d)
Sample size	20	10	5	5
Body length	316 (262 - 361)	499 (393 - 635)	1080 (953 - 1164)	1040 (786 - 1194)
Body width	93 (84 - 103)	124 (118 - 129)	198 (182 - 220)	389 (318 - 469)
Body depth	90 (84 - 95)	108 (80 - 122)	182 0	-
Anterior to V.S.(fore-body)	157 (122 - 171)	230 (197 - 273)	333 (287 - 393)	293 (249 - 340)
V.S.to posterior (hind-body)	106 (87 - 124)	186 (136 - 257)	602 (529 - 696)	526 (393 - 650)
Oral sucker length	49 (44 - 53)	68 (61 - 80)	110 (106 - 118)	109 (91 - 125)
Oral sucker width	41 (40 - 46)	57 0	92 (87 - 99)	125 (106 - 152)
Oral sucker depth	43 (40 - 46)	59 (57 - 61)	87 0	-
Prepharynx length	20 (15 - 25)	18 (11 - 27)	0 0	-
Pharynx length	29 (27 - 30)	43 (38 - 46)	76 (65 - 87)	84 (65 - 106)
Pharynx width	24 (21 - 27)	46 (42 - 53)	79 (65 - 103)	89 (76 - 106)
Pharynx depth	36 0	52 (42 - 61)	91 0	-
Oesophagus length	48 (34 - 57)	71 (53 - 91)	121 (106 - 133)	-
Ventral sucker length	57 (49 - 63)	93 (76 - 114)	147 (133 - 163)	184 (163 - 194)
Ventral sucker width	69 (65 - 72)	118 0	163 (148 - 171)	205 (171 - 228)
Ventral sucker depth	71 (67 - 76)	111 (91 - 133)	148 (137 - 160)	-
Ovary length	-	21 (19 - 23)	52 (42 - 65)	60 (46 - 76)
Ovary width	-	23 0	53 (46 - 57)	66 (61 - 76)
Ovary depth	-	23 0	44 (34 - 53)	-
Anterior testis length	-	34 (19 - 53)	138 (122 - 163)	100 (76 - 122)
Anterior testis width	-	38 0	133 (103 - 118)	161 (141 - 190)
Anterior testis depth	-	37 (23 - 65)	137 0	-
Posterior testis length	-	31 (19 - 57)	147 (118 - 175)	122 (87 - 152)
Posterior testis width	-	34 0	108 (87 - 118)	151 (122 - 190)
Posterior testis depth	-	31 (19 - 57)	125 0	-
Body length:body width ratio	3.40	4.02	5.45	2.67
O.S.(l+w):V.S.(l+w) ratio	0.71	0.59	0.65	0.60
Fore-body:hind-body ratio	1.48	1.24	0.55	0.56
Number of eggs	0	0	0	1.8 0.5 (1-2)

3.3.2 Adult (Figure 3.8)

The description of the morphology and anatomy of the adult (below), is based on ovigerous and non-ovigerous specimens recovered from experimentally infected ducklings. Unfortunately all of the ovigerous adults were fixed under coverslip pressure. Dimensions of both gravid and immature adults are shown in Table 3.8.

Description:

Elongate body, more or less cylindrical, covered by thick, aspinous tegument. Oral sucker oval, subterminal mouth opening ventrally. Prepharynx very short or absent, oval pharynx relatively large. Short oesophagus bifurcates anterior to well-developed ventral sucker, caeca extend to posterior end of body. O.S.:V.S. = 0.61 (0.59 - 0.65). Testes tandem, post-equatorial, equal, oval to rectangular. Cirrus pouch elongate, clavate, extends to mid-level of ovary. Seminal vesicle double-chambered, leads to coiled, folded, ejaculatory duct, which extends anteriad to genital pore. Genital pore median, anterior to ventral sucker, ventral or postero-ventral to gut bifurcation. Everted cirrus long, slender; length about 295μ, width at base about 63μ, width at tip about 27μ. Ovary oval, median to submedian, contiguous to base of cirrus pouch. Short oviduct leads to median ootype, surrounded by Mehlis' gland, between ovary and anterior testis. Laurer's canal not seen. Uterus passes anteriad from ootype to genital pore; containing few relatively large eggs. Vitellaria confined to hind-body, extending from ventral sucker to posterior end of body, lateral to gonads, meeting between, posterior to, testes. Vitelline ducts anterior to anterior testis, join to form medial vitelline reservoir. Fine, granule-filled subtegumental canals anastomose over fore-body, from oral sucker to mid level of ventral sucker; excretory pore subterminal ventral; flame-cell pattern not determined.

FIG. 3.8 Psilostomum sp.A

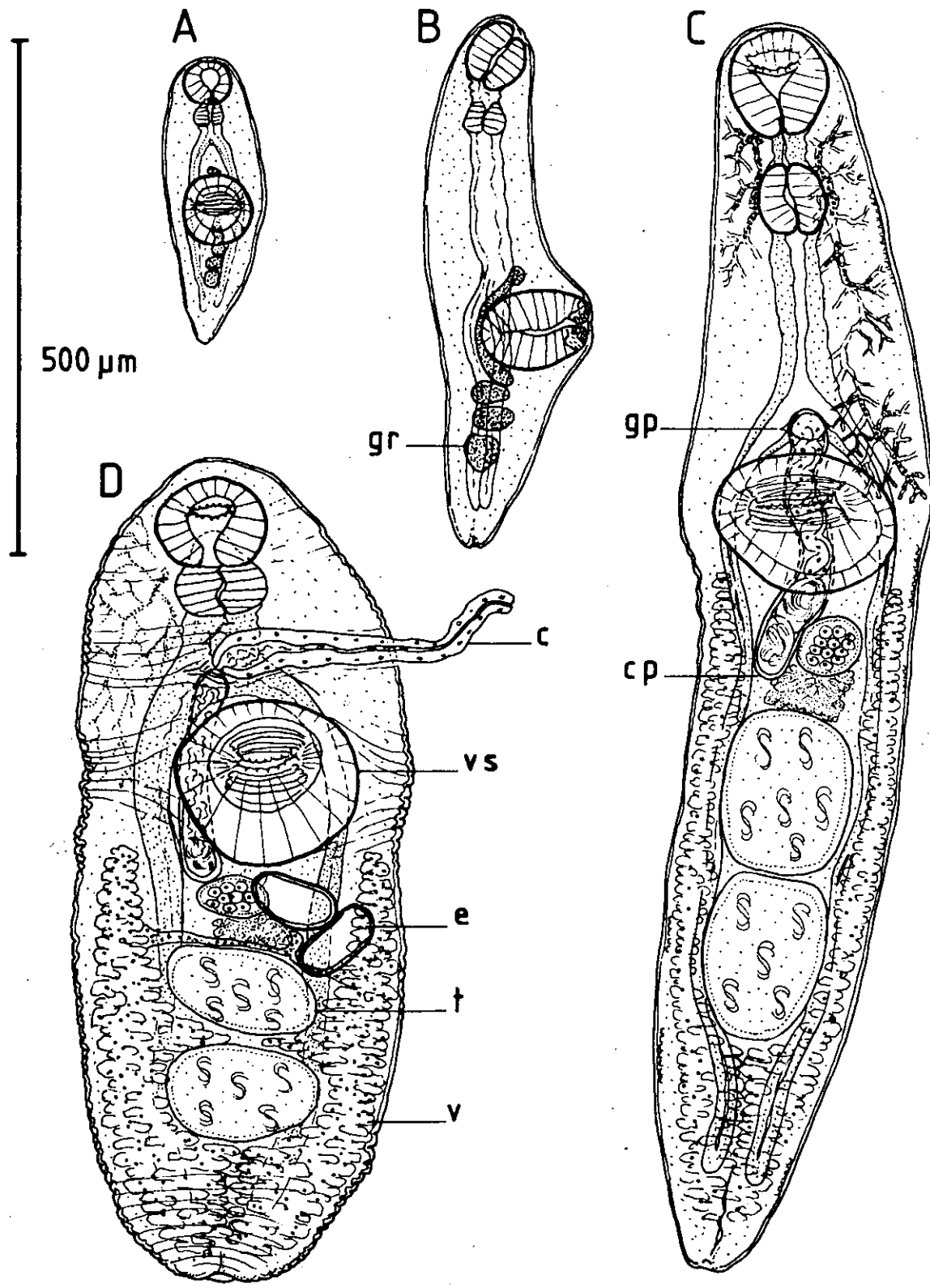


FIGURE 3.8 A, excysted metacercaria after 4 hours at 41°C; B, juvenile adult after 1,19 days in duckling; C, mature adult after 4,23 days in duckling, ventral view; D, gravid adult with everted cirrus after 9,0 days in duckling, ventral view, flattened under coverslip. (c: cirrus; cp: cirrus pouch; e: egg; gp: genital pore; gr: genital rudiment; t: anterior testis; v: vitellaria; vs: ventral sucker.)

Vertebrate hosts: *Anas platyrhynchos* L. (experimental host); *Poliocephalus poliocephalus* (Jardine and Selby); and possibly *Cygnus atratus* (Latham).

Habitat: Small intestine

Geographic location: Calvert's Lagoon

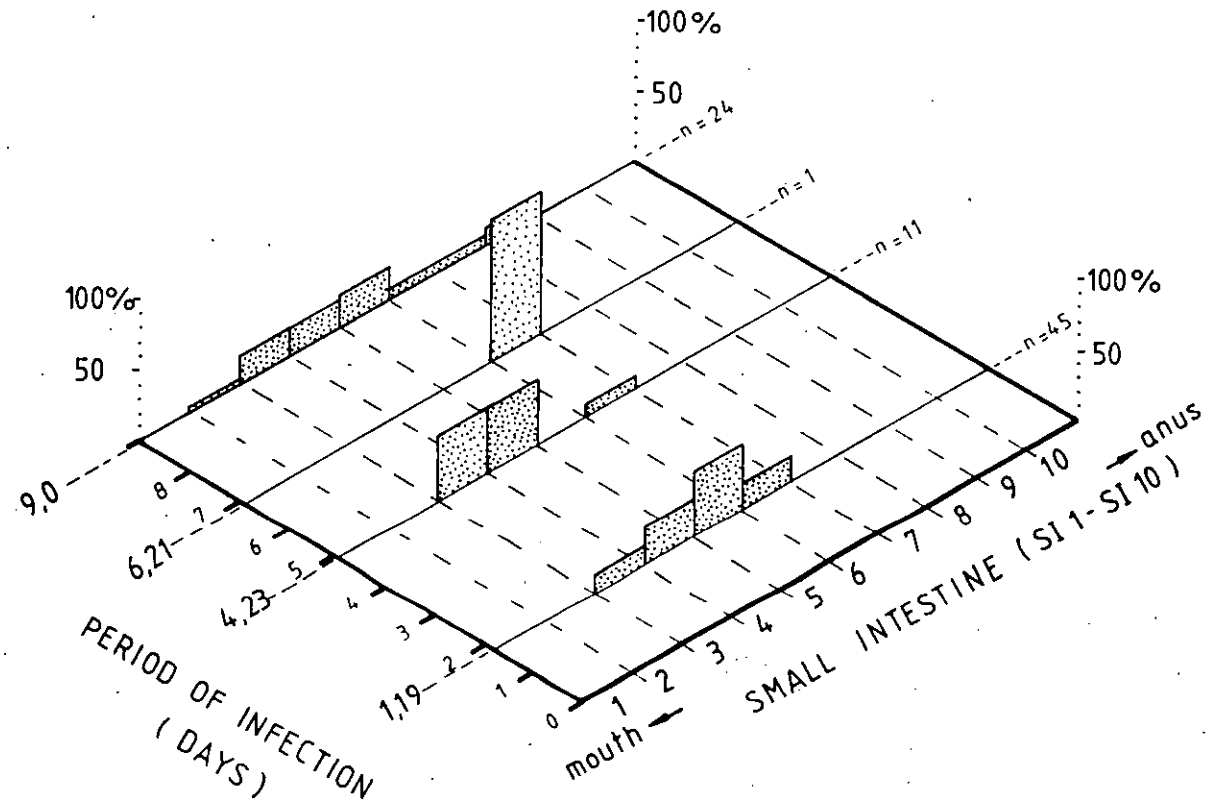
Material: Tasmanian Museum - K920, gravid adult (flattened); K921, immature adults; K922, excysted metacercariae.

Biology:

Metacercarial cysts taken from naturally infected snails were fed to laboratory ducklings which were sacrificed at different intervals from 1,0 to 11,0 days after infection. Fifty percent (5/10) of ducklings were infected with from 1 to 45 adults. The percentage of metacercariae that became established in the host's intestine varied from 5 to 56, and the greatest longevity recorded was 9,0 days.

The distribution of adults in the small intestines of laboratory ducklings did not vary greatly with time (Figure 3.9). The preferred habitat was the middle to upper region of the small intestine. Adults were concentrated in SI5 after 1,19 days, SI3 and SI4 after 4,23 days, SI6 after 6,21 days and SI5 after 9,0 days. Initially, excysted metacercariae were small and immature, and genital primordia were barely discernible in the small hind-body. The adult grew rapidly in the definitive host and as the reproductive system developed, the hind-body progressively became relatively larger. After 1,19 days the gonads were developed, but spermatogenesis had not commenced. After 4,23 days, spermatogenesis was advanced and mature sperm were present in the seminal vesicle. The vitellaria were well-developed, but not producing phenolic egg-shell precursors, except in a few advanced individuals. After 6,21 days, the vitellaria were producing phenolic egg-shell precursors, but egg production had not begun. After 9,0 days, 70% (17/24)

FIG. 3.9 Psilostomum sp. A. Distribution of adults in the gut of laboratory ducklings, at different intervals after infection, (n = no. of adults).



of flukes had produced eggs.

TABLE 3.9 Dimensions of adult flukes, believed to belong to *Psilostomum sp.A*, from natural infections of the hoary-headed grebe: (a) very immature specimens and (b) ovigerous specimens.

	(a)	(b)
Sample size	10	20
Body length	464 (302 - 620)	1786 (1450 - 2204)
Body width	171 (125 - 228)	304 (270 - 346)
Body depth	122 (106 - 129)	267 (182 - 327)
Anterior to V.S.(fore-body)	181 (125 - 243)	418 (348 - 522)
V.S. to posterior(hind-body)	153 (106 - 247)	1079 (754 - 1450)
Oral sucker length	87 (72 - 103)	186 (160 - 209)
Oral sucker width	78 (53 - 91)	143 (133 - 148)
Oral sucker depth	71 (65 - 84)	152 (114 - 190)
Pharynx length	71 (57 - 80)	129 (106 - 152)
Pharynx width	62 (49 - 76)	129 0
Pharynx depth	-	126 (114 - 148)
Ventral sucker length	141 (106 - 190)	240 (213 - 277)
Ventral sucker width	179 (122 - 209)	258 (236 - 304)
Ventral sucker depth	144 (106 - 190)	277 (228 - 319)
Ovary length	-	85 (61 - 110)
Ovary width	-	89 (84 - 95)
Ovary depth	-	86 (68 - 95)
Anterior testis length	27 0	168 (118 - 201)
Anterior testis width	27 0	151 (133 - 163)
Anterior testis depth	-	147 (133 - 167)
Posterior testis length	27 0	193 (144 - 239)
Posterior testis width	23 0	136 (110 - 160)
Posterior testis depth	-	150 (129 - 182)
Body length: body width ratio	2.71	5.88
O.S.(1+w):V.S.(1+w) ratio	0.52	0.66
Fore-body: hind-body ratio	1.18	0.39
Number of eggs	0	2.9 3.4(1-15)

Gravid and immature flukes of the genus *Psilostomum* inhabit the small intestine of the hoary-headed grebe and black swan at Calvert's Lagoon. At present it is not possible to distinguish, with certainty, all of the adults of *Psilostomum sp.A* and *Psilostomum sp.B*, because of the great variation in size of gravid, adult psilostomes, however some very immature specimens, from the hoary-headed grebe, were definitely of *Psilostomum sp.A* (Table 3.9). The vast majority of the adult flukes in the grebes were fairly uniform in size and the dimensions of ovigerous specimens are shown in Table 3.9. It is believed that these specimens were of *Psilostomum sp.A*, but that a few much larger specimens present in

the grebes and swans, were probably of *Psilostomum sp.B.* The dimensions of the latter are shown in Table 3.15.

3.3.3 Egg (Figure 3.8)

The operculate egg is relatively large, varying in shape from broadly oval to sub-reniform. The egg-shell is uniformly thick, except where it thins around the rim of the operculum and at the abopercular end, where it is slightly thickened. The dimensions are shown in Table 3.10.

TABLE 3.10 *Psilostomum sp.A.* Dimensions of eggs: (a) in 9 day old adults (fixed under coverslip pressure), from experimentally infected ducklings; and (b) in adults (fixed without flattening, by standard procedure), from a naturally infected hoary-headed grebe.

	No. of eggs	Length	Width
(a)	7	93 (80 - 103)	53 (46 - 61)
(b)	20	106 (95 - 118)	56 (49 - 70)

3.3.4 Redia (Figure 3.10)

Motile rediae, which produce cercariae, are distributed throughout the viscera of *Coxiella badgerensis*, but are concentrated in the gonad and between the lobes of the digestive gland. There is a gradation in size from small colourless specimens, containing only germ balls and very immature cercarial embryos, to large orange-pigmented specimens containing more mature cercariae. One infected snail contained 25 large, coloured rediae and 35 small, transparent rediae. The rediae are very similar in morphology and anatomy to those of *Psilochasmus oxyurus* (Figure 3.6). Their dimensions are shown in Table 3.11.

FIG. 3.10 Psilostomum sp.A

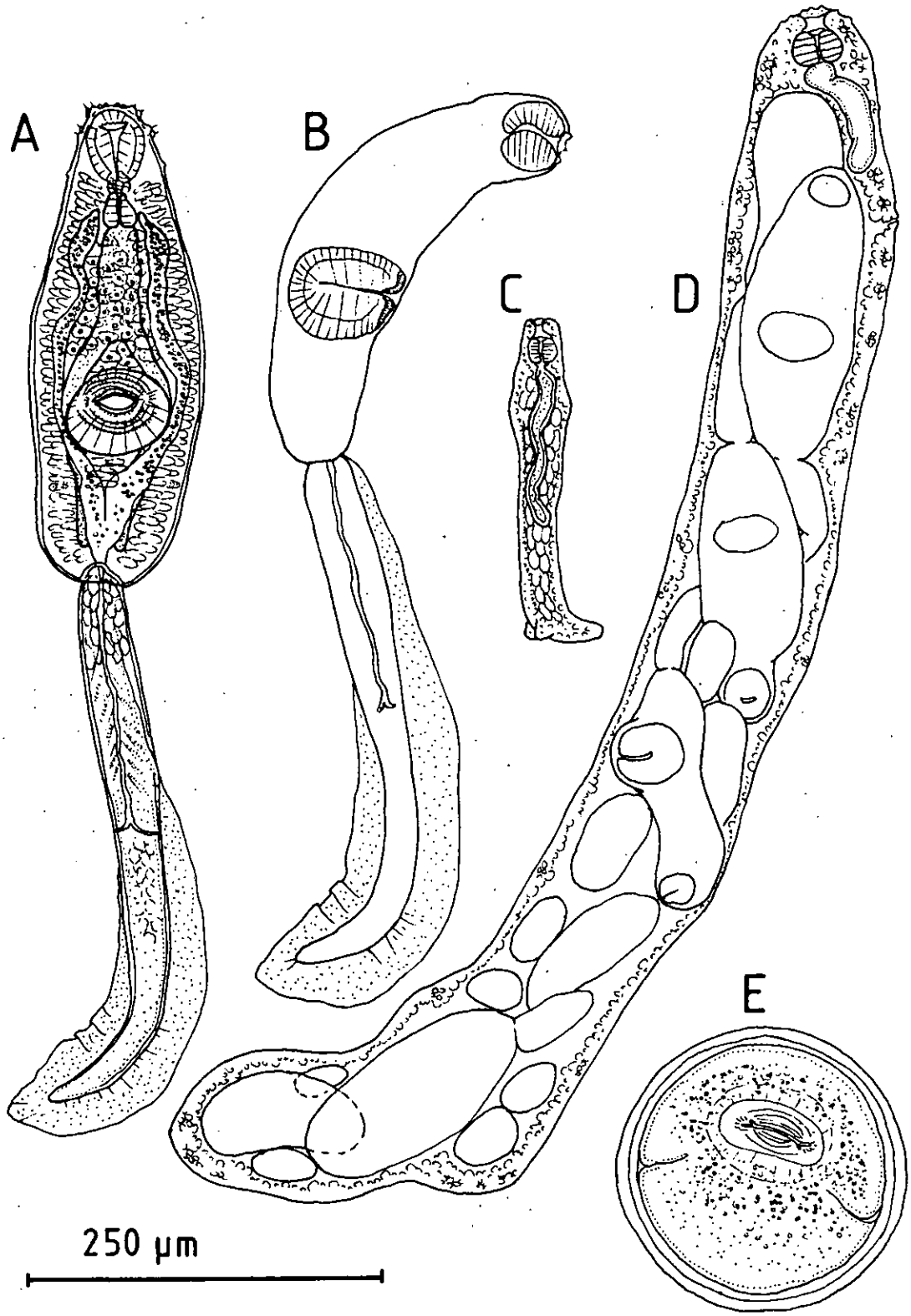


FIGURE 3.10 A, whole mature cercaria, ventral view; B, whole mature cercaria, lateral view; C, colourless immature daughter redia; D, mature pigmented daughter redia; E, metacercarial cyst after 14 days in experimental snail host.

TABLE 3.11 *Psilostomum sp.A.* Dimensions of colourless, immature rediae (a), and pigmented, mature rediae (b).

	(a)	(b)
Sample size	20	15
Body length	298 (266 - 323)	1648 (1436 - 1890)
Body width	53 (46 - 61)	162 (141 - 186)
Pharynx length	24 (23 - 27)	27 (27 - 30)
Pharynx width	28 (27 - 30)	35 (34 - 36)

3.3.5 Cercaria (Figure 3.10)

Morphology and anatomy:

The distomate cercaria is gymnocephalous and has well-developed dorso-ventral tail finfolds. Although much smaller than that of *Psilochasmus oxyurus*, it is very similar in morphology and anatomy. The granule-packed primary excretory vessels of this cercaria, however, only extend anteriorly as far as the pharynx, and do not have a short internal branch in the anterior region. The caudal excretory canal in this cercaria is relatively longer and extends about ½ the length of the tail. Dimensions of the mature, free-swimming cercaria are presented in Table 3.12.

Ecology and behaviour:

The cercaria emerges from the redia, completes its growth and development in the snail's viscera and then swims away from its primary intermediate host. Over a 4 day period in March, an average of 27 (23-31) cercariae emerged daily from one snail. Emergence was greatest in the afternoon or evening.

TABLE 3.12 *Psilostomum sp.A.* Dimensions of cercaria after emerging from the snail host (n = 20).

Body length	392 (333 - 423)
Body width	160 (141 - 182)
Body depth	120 (106 - 144)
Tail length	490 (423 - 529)
Tail width	49 (42 - 57)
Dorsal finfold length	322 (272 - 378)
Ventral finfold length	500 (438 - 529)
D.F.L.:V.F. L. ratio	0.64
Max. finfold width	86 (67 - 97)
Oral sucker length	67 (57 - 72)
Oral sucker width	56 (53 - 59)
Oral sucker depth	57 (51 - 67)
Ventral sucker length	68 (61 - 72)
Ventral sucker width	87 (80 - 95)
Pharynx length	38 0
Pharynx width	32 (27 - 38)

Within one minute of being placed in a crystal dish with a laboratory-bred snail, the free-swimming cercaria attached itself to the foot or head of the snail and crept quickly towards the mantle cavity. The powerful suckers were used in locomotion: the cercaria adhered to the snail by the ventral sucker, briefly swayed the anterior to and fro, attached the oral sucker and then drew the body fowards, like a caterpillar. The cercaria disappeared into the mantle cavity and within a few minutes the detached tail reappeared and swam away. Encystment occurred within one hour of the cercaria entering the mantle cavity. The cyst wall was initially very thin, but thickened rapidly, and 13 hours after invasion, the metacercarial cyst was fully formed (Table 3.13). In experimental infections cysts were always formed between the snail's shell and mantle; however, in naturally infected snails the cysts were usually embedded in the pericardium and only in very heavy infections did they occur between the shell and mantle.

Snails acting as the primary intermediate host can be reinvaded and act as the secondary intermediate host. This has been observed experimentally, and is also indicated by the relatively high incidence

of cysts of *Psilostomum sp.A* in snails acting as the primary host. The average number of cysts of this species in snails acting as both primary and secondary intermediate hosts was 60 (47 - 72).

The average number of cysts of *Psilostomum sp.A* per infected snail, in samples collected at Site 1 from August 1977 to September 1978, was only 5.4 (1 - 35). Four cercariae were exposed to both the 'parent' snail and a control snail in the same crystal dish and all encysted in the 'non-parent' snail. This observation, although not statistically significant, indicates that the cercaria of *Psilostomum sp.A* may be able to recognize the 'parent' snail, and tend to invade 'non-parent' snails.

3.3.6 Metacercaria

Metacercarial cyst: (Figure 3.8)

The cyst wall is about 2μ thick when first formed, but within 13 hours it is fully grown, about 15μ thick. The mature cyst is round, and small compared to those of *Psilochasmus oxyurus* and *Psilostomum sp.B*, which occur in the same site in the same snail. In optical section, the cyst wall appears to be composed of 2 equal layers: outer layer transparent; inner layer translucent, yellowish. Dimensions of cysts from natural and experimental infections are shown in Table 3.13.

TABLE 3.13 *Psilostomum sp.A.* Dimensions of metacercarial cysts from experimentally infected snails (a), and from naturally infected snails (b).

	Infection period (days)	No. cysts	External dimensions Length × Width	Cyst thickness (average)
(a)	0, 1	3	194 (190 - 198) 185 (182 - 186)	2
	0, 3	3	200 (196 - 201) 195 (190 - 201)	7
	0, 13	3	212 (209 - 213) 201 (198 - 203)	15
	1, 1	3	208 (205 - 209) 201 -	15
	2, 0	30	206 (198 - 217) 201 (190 - 209)	15
	7, 0	10	208 (201 - 220) 202 (198 - 205)	14
	14, 0	2	204 (203 - 205) 196 (190 - 201)	14
(b)	Wild snails	20	208 (194 - 220) 197 (179 - 217)	13

Excystment:

In vitro excystment occurred when cysts from naturally infected snails were incubated in 0.5% pancreatin in Hank's saline at 41°C: 25% of metacercariae had excysted after 2 hours, 49% after 3 hours and 70% after 11 hours. The process of excystment was stimulated by elevation of the temperature and exposure to digestive enzymes. The activated metacercaria escaped through a small hole, possibly with the aid of enzymes of parasite origin.

Excysted metacercariae: (Figure 3.8)

After encystment, metacercariae do not grow or develop significantly. Dimensions of excysted metacercariae are shown in Table 3.8. They are smaller than mature cercariae, probably due to evacuation of the extensive cystogenous glands during encystment. Genital primordia are indistinct in the hind-body, which is markedly smaller than the fore-body.

3.3.7 Discussion

Psilostumum sp.A appears to be a new species of this genus, most closely related to *P. brevicolle* (Creplin, 1829) (syn. *P. platyurum* (Mühling, 1896). The life-cycle of *P. brevicolle* was demonstrated by Loos-Frank (1968b). She found that the size of gravid adults varied greatly, as it does in *Psilochasmus oxyurus*, however, gravid adults of *P. brevicolle* recovered from *Larus argentatus* 6 days post infection, were much larger than flattened gravid adults of *Psilostomum sp.A* 9 days after infection of laboratory ducklings. The oral sucker to ventral sucker ratio of gravid adults of *P. brevicolle* from various hosts averaged 1.21 (Loos-Frank, 1968b), whereas that of the flattened adults of *Psilostomum sp.A* was only 0.60. The oral sucker to ventral sucker ratio of excysted metacercariae of *Psilostomum sp.A* was 0.71, and that of the small gravid adults, believed to be *Psilostomum sp.A*, infecting grebes at Calvert's Lagoon, was 0.66. There are also

differences in the life-histories of *Psilostomum sp.A* and *P. brevicolle*. The cercariae are very similar in size, however the primary excretory vessels of *P. brevicolle* extend to the oral sucker, whereas those of *Psilostomum sp.A* terminate at the level of the pharynx. The tail finfolds of the former species are very narrow, whereas those of the latter are expanded. The metacercarial cysts are similar in diameter, but that of *P. brevicolle* is only about 5μ thick, whereas that of *Psilostomum sp.A* is about 15μ thick. The cyst of *P. brevicolle* is formed in the digestive gland of marine mussels and occasionally in a marine snail, which does not serve as the primary intermediate host; whereas that of *Psilostomum sp.A* is formed in the pericardium, or between the mantle and shell, of the same species of brackishwater snail that serves as its primary intermediate host.

A specific determination for *Psilostomum sp.A* will be deferred until gravid adults, from experimentally infected ducklings, have been fixed by standard procedure without coverslip pressure.

3.4 Psilostomum sp.B

3.4.1 Life-cycle

The cyst of this species, which is significantly larger than those of *Psilochasmus oxyurus* and *Psilostomum sp.A*, is harboured by *Coxiella badgerensis* at Calvert's Lagoon. As this cyst was distinguished from that of *Psilochasmus oxyurus* only late in the study, its incidence in *C. badgerensis* is unknown. Nearly 200 psilostome cysts were dissected randomly from snails in December, 1979, and of these 13% were of *Psilostomum sp.B*, 29% were of *Psilochasmus oxyurus* and 58% were of *Psilostomum sp.A*. The life-cycle of *Psilostomum sp.B* is similar to those of *Psilochasmus oxyurus* and *Psilostomum sp.A*. The cercaria develops in a redia in *C. badgerensis* and, when fully developed, leaves the primary host and then reinvades the same snail, or invades a different snail, where it encysts in the pericardium, or between the shell and mantle. The adult is believed to infect

water birds feeding at Calvert's Lagoon.

3.4.2 Adult (Figure 3.11)

Immature adults of *Psilostomum sp.B* were discovered in the lower small intestine of an experimentally infected duckling, 1,7 days after it had been fed with large cysts collected from naturally infected snails from Calvert's Lagoon. At that time, the cyst of *Psilostomum sp.B* had not been distinguished from that of *Psilochasmus oxyurus*, and the duckling was also infected with immature adults of *P. oxyurus*. The adults of *Psilostomum sp.B* differed significantly, in size and morphology, from adults of *Psilostomum sp.A*, of a similar age (Tables 3.14 and 3.8). The bodies of the former specimens were much larger, and both oral and ventral suckers were very large, about twice the size of those of *Psilostomum sp.A*. Although the adults of both species were at a similar stage of reproductive development, viz. gonads were developed but spermatogenesis had not commenced, the hind-body of *Psilostomum sp.B* was larger than the fore-body, whereas the hind-body of *Psilostomum sp.A* was smaller than the fore-body.

The immature adults of *Psilostomum sp.B* were markedly larger than newly excysted metacercariae (Table 3.14). In the definitive host, the suckers and pharynx of *Psilostomum sp.B* had increased in size and gonads, which were barely discernible in excysted metacercariae, were larger and more distinct. The length of the hind-body, which contained the developing reproductive system, had increased greatly relative to the length of the fore-body.

Some unusually large *Psilostomum* specimens were found in the small intestine of hoary-headed grebes and a black swan at Calvert's Lagoon. They are believed to be adults of *Psilostomum sp.B* because of their great size and morphological similarity to immature specimens of *Psilostomum sp.B*. These flukes are described below and illustrated in Figure 3.11. Dimensions of some gravid specimens from a grebe are given in Table 3.15.

FIG. 3.11 Psilostomum sp.B

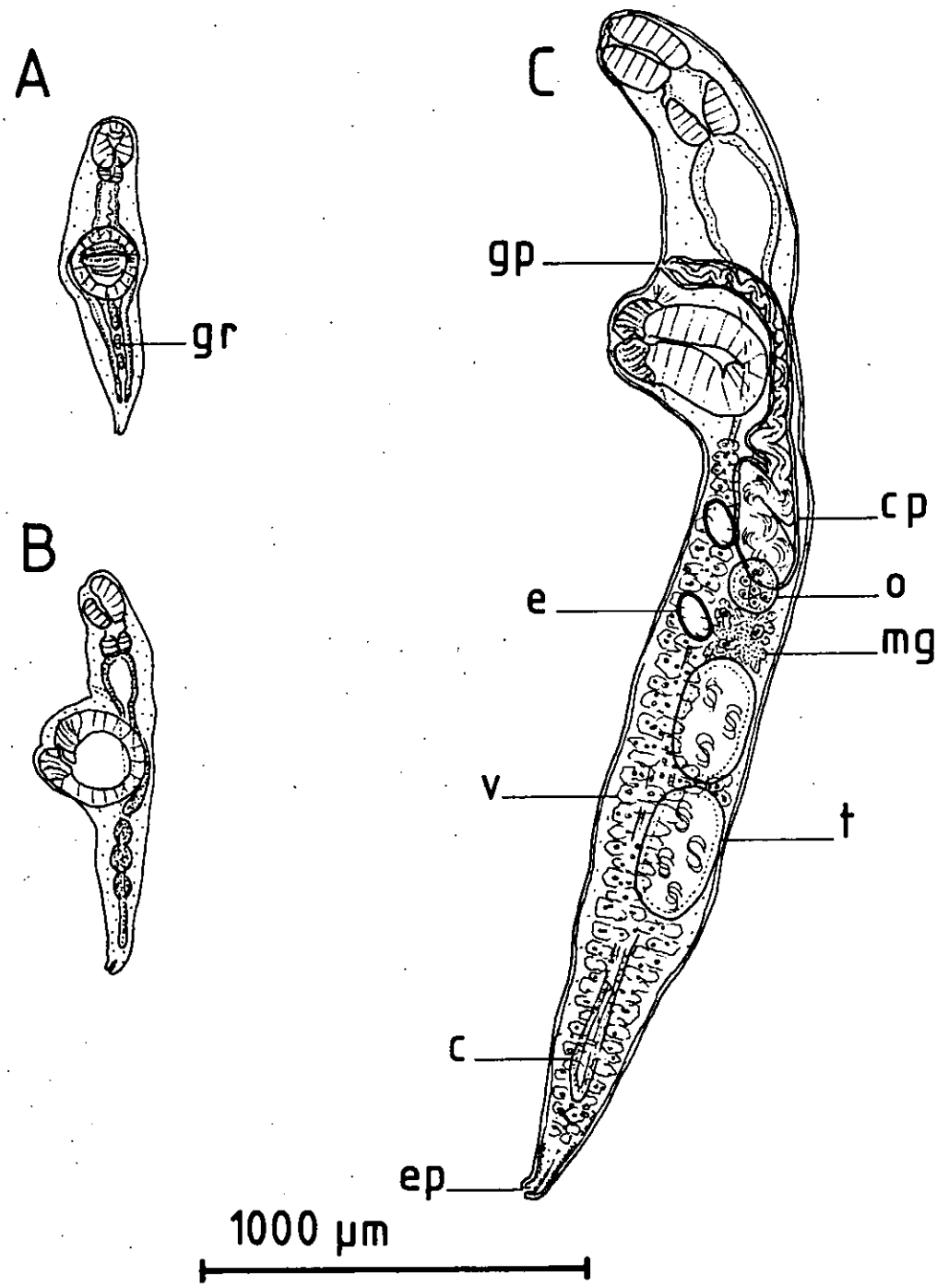


FIGURE 3.11 A, excysted metacercaria after 4 hours at 41°C; B, juvenile adult after 1,7 days in laboratory duckling; C, gravid adult from wild hoary-headed grebe. (c: caecum; cp: cirrus pouch; e: egg; ep: excretory pore; gp: genital pore; gr: genital rudiment; mg: Mehlis' gland; o: ovary; t: testis; v: vitellaria.)

TABLE 3.14 *Psilostomum sp.B.* Dimensions of metacercariae excysted *in vitro* after about 4 hours at 41°C (a); and dimensions of immature adults from an experimentally infected duckling 1,7 days after infection (b).

	(a)	(b)
Sample size	20	2
Body length	762 (718 - 832)	945
Body width	215 (201 - 228)	-
Body depth	229 (179 - 247)	155 (154 - 156)
Anterior to V.S. (fore-body)	322 (272 - 438)	355 (333 - 378)
V.S. to posterior (hind-body)	314 (272 - 348)	420 (408 - 431)
Oral sucker length	97 (87 - 110)	124 (118 - 129)
Oral sucker width	87 (76 - 103)	-
Oral sucker depth	83 (76 - 91)	91 (87 - 95)
Prepharynx length	18 (0 - 38)	0 0
Pharynx length	64 (53 - 80)	70 (65 - 76)
Pharynx width	52 (46 - 59)	-
Pharynx depth	73 (68 - 76)	84 0
Oesophagus length	133 (114 - 160)	131 (122 - 141)
Ventral sucker length	152 (99 - 224)	215 (213 - 217)
Ventral sucker width	168 (156 - 179)	-
Ventral sucker depth	192 (163 - 220)	283 (281 - 285)
Ovary length	-	48 (46 - 49)
Ovary depth	-	59 (57 - 61)
Anterior testis length	37 (34 - 38)	63 (61 - 65)
Anterior testis width	22 (23 - 27)	-
Anterior testis depth	33 (30 - 36)	59 (57 - 61)
Posterior testis length	37 (36 - 38)	59 (57 - 61)
Posterior testis width	20 (19 - 23)	-
Posterior testis depth	34 0	54 (53 - 55)
Body length:body width ratio	3.54	-
O.S.(l+w):V.S.(l+w) ratio	0.58	-
Fore-body:hind-body ratio	1.03	0.85

Description:

Body large, elongate, with thick, aspinous tegument. Fore-body more or less cylindrical, hind-body tapers from posterior testis to small, ventrally curved, non-muscular, terminal process. Protuberant ventral sucker much larger than oral sucker. Prepharynx short, pharynx well-developed, oesophagus bifurcates anterior to ventral sucker, caeca extending to near posterior extremity. Testes tandem, oval, equal in size; equidistant from ventral sucker, posterior end of body. Double-chambered seminal vesicle within

thin-walled, elongate cirrus pouch, extending to ovary. Genital pore at, or posterior to, oesophageal bifurcation. Ovary median, more or less round, 2/3rds of way from ventral sucker to anterior testis. Ootype between ovary and anterior testis, surrounded by extensive Mehlis' gland. Proximal part of uterus expanded, acting as 'receptaculum seminis uterinum'. Few eggs present in uterus. Vitellaria extend length of hind-body, from posterior end to ventral sucker. Large vitelline ducts unite medially, anterior to anterior testis, forming vitelline reservoir. Network of fine, granule-filled canals over fore-body, from oral sucker to ventral sucker; excretory pore terminal; flame-cell pattern not observed.

Vertebrate hosts: *Anas platyrhynchos* L. (experimental host); *Cygnus atratus* (Latham); and *Poliocephalus poliocephalus* (Jardine and Selby).

Habitat: Small intestine

Geographic location: Calvert's Lagoon

Material: Tasmanian Museum - K923, gravid adults; K924, adults (ringed); and K925, excysted metacercariae.

TABLE 3. 15 Dimensions of gravid flukes believed to belong to *Psilostomum sp.B:* (a) from the hoary-headed grebe, Calvert's Lagoon, (b) from the black swan, Lake Bookar, Queensland.

	(a)	(b)
Sample size	3	2
Body length	2859 (2726 - 3132)	5440 (4350 - 6525)
Body width	-	892 (741 - 1043)
Body depth	285 (251 - 331)	-
Anterior to V.S.(fore-body)	771 (754 - 812)	1293 (1160 - 1421)
V.S. to posterior (hind-body)	1873 (1740 - 2088)	3335 (2726 - 3944)
Oral sucker length	217 (201 - 236)	378 (363 - 393)
Oral sucker width	-	393
Oral sucker depth	171 (167 - 175)	272
Prepharynx length	39 (38 - 42)	-
Pharynx length	129 (118 - 137)	249 (242 - 257)
Pharynx width	-	227
Pharynx depth	165 (156 - 179)	212
Oesophagus length	296 (281 - 323)	-
Ventral sucker length	242 (227 - 257)	514 (423 - 605)

TABLE 3.15 (continued)

	(a) 3	(b) 2
Ventral sucker width	-	537 (469 - 605)
Ventral sucker depth	367 (363 - 378)	-
Ovary length	122 (114 - 129)	197
Ovary width	-	242
Ovary depth	124 (122 - 125)	-
Anterior testis length	298 (255 - 342)	627 (499 - 756)
Anterior testis width	-	378 (302 - 454)
Anterior testis depth	169 (156 - 182)	-
Posterior testis length	359 (353 - 365)	771 (650 - 892)
Posterior testis width	-	355 (318 - 393)
Posterior testis depth	162 (152 - 171)	-
Body length:body width ratio	-	6.10
O.S. (l+w): V.S. (l+w) ratio	-	0.73
Fore-body:hind-body ratio	0.41	0.39
No. of uterine eggs	1.5 (1-2)	28 (12-44)
Dimensions of eggs: (n = 20)		
length	101 (99 - 106)	103 (95 - 114)
width	56 (49 - 65)	65 (53 - 76)

3.4.3 Egg (Figure 3.11C)

The gravid adults described above contained eggs that were similar in size and morphology to the egg of *Psilostomum sp.A*. Dimensions of these eggs are shown in Table 3.15.

3.4.4 Redia (Figure 3.12)

Intramolluscan developmental stages of *Psilostomum sp.B*, other than metacercarial cysts, are rare, only 2 infected snails having been found in the course of the present study. The rediae vary in size and maturity, from small, transparent specimens containing only germ balls, to large, translucent specimens containing germ balls, and cercariae at different stages of development. The dimensions of these 2 classes of rediae are shown in Table 3.16. All of the rediae can creep actively. They are elongate, with procruscula protruding posterolaterally. The terminal mouth is surrounded by a muscular lip, or oral sucker. A well-developed pharynx underlies the mouth and leads to a thin-walled, colourless intestine, which becomes relatively shorter as the redia grows and develops. A slight 'collar' is situated just posterior to the pharynx. One infected snail contained 20 rediae, of varying sizes, but no free cercariae. Mature cercariae had been released intermittently from this particular snail over a period of 71 days.

FIG. 3.12 Psilostomum sp.B

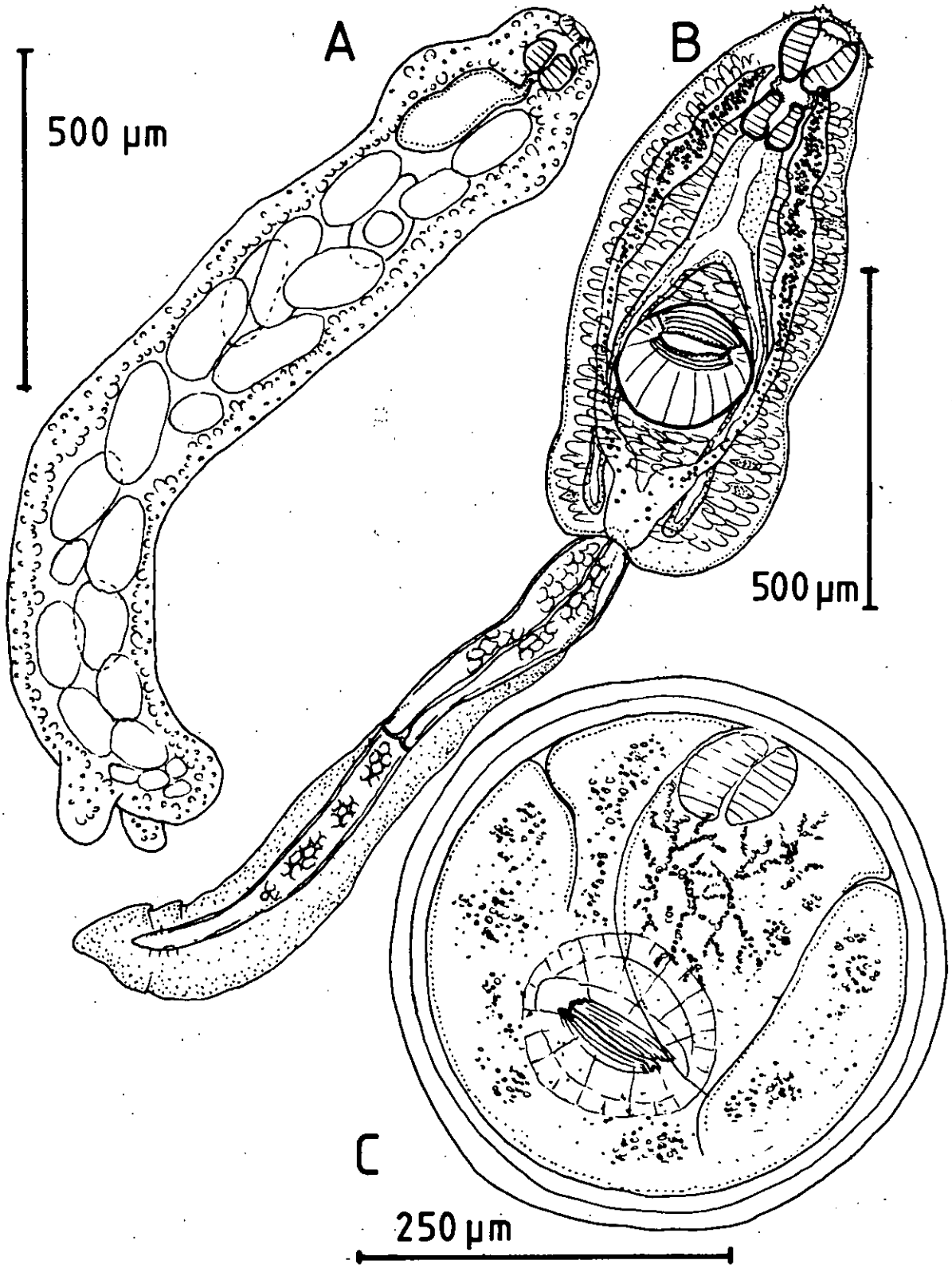


FIGURE 3.12 A, daughter redia; B, whole mature cercaria, ventral view; C, metacercarial cyst after 11 hours in experimental snail host. Note - for comparison, the metacercarial cysts of *Psilochasmus oxyurus*, *Psilostomum sp.A* and *Psilostomum sp.B* are drawn to the same scale in Figures 3.7, 3.10 and 3.12 respectively.

TABLE 3.16 *Psilostomum sp.B.* Dimensions of live transparent, immature rediae (a), and live, translucent, mature specimens (b).

	(a)	(b)
Sample size	8	11
Body length	454 (364 - 560)	1120 (840 - 1400)
Body width	84 (70 - 98)	130 (98 - 168)
Pharynx length	38 0	57 0
Pharynx width	38 0	57 0

3.4.5 Cercaria (Figure 3.12)

Morphology and anatomy:

The cercaria is distomate, gymnocephalous and has dorso-ventral finfolds. Dimensions are only available from 4 live specimens; however, these cercariae were much larger than fixed cercariae of *Psilochasmus oxyurus* and *Psilostomum sp.A* (Table 3.17). Although all three psilostome cercariae are similar in morphology and anatomy, the cercaria of *Psilostomum sp.B* is closer to that of *Psilostomum sp.A*. The primary excretory vessels of both *Psilostomum* species, unlike *Psilochasmus oxyurus*, do not have a short anterior branch. In both *Psilostomum* species the caudal excretory canal extends nearly ½ the length of the tail, whereas in *Psilochasmus oxyurus* it extends only ¼ the length of the tail.

TABLE 3.17 *Psilostomum sp.B.* Dimensions of live cercariae, after emerging from the snail host (n = 4).

Body length	901 (801 - 983)
Body width	404 (348 - 454)
Tail length	945 (907 - 983)
Tail width	95 0
Dorsal finfold length	529 0
Ventral finfold length	907 0
D.F.L.:V.F.L. ratio	0.58
Oral sucker length	132 (84 - 179)
Oral sucker width	160 (118 - 198)
Ventral sucker length	272 (190 - 350)
Ventral sucker width	246 (198 - 285)
Pharynx length	85 (65 - 95)
Pharynx width	89 (76 - 106)

Behaviour and ecology:

When exposed to its second intermediate host, *Coxiella badgerensis*, the cercaria of *Psilostomum sp.B* behaves like the other psilostome cercariae described. Within one minute of being introduced into the same crystal dish, it attached itself to a laboratory-bred snail, rapidly crept into the mantle cavity and soon shed its tail. Encystment occurred within one hour.

Cysts formed between the shell and mantle of laboratory hosts, but in the pericardium of natural hosts. The cyst was fully formed within 11 hours of invasion of the snail (Table 3.18). Sixteen cysts of *Psilostomum sp.B* were found in a snail serving as the primary host, whereas the average number of cysts of *Psilostomum sp.B* and *Psilochasmus oxyurus*, per infected snail collected at Site 1 from August 1977 to September 1978, was only 1.7 (1 - 6). This indicates that the cercaria of *Psilostomum sp.B* can reinvade the snail serving as the primary host.

3.4.6 Metacercaria

Metacercarial cyst: (Figure 3.12)

Dimensions of cysts from experimental and natural hosts are shown in Table 3.18. After an hour, the cyst measured 372 × 376µ, much larger than the cyst of *Psilochasmus oxyurus*. The cyst wall rapidly increased in thickness, while the overall size of the cyst did not change. After 11 hours the large, round cyst had a resilient wall, about 25µ thick, composed of 2 translucent, equal layers.

TABLE 3.18 *Psilostomum sp.B*. Dimensions of metacercarial cysts from experimentally infected snails (a); and from naturally infected snails (b).

	Infection period (days)	No. cysts	External diameter Length × Width	Cyst thickness
(a)	0, 1	1	376 × 372	8
	0, 11	1	376 × 374	25
(b)	Wild snails	20	377 9(357 - 391) × 371 9(353 - 388)	26

Excystment:

In vitro excystment of metacercariae occurred during incubation in 0.5% pancreatin in Hank's saline, at 41°C: 12% had excysted after 2 hours, 40% had excysted after 4 hours and 60% had excysted after 11 hours. The process of excystment was stimulated by exposure to elevated temperature and digestive enzymes. Escape of the actively stretching metacercaria, through a small hole in the apparently uniform cyst wall, is possibly aided by enzymes of parasite origin.

Excysted metacercaria: (Figure 3.11)

Dimensions of excysted metacercariae are shown in Table 3.14. The metacercaria undergoes little development within the cyst, the excysted metacercaria being very similar to the cercaria. It is elongate, with a more or less cylindrical fore-body and a smaller, tapering hind-body. The tegument is thick and aspinous and sensory papillae are prominent around the mouth. The ventral sucker is very large and protuberant. Gonads are discernible in some specimens.

3.4.7 Discussion

Psilostomum sp.B is most likely a new species; however, designation of a specific name has been deferred until ovigerous adults have been obtained under controlled conditions. Very large flukes of the genus *Psilostomum*, infecting the black swan and hoary-headed grebe at Calvert's Lagoon, are believed to be adults of this species. They are similar to *P. brevicolle*; however, they have a relatively larger, more protuberant ventral sucker than *P. brevicolle*, and, unlike *P. brevicolle*, they have a smaller oral than ventral sucker and a hind-body that tapers from the posterior testis to a small, ventrally curved, non-muscular terminal process (similar to but distinct from the retractable muscular 'horn' of *Psilochasmus*). In many ways, the life-histories of *Psilostomum sp.B* and *P. brevicolle* differ. The cercaria, metacercaria and metacercarial cyst

of the former are very much larger than the corresponding stages of *P. brevicolle*. The sites of encystment and the identities of the intermediate and definitive hosts also differ.

The dimensions of 2 large gravid *Psilostomum* adults, taken from a black swan at Lake Bookar, Queensland, are shown in Table 3.15. Although much larger than specimens infecting the hoary-headed grebe at Calvert's Lagoon, they are morphologically and anatomically very similar and may belong to *Psilostomum sp.B*.

3.5 General Discussion

Mathias (1924, 1925), who published the earliest account of a psilostome life-history, reported that the life-cycle of *Psilotrema spiculigerum* is absolutely parallel to that of *Fasciola hepatica*, and he believed "all members of the family Psilostomidae have the identical life-cycle". In fact the members of this family have varied life-cycle patterns from those with metacercariae that encyst free in the environment, to those with metacercariae that encyst in the tissues of a second intermediate host.

The Psilostomidae are placed in the superfamily Echinostomatoidae along with the Echinostomidae, and these 2 families have much in common. Pearson (1972), has presented a convincing argument to show that a 2 host life-cycle evolved in the Digenea prior to the development of a metacercarial cyst, and thus, before the acquisition of a second intermediate host. He suggested that the various life-cycle patterns in the Echinostomidae represent one way in which the second intermediate host was added to the digenean life-cycle. According to this scheme, encystment was initially free in the environment and then on the surface of animals, particularly snails. The next stage in the phylogeny was for the cercaria to enter a body opening, the kidney orifice of a snail, and encyst in the kidney. The cercaria could migrate from the kidney via the renopericardial duct to the pericardium and encyst there, or penetrate

out of the pericardium and encyst within other tissues. Pearson (1972), pointed to the similar development of life-cycles in the closely related Psilostomidae. Two evolutionary trends can be seen in the 10 psilostome life-cycles that have now been elucidated. Firstly there is the gradual acquisition of a second intermediate host, as outlined in the Echinostomidae. Parallel to this is the development of finfolds on the tail of the cercaria. *Psilotrema spiculigerum* and *P. oligoon* have simple tailed cercariae which encyst on vegetation or on stones (Mathias, 1925; Pike, 1969). *Sphaeridiotrema macrocotyla* has a simple-tailed cercaria which encysts on the surface of a crayfish (Macy and Bell, 1968). The cercaria of *S. globulus* has crenulate lateral margins and it encysts on the inner surface of the shell of its snail host (Szidat, 1937; Macy and Ford, 1964). The cercaria of *S. spinoacetabulum* has clear margins said to "suggest short lateral finfolds", and it encysts between the shell and mantle of its primary host, or another snail species (Burns, 1961b). The cercaria of *Psilochasmus oxyurus* has well-developed dorsoventral finfolds and encysts between the mantle and shell, in the pericardium, or in the "visceral sac" of the snail species serving as primary host, or another snail species (Szidat, 1957; Wisniewski, 1958b). *P. aglyptorchis* has a similar cercaria which encysts between the mantle and shell of the primary host (Loos-Frank, 1968a). The cercariae of *Psilostomum spp.A* and *B* have well-developed finfolds, and encyst between the shell and the mantle, or in the pericardium, of *Coxiella badgerensis*. *P. brevicolle* has a cercaria with dorsoventral finfolds, which encysts in the digestive gland of a species of snail, different from that serving as the primary host, and in the digestive gland of mussels (Loos-Frank, 1968b). The development of cercarial finfolds thus seems to be an adaptation that is related to the acquisition of a second intermediate host in this family. It would be of selective advantage for psilostome cercariae to be able to swim strongly and directly towards a potential second intermediate host, and the evolution of dorsoventral finfolds may

have satisfied these demands.

During the present study, the incidence of primary infections of *Coxiella badgerensis* with the 3 psilostome species was extremely low, less than 0.1%; however, the incidence of psilostome metacercarial cysts in *C. badgerensis* was consistently high (Figure 7.9). The incidence of cysts was directly related to the age of the snail host (Figure 7.10), indicating that snails gradually accumulate psilostome cysts. In one sample, collected in November 1977, 72% of adult snails (aperture length greater than 2 mm), harboured psilostome cysts. Limited laboratory observations of snails harbouring primary infections of *Psilochasmus oxyurus* and *Psilostomum sp.A*, indicated that few of the large psilostome cercariae were released daily. An average of 6 cercariae of *Psilochasmus oxyurus* and 27 cercariae of *Psilostomum sp.A*, emerged daily from the infected snails during the period of observation. The high level of secondary infections with psilostome cysts in snails at Calvert's Lagoon indicates that the psilostome cercariae continue to develop in, and emerge from their primary hosts over a long period, and, perhaps, that a high percentage of psilostome cercariae successfully encyst in second intermediate hosts. If the latter is true, it may be related to the possession of well-developed dorsoventral tail finfolds by each of the psilostome cercariae developing in *C. badgerensis*.

Chapter 4 THE NOTOCOTYLIDAE LUHE, 1909

4.1 General Introduction

The developmental stages of 4 species of notocotylid trematodes (subfamily Notocotylinae Kossack, 1911), were found infecting *Coxiella badgerensis* at Calvert's Lagoon. The cercariae of these species leave the molluscan host and encyst free in the aquatic environment. Pre-adult stages of notocotylid species are distinguished principally by the form of the 'excretory ring' of mature cercariae, and by the form and size of metacercarial cysts. An excretory ring, filled with excretory concretions or granules, is formed in these cercariae by coalescence, anteriorly and posteriorly, of the primary excretory ducts. Rothschild (1938), proposed that notocotylid cercariae be classified into 3 groups, according to the form of this excretory ring: Imbricata, Yenchingensis and Monostomi. The cercariae of 2 species at Calvert's Lagoon belong to the Imbricata Group, one belongs to the Yenchingensis Group and one belongs to the Monostomi Group.

In the laboratory, free swimming notocotylid cercariae encysted on the sides of glass 'crystal dishes', and on snail shells, within minutes of emerging from their snail hosts. All attempts to induce *in vitro* excystment of the metacercariae were unsuccessful, including the technique used by Dixon (1964), to excyst metacercariae of *Fasciola hepatica*, another species that encysts free in the aquatic habitat of the primary host. Cysts of the 4 notocotylid species at Calvert's Lagoon were fed to laboratory ducklings, but the adults of only 2 of the species became established in the alimentary tracts of these birds. They are both new species of the genus *Paramonostomum*. The species are named after their habitats in the definitive hosts: *Paramonostomum caecai* n.sp. inhabits the intestinal caeca, and *P. bursae* n.sp. inhabits the bursa fabricius. There have been few reports of notocotylids in birds of the Australasian region: *Catatropis gallinulae* was recorded in

the dusky moorhen in South Australia (Johnston, 1928); an unidentified notocotylid was recorded in the mallard in Tasmania (Angel, pers. comm. in Munday and Green, 1972); and *Uniserialis gippyensis, Catatropis* sp. and *Notocotylus* sp., were recorded in various anatids in New Zealand (Rind, 1974). In 1975, specimens of an unidentified notocotylid were found in the black swan at Lake Bookar, Queensland (Palmer, pers. comm., 1979).

THE IMBRICATA GROUP

Each cercaria in this group has an excretory ring with an anterior loop between the lateral eyespots, which passes anterior to the median eyespot and cerebral ganglion.

Genus PARAMONOSTOMUM Luhe, 1909

4.2 Paramonostomum caecai n.sp.

4.2.1 Life-cycle (Figure 4.1)

The primary intermediate host is *C. badgerensis*. Cercariae leave the snail and encyst on its shell and operculum and on other submerged surfaces, such as the valves of ostracods, cuticle of amphipods and possibly plants. The hoary-headed grebe, black swan and black duck serve as definitive hosts.

4.2.2 Adult (Figure 4.2)

Adults were recovered from laboratory ducklings that had been fed with round cysts, formed in crystal dishes by one of the 2 types of Imbricata cercariae developing in *C. badgerensis*. Unfortunately, the gravid specimens were all fixed under coverslip pressure. The dimensions of gravid and immature adults from these experimental hosts are shown in Table 4.1. Adult notocotylids inhabiting the caeca of the hoary-headed grebe, black swan and black duck at Calvert's Lagoon, are believed to

FIG. 4.1 Paramonostomum caecai n.sp.

Life-cycle

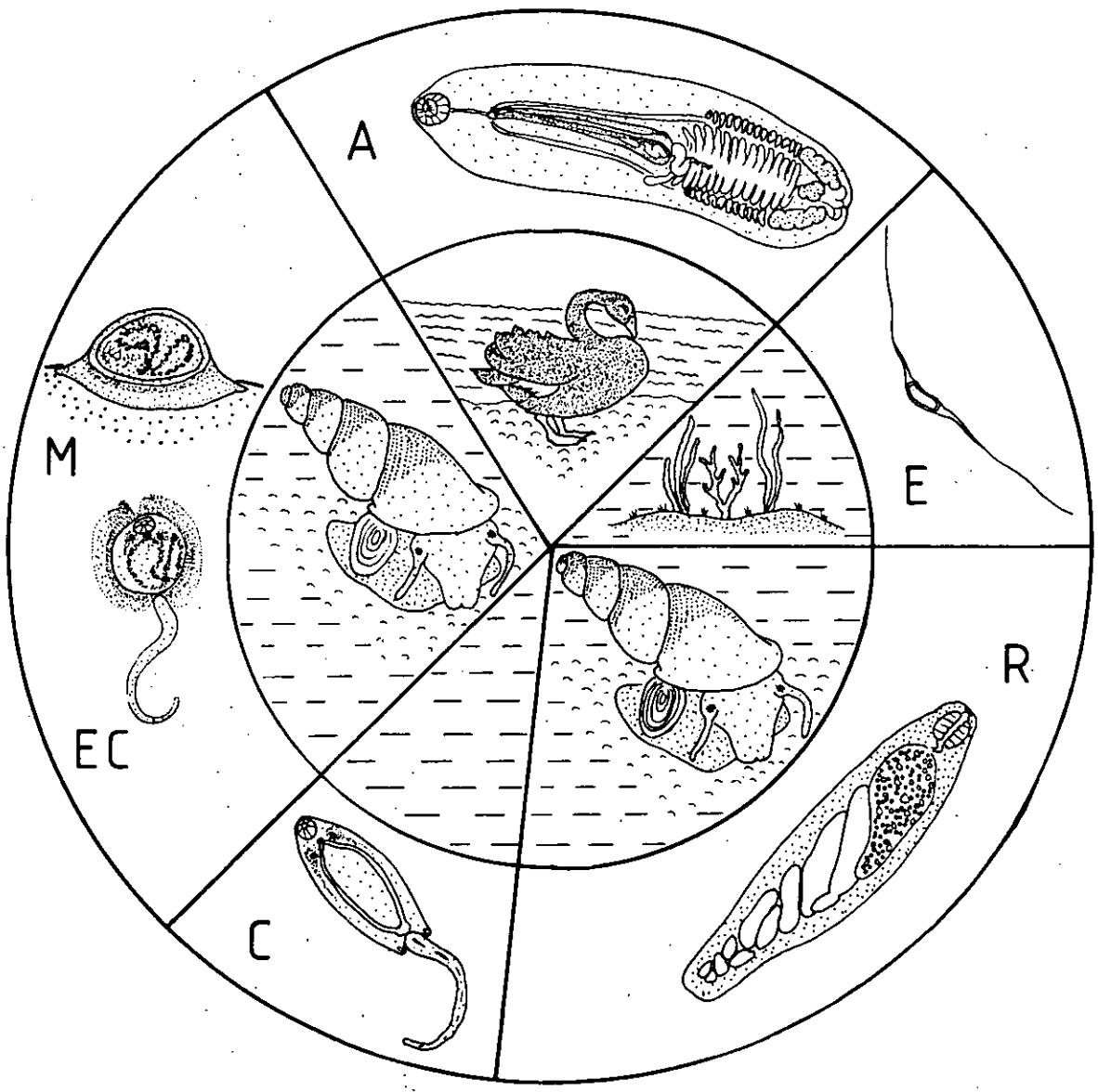


FIGURE 4.1 A, gravid adult; E, egg; R, daughter redia; C, mature cercaria; EC, encysting cercaria; M, metacercarial cyst.

belong to *P. caecai* n.sp. Dimensions of the holotype, and other gravid and immature adults from the hoary-headed grebe, fixed unflattened by standard methods, are shown in Table 4.2. Studies of live and fixed adults from experimental and natural hosts, using standard and phase contrast light microscopes, revealed no evidence of ventral glands or glandular ridges. The absence of such glands and glandular ridges is characteristic of the genus *Paramonostomum*.

Description:

Dorsoventrally flattened, elongate-spatulate body, margins slightly curled ventrally. Tegumental spines on ventral surface, diminish in size posteriorly; dorsal surface aspinous. Brown-black pigment dispersed in anterior 1/3 of body. No ventral glands or glandular ridges. Oval oral sucker terminal, mouth terminal or subterminal ventral. Pharynx absent; oesophagus short; simple caeca extend posteriorly between vitellaria and uterine loops, then ovary and testes, terminating medial to posterior lobes of testes. Excretory ducts ramify throughout body, connected to closed excretory ring. Large, lobate excretory bladder opens through dorsal excretory pore. Posterolateral elongate testes deeply lobed. Sperm ducts not seen. Convoluted, expanded vas deferens, serves as external seminal vesicle, enters base of cirrus pouch. Internal seminal vesicle bulbous; pars prostatica well-developed, leading to ejaculatory duct within long, slender, coiled cirrus. Everted cirrus, about 171 × 19μ, covered in small tubercles. Elongate, clavate cirrus pouch extends to near middle of body. Median genital pore overlying or anterior to oesophageal bifurcation. Lobate ovary, intercaecal, at level of anterior part of testes. Short oviduct leads anteriorly to ootype, surrounded by Mehlis' gland. Expanded proximal uterus serves as 'seminis receptaculum uterinum'. Uterus initially passes posteriorly, then forms 12 - 14 transverse loops between transverse vitelline

FIG. 4.2 Paramonostomum caecai n.sp.

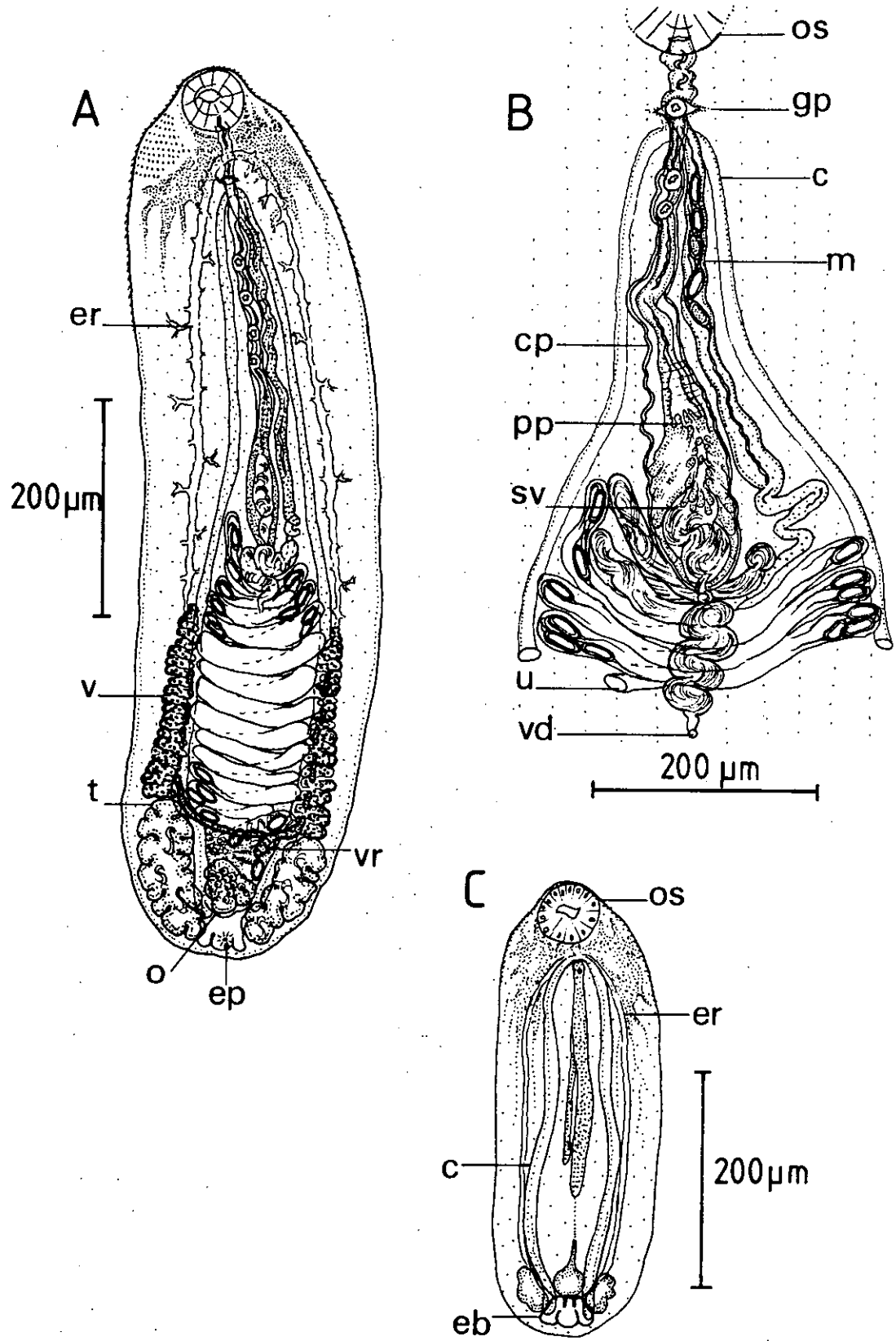


FIGURE 4.2 A, gravid adult from hoary-headed grebe, ventral view; B, detail of cirrus pouch region of gravid adult after 13 days in laboratory duckling, ventral view, flattened under coverslip; C, immature adult after 2,6 days in laboratory duckling, dorsal view. (c: caecum; cp: cirrus pouch; eb: excretory bladder; ep: excretory pore; er: excretory ring; gp: genital pore; m: metraterm; o: ovary; os: oral sucker; pp: pars prostatica; sv: seminal vesicle; t: testis; u: uterus; v: vitellaria; vr: vitelline receptacle; vd: vas deferens.)

ducts and cirrus pouch. Well-developed metraterm opens with cirrus pouch at ventral genital pore. Metraterm length to cirrus pouch length ratio = 0.9. Extracaecal vitelline glands longitudinal, extending from testes to 2/5 body length from posterior end of body i.e. about level of 10th uterine loop; never reaching middle of body. Transverse vitelline ducts unite between caeca, forming sinistral vitelline reservoir, which leads posteriorly to ootype, anteromedial to ovary. Hundreds of large eggs occupy uterus of gravid adult.

Vertebrate hosts: *Anas platyrhynchos* L. (experimental), *A. superciliosa* Gmelin; *Cygnus atratus* (Latham); and *Poliocephalus poliocephalus* (Jardine and Selby).

Habitat: Mainly intestinal caeca, also lower small intestine and rectum

Geographic location: Calvert's Lagoon

Type material: Tasmanian Museum - K907, holotype (ringed), gravid adult; K907, paratypes (not ringed), gravid adults; K908, K909, K910, and K911, paratypes, adults.

TABLE 4.1 *Paramonostomum caecai* n.sp. Dimensions of adults from experimentally infected ducklings: (a) 2,6 days P.I., (non-ovigerous); and (b) 13,0 days P.I., (ovigerous).

	(a)	(b)*
Sample size	10	5
Body length	364 (310 - 416)	1194 (983 - 1467)
Body width	157 (144 - 166)	479 (423 - 575)
Oral sucker length	44 (42 - 49)	81 (76 - 87)
Oral sucker width	52 (46 - 53)	89 (80 - 94)
Oesophagus length	4 (4 - 8)	-
Cirrus pouch length	-	505 (460 - 551)
Cirrus pouch width	-	74 (72 - 76)
Ovary length	23 (19 - 27)	109 (95 - 129)
Ovary width	19 (15 - 23)	49 (46 - 57)
Left testis length	38 (34 - 42)	173 (163 - 182)
Left testis width	22 (19 - 23)	89 (87 - 91)
Right testis length	40 (38 - 42)	182 (-)
Right testis width	25 (1 - 38)	87 (84 - 91)
Body length:body width ratio	2.32	2.49
Anterior to cirrus pouch base: body length ratio	-	0.52 (0.50 - 0.54)
Posterior to vitellaria tip: body length ratio	-	0.35 (0.32 - 0.38)

(* fixed under coverslip pressure)

TABLE 4.2 *Paramonostomum caecai* n.sp. Dimensions of adults from a naturally infected hoary-headed grebe: (a) ovigerous specimens; and (b) non-ovigerous specimens. Dimensions of the holotype, a gravid specimen, are also shown, (c).

	(a)	(b)	(c)
Sample size	10	10	1
Body length	736 (597 - 907)	475 (333 - 590)	816
Body width	189 (151 - 219)	133 (83 - 197)	219
Oral sucker length	53 (46 - 61)	47 (38 - 59)	51
Oral sucker width	59 (49 - 67)	47 (34 - 55)	57
Oesophagus length	12 (0 - 46)	6 (4 - 8)	53
Cirrus pouch length	331 (293 - 395)	224	361
Cirrus pouch width	33 (30 - 38)	19	32
Metraterm length	287 (228 - 369)	-	312
Metraterm width	16 (11 - 19)	-	14
Ovary length	61 (53 - 87)	38 (27 - 49)	57
Ovary width	38 (27 - 42)	23 (19 - 27)	38
Left testis length	108 (84 - 125)	93 (84 - 103)	110
Left testis width	45 (42 - 49)	40 (38 - 42)	49
Right testis length	108 (87 - 122)	82 (68 - 95)	122
Right testis width	52 (42 - 65)	46 (42 - 49)	65
Body length:body width ratio	3.89	3.57	3.73
Anterior to cirrus pouch base: body length ratio	0.55 (0.52 - 0.58)	-	0.56
Posterior to vitellaria tip: body length ratio	0.37 (0.33 - 0.42)	-	0.39

Relationships:

Paramonostomum caecai n.sp. clearly belongs to the *elongatum* group of Harwood (1939) and is most similar to the group of species: *P. elongatum, P. bucephalae* and *P. malerischi,* that Stunkard (1967) said were characterized by "linear, spatulate bodies, very long cirrus sacs that extend to the middle of the body, and short vitelline zones". From the position of the genital pore, overlying or anterior to the oesophageal bifurcation, and the relatively large eggs, *P. caecai* n.sp. appears closest to *P. malerischi,* a fluke inhabiting the caeca of the Emperor goose, *Philacte canagica,* in Alaska (Dunagan, 1957). Apart from the major differences in size, (*P. malerischi* is more than 3 times longer than the longest specimen of *P. caecai* n.sp.), and different hosts, the tegument of *P. malerischi* is aspinous and its cirrus is "unarmed", whereas the tegument of *P. caecai* n.sp. bears spines in the anterior ventral region, and its cirrus is covered with distinct tubercles.

Biology:

Cysts varying in age from 11 to 42 days, were found to be infective to laboratory ducklings. Forty four percent (4/9), of exposed ducklings became infected with from 1 to 28 flukes. Up to 80% of metacercariae were recovered as adults, all of them living in the intestinal caeca. The adults were established in the caeca after 22 hours and can live there for at least 13 days.

This fluke naturally inhabits the caeca, and lower alimentary tract, of several bird species at Calvert's Lagoon. One out of 2 black ducks harboured 7 adults; 2 out of 2 black swans harboured 20 and 70 adults and 5 out of 5 hoary-headed grebes were heavily infected, each harbouring hundreds of adults.

The metacercaria undergoes considerable growth and development within the bird host. After 2,6 days the genital primordia could be distinguished, and by 5,3 days eggs were being formed and occupied the proximal 11 loops of the uterus. After 13,0 days, the adult had grown considerably and eggs occupied 12 to 14 uterine loops. Adults removed from an experimentally infected duckling after 13,0 days, were maintained *in vitro* at 41°C for 2 days in 40% foetal calf serum in Eagles' M.E.M. Large numbers of eggs were shed into the culture medium.

4.2.3 Egg (Figure 4.3)

The operculate egg is without filaments when formed and is oblong to reniform in shape. Long polar filaments develop as it passes through the uterus, one filament forming at each end of the egg. The egg-shell is transparent, colourless and uniform in thickness, except where it thins at the operculum rim. A miracidium was discernible within the egg after 3 days in lagoon water, but had not hatched after 6 days under normal light and temperature conditions. Eggs were fed to 10 uninfected snails, *Coxiella badgerensis;* however, none were infected when dissected 49 days later. Live eggs measure about 26 × 11μ, and polar filaments

FIG. 4.3 Paramonostomum caecai n.sp

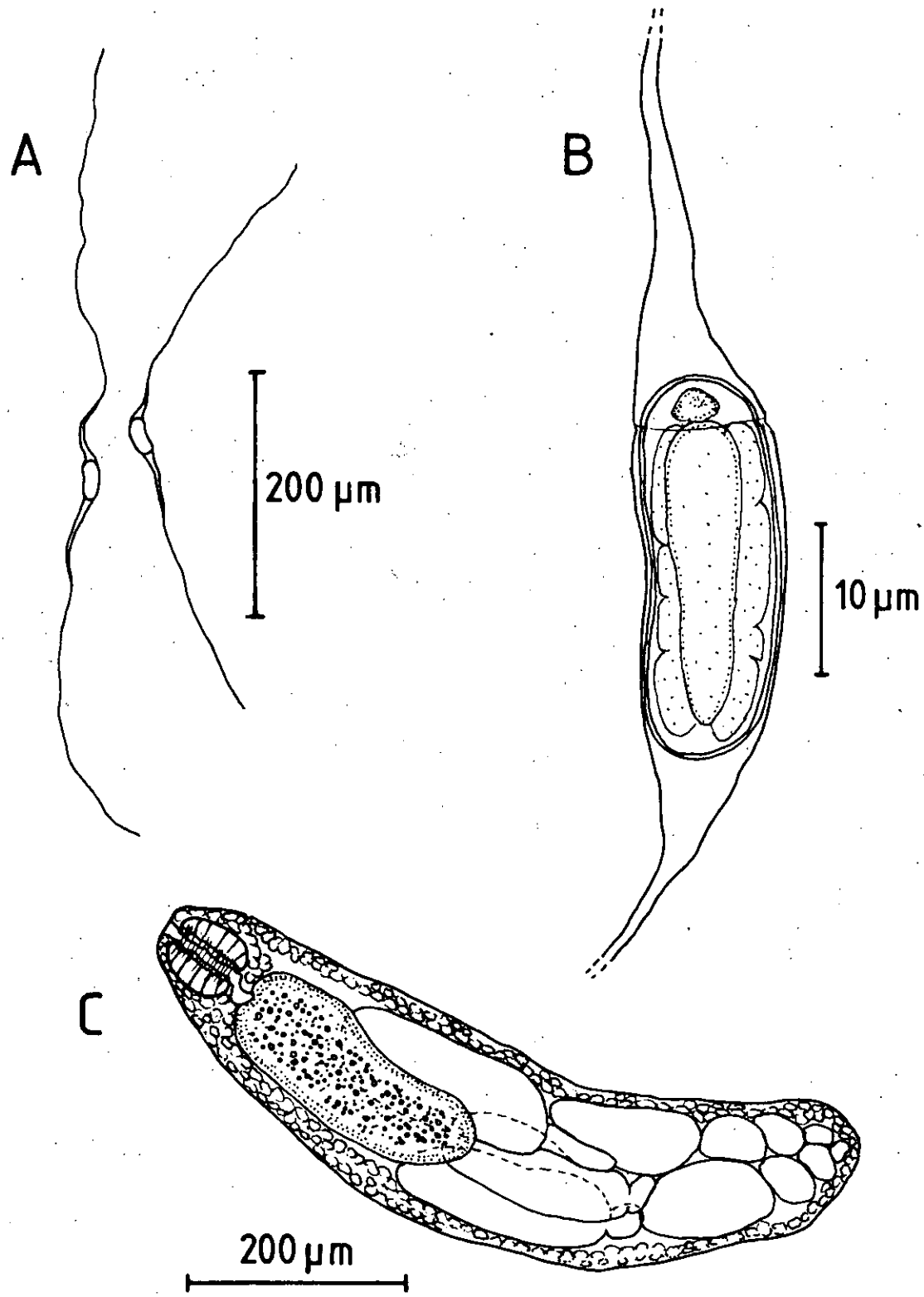


FIGURE 4.3 A, eggs deposited *in vitro* by fluke taken from experimentally infected duckling, 13 days post infection; B, detail of same egg after 3 days in lagoon water at room temperature; C, daughter redia.

extend up to 333μ. Dimensions of fixed eggs (not including the polar filaments), are shown in Table 4.3.

TABLE 4.3 *Paramonostomum caecai* n.sp. Dimensions of uterine eggs: (a) from a naturally infected hoary-headed grebe; and (b)* from an experimentally infected duckling, 13,0 days P.I.

Host	No. eggs	Length	Width
(a)	10	25 (23 - 29)	11 (8 - 14)
(b)	10	26 (24 - 27)	12 (11 - 12)

(* fixed under coverslip pressure)

4.2.4 Redia (Figure 4.3)

The rediae are more or less cylindrical to sausage-shaped, maximum width occurring near the middle and minimum width occurring at the posterior end. They range in size from very small individuals containing only germ balls, to the largest, containing germ balls and immature cercariae. The tegument is transparent and colourless, however the intestine appears brown-black, due to contained decomposing snail tissue. A terminal mouth opens into a large, barrel-shaped, muscular pharynx. A short sinuous oesophagus leads to the simple intestine, which is about ½ the body length in small rediae, but becomes relatively shorter as cercariae develop in the "brood chamber". Cercariae leave the redia through a birth pore situated at the mid-level of the pharynx and complete their growth and development free in the snail tissues.

The number of rediae found per infected snail, varied from 11 to 38. They occurred throughout the host's viscera, but were concentrated in the hepatopancreas. Dimensions of daughter rediae are shown in Table 4.4.

TABLE 4.4 *Paramonostomum caecai* n.sp. Dimensions of daughter rediae selected at random (n = 15).

Body length	597 (257 - 696)
Body width	160 (129 - 189)
Pharynx length	66 (61 - 76)
Pharynx width	62 (55 - 68)

4.2.5 Cercaria (Figure 4.4)

The body of this monostome cercaria is broadly oval to elongate, slightly concave ventrally. The tegument is speckled with numerous minute papillae, or spines. Under S.E.M. they are seen to be interconnected by an irregular network of fine mucoid threads. The tail is simple, usually longer than the body. Both the body and tail are very contractile. The tail is joined to the body by a slender peduncle, that is attached terminally or slightly postero-dorsally. The peduncle is flanked by 2 eversible, postero-lateral locomotory appendages, each with an adhesive sucker or gland at its tip. A narrow excretory duct extends about 3/4 of the length of the tail. A round to oval oral sucker protrudes anteriorly. The mouth is subterminal ventral, surrounded by a ring of about 32 ciliated papillae. The oesophagus bifurcates posterior to the lateral eyespots, forming simple caeca which extend to the level of the excretory bladder. The parenchyma is packed, from the mid-level of the oral sucker to the excretory bladder, with unicellular cystogenous glands, about 11μ diameter, each filled with bacilliform rods, about 2 - 3μ long. These glands make the body relatively opaque and obscure the anatomy. The excretory ring can only be seen when the body is flattened. It is of the Imbricata type, having an anterior loop that extends anteriad to the cerebral ganglion, overlapping, and sometimes extending anterior to the median eyespot. The ring is without diverticula and contains excretory granules, 5 - 8μ diameter, along its length. There are about 4 granules across the posterior part of the ring, where it is about 20μ wide, and from 1 to 3 granules across the anterior extremity of the ring, where it is only about 10μ wide. The lateral eyespots are oval to round, composed of densely packed black pigment granules. The median eyespot is annular, composed of more dispersed brown pigment granules. This pigment is also distributed in dendritic strands around, and extending posteriad from, the lateral eyespots.

FIG. 4.4 Paramonostomum caecai n.sp.

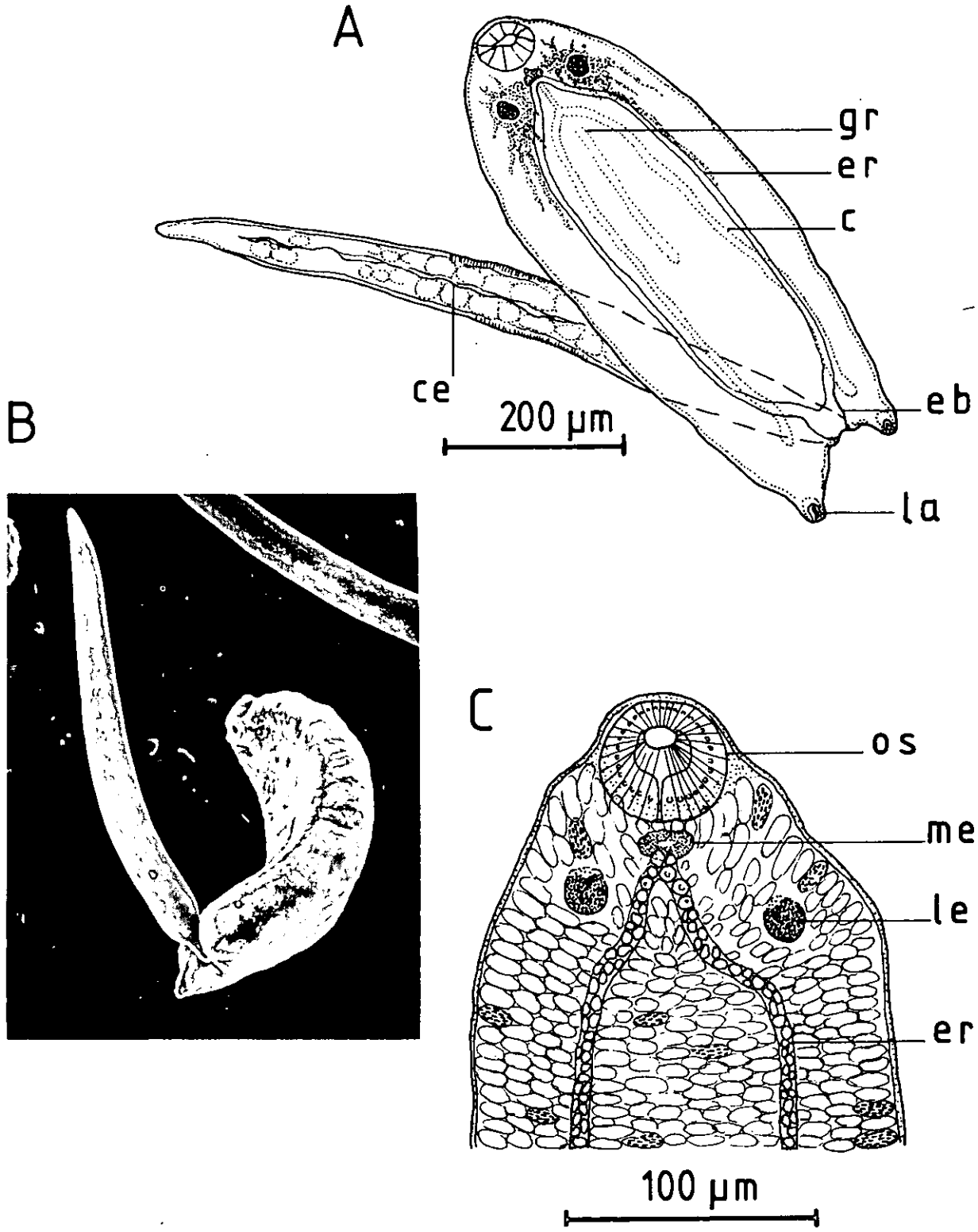


FIGURE 4.4 A, whole mature cercaria, dorsal view; B, S.E.M. photograph of cercaria, swimming position, ×200; C, detail of anterior region of mature cercaria, ventral view. (c: caecum; ce: caudal excretory duct; eb: excretory bladder; er: excretory ring; gr: genital rudiment; la: locomotory appendage; le: lateral eyespot; me: median eyespot; os: oral sucker.)

Under natural light and temperature conditions, mature cercariae emerged from the snail host between dawn and midday. Under controlled conditions, emergence occurred when the host snail was exposed to bright light, following a period of darkness. When snails were kept in constant darkness, no cercariae emerged. Free-swimming cercariae were positively phototactic, and in the laboratory swam near the water surface, encysting within an hour of emergence from the primary host. Although some cercariae encysted on the snail shell, most encysted on the side of the glass container. The cercaria swam an erratic course, the anterior leading, and the tail describing the infinity symbol, in the vertical plane.

Dimensions of the cercaria are shown in Table 4.5.

TABLE 4.5 *Paramonostomum caecai* n.sp. Dimensions of mature cercariae, after emergence from *Coxiella badgerensis* (n = 15).

Body length	534 (454 - 612)
Body width	165 (166 - 197)
Body depth	109 (91 - 144)
Tail length	655 (499 - 771)
Tail width	54 (45 - 68)
Oral sucker length	47 (42 - 53)
Oral sucker width	49 (46 - 51)
Oral sucker depth	48 (44 - 51)
Eyespot diameter	17 (15 - 19)

4.2.6 Metacercaria (Figure 4.5)

When the cercaria stops swimming, it creeps briefly, pauses, and then encystment commences. Cystogenous glands secrete bacilliform granules through the tegument, forming a flimsy outer cyst, which adheres to the substrate. The body contracts into a round shape and slow rhythmic contractions pass anteriorly along it. After a few minutes the body moves independently within the initial cyst wall. At this time the tail breaks free and swims away. The body rotates back and forth, as more cystogenous secretions are added internally to the cyst wall. The mature cyst is more or less hemispherical, with a thin flange around the base. Adherence to the substrate is by this peripheral flange, not by the ventral cyst wall. The enclosed metacercaria appears light

FIG. 4.5 Notocotylid metacercarial cysts

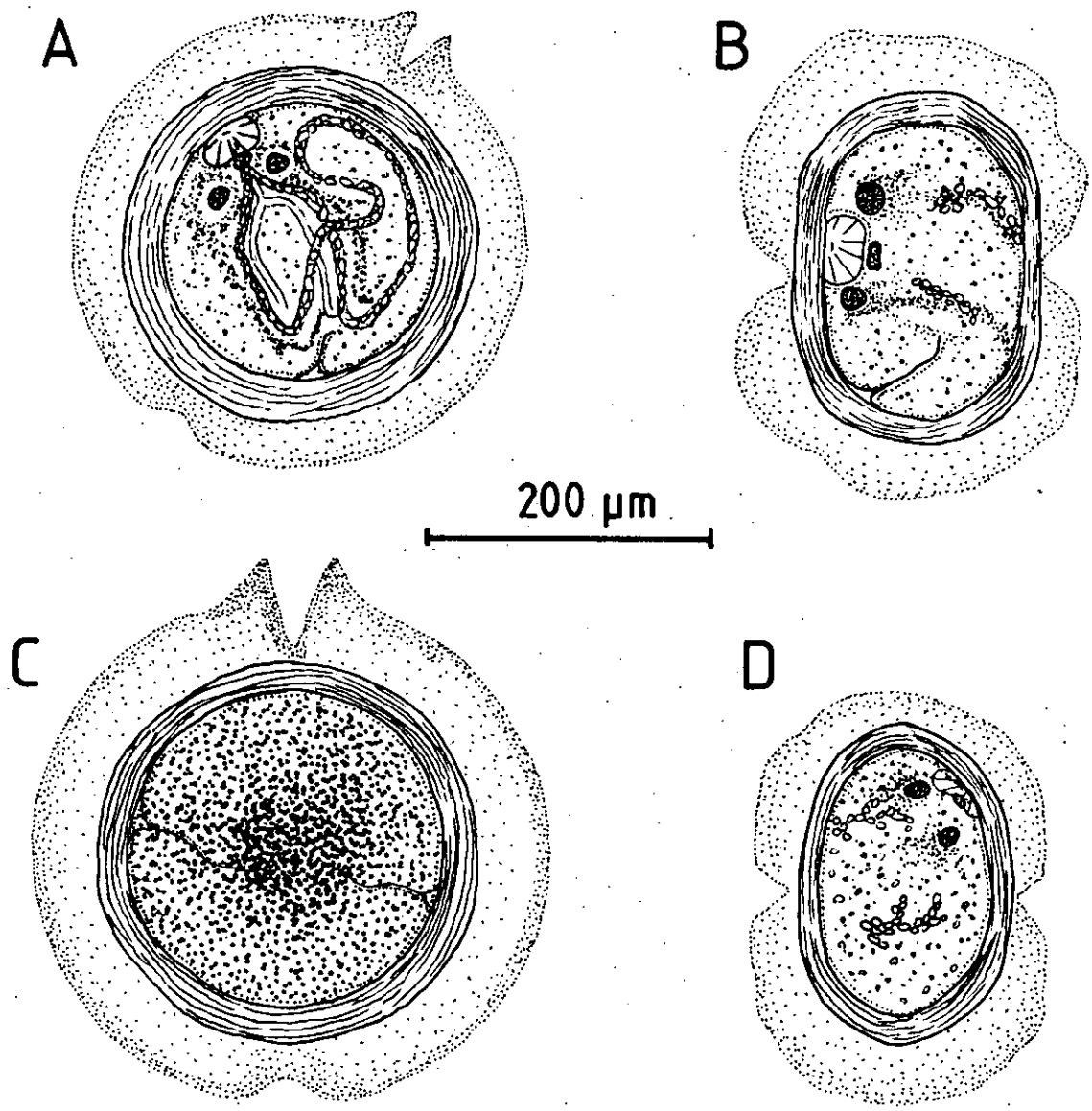


FIGURE 4.5 A, *Paramonostomum caecai* n.sp.; B, *Notocotylid sp.A;* C, *Paramonostomum bursae* n.sp.; D, *Notocotylid sp.B.*

brown, with darker stripes, due to the conspicuous granular excretory ring. The cysts formed by cercariae from the same snail are similar in size, however there is variation between cysts formed by cercariae from different snails. Dimensions of metacercarial cysts are shown in Table 4.6.

TABLE 4.6 *Paramonostomum caecai* n.sp. Dimensions of metacercarial cysts, formed by cercariae from 3 different snails: (a) n = 10, (b) n = 5, (c) n = 5.

		(a)	(b)	(c)
Interior	Length	187 (171 - 198)	191 (186 - 194)	198 (194 - 201)
	Width	-	192 (190 - 194)	195 (190 - 201)
Exterior	Length	-	253 (247 - 258)	252 (247 - 258)
	Width	-	255 (251 - 262)	248 (243 - 255)
Flange	Length	288 (255 - 315)	306 (300 - 312)	327 (319 - 346)
	Width	-	310 (304 - 323)	304 (-)

4.3 Notocotylid sp.A

The cercaria of this species closely resembles that of *Paramonostomum caecai* n.sp., however it forms a distinctive oblong cyst. One attempt was made to infect a laboratory duckling with these cysts, but was unsuccessful.

4.3.1 Redia (Figure 4.6)

The redia resembles that of *P. caecai* n.sp., in shape and size, however the pharynx of this species is significantly larger. Very small specimens, containing only germ balls, are colourless, however larger ones, containing germ balls and developing cercariae, have patches of yellow-orange pigment in the outer tegument, and brownish-grey, decomposing snail tissue within the intestine. Cercariae emerge, when immature, through a birth pore lateral to the pharynx.

When dissected, one infected snail contained 33 rediae, 65 immature cercariae, and one mature cercaria capable of free swimming. Dimensions of daughter rediae from one infected snail are shown in Table 4.7.

FIG. 4.6 Notocotylid sp.A

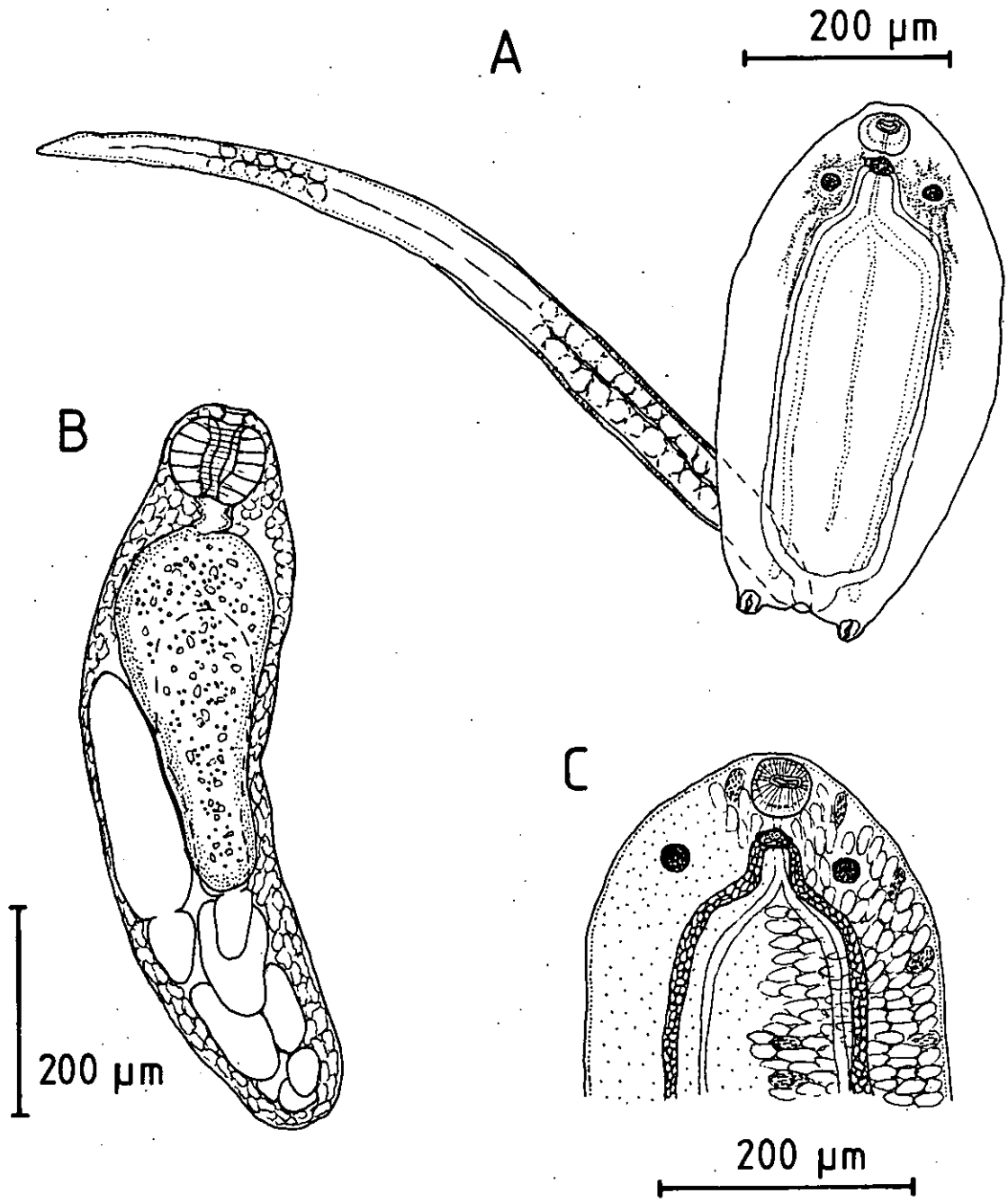


FIGURE 4.6 A, whole mature cercaria, dorsal view; B, daughter redia; C, detail of anterior region of mature cercaria.

TABLE 4.7 *Notocotylid sp.A.* Dimensions of daughter rediae selected at random (n = 15).

Body length	626 (575 - 680)
Body width	161 (148 - 167)
Pharynx length	71 (68 - 76)
Pharynx width	79 (76 - 82)

4.3.2 Cercaria (Figure 4.6)

The cercaria is very similar in morphology and anatomy to that of *Paramonostomum caecai* n.sp., however, the body and oral sucker of *Notocotylid sp.A* are slightly smaller and the tail slightly longer. The oesophagus bifurcates posterior to the lateral eyespots, and the caeca extend posteriorly, terminating near the excretory bladder. Genital primordia are barely discernible. The excretory ring, of the Imbricata type, has a distinctive inverted U-shaped anterior loop. It extends between the lateral eyespots and overlaps, or passes anterior to, the median eyespot. The ring is packed with irregularly shaped granules that increase in size anteriorly, from about 3 to 6μ long. There are about 7 granules across the posterior part of the ring, where it is 22μ wide, and about 3 granules across the anterior extremity, where it is 10μ wide.

Mature cercariae emerged from the host snail, under natural ight and temperature conditions, between dawn and midday, with a peak at about 9 a.m. The stimulus for emergence as with *P. caecai* n.sp., was exposure of the host to bright light following a long period of darkness. The positively phototactic cercariae swam away from the snail and encysted near the water level. In the laboratory, encystment occurred on the side of the glass container nearest the light source. No metacercarial cysts were formed on the shell of *C. badgerensis*.

Dimensions of the cercaria are shown in Table 4.8.

TABLE 4.8 *Notocotylid sp.A.* Dimensions of mature cercariae, after emergence from *Coxiella badgerensis* (n = 14).

Body length	458 (333 - 575)
Body width	184 (156 - 198)
Body depth	104 (87 - 129)
Tail length	715 (575 - 816)
Tail width	47 (42 - 49)
Oral sucker length	40 (38 - 46)
Oral sucker width	41 (38 - 42)
Oral sucker depth	40 (38 - 42)
Eyespot diameter	18 (15 - 19)

4.3.3 Metacercaria (Figure 4.5)

Before encysting, the cercaria stops swimming and creeps briefly on the substrate. The body contracts to a transversely oblong shape and cystogenous secretions ooze through the tegument, forming the initial thin, sticky cyst. The process of cyst formation is similar to that described for *Paramonostomum caecai* n.sp. When fully formed the light brown cyst is characteristically rectangular, and has a thin basal flange.

Dimensions of metacercarial cysts formed by cercariae from different snails, are shown in Table 4.9.

TABLE 4.9 *Notocotylid sp.A.* Dimensions of metacercarial cysts formed by cercariae from 2 different snails: (a) n = 5 and (b) n = 3.

		(a)	(b)
Interior	Length	198 (190 - 205)	215 (209 - 220)
	Width	154 (144 - 160)	149 (144 - 152)
Exterior	Length	241 (236 - 247)	269 (255 - 277)
	Width	196 (182 - 205)	189 (182 - 194)
Flange	Length	-	332 (315 - 342)
	Width	-	261 (255 - 266)

THE YENCHINGENSIS GROUP

Each cercaria in this group has an excretory ring with an anterior unpaired, finger-like diverticulum, which is filled with excretory granules. The diverticulum extends anteriorly as far as, or beyond, the median eyespot.

Genus PARAMONOSTOMUM LUHE, 1909

4.4 Paramonostomum bursae n.sp.

4.4.1 Life-cycle (Figure 4.7)

The life-cycle of *P. bursae* n.sp. is similar to that of *P. caecai* n.sp. The primary intermediate host is *C. badgerensis*. Cercariae encyst on the shell and operculum of this snail and on other submerged surfaces, like plant stems. The black duck serves as the definitive host.

4.4.2 Adult (Figures 4.8 and 4.9)

Adults were recovered from laboratory ducklings that had been fed with round cysts, formed in crystal dishes by the only Yenchingensis type of cercaria developing in *C. badgerensis*. A black duck killed at Calvert's Lagoon, was also found to harbour the adult of *P. bursae* n.sp. Dimensions of immature adults fixed by standard methods, from experimental hosts, are shown in Table 4.10. Unfortunately, gravid adults from both experimental hosts and the black duck, were fixed under coverslip pressure. One gravid adult from a laboratory duckling was designated as the holotype, and its dimensions are shown, with those of other gravid flukes, in Table 4.11. Studies of live and fixed adults, using standard and phase contrast light microscopes, revealed no evidence of ventral glands or glandular ridges. The absence of such glands and glandular ridges is characteristic of the genus *Paramonostomum*.

Description:

Body oval to pyriform, flattened, leaf-like; margins sometimes folded ventrally. Tegument aspinous. Black-brown pigment grains distributed extensively in anterior region. No ventral glands or glandular ridges. Round oral sucker terminal, mouth terminal or subterminal-ventral, pharynx absent, oesophagus short. Simple, convoluted caeca

FIG. 4.7 Paramonostomum bursae n.sp.

Life-cycle

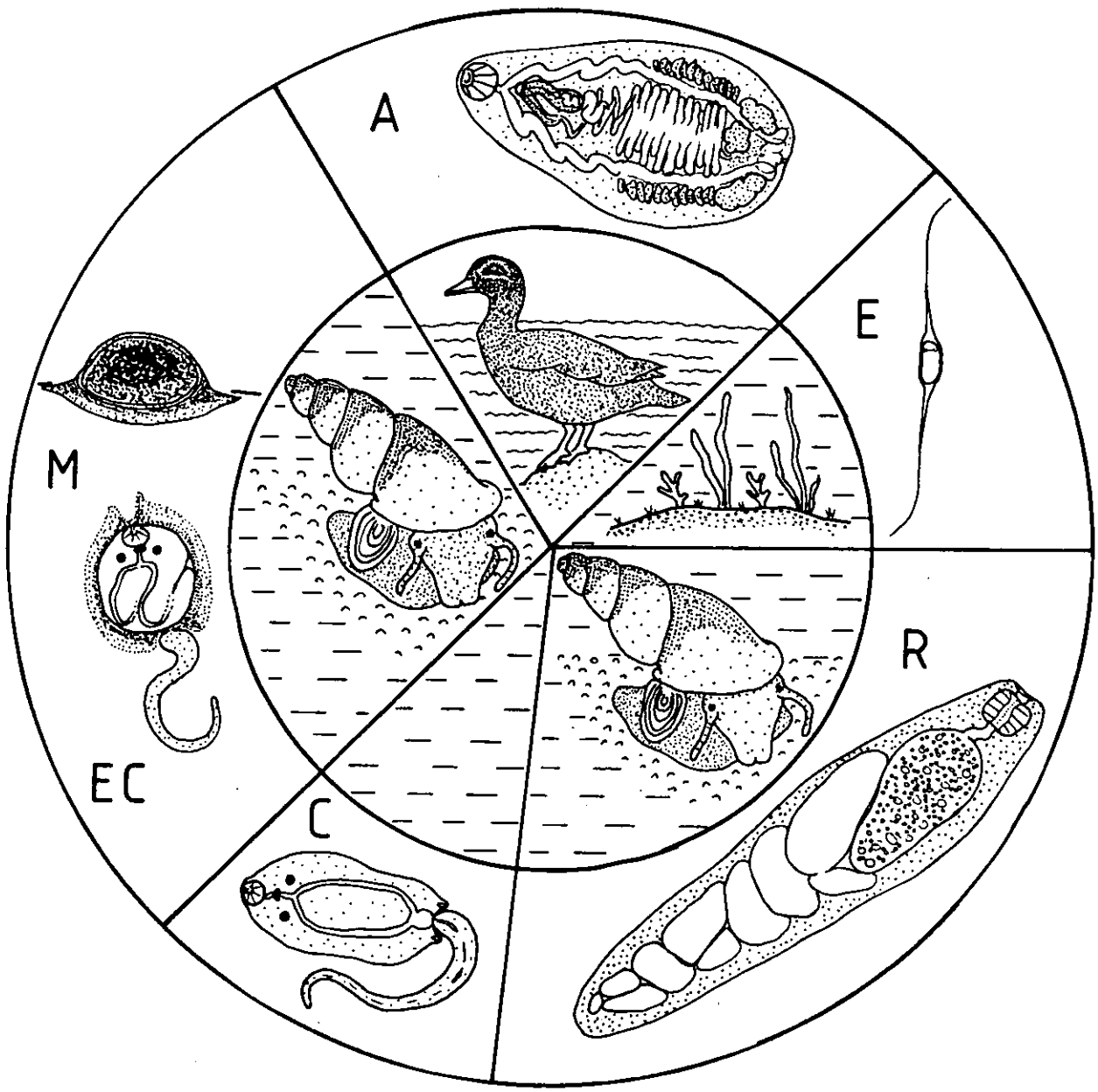


FIGURE 4.7 A, gravid adult; E, egg; R, daughter redia; C, cercaria; EC, encysting cercaria; M, metacercarial cyst.

extend posteriorly between vitellaria and uterus, ovary and testes, terminating posterior to testes. Excretory ducts anastomose throughout body, from excretory ring. Lobate excretory bladder opens through dorsal excretory pore. Testes lobate, posterolateral. Sperm ducts not seen. Convoluted, expanded vas deferens, serves as external seminal vesicle, entering proximal end of cirrus pouch. Internal seminal vesicle leads via well-developed pars prostatica to ejaculatory duct, within cirrus. Everted cirrus, elongate, about 300 × 35μ, covered in large tubercles. Clavate cirrus pouch extends posteriorly to between 1/3rd and 2/5ths body length from anterior end. Median genital pore immediately posterior to oesophageal bifurcation. Ovary lobate, sub-median dextral, intercaecal; mid-level anterior to mid-level of testes; usually extends anterior to testes, never posterior. Short oviduct passes posterolaterad to ootype, surrounded by median Mehlis' gland. Proximal uterus filled with sperm. Uterus convoluted, forming 12 to 15 transverse, intercaecal loops. Metraterm opens with cirrus pouch at ventral genital pore. Metraterm length to cirrus pouch length ratio = 0.6 - 0.8. Vitelline glands extracaecal, longitudinal; extend from testes to middle of body, about level of 11th uterine loop. Transverse vitelline ducts unite to form longitudinal vitelline reservoir, that leads posteriorly to ootype. Hundreds of relatively large eggs occupy uterus of mature adult.

Vertebrate hosts: *Anas platyrhynchos* L. (experimental), *A. superciliosa* Gmelin

Habitat: Bursa fabricius

Geographical location: Calvert's Lagoon

Type material: Tasmanian Museum - K912, holotype, gravid adult (flattened); K913, paratypes, gravid adults (flattened); K914 and K915, paratypes, immature adults.

FIG. 4.8 Paramonostomum bursae n.sp.

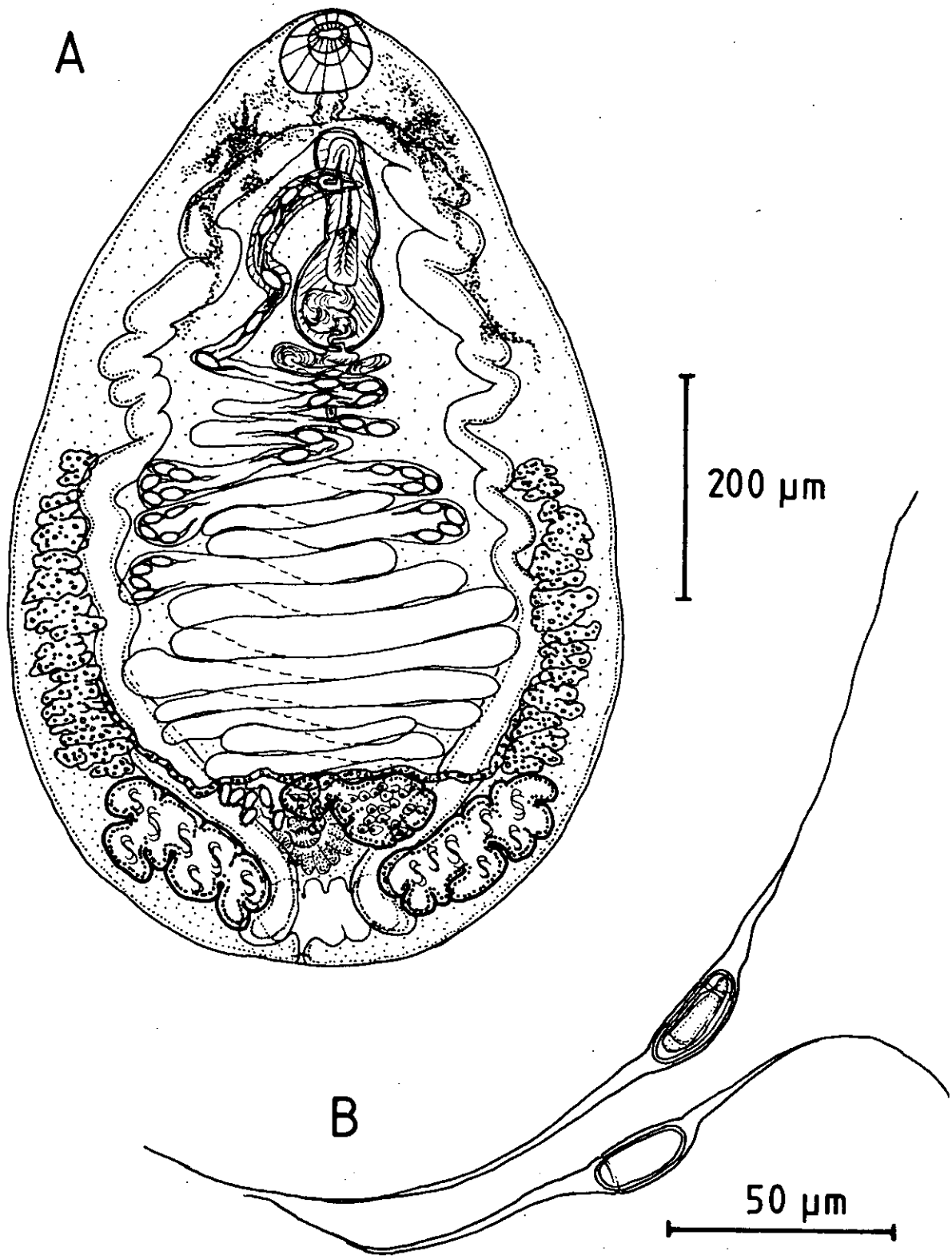


FIGURE 4.8 A, holotype, gravid adult from laboratory duckling 9 days post-infection, dorsal view, flattened under coverslip; B, eggs deposited *in vitro* by fluke from black duck.

TABLE 4.10 *Paramonostomum bursae* n.sp. Dimensions of adults from experimentally infected ducklings: (a) 2,12 days P.I. and (b) 5,3 days P.I. with newly formed eggs, lacking filaments, in the proximal uterus.

	(a)	(b)
Sample size	15	4
Body length	443 (378 - 537)	764 (756 - 771)
Body width	181 (136 - 212)	314 (295 - 333)
Oral sucker length	49 (46 - 53)	68 (67 - 68)
Oral sucker width	49 (46 - 51)	70 (68 - 72)
Oesophagus length	58 (57 - 61)	49 (38 - 61)
Cirrus pouch length	-	192 (171 - 213)
Cirrus pouch width	-	53 (49 - 57)
Metraterm length	-	148 (144 - 152)
Metraterm width	-	23 (-)
Ovary length	-	60 (53 - 67)
Ovary width	-	72 (-)
Left testis length	38 (-)	97 (87 - 103)
Left testis width	38 (34 - 42)	95 (87 - 103)
Right testis length	47 (38 - 53)	108 (106 - 110)
Right testis width	38 (34 - 46)	105 (103 - 106)
Body length:body width ratio	2.45	2.43

Relationships:

The broad, oval to pyriform body of *Paramonostomum bursae* n.sp. is characteristic of the *alveatum* group of the genus (Harwood, 1939), however unlike most species of this shape, the vitellaria do not extend anteriad as far as the cirrus pouch, and in fact rarely extend into the anterior half of the body. In morphology and anatomy *P. bursae* n.sp. appears most similar to *P. alveatum* and *P. parvum*. Like these species, it has a relatively short cirrus pouch, which extends only up to 2/5 body length from the anterior end. It differs from both of these species in the distribution of vitellaria, which extend into the anterior 1/3rd of the body in *P. alveatum* and *P. parvum*, according to Stunkard (1967). The position of the ovary also differs, being dextral and extending anterior to the testes in *P. bursae* n.sp.; and median, at the level of the posterior part of the testes in *P. alveatum* and *P. parvum*. The number of uterine loops of *P. bursae* n.sp. is 12 - 15, and that of *P. alveatum* and *P. parvum* is 10 - 12. *P. alveatum* and *P. parvum* inhabit the lumen of the intestine of ducks in North America (Stunkard and Dunihue, 1931;

FIG. 4.9 Paramonostomum bursae n.sp.

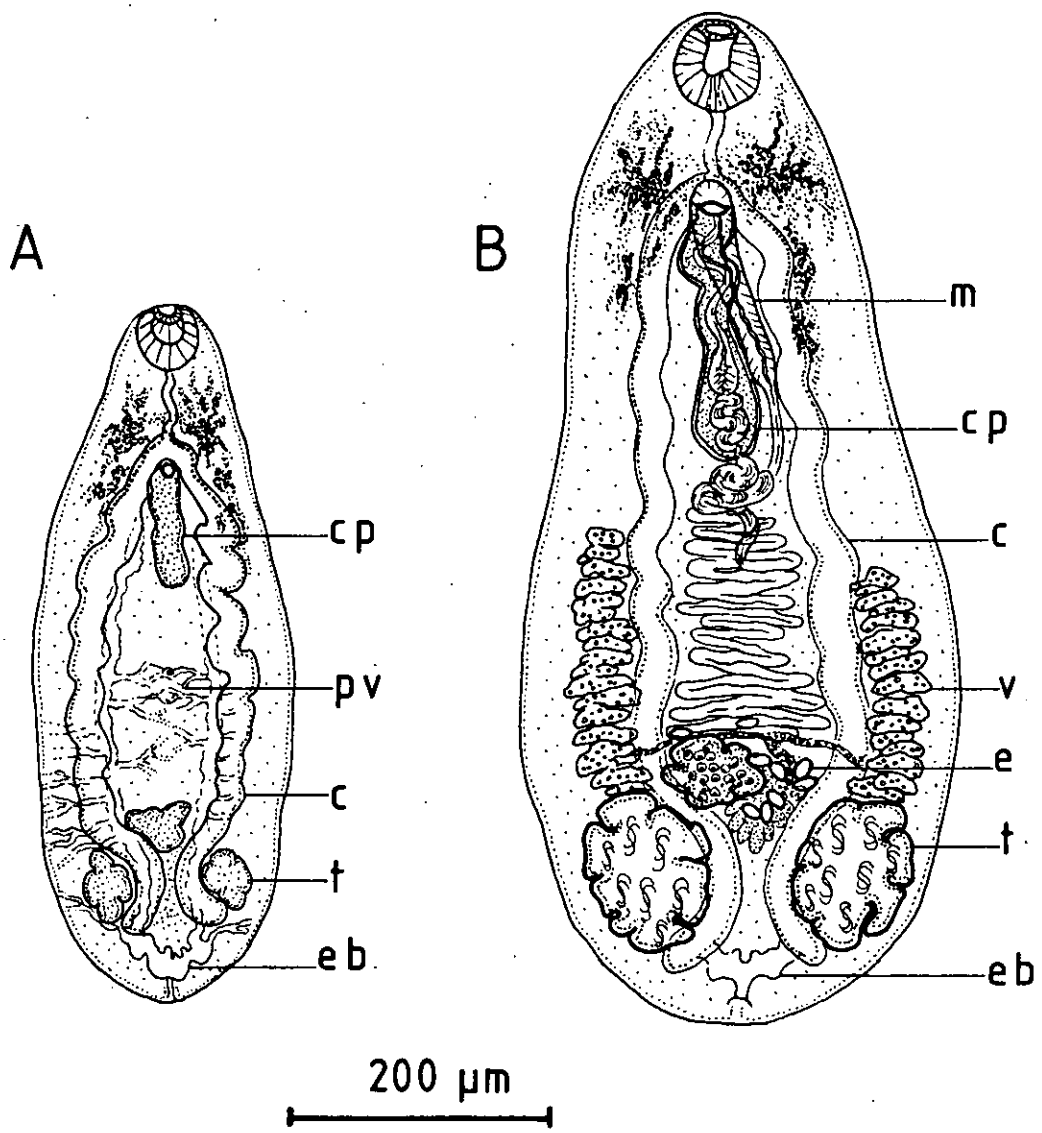


FIGURE 4.9 A, juvenile adult after 2,12 days in laboratory duckling, ventral view; B, mature adult with incompletely formed eggs in the proximal uterus after 5,3 days in laboratory duckling. (c: caecum; cp: cirrus pouch; e: egg; eb: excretory bladder; m: metraterm; pv: paranephridial vessels; t: testis; v: vitellaria.)

Stunkard, 1967) and *P. alveatum* also occurs in anatids in Europe (Yamaguti, 1971). *P. bursae* n.sp. inhabits the bursa fabricius of ducks at Calvert's Lagoon. The cercariae of *P. alveatum* and *P. parvum* are of the Monostomi type (Stunkard, 1967), whereas the cercaria of *P. bursae* n.sp. is of the Yenchingensis type.

TABLE 4.11 *Paramonostomum bursae* n.sp. Dimensions of gravid adults: (a) from an experimentally infected duckling 9,0 days P.I.; and (b) from a naturally infected black duck. Dimensions of the holotype, (from (a)), are also shown, (c).

	(a)*	(b)*	(c)*
Sample size	4	10	1
Body length	650 (469 - 832)	1338 (953 - 1648)	832
Body width	416 (318 - 544)	764 (605 - 907)	544
Oral sucker length	56 (49 - 65)	95 (76 - 106)	65
Oral sucker width	74 (72 - 80)	114 (87 - 129)	80
Cirrus pouch length	151 (95 - 201)	267 (156 - 327)	201
Cirrus pouch width	62 (46 - 80)	103 (68 - 114)	80
Metraterm length	152	169 (125 - 239)	152
Metraterm width	27	37 (30 - 42)	27
Ovary length	48 (38 - 61)	133 (76 - 182)	61
Ovary width	84 (76 - 95)	151 (122 - 198)	95
Left testis length	127 (99 - 179)	208 (148 - 266)	171
Left testis width	60 (49 - 72)	164 (114 - 228)	68
Right testis length	117 (80 - 171)	211 (148 - 239)	186
Right testis width	61 (49 - 76)	165 (129 - 209)	76
Body length:body width ratio	1.56	1.75	1.53
Anterior to cirrus pouch base:body length ratio	0.38 (.35 - .39)	0.35 (.29 - .39)	0.36
Posterior to vitellaria tip:body length ratio	0.53 (.51 - .55)	0.55 (.50 - .61)	0.52

(*fixed under coverslip pressure)

Biology:

Cysts up to 24 days old, proved to be infective to laboratory ducklings. Every adult fluke inhabited the bursa fabricius of its host. Sixty three percent (5/8) of ducklings that were exposed to infection, harboured adults of *P. bursae* n.sp. From 2 to 22 adults were recovered from each infected bird. Up to about 60% of metacercarial cysts were infective. The maximum recorded longevity of *P. bursae* n.sp. in the laboratory duckling was 9,0 days.

Considerable growth and development occur within the definitive host. After 1,23 days, the adult had reached the bursa fabricius. Its body was small and elongate and genital primordia were barely discernible. Black pigments were still concentrated in the regions of the cercarial eyespots. After 2,22 days, the body was broader and more pyriform. Black pigments were dispersed in the anterior region and the gonads and cirrus pouch had developed. The juvenile fluke doubled in size over the next 3 days and 5,3 days post-infection the reproductive system was well developed, and egg production had just commenced. After 9,0 days, the body had grown markedly, and the uterus was filled with eggs. Adults of *P. bursae* n.sp. were usually found deeply buried in the plicate lining of the bursa fabricius, their presence revealed by superficial white patches in the otherwise pink host tissue. When not buried, the dark anterior pigments made the flukes conspicuous.

4.4.3 Egg (Figure 4.8)

The operculate egg, like that of *P. caecai* n.sp., is formed without polar filaments. Filaments form as the clear, colourless egg passes through the uterus. Dimensions of uterine eggs, not including the polar filaments, are shown in Table 4.12. They are markedly smaller than those of *P. caecai* n.sp. Polar filaments are about 114μ long.

TABLE 4.12 *Paramonostomum bursae* n.sp. Dimensions of uterine eggs: (a)* from an experimentally infected duckling, 9,0 days P.I.; and (b)* from a naturally infected black duck.

Host	No. eggs	Length	Width
(a)	10	20 (19 - 21)	11 (8 - 11)
(b)	10	21 (18 - 23)	11 (10 - 12)

(* fixed under coverslip pressure)

A miracidium was evident in the egg after 2 days in lagoon water at room temperature, under normal light conditions. No miracidia, however, hatched from eggs maintained under these conditions for 30 days. Eggs incubated in lagoon water for 2 days were fed to 10 laboratory-bred

snails, *Coxiella badgerensis*. Five snails were dissected after 34 days, and 5 after 69 days; however, none were infected.

4.4.4 Redia (Figure 4.10)

Rediae are cylindrical to sausage-shaped, maximum width occurring in the anterior 1/3rd of the body. The terminal mouth opens into a relatively small pharynx and a short convoluted oesophagus leads to the simple intestine, which in larger specimens is filled with black-brown, granular, decomposing snail tissue. The gut becomes relatively shorter as the redia grows. Immature cercariae emerge through a birth pore lateral to the pharynx.

Rediae are found throughout the host viscera, mainly in the hepatopancreas. In one snail, 18 rediae were found, 8 relatively mature specimens containing germ balls and developing cercariae, and 10 immature specimens containing only germ balls. Dimensions of daughter rediae are shown in Table 4.13.

TABLE 4.13 *Paramonostomum bursae* n.sp. Dimensions of daughter rediae, selected at random (n = 10).

Body length	803 (544 - 953)
Body width	194 (151 - 257)
Pharynx length	51 (38 - 61)
Pharynx width	54 (38 - 61)

4.4.5 Cercaria (Figure 4.10)

Mature cercariae are similar in morphology and anatomy, but larger than, those of *Paramonostomum caecai* n.sp. and *Notocotylid sp.A*. The tegument is speckled with fine papillae, or spines. Two lateral eyespots are composed of dense concentrations of black pigment grains. A third, median eyespot is composed of more dispersed brown pigment grains, which are extensively distributed in dendritic strands around the eyespots and extend posteriorly, dorsal to the excretory ring. The tail is simple and attached terminally, between posterolateral, locomotory appendages.

FIG. 4.10 Paramonostomum bursae n.sp.

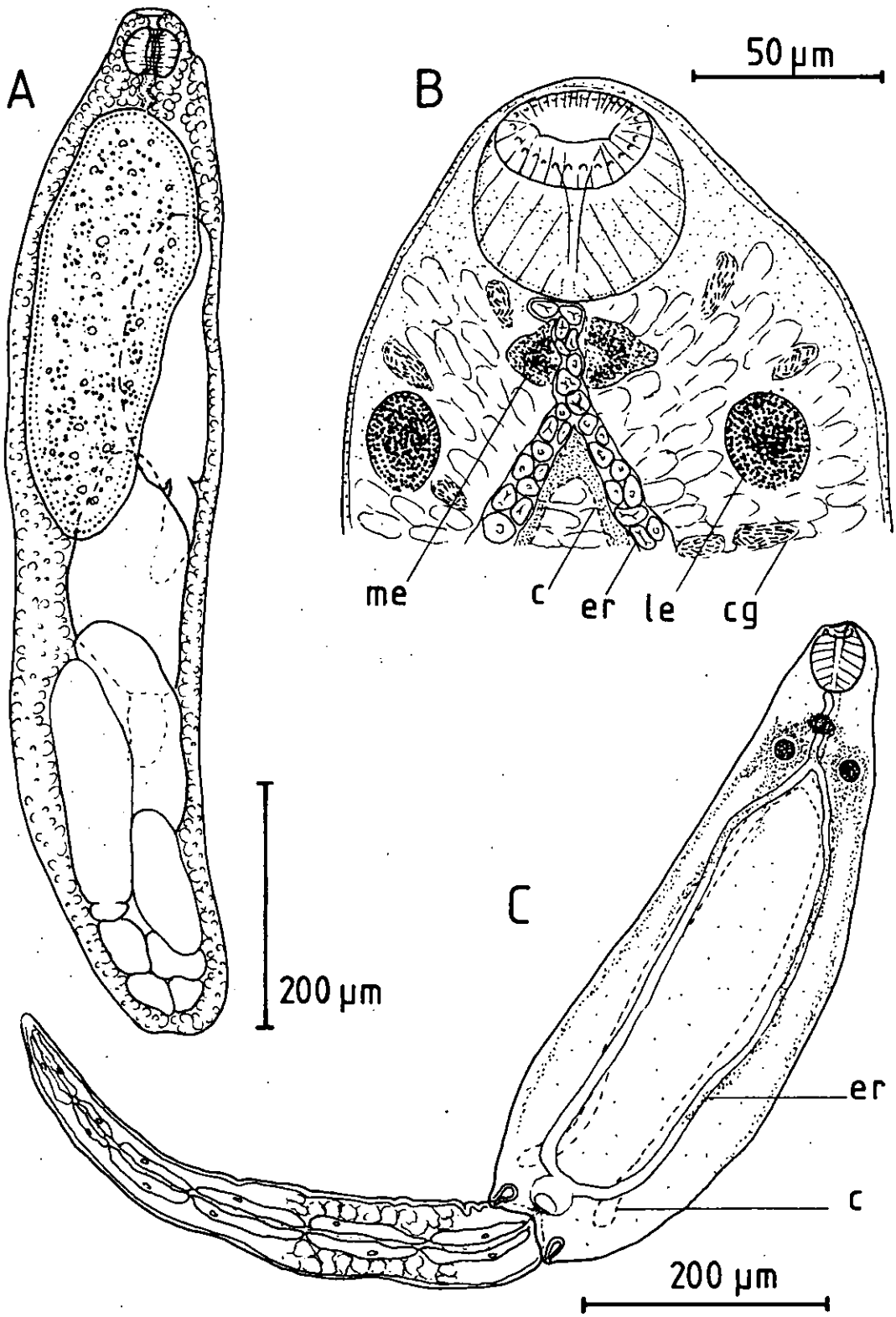


FIGURE 4.10 A, daughter redia; B, detail of anterior region of mature cercaria, ventral view; C, whole mature cercaria, dorsal view. (c: caecum; cg: cystogenous gland; er: excretory ring; le: lateral eyespot; me: median eyespot.)

A caudal excretory duct extends through the middle of the tail, flanked by 6 pairs of large cells, stained intensely by neutral red. The round oral sucker protrudes slightly anteriorly; the mouth is terminal to subterminal ventral, encircled by a ring of prominent sensory papillae. The oesophagus and caeca are developed and the body densely packed with round cystogenous glands, containing bacilliform granules, up to 5μ long. The distinguishing characteristic of this cercaria is the excretory ring of the Yenchingensis type. It has a large single anterior diverticulum, that arises between the lateral eyespots and extends anterior to the median eyespot, sometimes as far as the oral sucker. The excretory ring is packed with granules which increase in diameter anteriorly, from 5 up to 11μ. There are about 4 grains across the ring near the excretory bladder, and only 1 or 2 across the anterior part of the ring and diverticulum.

In the laboratory, mature cercariae emerged from the snail host during mid-morning. After a variable period of swimming, from about 1 minute to 1 hour, the positively phototactic cercariae encysted on the sides of the glass container, or on the host's shell and operculum. Dimensions of the cercaria are shown in Table 4.14.

TABLE 4.14 *Paramonostomum bursae* n.sp. Dimensions of mature cercariae, after emergence from the snail host (n = 15).

Body length	534 (408 - 620)
Body width	180 (163 - 201)
Body depth	147 (106 - 179)
Tail length	650 (559 - 786)
Tail width	58 (49 - 65)
Oral sucker length	51 (49 - 57)
Oral sucker width	46 (38 - 49)
Oral sucker depth	49 (-)
Eyespot diameter	24 (21 - 27)

4.4.6 Metacercaria (Figure 4.5)

Encystment occurs in the same manner as described for the other notocotylid species. The cyst is more or less hemispherical, with a thin basal flange. It is round, in plan view, with a markedly greater

diameter than that of the cyst of *Paramonostomum caecai* n.sp. The cyst contents of *P. bursae* n.sp. appear uniformly dark brown, and almost opaque, whereas those of *P. caecai* n.sp. appear striped, due to the translucent light brown parenchyma and opaque excretory ring. Dimensions of metacercarial cysts are shown in Table 4.15.

TABLE 4.15 *Paramonostomum bursae* n.sp. Dimensions of metacercarial cysts, formed by cercariae from 2 different snails: (a) n = 5, (b) n = 4.

		(a)	(b)
Interior	Length	214 (209 - 220)	224 (220 - 228)
	Width	215 (209 - 224)	220 (213 - 228)
Exterior	Length	-	280 (270 - 285)
	Width	-	282 (274 - 289)
Flange	Length	345 (342 - 350)	365 (361 - 372)
	Width	350 (342 - 372)	367 (361 - 376)

THE MONOSTOMI GROUP

The anterior transverse portion of the excretory ring of each cercaria in this group is situated posterior to the median eyespot.

4.5 Notocotylid sp.B

This species was the most common of the notocotylids infecting *Coxiella badgerensis* during the present study. The cercaria of *Notocotylid sp.B* has an unusual diverticulate excretory ring and forms characteristic small, oval cysts. Attempts to infect 8 laboratory ducklings with these cysts all failed. Cysts ranging in age from 1 to 62 days were used and the ducklings were killed at intervals from 1 to 26 days after ingestion of the cysts.

4.5.1 Redia (Figure 4.11)

The cylindrical to sausage-shaped redia is widest in the anterior 1/3rd of the body, tapering slightly posteriorly. The terminal mouth

FIG. 4.11 Notocotylid sp.B

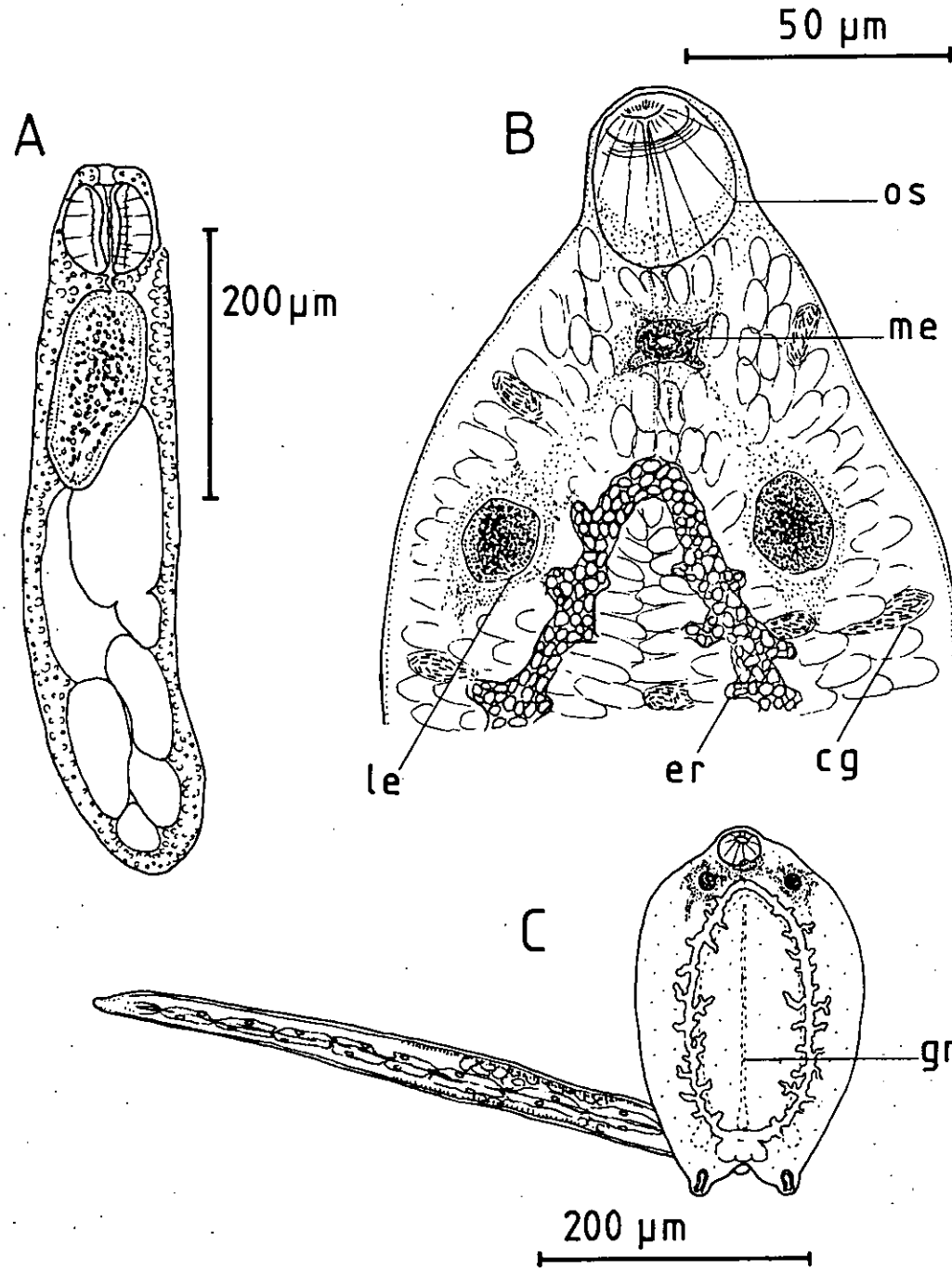


FIGURE 4.11 A, daughter redia; B, detail of anterior region of mature cercaria, ventral view; C, whole mature cercaria, dorsal view. (cg: cystogenous gland; er: excretory ring; gr: genital rudiment; le: lateral eyespot; me: median eyespot; os: oral sucker.)

opens into a relatively large pharynx. The oesophagus is short, leading to a sac-like intestine, which, in mature rediae, is filled with dark, granular yellow-brown material. Mature rediae contain germ balls and developing cercariae. Immature cercariae emerge through a birthpore, situated posterolateral to the pharynx.

The rediae are quite abundant throughout the viscera of infected hosts. An average of 60 (53 - 64) was found in 3 snails. Dimensions of daughter rediae are shown in Table 4.16.

TABLE 4.16 *Notocotylid sp.B.* Dimensions of daughter rediae selected at random (n = 15).

Body length	488 (280 - 680)
Body width	171 (129 - 239)
Pharynx length	73 (61 - 87)
Pharynx width	74 (65 - 84)

4.5.2 Cercaria (Figure 4.11)

The mature cercaria is similar in morphology and anatomy to other notocotylid cercariae infecting *Coxiella badgerensis;* however it is smaller in all dimensions (Table 4.17). The tegument is speckled with very fine spines or papillae and locomotory appendages protrude posterolaterally. A caudal excretory duct extends through the tail, flanked by large cells. The small round oral sucker protrudes anteriorly. A short oesophagus extends posteriad, however, no caeca are discernible. Two round to oval lateral eyespots are composed of densely packed black grains. The annular median eyespot is composed of dispersed brown pigment grains. The body is obscured by dense round cystogenous gland cells, packed with bacilliform grains, 2 - 3μ long. The excretory ring is of the Monostomi type. Conspicuous concretion-filled diverticula, which are not present in any other type of notocotylid cercaria infecting *C. badgerensis*, extend medially and laterally from the ring. There are 4 - 7 excretory concretions, each about 2 - 3μ diameter, across the anterior part of the ring.

In the laboratory, mature, positively phototactic cercariae emerged from the host between dawn and midday, with a peak at about mid-morning. Encystment occurred near the water surface after a short period of swimming. Most cercariae encysted on the glass wall of the container, but a few encysted on the snail shell.

TABLE 4.17 *Notocotylid sp.B.* Dimensions of cercariae, after emerging from the snail host (n = 15).

Body length	310 (285 - 365)
Body width	155 (133 - 167)
Body depth	104 (84 - 125)
Tail length	513 (484 - 559)
Tail width	38 (34 - 42)
Oral sucker length	33 (30 - 34)
Oral sucker width	33 (30 - 38)
Oral sucker depth	38 (-)
Eyespot diameter	15 (-)

4.5.3 Metacercaria (Figure 4.5)

The encystment process is similar to that described in other notocotylid species. As encystment commences, the body of the cercaria contracts into a transversely oval shape, thus forming an oval cyst. The eyespots and granular excretory ring, are just discernible within the fully developed yellow-brown cyst. Dimensions of cysts are shown in Table 4.18. There is slight variation in the size of cysts formed by cercariae from different snails.

TABLE 4.18 *Notocotylid sp.B.* Dimensions of metacercarial cysts, formed by cercariae from 3 different snails: (a) n = 4, (b) n = 4, (c) n = 4.

		(a)	(b)	(c)
Interior	Length	189 (182 - 198)	201 (198 - 205)	192 (190 - 194)
	Width	127 (118 - 133)	125 (122 - 129)	118 (110 - 122)
Exterior	Length	220 (209 - 228)	239 (236 - 239)	228 (-)
	Width	154 (152 - 156)	156 (148 - 160)	148 (144 - 152)
Flange	Length	-	-	295 (285 - 296)
	Width	-	-	194 (190 - 201)

4.6 Discussion

Infection of one snail species by a number of notocotylid trematodes has been previously recorded. Rothschild (1941), reported a 5 year study of the infection of *Peringia ulvae* Pennant in England by 6 notocotylid species. Three of the species belonged to the Yenchingensis Group and 3 to the Monostomi Group. Stunkard (1967), reported the discovery of 5 notocotylid species infecting *Hydrobia salsa* in the U.S.A. One of the species belonged to the Imbricata Group, 2 to the Yenchingensis Group and 2 to the Monostomi Group.

Although the life-cycles of 2 of the notocotylids at Calvert's Lagoon, *Paramonostomum caecai* n.sp. and *P. bursae* n.sp., have been elucidated, further studies are needed to identify the other 2 species. It is believed that a diminutive oval unidentified notocotylid, that was discovered in large numbers in the intestines of 2 black swans killed at Calvert's Lagoon, may be the adult of *Notocotylid sp.B*.
The swans had been feeding almost exclusively on the dominant hydrophytes at Calvert's Lagoon. Cysts of *Notocotylid sp.B* were found in the alimentary tracts of both birds, one cyst being attached to a plant stem, *Ruppia maritima*. In January 1975, small oval immature notocotylids were found in black swans at Lake Bookar, Queensland, and some specimens were kindly sent to the author by Dieter Palmer, of James Cook University. They closely resemble the unidentified notocotylid that infects black swans at Calvert's Lagoon. Dimensions of the flukes from Calvert's Lagoon and Lake Bookar, are shown in Table 4.19. The small size of these flukes made identification to the generic level difficult. No ventral glands or glandular ridges could be seen in fixed specimens; however, no studies were made of live specimens. Identification is deferred until the morphology of these adults has been studied under a scanning electron microscope.

TABLE 4.19 Dimensions of flukes suspected of being the adults of *Notocotylid sp.B*, that were found in the intestines of black swans: (a) gravid and (b) immature flukes from Calvert's Lagoon; and (c) immature flukes from Lake Bookar, Queensland.

	(a)	(b)	(c)
No. of flukes	15	15	15
Length	296 (257 - 340)	251 (212 - 272)	198 (174 - 257)
Width	177 (151 - 197)	142 (121 - 159)	178 (151 - 212)

Rothschild (1941), found that 2 species of the Yenchingensis group developed into adults of the genus *Paramonostomum*, in the intestinal caeca of ducks. Stunkard (1967), found that the cercariae of *P. alveatum* and *P. parvum*, belong to the Monostomi group, and that the corresponding adults inhabit the lumen of the intestine of their bird host. Velasquez (1969), reported that the cercaria of *P. philippinensis* is of the Yenchingensis group, although his description and illustration indicate that it belongs to the Imbricata group. The adult of this species inhabits the intestinal caeca of the bird host. At Calvert's Lagoon, the cercaria of *P. caecai* n.sp. belongs to the Imbricata group, and the adult inhabits the intestinal caeca of ducks, whereas that of *P. bursae* n.sp. belongs to the Yenchingensis group, and the adult inhabits the bursa fabricius of ducks. The cercariae of species currently classified in the genus *Paramonostomum*, thus belong to the Imbricata, Yenchingensis and Monostomi groups. Odening (1966), reported that the cercariae of 5 *Notocotylus* species belonged to the Monostomi group, whereas Stunkard (1960; 1966), found that the cercariae of 2 *Notocotylus* species belonged to the Yenchingensis group. A cercaria of the Yenchingensis group (Szidat and Szidat, 1933), and one of the Monostomi group (Yamaguti, 1938), are reported to develop into the same adult, *Notocotylus attenuatus*.

The lack of correlation between cercarial types and notocotylid genera throws doubt on the validity of the existing classification within the family *Notocotylidae*, based, as it is, on the presence, number and

distribution of ventral glands in the adult. In his review of the genus *Paramonostomum*, Stunkard (1967), emphasized the importance of the discovery of life-cycles and the description of larval stages, to the taxonomy of the *Notocotylidae*. The present studies add support to that view. Any attempt towards a more natural classification of notocotylids should consider all life-history stages.

Chapter 5 THE SCHISTOSOMATIDAE, RENICOLIDAE, HETEROPHYIDAE AND STRIGEIDAE

The cercariae of one schistosome, one renicolid, three heterophyid and one strigeid species, develop in and emerge from *Coxiella badgerensis*. An encysted metacercaria, which infects this snail, appears to belong to another renicolid species, one whose cercariae encyst without emerging from the primary intermediate host. No stages in the life-cycles of these 7 species have been experimentally demonstrated. Circumstantial evidence indicates that the strigeid species is *Apatemon (Apatemon) gracilis* (Rudolphi, 1819); however the identities of the remaining 6 species are not known.

5.1 Family SCHISTOSOMATIDAE

5.1.1 Schistosome sp.A

Sporocyst: (Figure 5.1)

Mature daughter sporocysts are long, vermiform and colourless and difficult to separate intact from snail tissue. They extend throughout the host's viscera, but are concentrated around the hepatopancreatic tubules. Sporocysts containing germ balls and cercariae vary in size up to about 8000 × 114μ. The posterior end is sessile, lodged deeply in the hepatopancreas and the narrower anterior end is motile and makes slow stretching movements. The tegument is thin and traversed by a birth canal at the anterior end. This canal opens through a small, terminal birth pore. There is a gradation in cercarial development from undifferentiated germ balls at the posterior end, to active fully-developed cercariae near the birth pore. The mature cercariae creep actively through the birth canal and emerge body first.

Cercaria: (Figures 5.1 and 5.2)

The cercaria is an apharyngeate, brevifurcate, furcocercous

FIG. 5.1 Schistosome sp.A

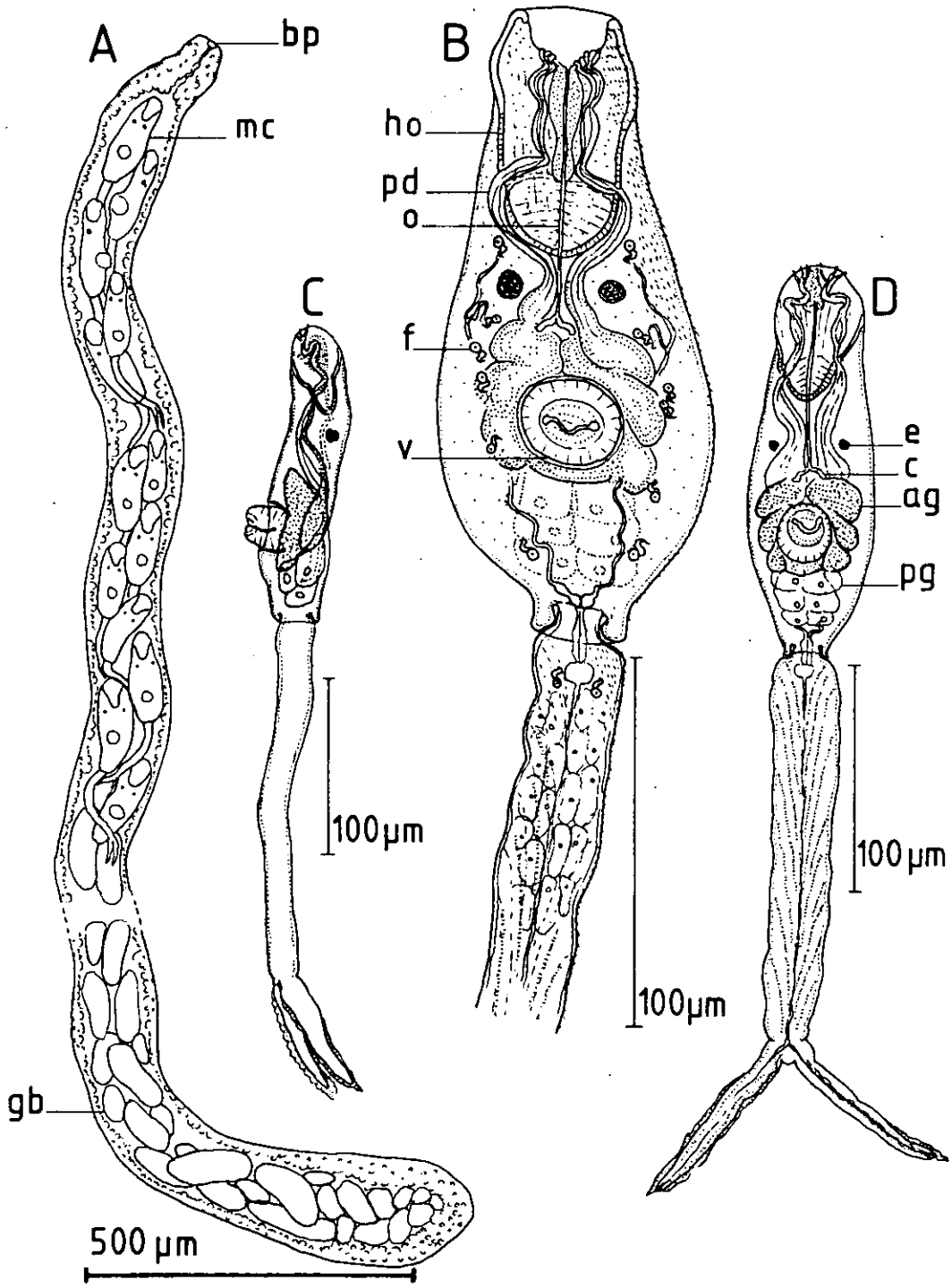


FIGURE 5.1 A, daughter sporocyst; B, cercaria, anterior retracted, showing distribution of flame-cells, ventral view; C, whole cercaria, lateral view; D, whole cercaria, anterior everted, ventral view. (ag: anterior penetration gland; bp: birth pore; c: caecum; e: eyespot; f: flame-cell; gb: germ ball; ho: head organ; mc: mature cercaria; pd: penetration gland ducts; pg: posterior penetration gland; o: oesophagus; v: ventral sucker.)

distome, with pigmented eyespots. Its dimensions are shown in Table 5.1.

TABLE 5.1 *Schistosome sp.A.* Dimensions of cercariae after emerging from the snail host (n = 15).

Body length	170 (152 - 205)
Body width	66 (57 - 72)
Body depth	55 (49 - 57)
Tail stem length	212 (186 - 239)
Tail stem width	28 (25 - 32)
Tail furca length	79 (61 - 103)
Tail furca width	13 (10 - 19)
Head organ length	68 (53 - 76)
Ventral sucker diameter	24 (19 - 27)

The body is more or less oval, maximum width occurring near the level of the ventral sucker. Fine simple tegumental spines occur on the body, where they are more densely distributed anterior to the ventral sucker, and on the tail stem and tail furcae. A head organ is separated from the body parenchyma by a distinct wall. The posterior part contains a powerful oval muscle and the anterior part may be invaginated, or evaginated into a dome-shape. A finely granular yellow body is contained in the anterior part of the head organ. A narrow medial oesophagus passes through the head organ and bifurcates just posterior to the level of the eyespots, giving rise to 2 short caeca. The ventral sucker is protrusible and very strong. It is important in the rapid creeping movements of the cercaria on solid substrates and is used to attach the cercaria to the underside of the air-water interface. There are 5 pairs of penetration glands: the anterior 2 pairs, situated anterior and dorsal to the ventral sucker, are coarser grained than the 3 larger post-acetabular pairs. The anterior pairs of glands are stained bright pink by saturated purpurin solution in 95% ethanol, and the posterior pairs are not stained. The 5 pairs of ducts from the penetration glands pass sinuously anteriad. They pass dorsal to the ventral sucker and ventro-medial to the eyespots, before entering the head organ posterolaterally. Each duct opens through a separate pore

FIG. 5.2 Schistosome sp.A - cercaria

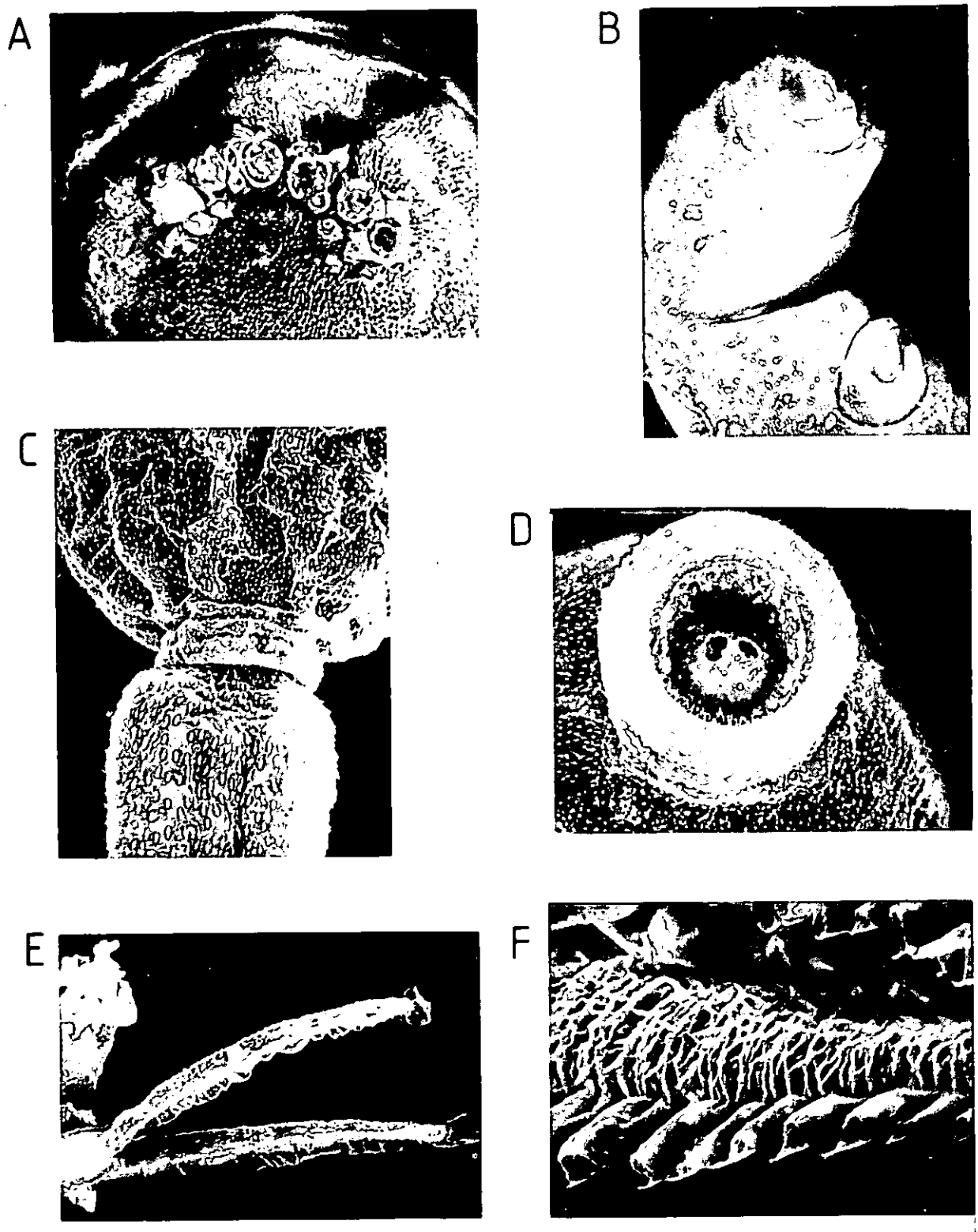


FIGURE 5.2 S.E.M. photographs - A, everted anterior end of cercaria, showing arc of penetration gland duct openings and ciliated sensory papillae, ×3,000; B, forebody, showing prominent ventral sucker and retracted anterior end, ×1,200; C, junction of body and tail, showing tegumental spines, ×3,000; D, interior of terminal 'socket' of body, tail removed, showing openings of two excretory ducts, ×3,000; E, tail furcae, ×500; F, detail of tail furca, showing regular convolutions of finfold, and tegumental spines, ×3,000.

at the anterior extremity of the body. The excretory system consists of 6 pairs of flame-cells in the body and one pair on the anterior part of the tail stem. In the body, one pair lies anterior to the eyespots, a second is between the eyespots and ventral sucker, a third and fourth pair are at the anterior and equatorial level of the ventral sucker respectively, a fifth pair lies posterolateral to the ventral sucker and the sixth pair lies near the junction with the tail. The third and fourth pairs are greatly obscured by the anterior penetration glands. The primary collecting ducts are slightly expanded at the posterior end of the body, to form small, separate, excretory bladders. The collecting ducts unite in the tail stem, to form a small bladder and then continue as a single caudal excretory duct which bifurcates at the base of the tail stem and eventually opens through the tip of each furca. The lanceolate furcae have convoluted dorsoventral finfolds which extend along their entire length. The finfolds increase in width posteriorly, to about 7μ wide near the tip of the furca.

In the laboratory, cercariae emerged from the host snail during the evening. After swimming erratically in the lagoon water, they attached themselves strongly by the ventral sucker to the water surface, or to the walls of the glass container, near the surface. They were concentrated nearest to a light source that illuminated one side of the dish, and were difficult to dislodge mechanically. When swimming, the tail usually led and the rapidly beating tail and body produced a standing wave, like a figure-of-8.

5.1.2 Discussion

Yamaguti (1975), briefly compares the cercariae of the family *Schistosomatidae*. Cercariae of the genera *Heterobilharzia*, *Bilharziella*, *Trichobilharzia* and *Gigantobilharzia*, have finfolds on the tail furcae and eyespots, as does the cercaria of *Schistosome sp.A*. The numbers of flame-cells of the cercariae of these 4 genera are 12, 14, 14 and 10-14

respectively. The cercaria of *Schistosome sp.A* has 14 flame-cells. In general morphology and anatomy, the developmental stages of *Schistosome sp.A* in *Coxiella badgerensis* appear closest to those of the genera *Trichobilharzia* and *Gigantobilharzia* (Wu, 1953; Najim, 1956; and Yamaguti, 1975).

5.2 Family RENICOLIDAE

5.2.1 Renicolid sp.A

Sporocyst: (Figure 5.3)

The daughter sporocyst is thin-walled, oval and transparent and lacks a birth pore. It is intimately associated with snail gonadal tissue and is difficult to dissect intact. Sporocysts containing one or two mature cercariae, a few developing cercariae and a few germ balls, measured 213 - 323μ long × 133 - 209μ wide.

Cercaria: (Figures 5.3 and 5.4)

The cercaria is a pharyngeate, leptocercous, distomate xiphidiocercaria. Its dimensions are shown in Table 5.2.

TABLE 5.2 *Renicolid sp.A*. Dimensions of cercariae after emerging from the snail host (n = 15).

Body length	264 (224 - 312)
Body width	81 (76 - 91)
Body depth	55 (46 - 61)
Tail length	228 (201 - 251)
Tail width	25 (23 - 27)
Oral sucker length	40 (38 - 42)
Oral sucker width	36 (30 - 42)
Oral sucker depth	35 (32 - 38)
Ventral sucker length	40 (34 - 46)
Ventral sucker width	40 (38 - 44)
Ventral sucker depth	22 (19 - 27)

The body is elongate oval, convex dorsally and slightly flattened ventrally, maximum width occurs at about the ventral sucker level. Small, peg-like tegumental spines are distributed over the body, however, both

FIG. 5.3 Renicolid sp.A

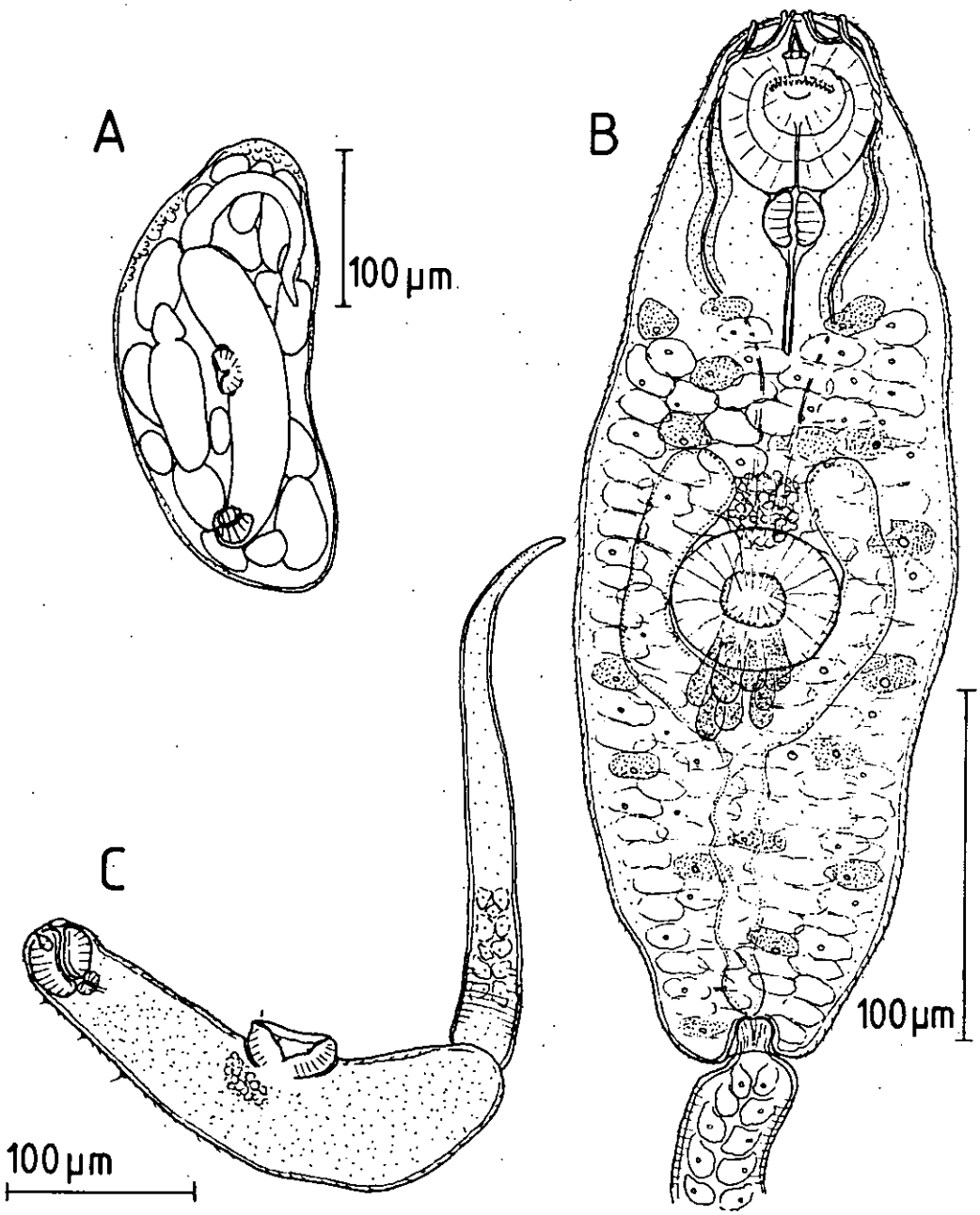


FIGURE 5.3 A, daughter sporocyst; B, body of mature cercaria, ventral view; C, whole cercaria, swimming position, lateral view.

oral and ventral suckers are aspinous, except for two rows of spines on their internal surfaces. The tail is generally slightly shorter than the body. The oral sucker is oval, and the subterminal-ventral mouth is surrounded by a raised ring of 14 large ciliated sensory papillae. A stylet, located in the antero-dorsal part of the oral sucker, can be protruded through a terminal aperture. The stylet measures 8 × 5μ, with the thickened point being about 6μ long. A pre-pharynx is short or absent, and the poorly developed pharynx is about 8μ diameter. The oesophagus extends posteriorly and is obscured by cystogenous glands about midway between the pharynx and protrusive ventral sucker. The rim of the ventral sucker is thrown into radial folds, within which are the two rows of spines. The ventral surface and the stem of the sucker are pitted but aspinous. Eight large ciliated sensory papillae are uniformly distributed on the internal surface of the sucker. About 4 pairs of glands (either penetration or frontal glands), lie in two groups immediately posterior to the ventral sucker. Their ducts extend anteriorly in two bundles lateral and dorsal to the oral sucker. Two pairs of gland ducts open medially near the tip of the stylet and the other pairs open anterolaterally. Cystogenous glands, filled with fine granules, are densely packed in the body, from midway between the suckers to the posterior extremity. Their contents are stained intensely by neutral red and brilliant cresyl blue. Flame-cells and collecting ducts are obscured in mature cercariae. The excretory bladder is Y-shaped, the long stem branching immediately posterior to the ventral sucker, and the arms, of variable length, extending about 1/3 to 1/2 of the distance to the oral sucker.

In the laboratory, the cercaria emerged from its snail host in the evening and swam almost continuously, until exhausted. The cercaria swam upside down, with the tail lashing in a plane perpendicular to the body, and showed no phototaxis. When not swimming, it crept strongly on the substrate, using the well developed suckers.

FIG. 5.4 Renicolid sp.A (cercaria) and Renicolid sp.B (metacercaria)

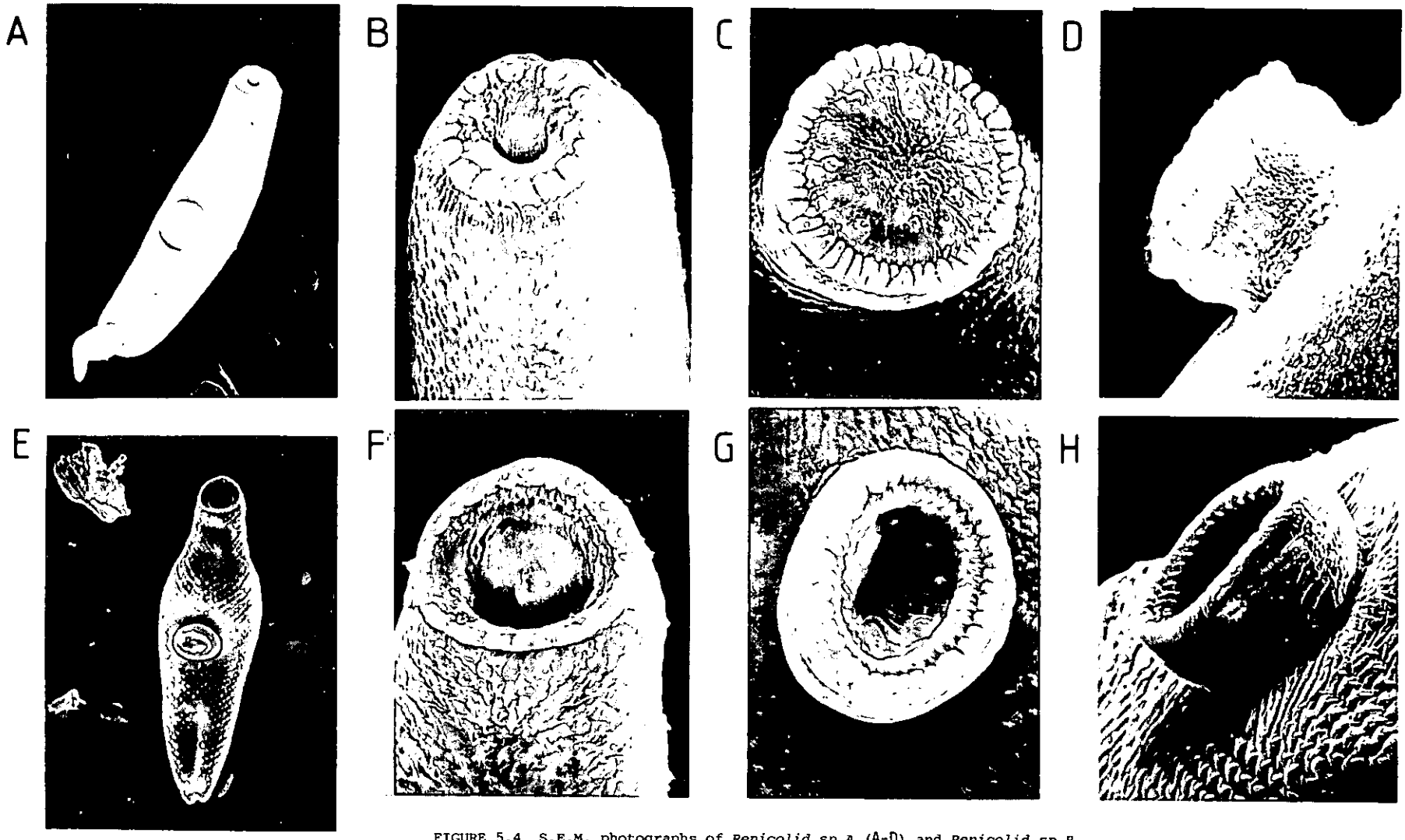


FIGURE 5.4 S.E.M. photographs of *Renicolid sp.A* (A-D) and *Renicolid sp.B* (E-H) - A, whole cercaria, ×400; B, oral sucker region of cercaria, ×2,000; C, ventral sucker of cercaria, ventral view, ×2,250; D, ventral sucker of cercaria, lateral view, ×2,200; E, whole excysted metacercaria, ×250; F, oral sucker region of metacercaria, ×1,400; G, ventral sucker of metacercaria, ventral view, ×1,700; H, sucker of metacercaria, lateral view, ×1,600.

5.2.2 *Renicolid sp.B*

Metacercaria: (Figures 5.4 and 5.5)

The cyst is round and colourless; dimensions are given in Table 5.3. The encysted metacercaria appears striped due to almost opaque granules in the elongate excretory bladder. The uniform cyst wall is composed of two layers, the inner layer being thicker and more transparent than the outer layer. Cysts of this species, which always occur in large numbers, are localized in the proximal part of the visceral hump of the snail host, adjacent to both gonadal and hepatopancreatic tissue. One snail contained a cluster of 290 cysts.

TABLE 5.3 *Renicolid sp.B*. Dimensions of metacercarial cysts from two naturally infected snails.

Host	No. cysts	External dimensions Length	Width	Cyst thickness (average
Snail 1	10	169 (160 - 179)	163 (160 - 171)	12
Snail 2	10	179 (171 - 186)	168 (160 - 175)	16

In vitro excystment occurs, after 1 or 2 hours, in 0.5% pancreatin in Hank's saline at 41°C. The excysted metacercaria is larger than the body of the cercaria of *Renicolid sp.A*, however they are very similar in morphology and anatomy. Dimensions of the former are shown in Table 5.4. The body is elongate oval, and slightly concave ventrally. The distinctive protrusive ventral sucker is like that of the cercaria of *Renicolid sp.A*. The tegument is spinous, bearing peg-like spines, except on the suckers and the ventral surface posterior to the ventral sucker. Two rows of spines are present on the outer rim of the internal surface of each sucker. The mouth is subterminal-ventral, prepharynx absent and pharynx small. The oesophagus and caeca are not visible. Finely granular frontal gland cells occupy the middle 1/3 of the body. About 8 gland ducts are discernible, extending anteriorly and opening antero-dorsal to the oral sucker. The reproductive system is not

FIG. 5.5 Renicolid sp.B

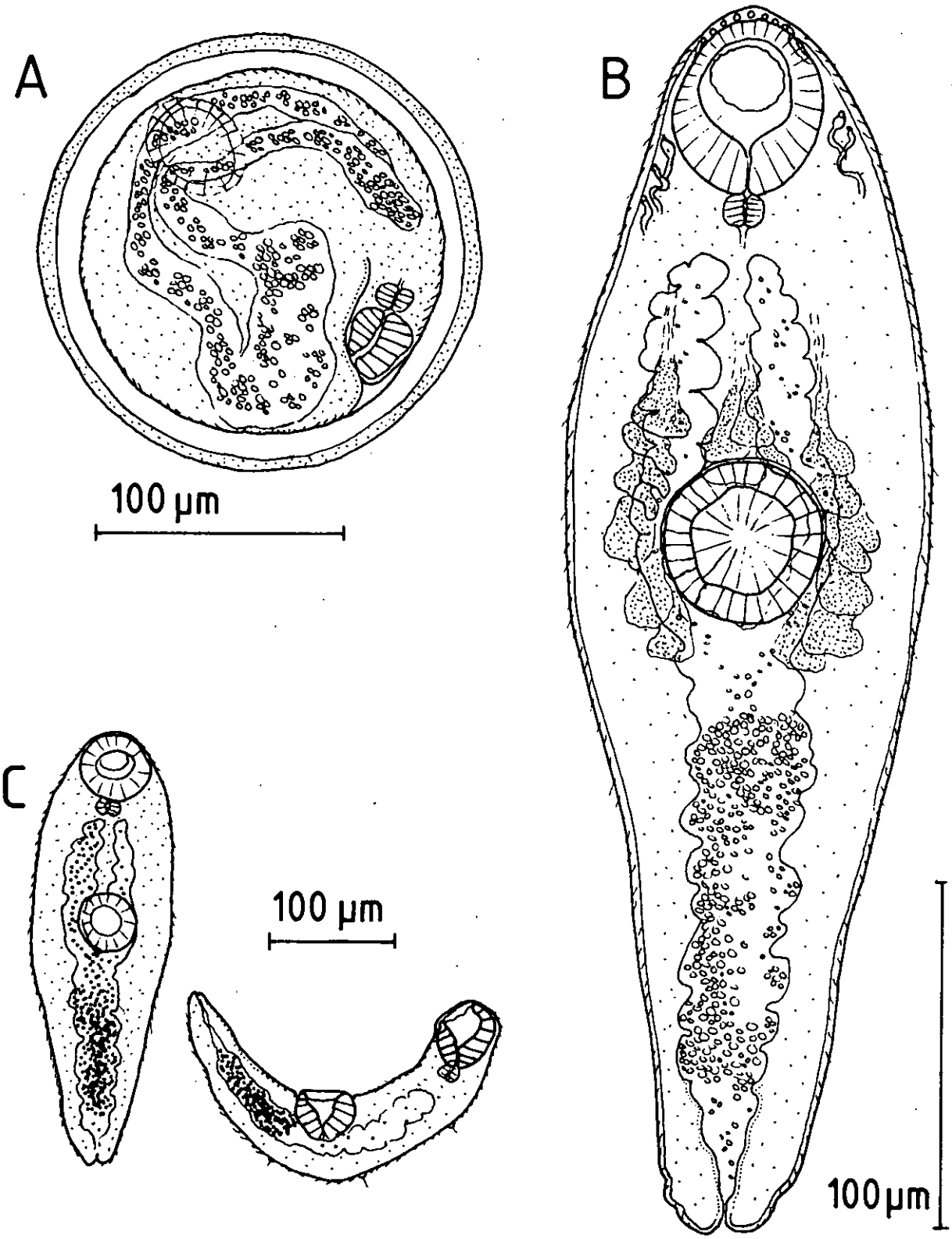


FIGURE 5.5 A, metacercarial cyst; B, excysted metacercaria after 1½ hours at 41°C, slightly flattened under coverslip pressure, ventral view; C, characteristic form of excysted metacercaria, ventral and lateral views.

developed. The Y-shaped excretory bladder is filled with refractile excretory granules; the arms of the bladder, variable in length, extend from just posterior to the ventral sucker, sometimes reaching almost to the level of the pharynx.

Two juvenile flukes, similar in size, morphology and degree of maturity to the excysted metacercaria of *Renicolid sp.B*, were found in the caeca of an hoary-headed grebe at Calvert's Lagoon.

TABLE 5.4 *Renicolid sp.B.* Dimensions of excysted metacercariae (n = 10).

Body length	356 (258 - 429)
Body width	114 (108 - 118)
Body depth	76 (-)
Oral sucker length	59 (46 - 68)
Oral sucker width	53 (48 - 55)
Pharynx length	14 (11 - 17)
Pharynx width	16 (15 - 19)
Ventral sucker length	49 (46 - 53)
Ventral sucker width	51 (46 - 55)
Anterior body to ventral sucker	156 (87 - 205)
Posterior body to excretory bladder extremity	287 (205 - 331)

5.2.3 Discussion

The Renicolidae is an unusual trematode family, as the morphology of the adults (kidney flukes of birds), is very uniform, but different species may have widely different life-cycles and cercariae (Wright, 1971; Pearson, 1972). Within this family cercariae vary from the large Rhodometopa type, with a branched, Y-shaped excretory bladder, to a small xiphidiocercaria, with a simple, Y-shaped excretory bladder (Cable, 1965; Pearson, 1972). Stunkard (1964), experimentally demonstrated that the cercaria of *Renicola thaidus*, developing in marine snails in Massachusetts, U.S.A., was a pharyngeate, leptocercous, distomate xiphidiocercaria. It emerged from the snail host and encysted in bivalve molluscs. He noted that the cercaria was very similar to *Cercaria parvicaudata*, also found in marine snails in Massachusetts (Stunkard and Shaw, 1931), and *C. roscovita*, found in snails on the Brittany coast of

France (Stunkard, 1932). James (1969), described the developmental stages of 3 species of "plagiorchioid distome xiphidiocercariae", *Cercaria roscovita, C. brevicaudata* and *C. emasculans*, that infected the marine snail, *Littorina saxatilis*, at Cardigan Bay, Wales. These cercariae were similar to that of *Renicola thaidus*. *C. roscovita* left the primary intermediate host and invaded another specimen of the same species, or re-entered the first host. It also invaded other snail species, and more rarely, a crab. The post-cercaria migrated to, and encysted in, the haemocoel and gonad of the snail host. *C. brevicaudata* usually escaped from the daughter sporocyst and encysted in the primary host's visceral haemocoel, but sometimes encysted in the daughter sporocyst. *C. emasculans* left the primary intermediate host and encysted in crabs and fish. Werding (1969) found *C. roscovita* infecting *Littorina littorea* in the North Sea. He experimentally showed that the cercaria left the snail host and encysted either in snails of the same species, or in mussels; and that the adult, *Renicola roscovita*, infected gulls.

Pearson (1979), reported the occurrence of distome xiphidiocercariae of 5 renicolid species in a freshwater prosobranch snail, in the Brisbane River, Queensland. All were characterised by a simple long-stemmed, Y-shaped excretory bladder. He experimentally demonstrated that one of these cercariae encysted in the native fish, *Melanotaenia*, and that the adult fluke, a species of *Renicola*, inhabited the kidney of the Australian pelican.

At Calvert's Lagoon, trematode developmental stages that resemble the renicolid xiphidiocercariae described by Stunkard (1964), Werding (1969) and Pearson (1979), develop in *Coxiella badgerensis*. The cercaria of *Renicolid sp.A* emerges from its snail host, however, attempts to infect laboratory bred specimens of *C. badgerensis* with the free swimming cercaria have failed. The metacercaria of *Renicolid sp.B* is morphologically similar to, but larger than, the body of the cercaria of

Renicolid sp.A. Both trematodes have a distinctive ventral sucker and double rows of spines around the openings of the oral and ventral suckers, like the cercaria of *Renicola thaidus* (Stunkard, 1964). The primary collecting tubules of the protonephridial system, which characteristically branch from the main stem of the excretory bladder of renicolid xiphidiocercariae, were not seen in either *Renicolid sp.A* or *B*. The cysts of *Renicolid sp.B* occur in a small region of the proximal part of the visceral hump of *C. badgerensis*, always in large numbers, up to about 300, which indicates that the cercaria of this species encysts without emerging from its primary intermediate host. Hence, it appears that either there is one renicolid species infecting *C. badgerensis* that has alternative life-cycle patterns; or, that there are 2 species: *Renicolid sp.A* with a 3 host life-cycle and *Renicolid sp.B* with an atypical 2 host life-cycle. The latter seems likely. To date no mature renicolid adults have been found in waterfowl living at Calvert's Lagoon; however, they may have been overlooked, as the kidneys of only a few birds were scrutinized during dissection.

5.3 Family HETEROPHYIDAE

5.3.1 Heterophyid sp.A

Redia: (Figure 5.6)

The motile daughter redia is elongate, sausage-shaped and slightly attenuated anteriorly. Its mouth is small and terminal; the round pharynx is relatively small and the simple intestine short and filled with yellow-brown granular material. The colourless tegument is thin, except at the extremities. Large numbers of germ balls and developing cercariae, are contained within the brood chamber, the degree of cercarial development increasing anteriorly. A small birth pore is situated posterolateral to the pharynx. The rediae are concentrated in the hepatopancreas and gonad. Their dimensions are shown in Table 5.5.

FIG. 5.6 Heterophyid sp.A

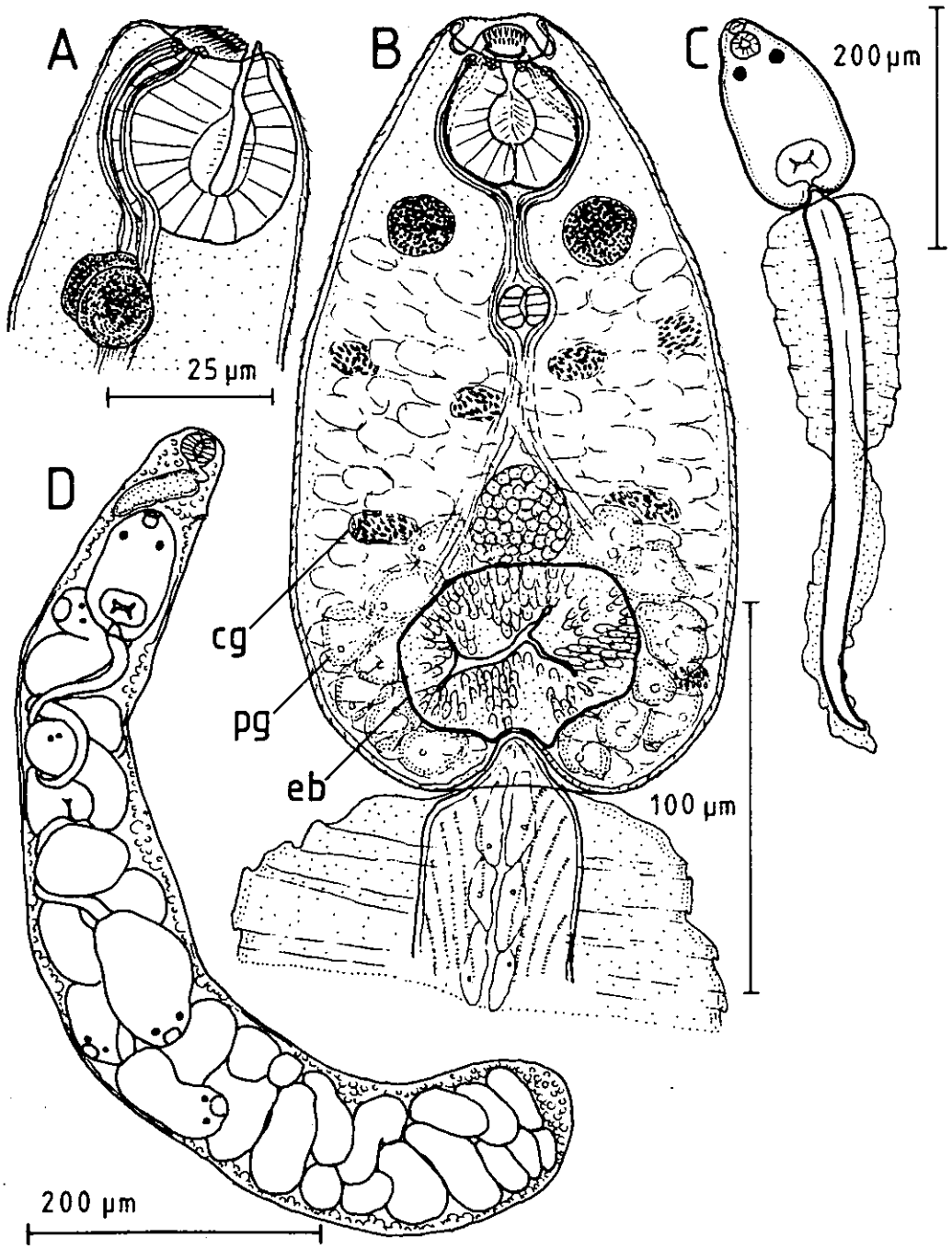


FIGURE 5.6 A, oral sucker region of cercaria, lateral view; B, mature cercaria, slightly flattened, ventral view; C, whole cercaria, dorsal view; D, daughter redia. (cg: cystogenous gland; eb: excretory bladder; pg: penetration gland.)

TABLE 5.5 *Heterophyid sp.A.* Dimensions of rediae, selected at random (n = 10).

Body length	689 (544 - 847)
Body width	104 (83 - 129)
Pharynx length	29 (25 - 32)
Pharynx width	30 (27 - 32)

Cercaria: (Figures 5.6 and 5.7)

The cercaria is monostomate and oculate, with para-vesicular penetration glands and both lateral and dorso-ventral finfolds. The body shape is variable, but usually pyriform. Round, black pigmented eyespots are situated about 1/3 of the body length from the anterior end. Stout, peg-like tegumental spines are distributed over the body, diminishing in size posteriorly. The round, well-developed oral sucker is recessed, its anterior part forming an eversible, conical protuberance. The mouth is on the ventral surface of this protuberance. Large, "pre-oral" or "penetration" spines are arranged in two rows, of 7 and 9, dorsal to the mouth. The prepharynx is variable; the small, round pharynx, about 12μ diameter, usually lying immediately posterior to the eyespots. The oesophagus and caeca are not visible. Seven pairs of inconspicuous penetration glands are distributed postero-laterally, around the excretory bladder. Their ducts pass anteriorly, around the pharynx, between the eyespots and dorsally over the oral sucker. They separate into 4 bundles of 3, 4, 4 and 3 ducts, which open medially and laterally, near the mouth. The anatomy of the body is obscured by densely packed cystogenous gland cells, which extend from just posterior to the eyespots to the posterior end of the body. Under coverslip pressure, the granular contents of these cells ooze through the tegument and unravel, like scrolls. The genital primordium, a region of small uniform cells, about 14 × 18μ, is anterior to the excretory bladder, which is simple and very thick-walled. The long tail is attached to the body in a terminal socket. An anterior pair of lateral finfolds extends to the middle of the tail. The posterior pair of dorsoventral finfolds is asymmetrical, the dorsal part, which is

FIG. 5.7 Heterophyid sp.A (cercaria)

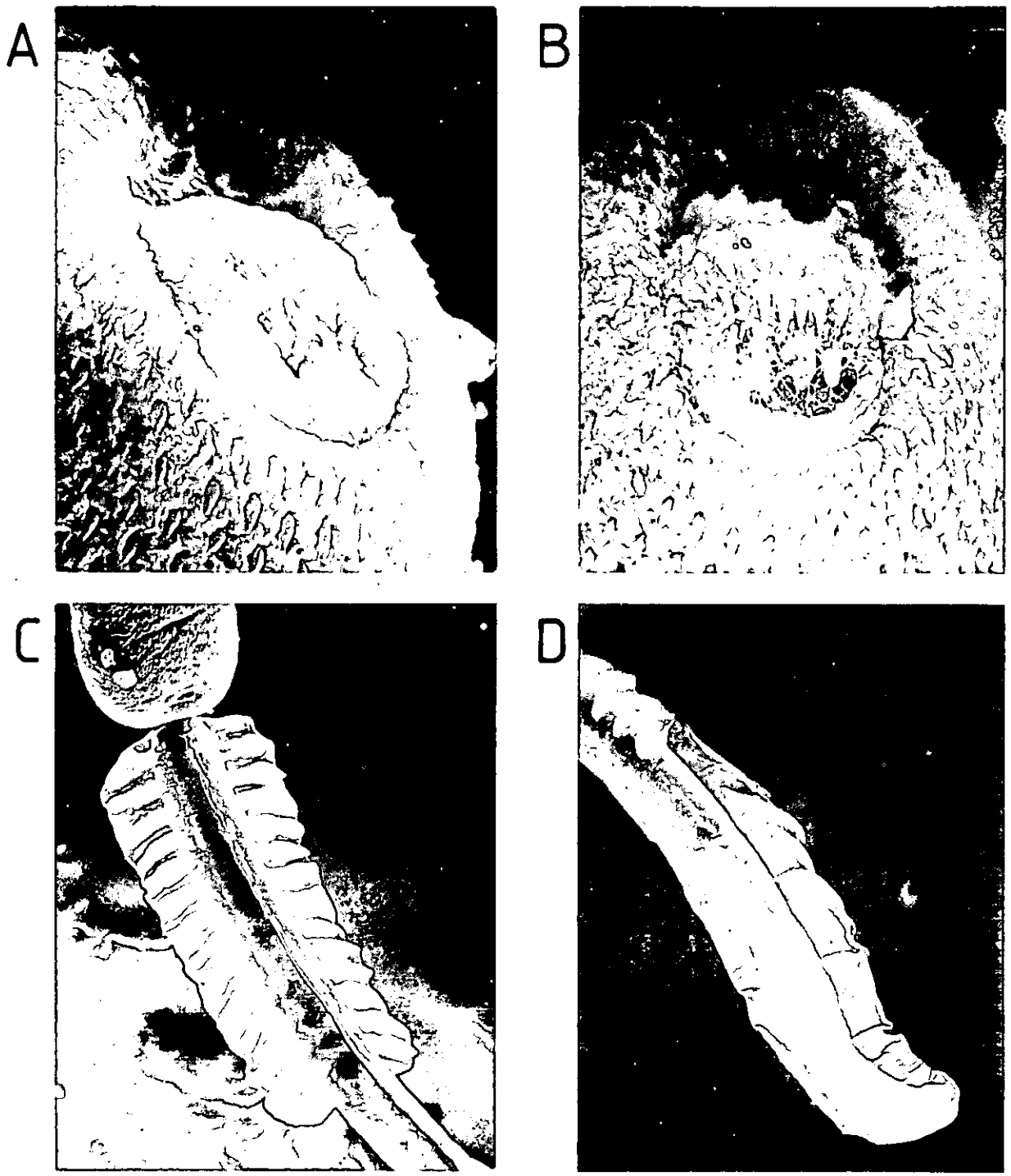


FIGURE 5.7 S.E.M. photographs - A, ventrolateral view of mouth and 'pre-oral' spines, ×5,250; B, ventral view of mouth, with distinctly raised lip, and 'pre-oral' spines, ×3,500; C, lateral tail finfolds, dorsal view, ×525; D, junction of lateral and dorso-ventral finfolds, lateral view, ×800.

longer than the ventral part, overlaps the anterior pair of finfolds. The latter are strengthened by fixed, regular convolutions, which are not present on the dorsoventral finfolds.

TABLE 5.6 *Heterophyid sp.A.* Dimensions of cercariae soon after emerging from the snail host (n = 10).

Body length	164 (137 - 201)
Body width	78 (68 - 87)
Body depth	55 (46 - 65)
Tail length	454 (423 - 484)
Tail width	30 (-)
Anterior finfold length	232 (217 - 247)
Oral sucker length	31 (30 - 34)
Oral sucker width	31 (27 - 34)
Eyespot diameter	14 (11 - 15)
Excretory vesicle length	43 (38 - 49)
Excretory vesicle width	58 (53 - 61)

In the laboratory the positively phototactic cercaria emerged from the host snail in the morning. It swam intermittently near the water surface, in brief bursts of up to 2 or 3 seconds. When swimming, the body was more or less horizontal, ventral uppermost, with the tail lashing from side to side, in a vertical plane. After swimming, the cercaria remained still and sank slowly, body leading, for up to several minutes. Swimming recommenced immediately if the cercaria, or the surrounding water, were physically disturbed. Preliminary investigations revealed that when cercariae are exposed to brown trout fingerlings, *(Salmo trutta)*, and mountain trout, *(Galaxias truttaceus)*, they attach themselves to the fish by the oral sucker and shed their tails within a few minutes. This response was not evidenced when, in separate experiments, cercariae were exposed to swimming amphipods, *Austrochiltonia australis*, and swimming ostracods, *Mytilocypris tasmanica*, or if they were prodded with a needle. The fish exposed to infection died within a few days, apparently due to the trauma of the experimental conditions and no metacercarial cysts were recovered.

5.3.2 Heterophyid sp.B

Redia: (Figure 5.8)

The elongate daughter redia is widest posteriorly and slightly attenuated anteriorly. It is quite motile and the tegument is pale yellow. A round terminal mouth opens into an oval pharynx; the short, saccate intestine is yellow. The brood chamber contains germ balls and developing cercariae - the degree of development increasing anteriorly. A birth canal opens through a pore, posterolateral to the pharynx. Large numbers of rediae occur throughout the host's viscera, from the hepatopancreas to the pallial oviduct, 210 being counted in one snail. The dimensions of the daughter rediae are shown in Table 5.7.

TABLE 5.7 *Heterophyid sp.B.* Dimensions of rediae, selected at random (n = 15).

Body length	505 (348 - 680)
Body width	87 (61 - 118)
Pharynx length	27 (25 - 30)
Pharynx width	30 (27 - 34)

Cercaria: (Figure 5.8)

The cercaria, similar to, but smaller than the cercaria of *Heterophyid sp.A*, is monostomate and oculate, with para-vesicular penetration glands and both lateral and dorso-ventral finfolds. Its dimensions are shown in Table 5.8. Body shape is variable, but usually elongate oval. The black pigmented eyespots are transversely elongate, situated about 1/3 body length from the anterior end. Peg-like tegumental spines are prominent on the anterior of the body, but diminish in size posteriorly. The oval oral sucker is recessed terminally, the anterior part forming a small conical protuberance. The mouth is on the ventral part of this protuberance and two transverse rows of large, closely-spaced "pre-oral" spines cover the dorsal part. The small round pharynx, about 8μ diameter, usually lies just posterior to the eyespots,

FIG. 5.8 Heterophyid sp.B

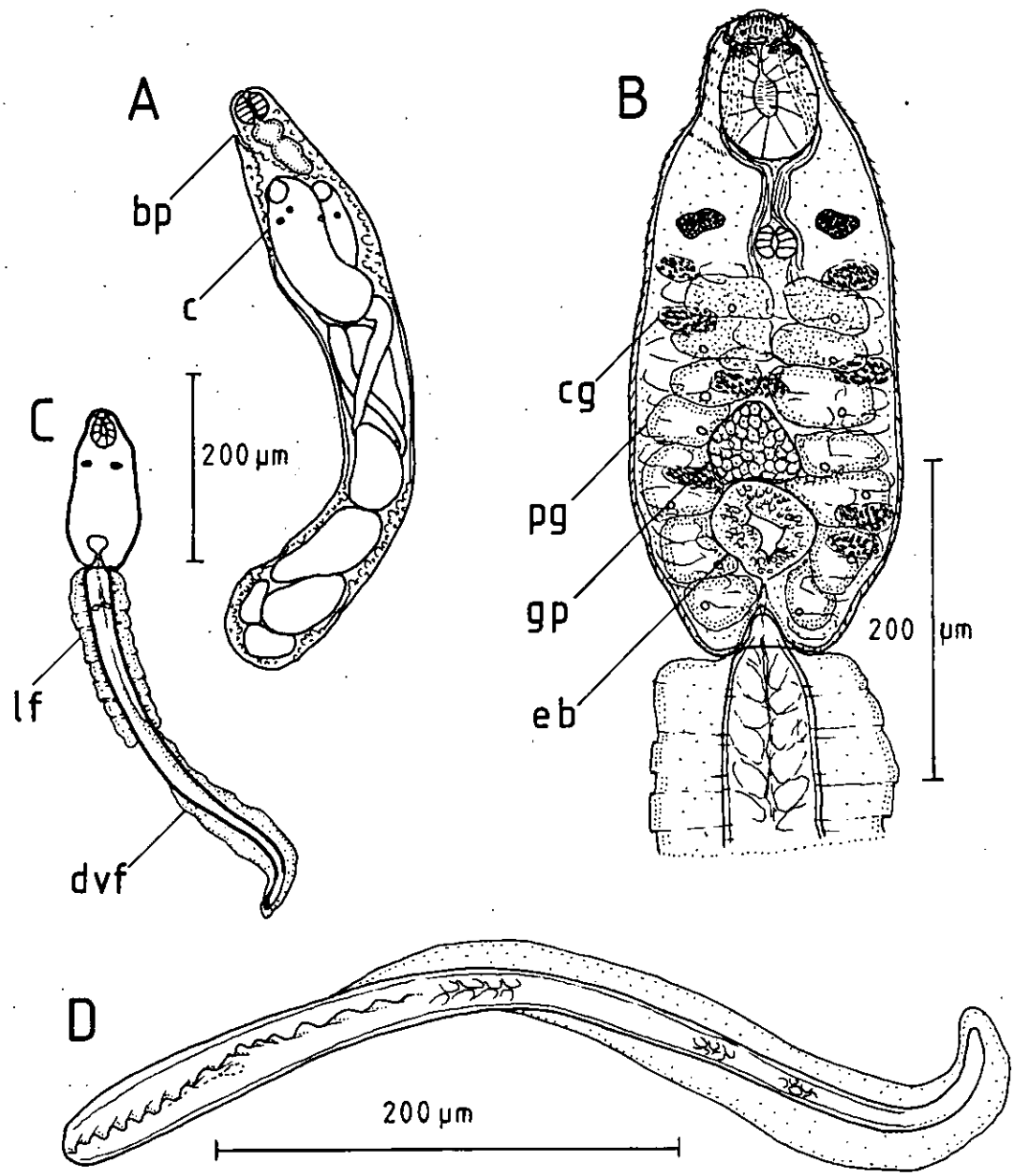


FIGURE 5.8 A, daughter redia; B, body of cercaria, slightly flattened; C, whole cercaria; D, tail of cercaria, lateral view. (bp: birth pore; c: cercaria; cg: cystogenous gland; dvf: dorso-ventral finfold; eb: excretory bladder; gp: genital primordium; lf: lateral finfold; pg: penetration gland.)

but is sometimes adjacent to the oral sucker. The oesophagus and caeca are not visible. Seven pairs of conspicuous penetration glands are arranged symmetrically in longitudinal rows on each side, from posterior to the eyspots, to the posterior end of the body. Their ducts pass anteriorly around the pharynx, between the eyespots and dorsal to the oral sucker. They separate over the oral sucker into 4 bundles, in a 3-4-4-3 arrangement. The inner bundles open medially and the outer bundles laterally, near the mouth. Granular cystogenous gland cells are packed into the body between the eyespots and the posterior extremity. The excretory bladder is more or less round and thick-walled. The caudal excretory duct is shaped like a short, inverted T. The genital primordium, adjacent to the anterior border of the bladder, measures about 17 × 14μ. The tail is slender and attached to the body in a terminal socket. Anterior, lateral finfolds extend only about 2/5 of the tail length from the junction with the body. Asymmetrical dorso-ventral finfolds extend along the posterior part of the tail. The dorsal part of the finfold is longer than the ventral part and overlaps the anterior pair of finfolds. The anterior finfolds have fixed regular folds, which look like rays or bristles, at low magnification.

The patterns of emergence, and swimming behaviour, were the same as those of the cercaria of *Heterophyid sp.A*.

TABLE 5.8 *Heterophyid sp.B*. Dimensions of cercariae, soon after emerging from the snail host (n = 15).

Body length	136 (122 - 160)
Body width	53 (49 - 57)
Body depth	38 (34 - 42)
Tail length	366 (325 - 431)
Tail width	23 (21 - 25)
Anterior finfold length	150 (141 - 167)
Oral sucker length	22 (19 - 23)
Oral sucker width	19 (15 - 23)
Eyespot length	7 (5 - 10)
Excretory vesicle length	20 (15 - 23)
Excretory vesicle width	17 (13 - 23)

5.3.3 *Heterophyid sp.C*

Redia: (Figure 5.9)

The daughter redia is elongate and cylindrical. Its tegument is slightly yellow. The terminal mouth opens into a small, round pharynx; the intestine is short and inconspicuous. The brood chamber contains germ balls and developing cercariae. No birth canal was seen. Rediae occur throughout the viscera of the host snail. Their dimensions are shown in Table 5.9.

TABLE 5.9 *Heterophyid sp.C.* Dimensions of rediae selected at random (n = 10).

Body length	369 (312 - 403)
Body width	76 (53 - 114)
Pharynx length	20 (19 - 23)
Pharynx width	19 (15 - 21)

Cercaria: (Figure 5.9)

The cercaria is monostomate and oculate, with pre-vesicular penetration glands and no finfolds. It is similar in anatomy and morphology to the cercariae of *Heterophyid spp.A* and *B*, except for the absence of finfolds on the tail. The body is elongate oval to pyriform. Transversely elongate, black pigmented eyespots, about 10 × 6μ, are situated about 1/3 body length from the anterior end. Small, simple tegumental spines are distributed over the body and are more prominent anteriorly. The oral sucker is round and the mouth subterminal-ventral. Two rows of 5 or 6 large, "pre-oral" spines are located anterior to the mouth. The pharynx is weakly developed. Seven pairs of penetration gland cells are located in two clusters in the middle 1/3 of the body. Their ducts pass anteriorly between the eye-spots and dorsally over the oral sucker. They separate into two bundles, in a 3-4-4-3 arrangement and open anterodorsally to the mouth. Round cystogenous glands, about 8μ diameter, containing coarse, oblong

FIG. 5.9 Heterophyid sp.C

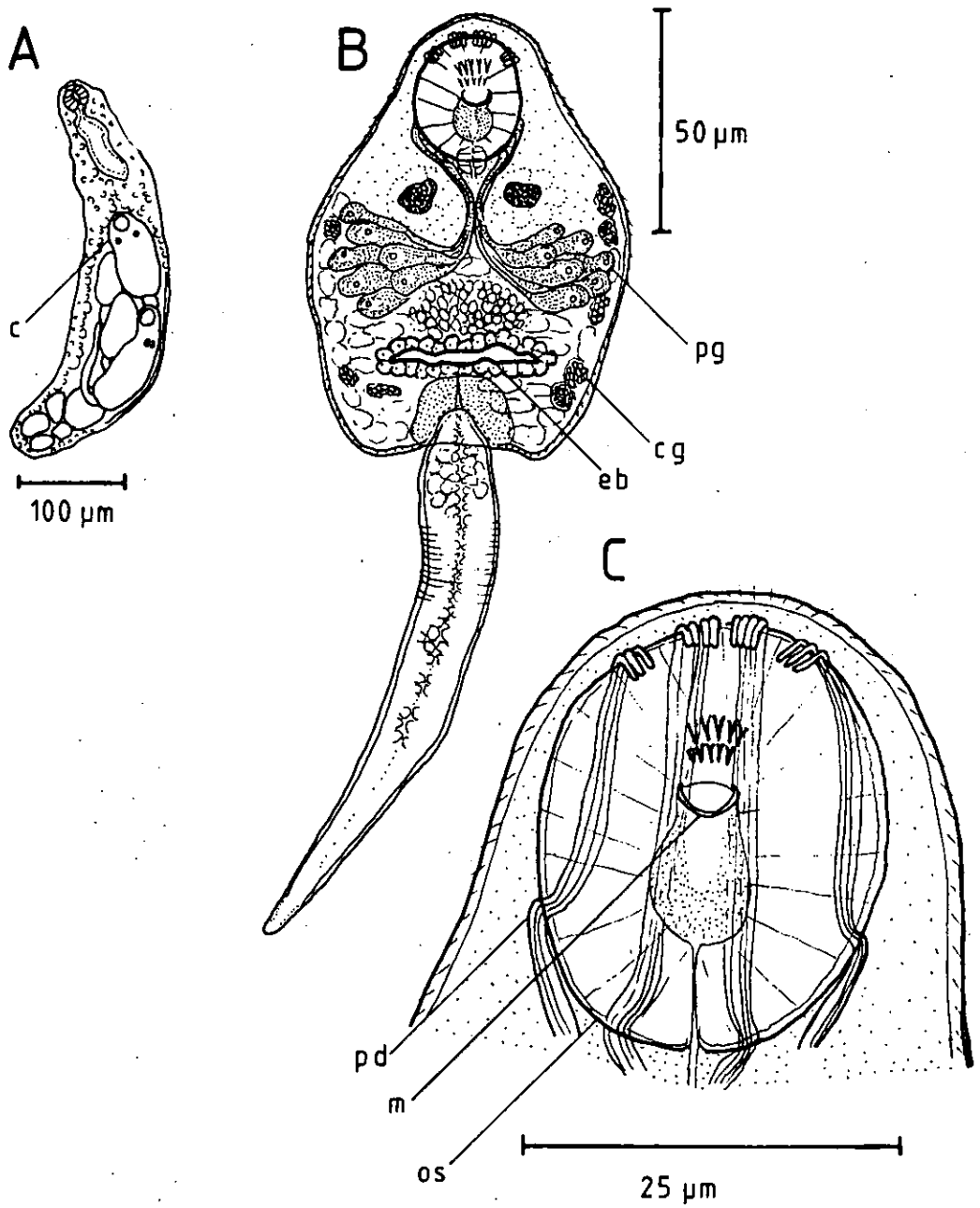


FIGURE 5.9 A, daughter redia; B, whole cercaria, ventral view, slightly flattened; C, oral sucker region of cercaria, ventral view, showing terminal part of penetration gland ducts. (c: cercaria; cg: cystogenous gland; eb: excretory bladder; m: mouth; os: oral sucker; pd: penetration gland ducts; pg: penetration gland.)

grains, are distributed from the level of the eyespots to the posterior end of the body. The genital primordium, about 18μ diameter, is situated just anterior to the thick-walled excretory bladder, which varies from V to X-shaped. The tail, which has fine tegumental annulations, is slightly longer than the body and joins the body in a subterminal-ventral socket.

TABLE 5.10 *Heterophyid sp.C.* Dimensions of cercariae, soon after emerging from the snail host (n = 15).

Body length	92 (84 - 106)
Body width	63 (53 - 70)
Tail length	111 (99 - 120)
Tail width	16 (15 - 17)
Oral sucker length	24 (23 - 27)
Oral sucker width	23 (21 - 23)

Swimming behaviour was observed to be similar to that of the cercariae of *Heterophyid spp. A* and *B*, however the cercaria of *Heterophyid sp.C* spent more time swimming and less time resting and sinking, than the other species. Newly emerged cercariae swam for about 20 seconds and then rested, and sank, for only up to about 5 seconds. This difference in the activity of the heterophyid cercariae was probably related to the possession or absence of tail finfolds, with the simple-tailed cercaria of *Heterophyid sp.C* sinking at a faster rate than the cercariae of *Heterophyid spp.A* and *B*, which have lateral and dorso-ventral finfolds.

5.3.4 Discussion

The cercariae of *Heterophyid spp.A* and *B* are similar, but differ in several respects. The former is more massive and its oral sucker, eyespots and excretory bladder, are relatively larger than those of the latter. Other differences between these cercariae are in the arrangement of the penetration glands and the relative length of the lateral finfolds on the tail. Several species in the genera *Euhaplorchis*, *Haplorchis* and *Procerovum* are known to have cercariae, like those of

Heterophyid spp.A and *B*, with para-vesicular penetration glands and both lateral and dorso-ventral tail finfolds (Pearson, 1973; Yamaguti, 1975). The cercaria of *Heterophyid sp.A* closely resembles that of *Euhaplorchis californiensis*, (Martin, 1950), which develops in a marine snail in California, encysts in a fish and infects gulls and other piscivorous birds. The cercaria of *Heterophyid sp.B* appears closest to that of *Haplorchis taichui*, (Martin, 1958), which develops in a freshwater snail in Hawaii, encysts in a fish, and infects herons.

The cercaria of *Heterophyid sp.C* is quite distinct from the other heterophyid cercariae developing in *C. badgerensis*, in that its tail is much shorter and devoid of finfolds. Several species of *Centrocestus* have been shown to have cercariae of this type (Yamaguti, 1975). The cercaria of *Heterophyid sp.C* closely resembles that of *C. formosanus*, a trematode which develops in freshwater snails, encysts in fish and frogs, and utilizes herons and rats as definitive hosts in China, Taiwan and Hawaii (Yamaguti, 1975).

5.4 Family STRIGEIDAE

5.4.1 *Apatemon (Apatemon) gracilis* (Rudolphi, 1819), Szidat 1928

Sporocyst: (Figure 5.10)

The daughter sporocyst is colourless, motile and vermiform, and is distributed throughout the viscera of the snail host. Each sporocyst contains large numbers of mature and developing cercariae and germ balls. The anterior end is very active, stretching and twisting among the snail tissues. The dimensions vary greatly, from 638 to 3480μ long, and from 99 to 133μ wide. No birth pore was seen, however an incomplete birth canal is evident at the anterior end.

Cercaria: (Figure 5.10)

The cercaria is a pharyngeate, longifurcate, furcocercous, distome,

FIG. 5.10 Apatemon gracilis

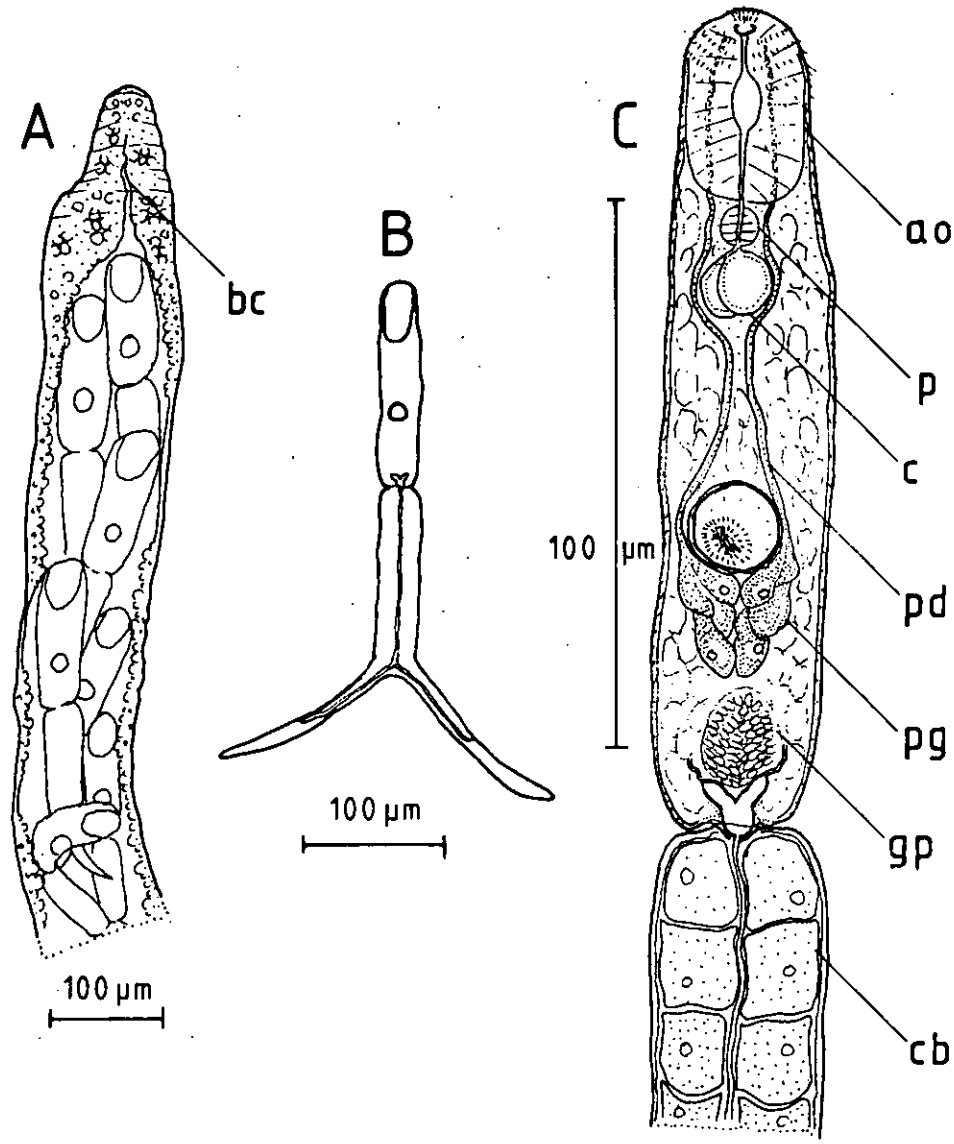


FIGURE 5.10 A, daughter sporocyst, anterior end; B, whole cercaria, ventral view; C, detail of body of cercaria and anterior part of tail stem. (ao: anterior organ; bc: birth canal; c: caecum; cb: caudal body; gp: genital primordium; p: pharynx; pd: penetration gland ducts; pg: penetration gland.)

without pigmented eyespots. Its dimensions are presented in Table 5.11.

TABLE 5.11 *Apatemon (Apatemon) gracilis*. Dimensions of cercariae, soon after emerging from the snail host (n = 10).

Body length	148 (137 - 160)
Body width	35 (32 - 38)
Tail stem length	131 (110 - 148)
Tail stem width	33 (30 - 36)
Tail furca length	149 (141 - 160)
Tail furca width	13 (11 - 15)
Anterior organ length	34 (30 - 38)
Anterior organ width	22 (19 - 25)
Ventral sucker length	17 (15 - 19)
Ventral sucker width	19 (17 - 19)

The body is elongate oblong. The anterior organ is oblong. A small subterminal ventral mouth opens into a narrow prepharynx which passes through the anterior organ to a small oval pharynx, about 8 × 10μ. Immediately posterior to the pharynx a short oesophagus gives rise to two bulbous caeca. The body tegument is spinous, spines being more prominent over the anterior half of the anterior organ. An 'apical tuft' of closely-spaced spines is arranged in an arc anterior to the mouth. No spines are evident on the tail. An indeterminate number of granular penetration glands are clustered posterolateral and posterior to the ventral sucker. Ducts from these glands pass anteriorly in two bundles, entering the anterior organ posterolaterally, and opening on either side of the mouth. The middle of the ventral sucker, which is situated in the posterior half of the body, is encircled by three concentric rings of large inwardly directed spines. The tail stem is similar in size to the body, and tail furcae are slightly longer than the tail stem. Flame-cells were not observed. The excretory bladder is Y-shaped, with short divergent arms. A caudal excretory canal passes axially along the length of the tail stem and then bifurcates and opens just over halfway along the margin of each furca. Six pairs of different-sized 'caudal bodies' are symmetrically arranged along the length of the tail stem. A zone of small, uniform cells anterior to the excretory bladder, may be the genital primordium.

After emerging from the snail host, the cercaria swam very strongly and directly, with the body and tail in the same plane, quite unlike the swimming behaviour of any other cercaria seen during the present study. A standing wave was caused by the oscillating tail, with nodes at the junctions of the tail stem with the body, and with the furcae. The cercaria slowly spiralled as it moved rapidly through the water like a torpedo. The cercaria, which was not phototactic, swam continuously for at least 43 hours in lagoon water at 15°C.

5.4.2 Discussion

Blair (1977), presented a key to the cercariae of British strigeoids, for which the life-cycles are known. The strigeoid cercaria developing in *Coxiella badgerensis* keys out to the subgenus *Apatemon (Apatemon)*, and corresponds very closely with the description of the cercaria of *A. (A.) gracilis*, presented by Blair (1976).

A black duck feeding at Calvert's Lagoon was found to be infected by adults of *A.(A.) gracilis*. Encysted metacercariae of this trematode infect a galaxiid fish at Lake Crescent, about 100 km from Calvert's Lagoon (Appendix 2). On the basis of this circumstantial evidence, it is concluded that the strigeid intramolluscan developmental stages in *C. badgerensis* probably belonged to *A. (A.) gracilis*. Such an infection of *C. badgerensis* was only found on one occasion during the present study.

Chapter 6 *IN VITRO* CULTURE OF SOME MICROPHALLID METACERCARIAE

6.1 Introduction

During the last quarter of a century, *in vitro* culture of helminths has increasingly been used as an experimental tool. The commercial production of defined culture media (e.g. Medium 199 and Eagles MEM), and complex undefined media components (e.g. foetal calf serum and red blood cells), has allowed *in vitro* culture to be readily undertaken in parasitological laboratories around the world, and to be taught in undergraduate courses. In particular, *in vitro* culture facilitates study of the physiology and biochemistry of helminths in isolation from their hosts; research into their reaction to the immune response of their hosts, and tests to determine the effects and usefulness of antihelminthic drugs (Davies, 1976). Another application of *in vitro* culture is to the elucidation of trematode life-histories. Adults of some trematode species can be cultured to egg production from metacercariae with relatively little expenditure of time, effort or resources. This technique is often more convenient than experimental infection of laboratory hosts. It also enables the live fluke to be studied directly, providing information about the parasite's possible definitive host, habitat and *in vivo* form and behaviour.

In the present study, the metacercariae of 7 microphallid species have been cultured *in vitro*: *Atriophallophorus coxiellae*, *Gynaecotyla hickmani* n.sp., *G. macrocotylata* n.sp., *Levinseniella tasmaniae*, *Maritrema calvertensis*, *M. eroliae* and *Microphallus paragrapsi* n.sp.

The literature on *in vitro* cultivation of digenetic trematodes has been reviewed by Silverman (1965), Clegg and Smyth (1966 and 1968), Smyth (1966 and 1976), Taylor and Baker (1968), Silverman and Hansen (1971) and Davies (1976). Attempts to culture metacercariae to maturity have had varied results depending largely on the degree of sexual development of the metacercariae at the time of excystment. There is a continuous

range of variation of digenetic trematodes, from species such as *Coitocaecum anaspidis*, in which eggs are produced within the metacercarial cyst in the crustacean intermediate host (Hickman, 1934), to *Diplostomum spathaceum*, in which the metacercaria is completely lacking in genital primordia (Kannangara and Smyth, 1974). Most examples of metacercariae developing *in vitro* to egg-producing adults have involved species whose metacercariae are sexually advanced, or at least possess pre-formed genital primordia, such as the psilostome *Sphaeridiotrema globulus*, (Berntzen and Macy, 1969), the strigeid *Cotylurus lutzi*, (Basch, DiConza and Johnson, 1973), and the gymnophallid *Parvatrema timondavidi*, (Yasuraoka *et al.*, 1974). However, the metacercariae of *Diplostomum phoxini* and *D. spathaceum*, which lack genital rudiments, have also been cultured to egg production in recent years (Kannangara and Smyth, 1974). The *in vitro* maturation of these metacercariae involves considerable growth and development, and requires complex media containing undefined components such as egg macerate. Culture of such indifferentiated metacercariae presents more fundamental problems than culture of metacercariae containing genital primordia (Smyth, 1976).

Excysted metacercariae of microphallid species are generally sexually advanced and of approximately adult size. Several microphallids have been cultured *in vitro* to egg production using relatively simple media. The most successful culture medium for *Gynaecotyla adunca* was found to be 1% seawater, even though various more complex media were tried (Hunter and Chait, 1952). Sperm appeared within the cirrus sac from 1 to 3 hours after excystment, eggs were formed after 10 to 12 hours incubation at 40°C, and normal eggs were shed after 80 hours. Cultured worms survived for up to 8 days. Smyth (1966), reported that *Microphallus papillorobustus* will produce eggs after 4 hours incubation in seawater. When *Microphallus pygmaeus* was cultured in Medium 199 plus 10% bovine serum at 37°C, egg production commenced after 5 days, and a

maximum of 20 eggs was produced after 8 days (James, 1971). *Microphalloides japonicus* has been cultured to egg production in a variety of media (Fujino *et al.*, 1977). In each case sperm appeared in the seminal vesicle after about 12 hours and eggs appeared after about 24 hours. The maximum production of eggs occurred in Eagles MEM plus 20% inactivated calf serum, with an average of 94 eggs per fluke after 5 days. *Microphallus similis* has been cultured *in vitro* in a wide variety of media, at 38 to 41°C (Davies and Smyth, 1979). In Hank's Balanced Salt Solution and NCTC 135, flukes died within 3 days; however gametogenesis and spermatogenesis occurred, and a few apparently normal eggs were produced in a small proportion of worms. When serum was present in the culture medium, the worms survived for up to 30 days. On the fourth day of culture, the greatest percentage of flukes with normal eggs was found in NCTC 135 plus 20% foetal calf serum, and the maximum number of normal eggs per fluke in this medium was 44. The addition of various other undefined components, such as mouse intestinal mucosal extract, red blood cells, yeast and egg, was not beneficial. When normal eggs were produced *in vitro* it was only for the first 1 or 2 days, and thereafter abnormal eggs were formed. It appeared that premature tanning of vitelline droplets occurred in the vitellaria and vitelline ducts which prevented the normal production of eggs. Similar observations were made during the cultivation of *Fasciola hepatica*,(Clegg, 1957), *Microphalloides japonicus*,(Fujino *et al.*, 1977) and *Meiogymnophallus minutus*,(Davies, 1976).

The basic aim of *in vitro* cultivation of metacercariae is to provide conditions that permit growth and development to proceed at normal rates, leading to the production of viable eggs. When the *in vivo* developmental process of a species is known it may be subdivided into stages that can be used as criteria for assessing *in vitro* development. Bell and Smyth (1958), suggested that these stages should be precisely definable, readily recognizable, and cover the whole range of the maturation

process. They defined 7 stages to assess the *in vitro* development of the strigeoid *Diplostomum phoxini:* cell multiplication, body shaping, organogeny, early gametogeny, late gametogeny, egg shell formation and vitellogenesis and oviposition. Kannangara and Smyth (1974), found that a slightly modified set of criteria were more useful in assessing the development of *D. phoxini* and *D. spathaceum*. Although the development criteria described for strigeoids can be applied to other trematodes, the maturation process of different trematodes is so varied that it is preferable to determine an appropriate set of criteria for each family. Davies (1976), assessed the *in vitro* development of the microphallid *Microphallus similis* according to 8 simple criteria:

1. The day when the vitellaria become positive for egg-shell precursors (i.e. positive when treated with Fast Red Salt B or with Catechol).
2. The day when sperm are first seen in the testes.
3. The day when sperm are first seen in the vesicula seminalis.
4. The day when the vitelline reservoir is formed.
5. The day when normal eggs are first produced.
6. The maximum number of normal eggs produced.
7. The maximum number of abnormal eggs produced.
8. The day oviposition first occurs.

The same system has been used in the present study of 7 microphallid species.

6.2 Results (Tables 6.1 to 6.7)

The excystment of metacercariae *in vitro* is described separately for each species with the descriptions of their life-histories (Chapter 2 and Appendix 3). In general, excystment of the microphallids studied occurs at an optimum temperature of about 40 to 42°C; the excystment process involves an initial passive phase, during which the cyst wall is

TABLE 6.1 *Maritrema calvertensis*

Medium	Replicates	Excysted Metacercariae	Longevity (days)	Criteria (p.290) 1	2	3	4	5	6	7	8	Comments
Eagles MEM	2	30	2	1	1	1	1	2	8	2	-	
Eagles MEM plus 20% FCS.	2	30	2	1	1	1	1	-	-	-	-	
Eagles MEM plus 40% FCS.	2	30	2	1	1	1	1	-	-	2	-	Vitellaria abnormal after 2 days - large tanned granules.
Medium 858 plus 30% FCS.	2	20	8	1	1	1	1	-	-	6	-	Vitellaria and eggs very abnormal after 2 days. Dispersed tanned granules in uterus.
In vivo development in ducklings	11	1650	19	1	1	1	1	1	182	-	-	On average, 18 eggs per fluke after 1 day.

TABLE 6.2 *Levinseniella tasmaniae*

Medium	Replicates	Excysted metacercariae	Longevity (days)	Criteria (p. 290) 1	2	3	4	5	6	7	8	Comments
Hank's BSS	10	100	2	1	1	1	1	1	175	8	-	
Eagles MEM	5	50	2	1	1	1	1	1	108	-	-	
Eagles MEM plus 20% FCS.	2	20	2	1	1	1	1	1	188	6	2	Relatively few abnormal eggs
Eagles MEM plus 40% FCS.	2	20	2	1	1	1	1	1	35	15	-	High percentage of abnormal eggs
Medium 858 plus 30% FCS.	2	20	8	1	1	1	1	1	105	42	-	Abnormal vitellaria after 3 days - tanned dispersed granules
In vivo development in ducklings	3	225	2	1	1	1	1	1	186	-	-	186 eggs were produced by one fluke

TABLE 6.3 *Atriophallophorus coxiellae*

Medium	Replicates	Excysted metacercariae	Longevity (days)	Criteria (p. 290) 1	2	3	4	5	6	7	8	Comments
Hank's BSS	2	50	2	1	1	1	1	2	3	1	-	
Eagles MEM	2	50	2	1	1	1	1	2	2	2	-	
Eagles MEM plus 30% FCS.	10	250	6	1	1	1	1	1	1	1	2	After 3 days vitellaria very abnormal - tanned dispersed granules
Medium 858 plus 30% FCS.	2	50	9	1	1	1	1	2	1	1	-	ditto
NCTC 135 plus 30% FCS.	5	125	7	1	1	1	1	2	3	2	-	ditto
In vivo development in ducklings	7	1400	12	1	1	1	1	1	62	-	-	On average, 12 eggs per fluke after 1 day

TABLE 6.4 *Gynaecotyla hickmani* n.sp.

Medium	Replicates	Excysted metacercariae	Longevity (days)	Criteria (p. 290) 1	2	3	4	5	6	7	8	Comments
Eagles MEM	10	100	6	1	1	1	1	1	12	36	4	Body and vitellaria normal after 6 days
Eagles MEM plus 20% FCS.	5	50	7	1	1	1	1	1	112	22	4	Flukes healthy after 6 days. Some abnormal eggs formed on Day 3.
Eagles MEM plus 40% FCS.	2	20	7	1	1	1	1	1	0	75	4	All eggs very abnormal. Flukes still active, normal after 6 days.

TABLE 6.5 *Gynaecotyle macrocotylata* n.sp.

Medium	Replicates	Excysted metacercariae	Longevity (days)	Criteria (p.290) 1	2	3	4	5	6	7	8	Comments
Eagles MEM plus 20% FCS.	2	10	7	1	1	1	1	1	20	28	-	After 3 days, most eggs abnormal.

TABLE 6.6 *Maritrema eroliae*

Medium	Replicates	Excysted metacercariae	Longevity (days)	Criteria (p.290) 1	2	3	4	5	6	7	8	Comments
Hank's BSS	5	25	2	1	1	1	1	1	75	0	-	
Eagles MEM	5	25	5	1	1	1	1	1	215	0	-	
Eagles MEM plus 20% FCS.	2	10	7	1	1	1	1	-	0	96	-	All eggs abnormal
Eagles MEM plus 40% FCS.	2	10	6	1	1	1	1	1	30	122	-	Most eggs abnormal. Some normal eggs formed initially.
In vivo development in ducklings	1	20	1	1	1	1	1	1	52	0	-	One fluke contained 52 eggs after 17 hours.

TABLE 6.7 *Microphallus paragrapsi* n.sp.

Medium	Replicates	Excysted metacercariae	Longevity (days)	Criteria (p.290) 1	2	3	4	5	6	7	8	Comments
Eagles MEM	5	100	4	1	1	2	-	-	0	0	-	
Eagles MEM plus 20% FCS.	4	80	7	1	1	2	2	2	4	9	-	Vitellaria normal after 2 days - tanned and dispersed after 6 days
Eagles MEM plus 40% FCS.	4	80	7	1	1	2	2	-	0	11	-	Vitellaria tanned after 2 days - all eggs very abnormal.
In vivo development in ducklings	3	300	2	-	-	-	-	-	0	0	-	One very immature metacercaria survived for 26 hours.

dissolved or weakened by exogenous digestive enzymes and/or bile salts, and an active phase, during which the cyst wall is ruptured by vigorous movements of the metacercaria, possibly aided by enzymes of parasite origin. The percentage of metacercariae excysting, and their rate of development *in vitro*, is related to the age of the cysts. The results presented below are based on the more advanced metacercariae of each species. The number of experiments performed was small, and the number of metacercariae of each species used in each experiment varied depending on their availability. The number of replicate cultures and the approximate total number of metacercariae used, is indicated with each set of results. The maximum recorded longevity (i.e. the longest period that any individual survived) in each culture medium is also shown. Where possible, the *in vivo* development of the microphallid species in laboratory ducklings is presented for comparison with the *in vitro* results.

6.3 Discussion

Changes in the behaviour and appearance of the microphallids occurred during the period of culture, similar to those described for *Microphallus similis* (Davies, 1976). Initially, the flukes were active, continuously stretching and creeping. Eventually, however, their movements became irregular and erratic, before they became moribund and usually curled ventrally (Figure 6.1). Cultured worms frequently became darker in appearance. Although this was partly related to their degree of contraction, it may also have been due to accumulation of lipid droplets in degenerating cells (Davis, 1976). "Tegumental bubbles" frequently seen by Davies (1976) on the surface of *Microphallus similis* during culture, were only seen in this study on flukes that were obviously moribund.

Although the excysted metacercariae of microphallids approximate adult size and are sexually relatively advanced, each of the species

FIG. 6.1 In vitro culture

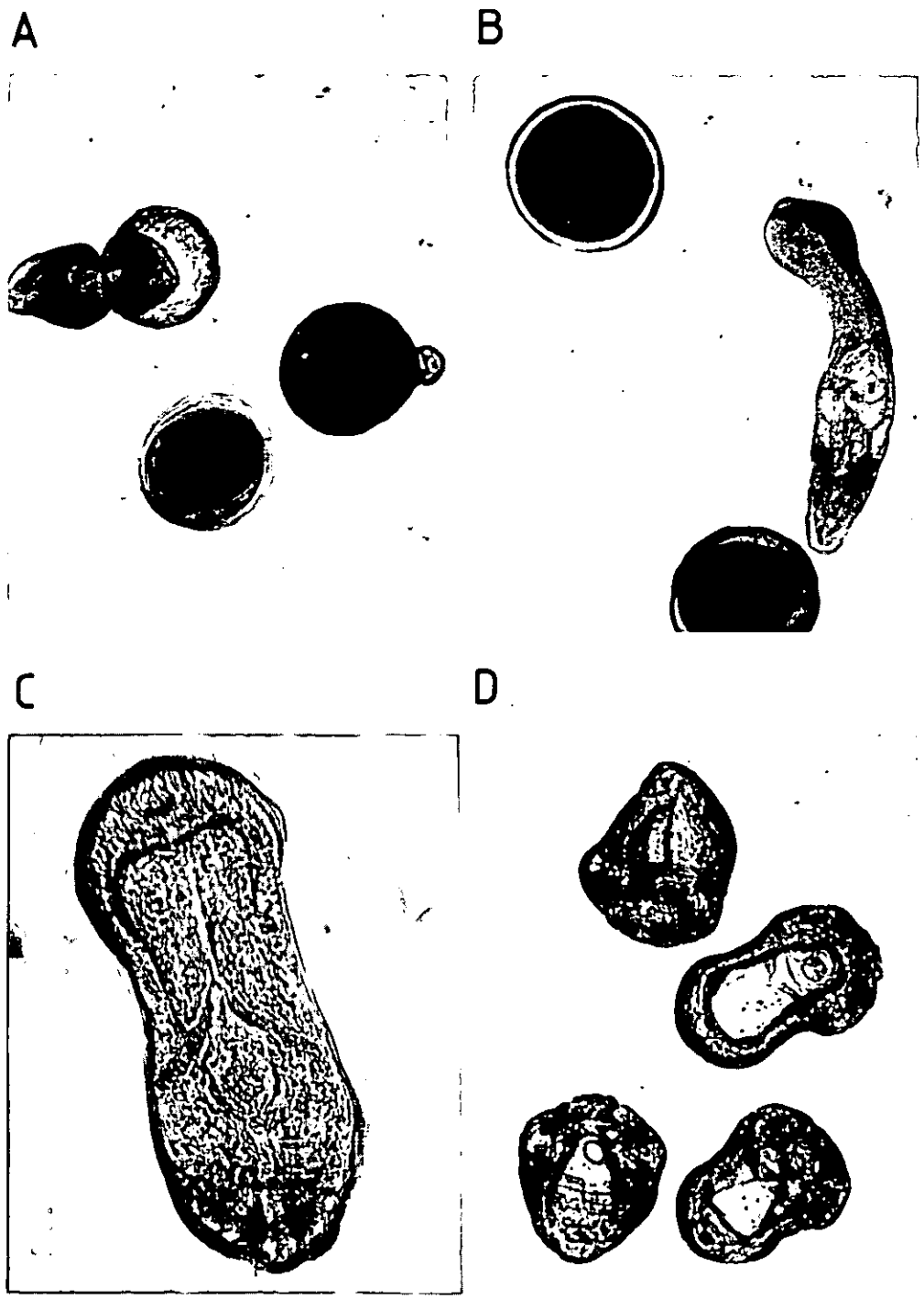


FIGURE 6.1 Photographs taken under inverted microscope at about 39°C - A, three cysts of *Gynaecotyla* sp., one metacercaria excysting, ×40; B, excysted metacercaria of *Gynaecotyla ? hickmani* n.sp., ×40; C, actively creeping excysted metacercaria of *Microphallus paragrapsi* n.sp., after 1 day in Eagles MEM, ×100; D, ventrally curled moribund specimens of *M. paragrapsus* n.sp., after 3 days in Eagles MEM, ×40.

studied exhibited some degree of *in vitro* development. Morphologically normal eggs were produced in one or more culture media by each species, however the viability of the eggs is unknown. Cell division of ova *in utero* was not observed. A similar lack of embryonation has been recorded with eggs produced *in vitro* by *Microphallus similis* (Davies and Smyth, 1979). In most cultures an initial period of producing apparently normal eggs was followed by production of abnormal eggs. In each case, production of abnormal eggs was associated with the appearance of yellow-orange, tanned granules within the vitelline ducts, and deformed granular vitelline glands. Although all 3 types of abnormal eggs described by Davies and Smyth (1979), were observed, the most common was "type 3", i.e. an unshelled mass of tanned vitelline materials with no associated ovum.

In general, the development and survival rates of worms were increased by the inclusion of foetal calf serum in the culture medium, with an optimum concentration of about 20%. High proportions of abnormal eggs were produced by all flukes cultured in 40% foetal calf serum, and preliminary experiments have shown that survival and development in 80% and 100% foetal calf serum is very poor. Davies and Smyth (1979), found that when defined culture media (Hank's BSS and NCTC 135), were supplemented by the addition of 20% foetal calf serum, *Microphallus similis* survived for longer periods (up to 30 days), had a more normal appearance and egg production was increased.

The *in vivo* patterns of development of the 3 species from Calvert's Lagoon, *Atriophallophorus coxiellae*, *Levinseniella tasmaniae* and *Maritrema calvertensis*, are known from experimentally infected ducklings. The longest recorded period of survival of specimens of *A. coxiellae* and *M. calvertensis* in laboratory ducklings was 12 and 19 days respectively, however, the longest recorded period of survival of specimens of *L. tasmaniae* in these hosts was only 2 days. The maximum recorded longevity of *L. tasmaniae in vitro* was 8 days; however, flukes were relatively

moribund after the first 2 or 3 days, and such loss of vigour *in vivo* probably results in expulsion from the host. *L. tasmaniae* produced eggs *in vitro* and *in vivo* at much faster rates than the other species. Egg production by *L. tasmaniae* occurred *in vitro* at a more or less normal rate, even in a balanced salt solution (Hank's BSS). The results indicate that *L. tasmaniae* relies on endogenous energy sources to produce eggs rapidly and briefly in the definitive host. The rates of egg production of *A. coxiellae* and *M. calvertensis* were far less *in vitro* than *in vivo*. These species inhabit the lower small intestine of the same bird hosts, and probably have similar habitat requirements, that were not met by the conditions of *in vitro* culture. In general, very short lived microphallid adults, like *L. tasmaniae*, probably rely on endogenous food reserves to produce eggs within the definitive host, whereas relatively long lived adults like those of *A. coxiellae* and *M. calvertensis* require food from the host for normal egg production.

The *in vivo* patterns of development of the 4 species from the estuarine crab *Paragrapsus gaimardii* are not known. They were, in general, not infective to laboratory ducklings (Appendix 3). *In vitro*, *M. eroliae* produced morphologically normal eggs rapidly in defined media (Hank's BSS and Eagles MEM), in fact, more rapidly without serum supplements than when serum was included. The adult of this species may, like *L. tasmaniae*, rely on endogenous food reserves to produce eggs rapidly after entering the definitive host, and be relatively short lived. Both *Gynaecotyla* species initially produced many apparently normal eggs in medium containing 20% serum. The results are too limited to suggest how long *G. macrocotylata* n.sp. lives in its definitive host; however, the observation that *G. hickmani* n.sp. produced eggs at a faster rate when the culture medium was supplemented with serum, indicates that it may be able to utilize an exogenous energy source, and may therefore be relatively long lived. *Microphallus paragrapsi* n.sp. produced a few morphologically normal eggs only when serum was included in the culture medium, which suggests that this species may also be relatively long lived in its

definitive host, and require complex food from its environment for normal egg production.

The results of these preliminary experiments on *in vitro* culture of 7 microphallid species show that although microphallid metacercariae are relatively easily cultured to egg production, there is considerable variation within and between species. Variation within species was largely due to the different age of metacercarial cysts used in experiments and consequent differences in the state of development of excysted metacercariae prior to culture. Variation between species was due to differences in the state of development reached by encysted metacercariae, and the habitat requirements of the adult stages. Microphallid species with adults that are relatively short lived, have simple, if any, nutritional requirements and are easily cultured *in vitro*. There is probably a continuous range of variation from such species to those with adults that live for much longer, have more complex nutritional and physical habitat requirements and are more difficult to culture *in vitro*. Future attempts to culture microphallids could give more consideration than was given in the present study, to the physical needs of adult microphallids, particularly long-lived species, perhaps by providing irregular artificial surfaces within the culture vessel to present a range of microhabitats similar to those experienced *in vivo*.

PART V

Chapter 7 EPIDEMIOLOGY OF TREMATODE INFECTIONS OF THE FAUNA AT CALVERT'S LAGOON

At Calvert's Lagoon, a variety of different invertebrates and birds serve as hosts in the life-cycles of trematodes. The only snail in the lagoon, *Coxiella badgerensis*, is primary intermediate host to all of the trematodes. It also serves as secondary intermediate host to some species whose cercariae either encyst without emerging from the snail, or emerge and then invade and encyst within the same, or another, snail. The amphipod *Austrochiltonia australis* and the ostracod *Mytilocypris tasmanica*, both serve as second intermediate hosts. Adult flukes inhabit the numerous bird species which live and feed at the lagoon.

7.1 Coxiella badgerensis (Johnston, 1878) Hedley, 1904

syn. *Pomatiopsis badgerensis* Johnston, 1878

(Order Prosobranchia; Superfamily Rissoidea; Family Hydrobiidae)

7.1.1 Description (Figure 7.1)

The original species description was based on shells of subrecent deposits on Badger Island, in the Furneaux Group, Bass Strait:

> "*Shell pyramidal, generally decollate, thin, scarcely opaque, pale fleshy white, inside tinted reddish brown; whorls, prior to being decollated, usually 7, subsequently average 5; decussate with irregularly raised lirae, and indistinct varices; suture deeply impressed, aperture roundly ovate, peristome continuous, margin somewhat thickened, inflated, and reflected; inner lip conspicuously reflected,*" (Johnston, 1878).

The shell is very similar to that of *Coxiella striata*, which lives in saline water bodies in the South East of mainland Australia. Smith and Kershaw (1979), consider *C. badgerensis* to be a synonym of *C. striata*. A Ph.D study of the taxonomy of *Coxiella* and the physiology and reproductive

FIG. 7.1 Coxiella badgerensis

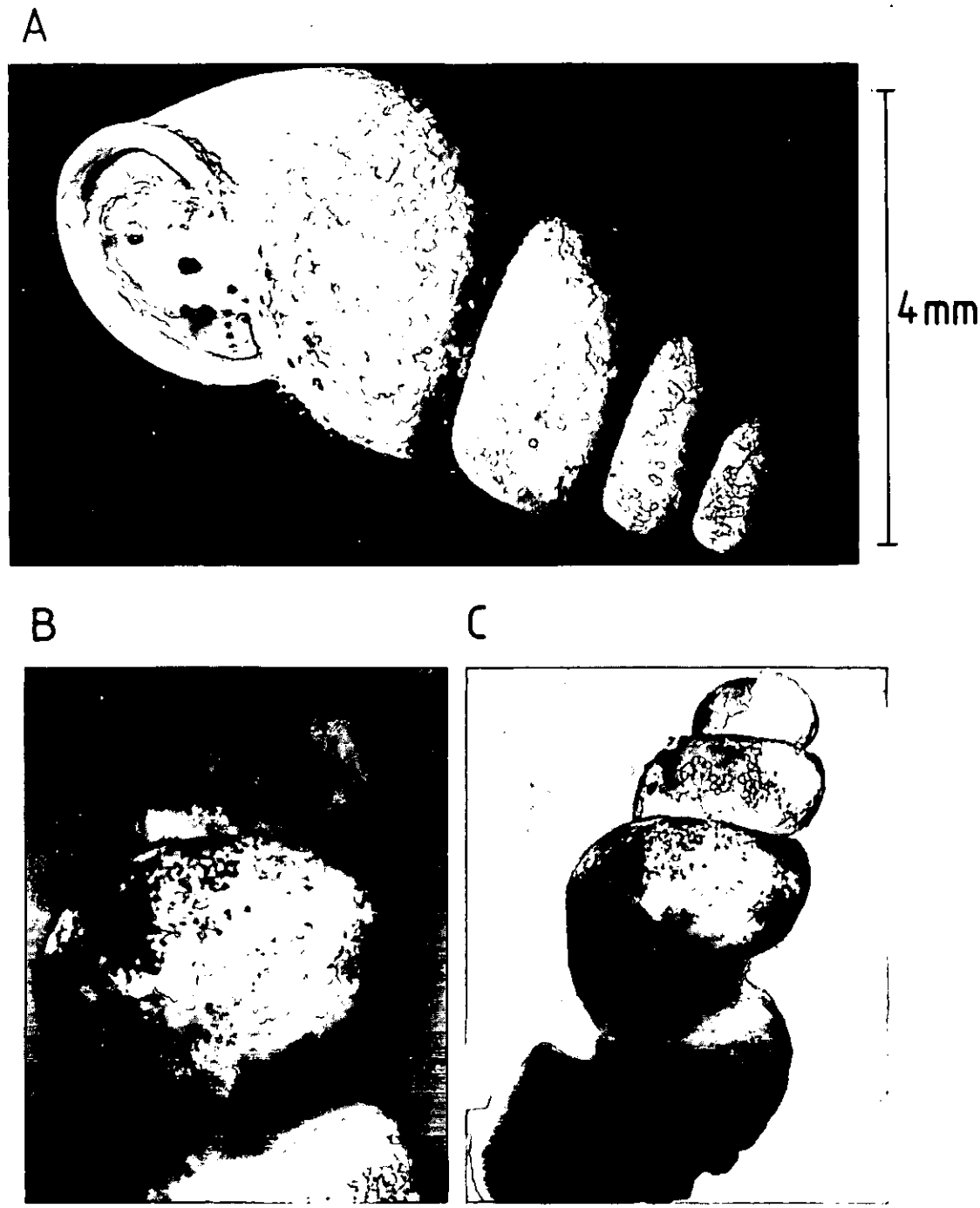


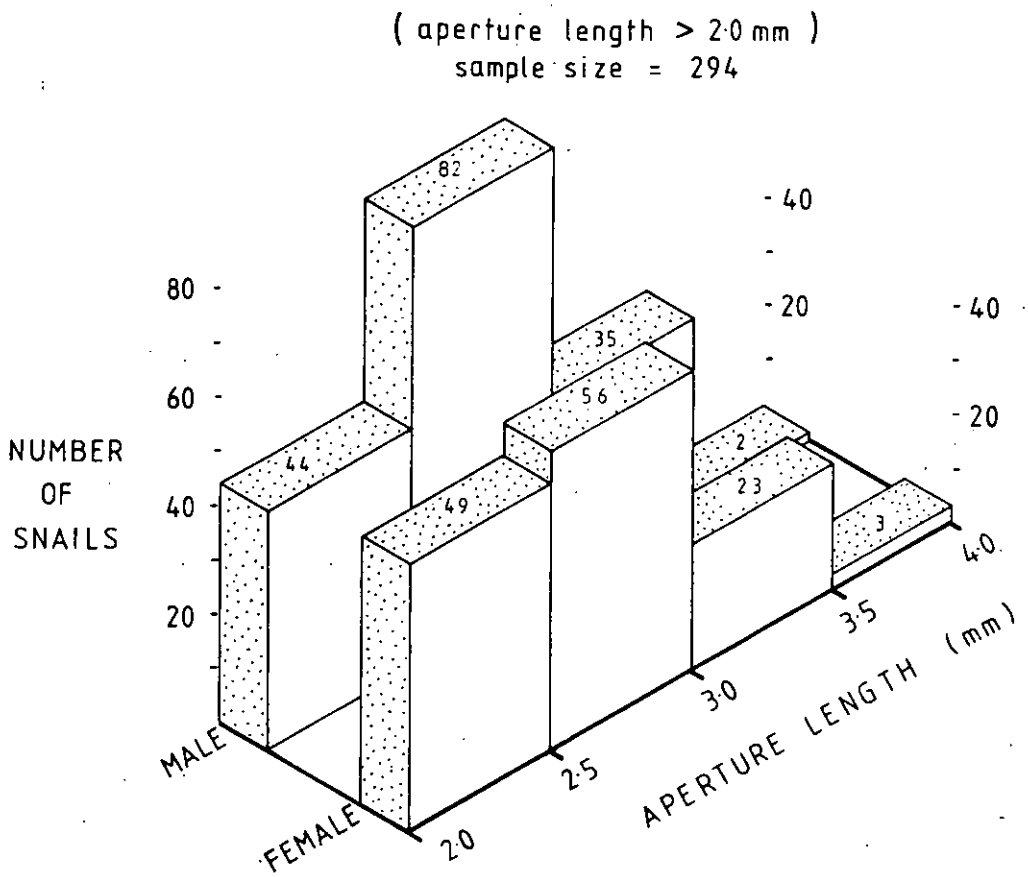
FIGURE 7.1 A, decussate adult snail shell from Calvert's Lagoon; B, detail of visceral hump of snail infected with hundreds of metacercarial cysts of *Atriophallophorus coxiellae*, shell removed; C, body of snail, shell removed, almost all viscera infected with cysts of *A. coxiellae*.

biology of *C. striata*, has been undertaken by Ross Kaires at the University of Adelaide, which may resolve this question of synonymy. For the present, the *Coxiella* species inhabiting Tasmanian brackish lagoons is referred to as *C. badgerensis*. As well as occurring on Badger Island and Calvert's Lagoon, this snail has been recorded from Cape Portland, Diana's Basin, Half Moon Bay Lagoon, Little Musselroe Bay Lagoon, Muddy Plains, Primrose Sands Lagoon, Troyheleener Lagoon and Tregaron Lagoons (Tasmanian Museum records, and personal observations). Thiele (1929), placed *Coxiella* in the family Hydrobiidae, subfamily Truncatellinae and this classification was accepted by Macpherson (1957). Grassé (1968), included the genus in the family Truncatellidae, subfamily Tomichiinae, however Smith and Kershaw (1979), returned it to the family Hydrobiidae.

At Calvert's Lagoon, the largest specimen found was 13.2 mm long, with an aperture length (A.L.) of 3.4 mm, however adults average about 7 mm in length, with an aperture length of about 2.6 mm. The dextrally coiled shell is generally decollate and varies from olive green to brown. Before losing the apex of the shell, the number of whorls per adult snail is 7 or 8, and subsequently averages 5. The operculum is concentric, thin, pale yellow-brown, with a raised central section.

C. badgerensis is dioecious and although there is no obvious sexual dimorphism, the sexes can be readily distinguished by the presence or absence of a penis, when the aperture length exceeds about 1.75 mm. Of 294 adults (A.L. greater than 2.0 mm), collected in monthly samples from Site 1 between November 1977 and September 1978, the proportion of males to females was 0.55 to 0.45, which does not differ significantly from a 50:50 sex ratio ($X^2_1 = 3.48$; $0.1>P>0.05$; N.S.). The relationship between the size and sex of these snails is shown in Figure 7.2. The proportion of males in each size class varied from 0.47 (A.L. 2.0 - 2.5 mm), to 0.59 (A.L. 2.5 - 3.0 mm), to 0.60 (A.L. 3.0 - 3.5 mm), to 0.40 (A.L. 35 - 4.0 mm). There were significantly more males than females in the size

FIG. 7.2 Coxiella badgerensis. Size distributions of male and female adults, collected at Calverts Lagoon, November 1977 - September 1978.



range A.L. 2.5 - 3.00 mm (X_1^2 = 4.90; 0.05>P>0.02*), however there was no significant difference between the proportions of males and females in the other size ranges: A.L. 2.0 to 2.5 mm, X_1^2 = 0.27, 0.8>P>0.5, N.S.; A.L. 3.0 to 3.5 mm, X_1^2 = 0.2>P>0.1, N.S. and A.L. 3.5 to 4.0 mm, X_1^2 = 0.200, 0.8>P>0.5, N.S. The possible relationship between this differential sex ratio and parasitism is discussed later.

In general, the anatomy of *C. badgerensis* is similar to that of the truncatellid *Truncatella kiusiuensis* (Kosuge, 1966), and the hydrobiids *Pomatiopsis lapidaria* and *Oncomelania hupensis formosana* (Davis, 1967). A brief account of the anatomy of *C. badgerensis* is presented here as a guide for dissection of the snail and examination for trematodes. The mantle cavity is exposed by cutting posteriorly along the right side of the mantle wall. The ctenidium, composed of a row of gill filaments, extends most of the length of the interior of the mantle wall. An elongate osphradium is located at the base of the gill filaments, near the anterior end of the mantle cavity. Narrowing posteriorly, the mantle cavity terminates adjacent to the pericardium and kidney. The kidney wall is contiguous to the pericardium. Within the pericardium the auricle and ventricle may be seen beating for a period after dissection. The kidney orifice, bounded by a pair of swollen, whitish lips, opens into the posterior mantle cavity. Kidney tissue is speckled whitish and amorphous, moulded around and between organs from the mantle cavity to the anterior of the stomach. The oesophagus passes posteriorly through the neck of the snail to the anterior chamber of the stomach. The style sac arises from this chamber and extends anteromedially to the pericardium. On dissection, a clear gelatinous cylinder, the "crystalline style", is readily freed intact from within the style sac. The intestine arises from the left ventral side of the style sac and passes anteriorly around its anterior tip, before passing dorsally and posteriorly along its length. The intestine then recurves sharply anteriorly, with a muscular 'faecal pellet compressor' at the flexure. The rectum, containing distinct

faecal pellets, extends from this flexure, along the border of the pallial gonoduct, opening through the anus near the right mantle edge. Brown lobes of the digestive gland, together with the gonad, form the spiral coils of the visceral hump. In females, the cream, lobate ovary is mainly located in the upper part of the visceral hump. Oocytes, diameter 191 (182 - 205)µ, pass along the oviduct to the posterior end of the pallial oviduct. The oocytes consist of a translucent ovum about 60µ diameter, surrounded by opaque material composed of fine grains, about 1.5µ diameter, and larger round bodies, about 8µ diameter, each containing one irregular refractile granule. The large cream pallial oviduct, or capsule gland, extends almost the length of the mantle cavity, adjacent to the rectum. A yellow body, presumed to be the albumen gland, is located at the posterior end of the pallial oviduct and is packed with fine granular material and bundles of motile sperm. Adjacent to the posterior end of the albumen gland is the bursa copulatrix, which is densely packed with sperm. In males, the lobed testis occupies the same position as the ovary in females. Sperm are stored in the creamy pink, convoluted posterior vas deferens, which leads to the posterior of the mantle cavity, where it enters the yellow prostate gland. The anterior vas deferens leaves the prostate gland and extends anteriorly to the penis, or verge, which arises on the right side of the neck. The simple, unflattened penis is curled over the neck. Passing through the middle of the penis, the anterior vas deferens narrows near the tip, which is without a papilla. Ciliated epithelium extends about ½ to 1/3rd of the length of the penis, from just behind the tip. Penis length is related to the age of the snail, but in adults is approximately 1.7 × 0.3 mm. Deformities occur rarely, with up to 3 off-shoots from the penis.

7.1.2 Growth

Initially, shell length was used as a guide to the age and maturity

of snails, however, decollation of shells in the lagoon made this method of age determination unreliable. Measurements of 125 wild and 125 laboratory-bred snails, none of which were decollate, showed a direct relationship between aperture length and shell length (Figure 7.3). There was highly significant regression of shell length on aperture length for wild snails ($Y = 1.7x - 0.7$; $F = 3202$; $P<0.001$) and for laboratory-bred snails ($Y = 1.6x - 0.6$; $F = 4307$; $P<0.001$). Thereafter, aperture length (A.L.) was used as a measure of the age of snails.

Young snails (ave. A.L. 1.06 mm; 4 whorls), taken from the lagoon in October 1977, were cultured in the laboratory, and their growth was monitored for more than a year (Figure 7.4). At the end of this period, the largest snail had an A.L. of 3.30 mm and had 8 whorls. In May 1978, after 30 weeks, the first generation of laboratory-bred snails (ave. A.L. 0.64 mm; 2-3 whorls) were noticed in the culture. These snails were separated, and their growth monitored for about 6 months, by which time the largest snail had an A.L. of 3.30 mm, and had 7 whorls (Figure 7.4). In August 1978, 16 weeks after the appearance of offspring in the first culture, a new generation of very young snails were discovered in the second culture. The smallest snail had an A.L. of only 0.28 mm and had 2 whorls. Breeding appeared to be continuous, as very young snails continuously appeared in both cultures after the first offspring were noticed. Although maturity may be reached in younger snails, the smallest mature male dissected, (with sperm stored in the posterior vas deferens), had an A.L. of 1.65 mm; and the smallest mature female dissected (with oocytes in the oviduct), had an A.L. of 2.35 mm. The growth curves of cultured snails, shown in Figure 7.4, indicate that snails grew at a constant, rapid rate for the first 3 to 4 months, after which growth continued at a much slower rate. Under laboratory conditions, males would thus be producing sperm when 2 to 3 months old, and females would be producing oocytes when about 4 months old. In the lagoon, similar rates of develop-

FIG. 7.3 Coxiella badgerensis. Relationship between shell length and aperture length.

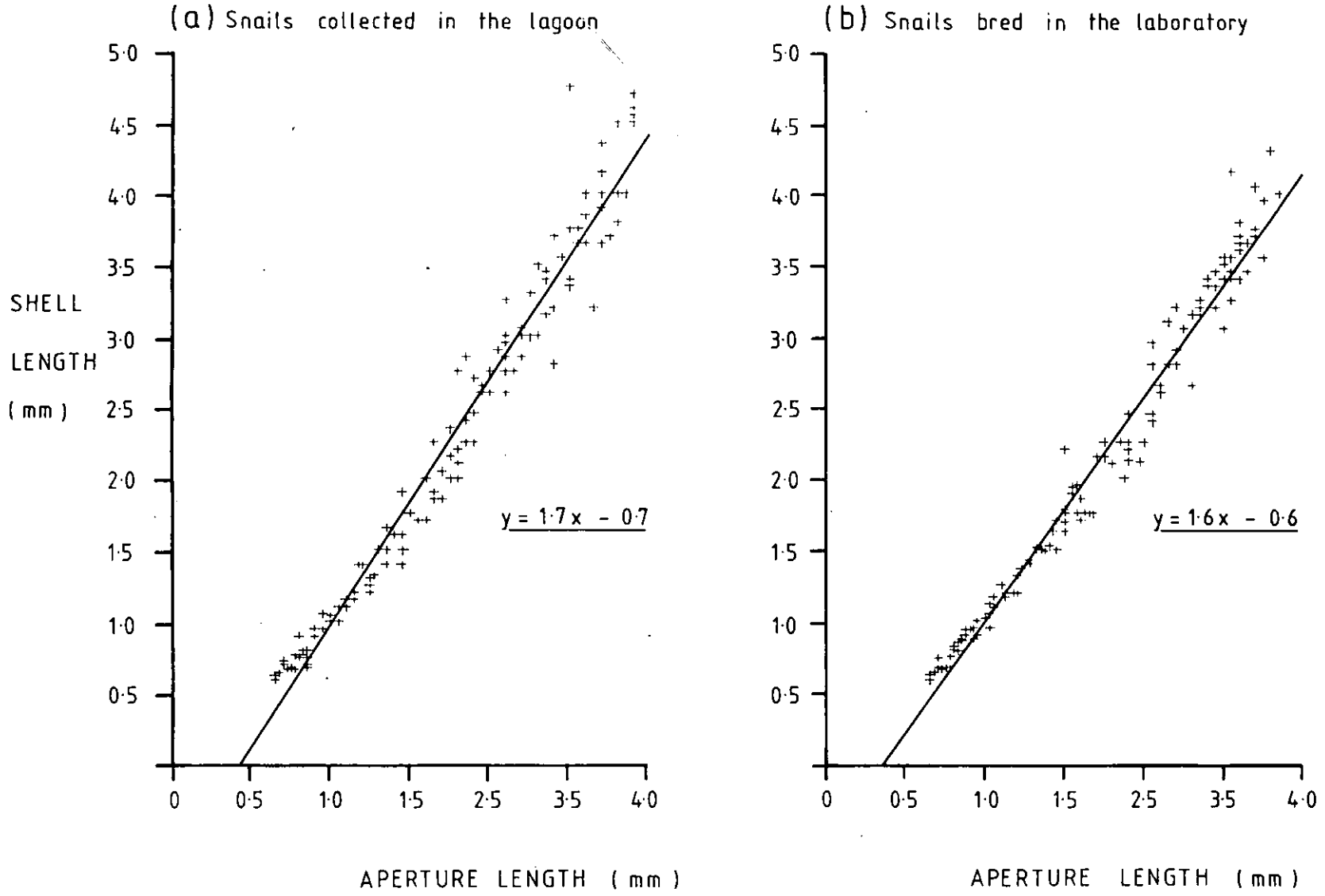
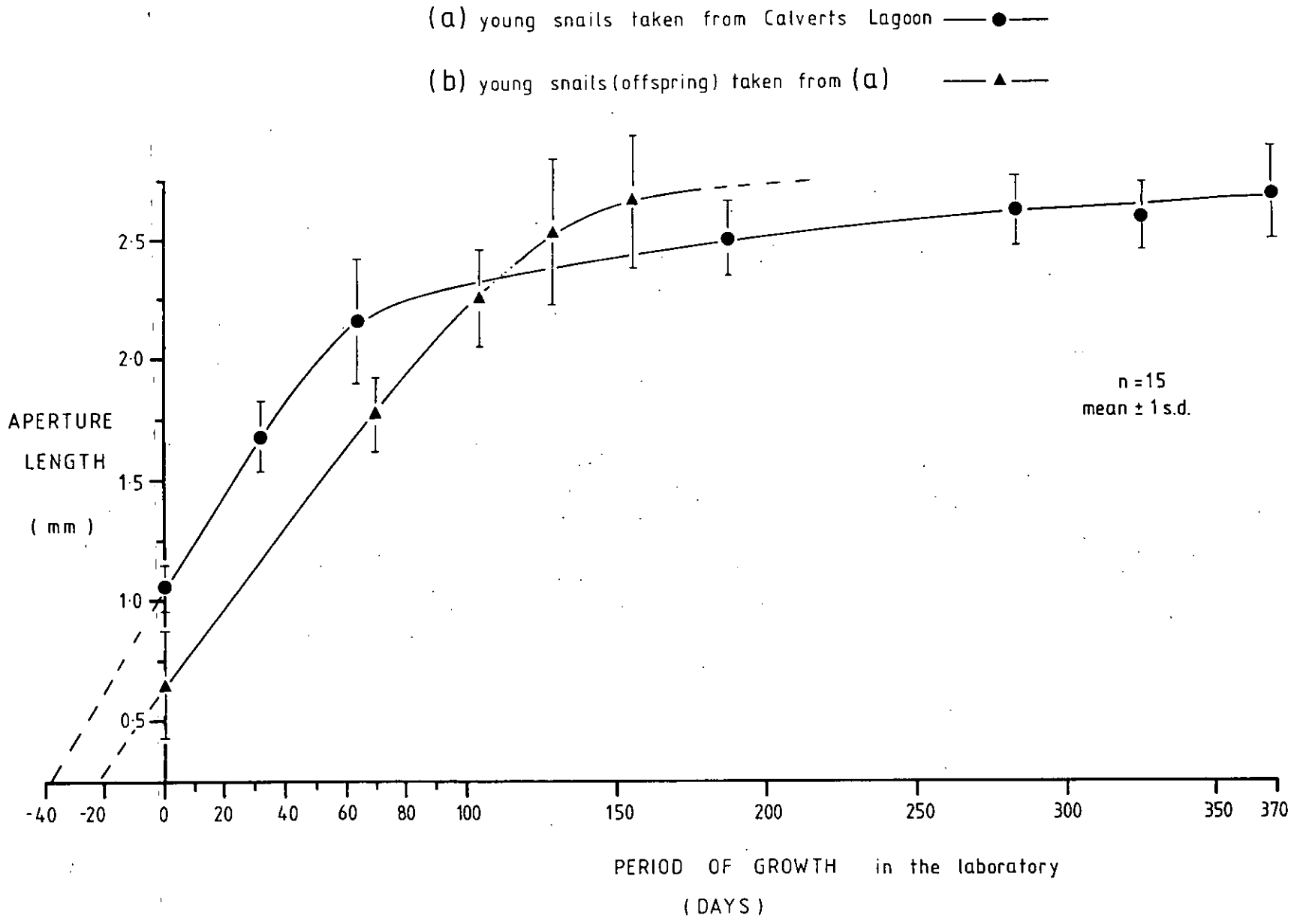


FIG. 7.4 Coxiella badgerensis. Growth of snails in the laboratory



ment may occur in spring and summer, when water temperature is high and food unlimited; however, growth and development would be much slower during autumn and winter. These results suggest that snails probably breed more or less continuously in the lagoon, with 2 and possibly 3, generations occurring each year and this is supported by the fact that very young snails (A.L. less than 0.50 mm), are present in the lagoon throughout the year. Seasonal variation in the size distribution of snails in the population (with A.L. greater than or equal to 0.50 mm), is shown in Figure 7.5. The observed size distribution of snails during the year is consistent with breeding occurring throughout the year, with a reduction in recruitment of young snails into the population from May to August. During this period water temperatures in the lagoon fall below 10°C for several months.

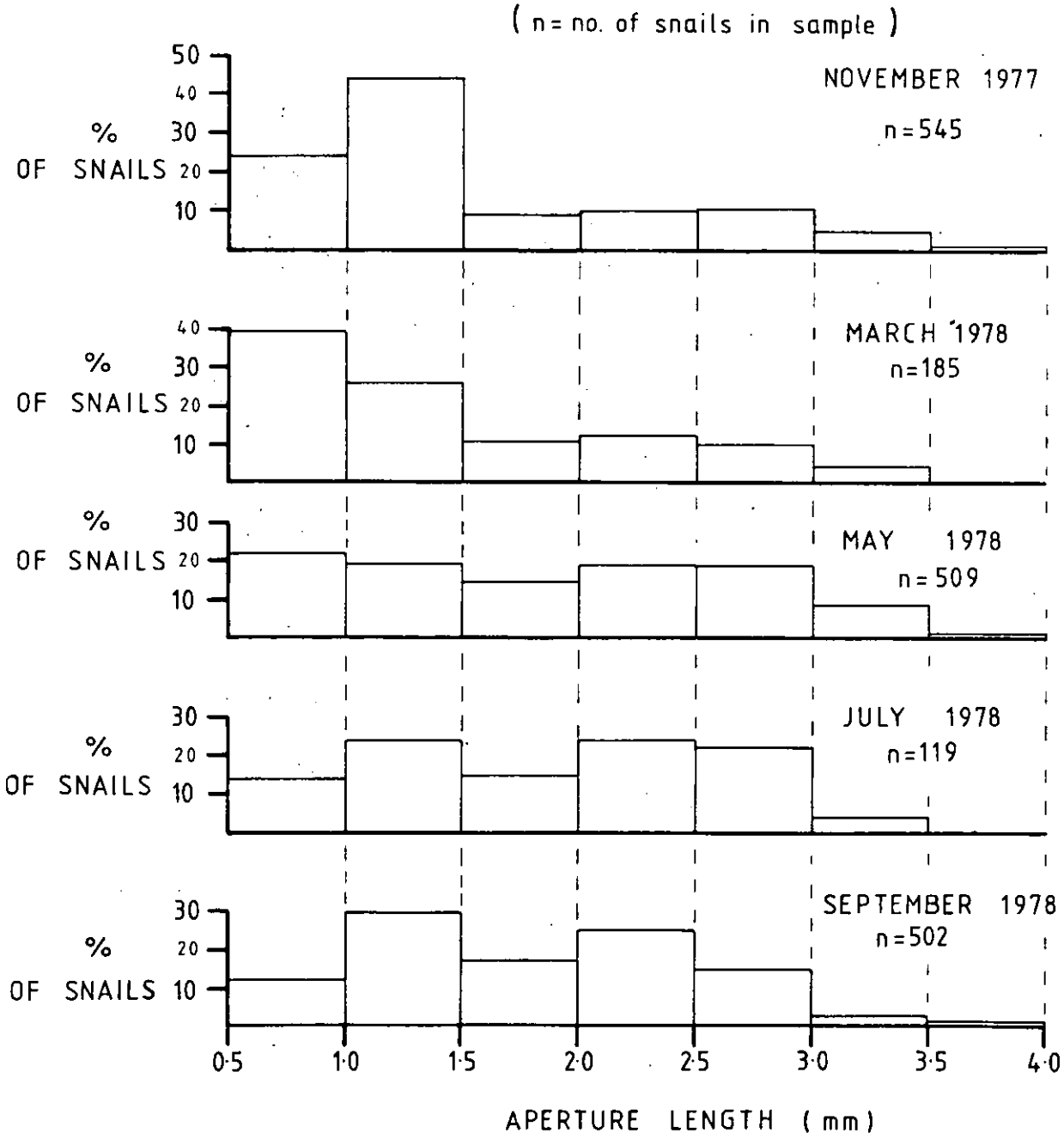
7.1.3 Infection

Primary infection:

The incidence of primary trematode infections (resulting from invasion by miracidia), in samples of 30 adult snails collected at Site 1 from August 1976 to September 1978, varied from 27 to 93%, at an average of 66%. In all snails examined during the present study, primary trematode infections caused by a total of 17 species from 7 families were found. The proportion of primary infections caused by each of the families in samples collected at Site 1, are shown in Figure 7.6.

The incidence of primary infections is directly related to the size, and presumably the age, of snails. Primary infections were present in 40% of 180 snails, with A.L. greater than 0.5 mm, collected in November 1977. Only 8% of the snails with A.L. less than 2 mm served as primary hosts, compared with 74% of the snails with A.L. greater than 2 mm. The smallest snail found to be serving as a primary intermediate host, had

FIG. 7.5 Coxiella badgerensis. Size distribution of snails in samples collected at Calverts Lagoon, November 1977 - September 1978.



an A.L. of 1.35 mm, and harboured immature sporocysts of *Schistosome sp.A*. The relationship between the incidence of primary infection and snail size is shown in Figure 7.7.

Male and female snails were found to be equally susceptible to primary infections. Of 125 adult males and 125 adult females (A.L. greater than 2.0 mm), collected in monthly samples from November 1977 to September 1978, 84% of the males and 81% of the females served as primary intermediate hosts ($X_1^2 = 0.22$; $0.8>P>0.5$; N.S.).

The incidence of primary infections caused by various trematode species in 360 snails, collected at Site 1 from August 1977 to September 1978, (12 samples of 30 snails), is shown in Table 7.1.

TABLE 7.1 The incidence of primary infections by trematode species in 360 adult snails, collected at Site 1 (August 1977 - September 1978).

Family	Species	Symbol*	No.	%
Microphallidae	*Maritrema calvertensis*	M	185	51.4
	Levinseniella tasmaniae	L	65	18.2
	Atriophallophorus coxiellae	A	51	14.2
Schistosomatidae	*Schistosome sp.A*	S	66	18.3
Notocotylidae	*Notocotylid sp.B*	N	17	4.7
	Paramonostomum caecai n.sp.	-	3	0.8
	Paramonostomum bursae n.sp.	-	2	0.6
	Notocotylid sp.A	-	0	0
Psilostomidae	*Psilostomum sp.A*	-	1	0.3
	Psilochasmus oxyurus	-	0	0
	Psilostomum sp.B	-	0	0
Renicolidae	*Renicolid sp.A*	-	2	0.6
	Renicolid sp.B	-	2	0.6
Heterophyidae	*Heterophyid sp.C*	-	1	0.3
	Heterophyid sp.A	-	0	0
	Heterophyid sp.B	-	0	0
Strigeidae	*Apatemon gracilis*	-	0	0

(* symbols used in Tables 7.2 and 7.3)

Three microphallid species, one schistosome and one notocotylid, were by far the most common of the trematodes. Variation in the incidence

FIG. 7.6 The incidence of primary trematode infections in samples of adult snails collected at Site 1, Calverts Lagoon, (April 1976 to September 1978).

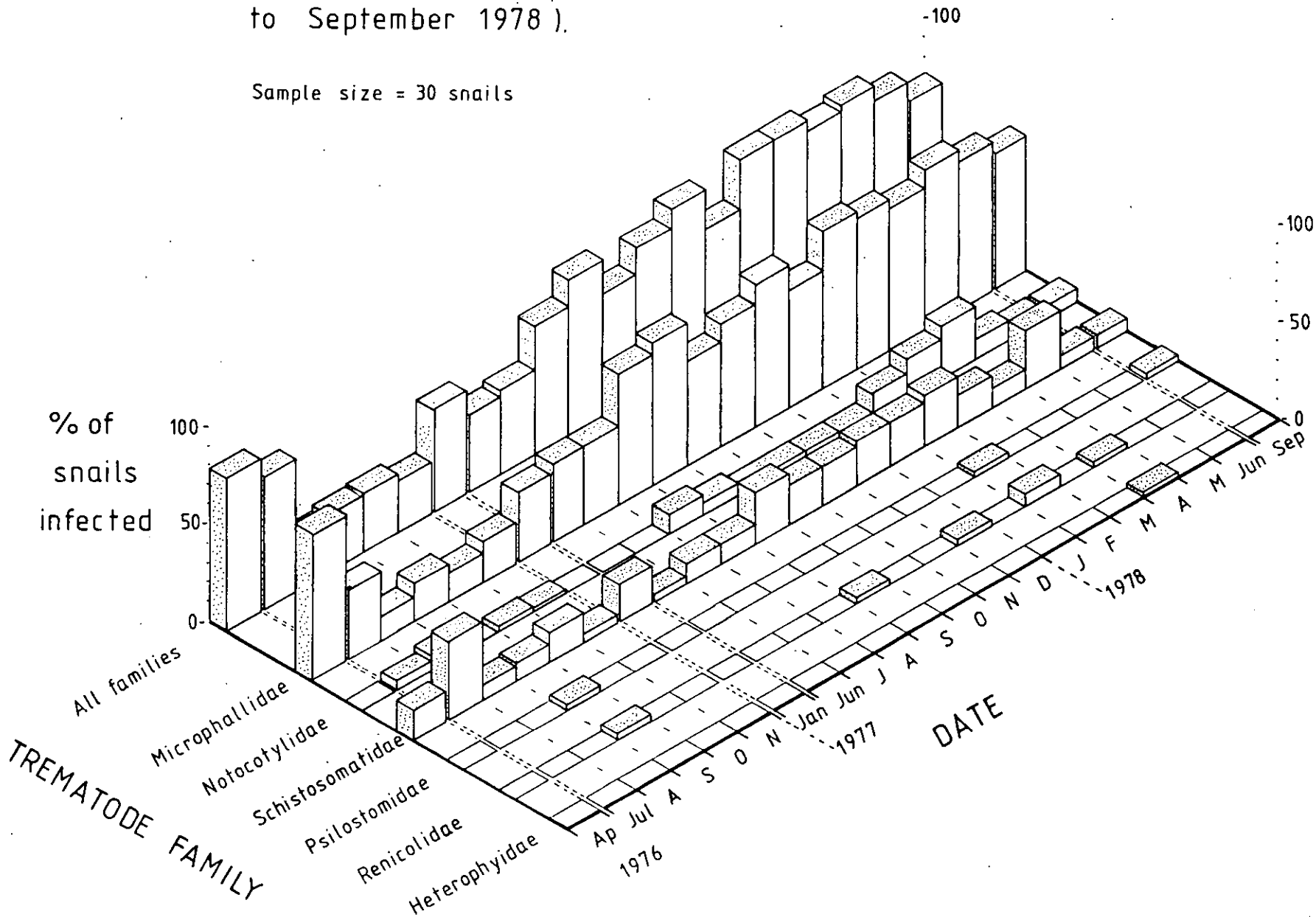


FIG. 7.7 Relationship between the size of snails and the incidence of primary trematode infections, in a sample collected at Calverts Lagoon, November 1977.

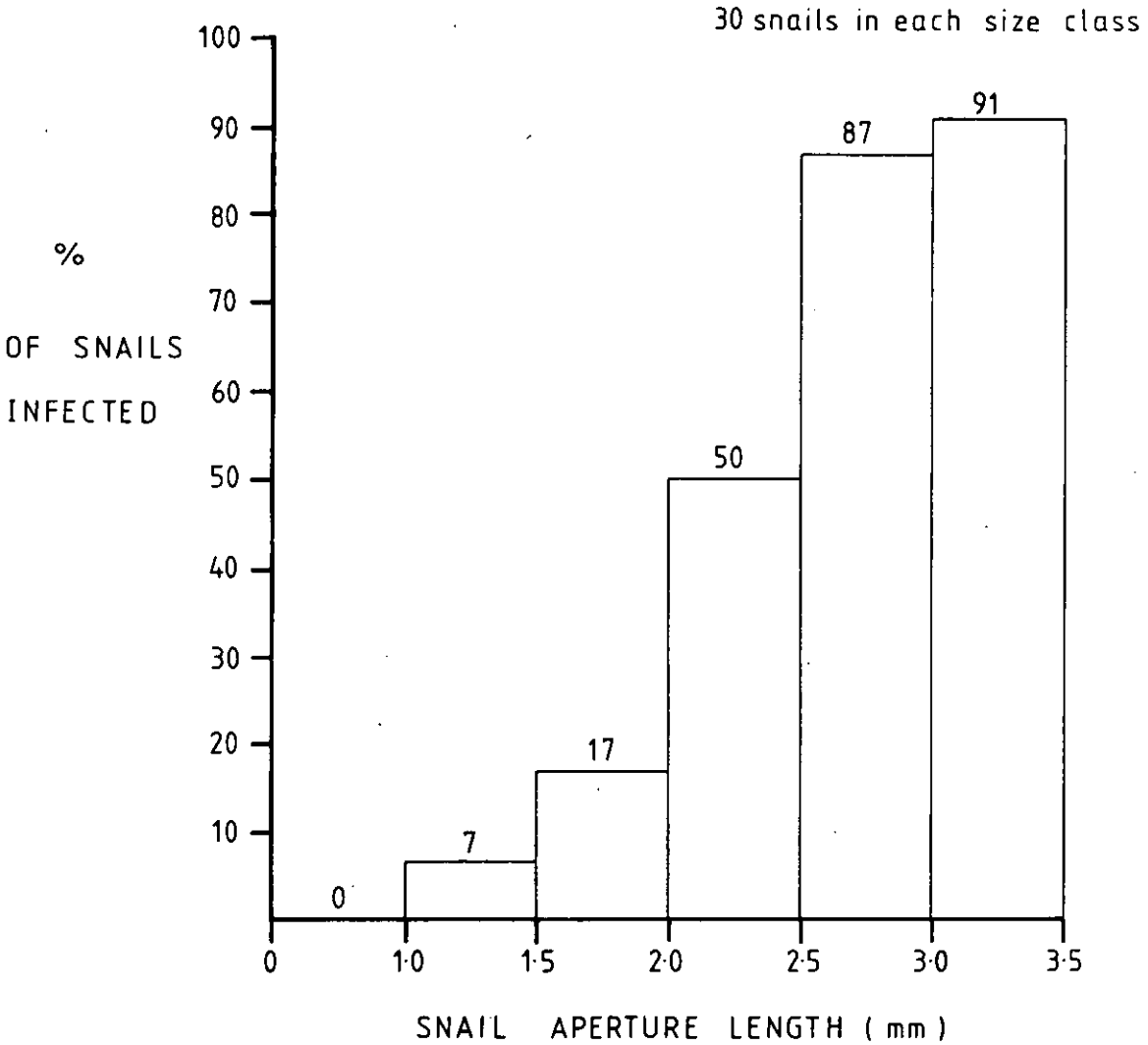
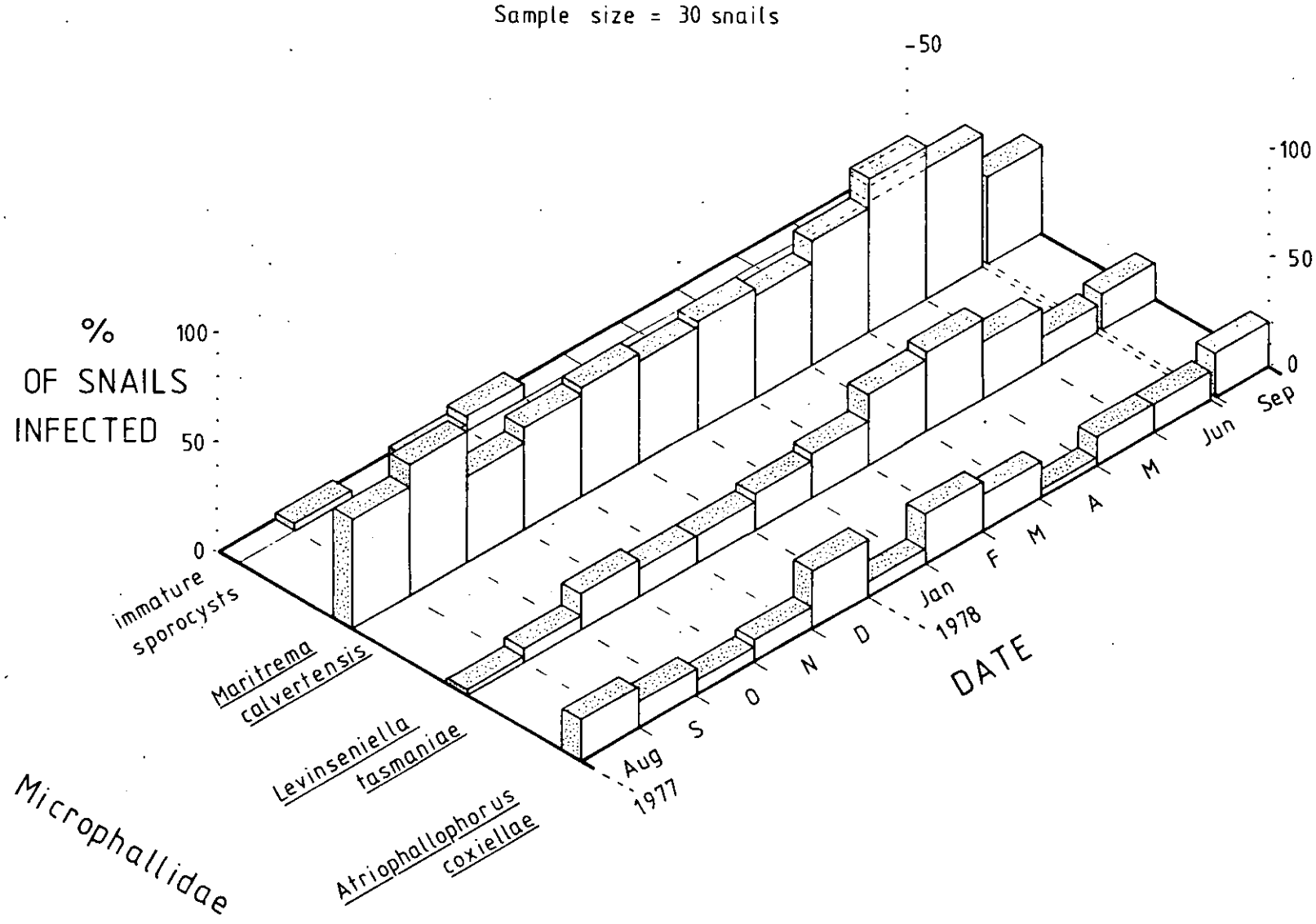


FIG. 7.8 The incidence of primary infections with microphallid trematodes, in samples of adult snails collected at Site 1 Calverts Lagoon, (August 1977 to September 1978).



of infection with the three microphallids is shown in Figure 7.8. Although the relative abundance of the species varied between samples, *Maritrema calvertensis* was always the most common microphallid parasite of *C. badgerensis*.

Many of the snails concurrently harboured developmental stages of 2 or 3 species. The observed incidences of double infections involving the 5 most abundant species are shown in Table 7.2, together with the incidences expected by chance alone (e.g. the expected incidence of concurrent infections by *Maritrema calvertensis* and *Levinseniella tasmaniae* is $\frac{51.4}{100} \times \frac{18.1}{100} \times 360 = 33.5$). All of the 10 possible double associations occurred. The incidences of 6 of the 10 combinations were not significantly different from those expected by chance, however the incidences of the other 4 combinations (none of which included the notocotylid species), were all significantly less that those expected by chance alone.

TABLE 7.2 Double infections in a sample of 360 snails

Infection trematode spp.*	Snails with the infection Observed	Expected	Snails without the infection Observed	Expected	X_1^2	P	Sig.
M×L	19	33.5	341	326.5	6.92	0.01>P>0.001	**
M×A	10	26.3	350	333.7	10.9	0.001>P	***
M×S	30	33.9	330	326.1	0.495	P>0.2	N.S.
M×N	6	8.7	354	351.3	0.859	P>0.2	N.S.
L×A	8	9.3	352	350.7	0.187	P>0.5	N.S.
L×S	5	11.9	355	348.1	4.14	0.05>P>0.01	*
L×N	3	3.1	357	356.9	0.003	P>0.9	N.S.
A×S	1	9.4	359	350.6	7.71	0.01>P>0.001	**
A×N	1	2.4	359	357.6	0.822	P>0.2	N.S.
S×N	1	3.1	359	356.9	1.43	P>0.2	N.S.

(* Symbols shown in Table 7.1)

The incidences of triple infections involving the same 5 species are shown in Table 7.3, together with the incidences expected by chance alone (e.g. the expected incidence of concurrent infections by *Maritrema calvertensis, Levinseniella tasmaniae* and *Atriophallophorus coxiellae* is $\frac{51.4}{100} \times \frac{18.1}{100} \times \frac{14.2}{100} \times 360 = 4.8$).

Six of the possible triple associations were observed. The

incidences of none of the combinations were significantly different from those expected by chance alone.

TABLE 7.3 Triple infections in a sample of 360 snails

Infection trematode spp.	Snails with the infection *Observed	Expected	Snails without the infection Observed	Expected	X_1^2	P	Sig.
M×L×A	2	4.8	358	355.2	1.66	P>0.05	N.S.
M×L×S	4	6.1	356	353.9	0.735	P>0.2	N.S.
M×L×N	1	1.6	359	358.4	0.226	P>0.5	N.S.
M×A×S	2	4.8	358	355.2	1.66	P>0.05	N.S.
M×A×N	0	1.2	360	358.8	1.20	P>0.2	N.S.
M×S×N	0	1.6	360	358.4	1.61	P>0.2	N.S.
L×A×S	1	1.7	359	358.3	0.290	P>0.5	N.S.
L×A×N	0	0.4	360	359.6	0.400	P>0.5	N.S.
L×S×N	0	0.6	360	359.4	0.601	P>0.2	N.S.
A×S×N	1	0.4	359	359.6	0.901	P>0.2	N.S.

(* Symbols shown in Table 7.1)

Seasonal variation in the incidence of primary infections at Site 1, from July 1977 to June 1978, was analysed statistically. The three monthly samples within each season were considered as replicates, thus the June, July and August samples were treated as replicates for winter; the September, October and November samples as replicates for spring etc. The percentages of snails infected by each trematode family in the 12 samples of 30 snails, were converted to angles using 'angular transformation' and analysis of variance was carried out on the transformed data (Table 7.4).

TABLE 7.4 Analysis of variance table showing the seasonal variation in the incidence of primary infections of *Coxiella badgerensis* by different trematode families at Site 1 Calvert's Lagoon.

Source of variation	d.f.	M.S.	F	P	Sig
Trematode family (T)	6	5041.7	215.5	P<0.001	***
Season (S)	3	156.5	6.7	P<0.001	***
Interaction (T-S)	18	44.2	1.9	0.05<P<0.02	N.S.
Residual	56	23.4			

There was a highly significant difference in the levels of infection between the seasons and, of course, between the trematode families.

The mean incidence of snails serving as primary hosts was highest in autumn (90%), followed by summer (82%) and then spring (73%), and was lowest in winter (64%). The mean infection rate for each trematode family was: Microphallidae 68.8%, Schistosomatidae 17.2%, Notocotylidae 6.1%, Renicolidae 0.5%, Heterophyidae and Psilostomidae 0.05% and Strigeidae 0%. There was no significant interaction between 'season' and 'trematode family', which indicates that the relative abundance of each trematode family was similar in each season.

Variation in infection of *C. badgerensis* around the lagoon was investigated by collecting quarterly samples of 30 adult snails from 4 widely separated sites, over a period of 12 months. Replicates at each site in each season were obtained by dividing each sample of 30 snails into 3 sub-samples of 10, using random number charts. In the sub-samples, the percentages of snails infected by each trematode family were 'normalized' by angular transformation, and subjected to analysis of variance (Table 7.5).

TABLE 7.5 Analysis of variance table showing seasonal variation in the incidence of primary infections of *Coxiella badgerensis* by different trematode families at different sites around Calvert's Lagoon.

Source of variation	d.f.	M.S.	F	P	Sig.
Trematode family (T)	6	14978	264.0	P<0.001	***
Season (Se)	3	352.7	6.2	P<0.001	***
Site (Si)	3	116.2	2.1	0.05<P<0.2	N.S.
T - Se	18	76.1	1.3	0.05<P<0.2	N.S.
T - Si	18	70.3	1.2	0.05<P<0.2	N.S.
Se - Si	9	107.4	1.9	0.05<P<0.1	N.S.
T - Se - Si	54	82.9	1.5	0.01<P<0.05	*
Residual	224	56.7			

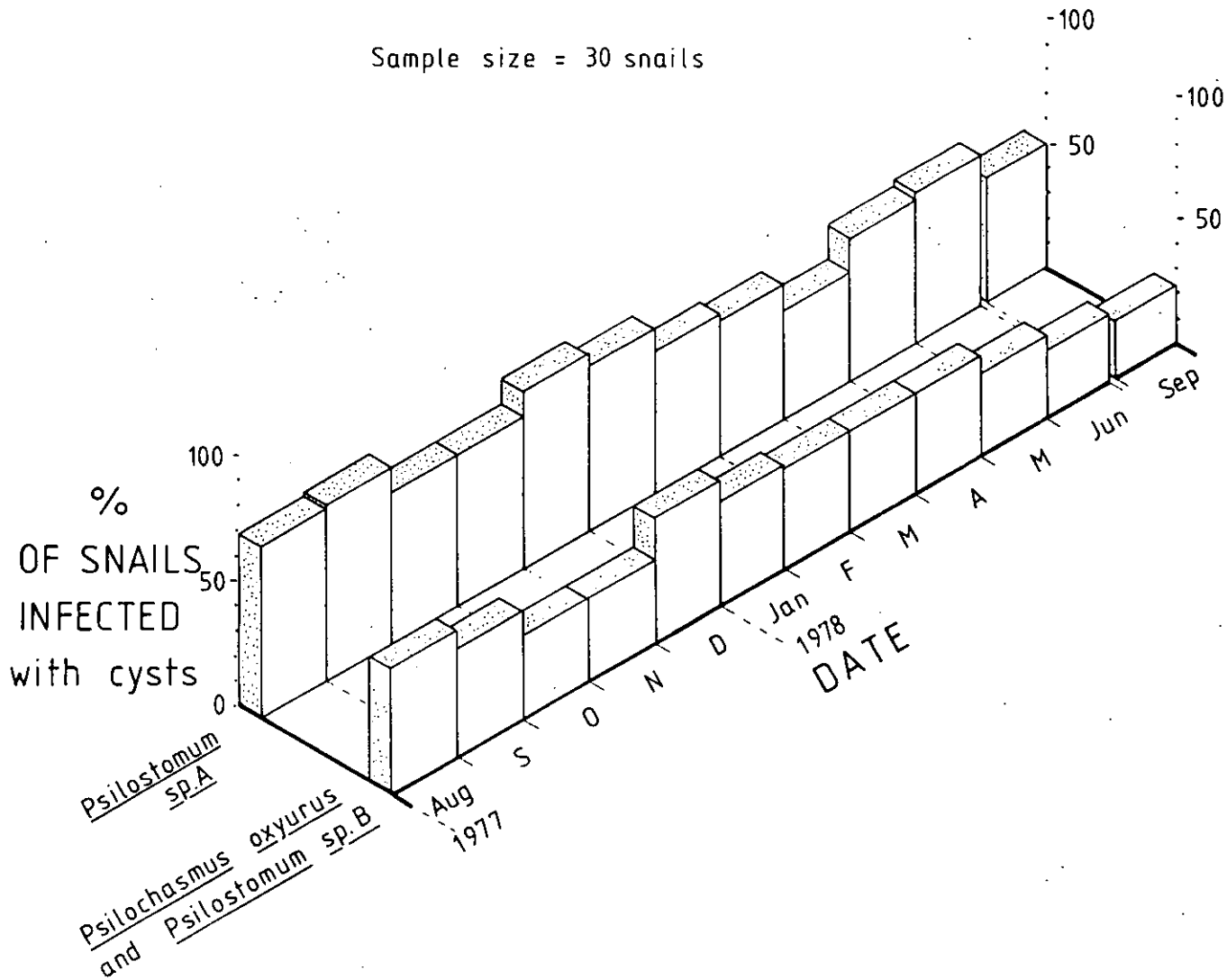
Three factor analysis of variance showed that there was highly significant variation in the incidence of primary infections between seasons and between trematode families; however, no significant variation was found between the 4 sampling sites. The mean incidence of snails serving as primary hosts was 65% at Site 1, 64% at Site 2, 61% at

Site 3 and 63% at Site 4. The mean infection rate for each family at all sites around the lagoon was: Microphallidae 56.9%, Schistosomatidae 5.6%, Notocotylidae 1.7%, Renicolidae 0.1%, Heterophyidae 0.05% and Psilostomidae and Strigeidae less than 0.05%. The relative abundance of the trematode families did not vary significantly between seasons ('trematode family-season' interaction), nor between sites ('trematode family-site' interaction). Differences between the seasonal variation in overall incidence of primary trematode infections at each site, were nearly significant at the 5% level ('season-site' interaction). This may reflect some seasonal movement of the bird hosts around the lagoon, associated perhaps with breeding activity, human interference (e.g. anglers, or surfers driving to Calvert's Beach along the road on the southern margin of the lagoon), or a seasonal food resource. The only interaction term that was a significant source of variation was 'trematode family-season-site', which indicates that the relative abundance of trematode families in each season varied differently at the 4 sites. This may reflect different seasonal movements around the lagoon by the various bird host species, each of which harbour different assemblages of parasites.

Secondary infection:

C. badgerensis serves as the secondary intermediate host to *Psilochasmus oxyurus*, *Psilostomum sp.A* and *Psilostomum sp.B*. The cercariae of these psilostome species emerge from the primary host and spend a period swimming in the lagoon before invading the same, or another snail. The percentage of snails infected with 'small' and 'large' psilostome cysts, in monthly samples collected at Site 1 from August 1977 to September 1978, are shown in Figure 7.9. The 'small' cysts were all those of *Psilostomum sp.A*, whereas the 'large' cysts belonged to *Psilochasmus oxyurus* and *Psilostomum sp.B*. The percentage of snails

FIG. 7.9 The incidence of secondary infections with psilostome trematodes, in samples of adult snails collected at Site 1 Calverts Lagoon, (August 1977 to September 1978).



infected with *Psilostomum sp.A* cysts varied from 43 to 70% (average 59%), and the average number of cysts per infected snail was 5.4. The percentage of snails infected with *Psilochasmus oxyurus* and/or *Psilostomum sp.B* cysts varied from 23 to 50% (average 38%), and the average number of cysts per infected snail was 1.7. Cysts of *Psilochasmus oxyurus* and *Psilostomum sp.B* were not distinguished until later in the study. In a sample of 200 'large' cysts collected from snails in December 1979, the ratio of *Psilochasmus oxyurus* to *Psilostomum sp.B* was 2.2:1.

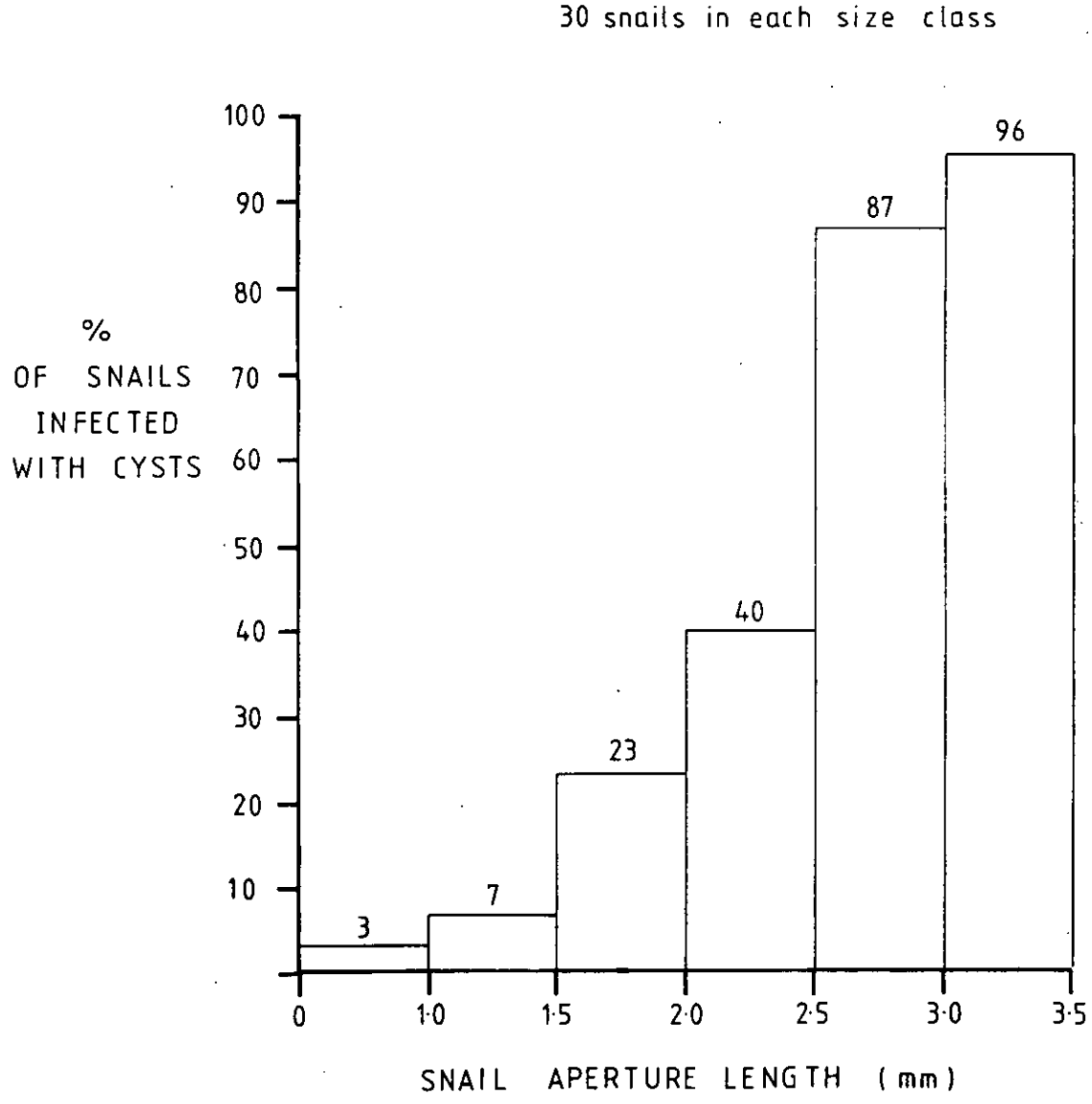
The incidence of secondary infections, resulting from invasion by psilostome cercariae, is directly related to the size and hence the age of *C. badgerensis* (Figure 7.10). Such infections were present in 40% of 180 snails, with A.L. greater than 0.5 mm, collected in November 1977. However, only 11% of the snails with A.L. from 0.5 to 2 mm harboured psilostome cysts, compared with 72% of those with A.L. greater than 2 mm. The smallest snail found to be serving as a secondary intermediate host had an A.L. of 0.9 mm, and harboured one cyst of *Psilostomum sp.A*.

Male and female snails were found to be equally susceptible to invasion by psilostome cercariae. Of 125 adult males and 125 adult females (A.L. greater than 2.0 mm), collected in monthly samples from November 1977 to September 1978, exactly the same proportion of males and females, 62%, harboured psilostome cysts.

7.1.4 Host-parasite relationships and pathology

The microphallid species infecting *C. badgerensis* invade various tissues. Large numbers of sporocysts and metacercariae of *Atriophallophorus coxiellae* develop in the digestive gland and gonad, and extend anteriorly into the kidney, and pallial oviduct (in females). Sporocysts of *Levinseniella tasmaniae* form characteristic clusters along the oviduct and posterior vas deferens; but, like the sporocysts of *Maritrema calvertensis*, are also widely distributed throughout the host's viscera. Rediae of the 3 psilostome species are concentrated

FIG. 7.10 Relationship between the size of snails and the incidence of secondary infection with psilostome trematodes, in a sample collected at Calverts Lagoon, (November, 1977).



in the gonad, but also extend between the lobes of the digestive gland and anteriorly into the kidney and capsule gland. Large free cercariae are distributed throughout the viscera of the host. Notocotylid rediae occur between the lobes of the digestive gland and in the gonad, as well as in the kidney and pallial oviduct. Free cercariae are widely distributed. Sporocysts of *Schistosome sp.A* are concentrated in the gonad, but extend throughout the viscera. The sporocysts of *Renicolid sp.A* are restricted to the gonad and metacercarial cysts of *Renicolid sp.B* are clustered near the base of the visceral hump. Rediae and free cercariae of the heterophyid species are widely distributed throughout the viscera, as are the sporocysts and cercariae of the strigeid *Apatemon gracilis*. All of these trematodes undergo great multiplication in the primary intermediate host, causing extensive disruption and breakdown of the invaded tissues. In all infections, the relative proportion of parasite to host tissue is high; sometimes (e.g. infection with *Atriophallophorus coxiellae*, Figure 7.1), most of the host's body volume is occupied by parasites. Localized cellular breakdown in the parasitized host tissues may result from histolytic secretions produced by daughter sporocysts (Erasmus, 1972). The rediae of 10 species (viz. the psilostomes, notocotylids and heterophyids), actively ingest host tissues, particularly the digestive gland.

There is no reliable external sign that a snail is infected by trematodes. Despite the impact that primary infections must have on the metabolism of the host, the snail's behaviour generally remains apparently normal. However, during periods of hot, still weather at Calvert's Lagoon, when oxygen tension in the lagoon would be relatively low, snails climb up water plants out of the water, or hang suspended from the water surface. The same behaviour is observed when snails are transferred from the lagoon to plastic containers in the laboratory, particularly when aeration is withheld. Examination of snails that had emerged from the water revealed that an unusually high proportion of them harboured hundreds

of cysts of *A. coxiellae*. The relationship between the positions, relative to water level, assumed by 114 adult snails maintained in an unaerated container for 24 hours after collection, and the incidence of infection with *A. coxiellae* is shown in Table 7.6.

TABLE 7.6 The relationship between the positions, relative to water level, assumed by *Coxiella badgerensis*, and the incidence of infection with *Atriophallophorus coxiellae*.

	Position of snail		
	emerged	floating	submerged
Number of snails (total = 114)	19	42	53
Observed: infected	13	7	6
not infected	6	35	47
*Expected: infected	4.3	9.6	12.1
not infected	14.7	32.4	40.9
X_1^2	22.8	0.91	3.98
P	0.001>P	0.5>P>0.2	0.05>P>0.02
Sig.	***	N.S.	*

(*expected numbers based on the proportion of snails infected with *A. coxiellae* in the whole sample i.e. 26/114 = 0.228).

Significantly more of the snails that emerged from the water were infected with *A. coxiellae* than would be expected by chance alone. The incidence of infection with *A. coxiellae* among the floating snails was not significantly different than that expected, whereas the incidence among the snails below the water surface was significantly less than that expected by chance. Most primary trematode infections cause depletion of their molluscan host's glycogen reserves and make infected snails less able to tolerate anaerobic conditions (Wright, 1971; Erasmus, 1972). Emergence of *C. badgerensis* from the water appears to be a response to low oxygen tension. Snails infected with *A. coxiellae* are less likely to be able to tolerate low oxygen tension than are healthy snails or snails with more benign infections, and thus are more likely to emerge. This behaviour probably increases the chance of transmission of *A. coxiellae*,

and, perhaps, other trematodes, by making the snail host more vulnerable to predation by birds.

Some snails have abnormally thin, and light brown, rather than dark green-brown, shells. With rare exceptions, these snails were found to harbour primary trematode infections and an unusually high proportion of them contained cysts of *A. coxiellae*. In several other studies, shell fragility has been linked with parasitism, and it is possible that trematode infections may interfere with the snail's normal calcium metabolism and that this is reflected in the condition of the shell (Wright, 1971). Environmental conditions, however, can also lead to shell fragility (Wright, 1971). Uninfected snails with fragile shells were rarely found at Calvert's Lagoon.

Developmental stages of many trematodes interfere with the reproductive capacity of the molluscan host, and may cause 'parasitic castration' (Cheng, 1967; Wright, 1971; and McArthur and Featherston, 1976). Wright (1971), reported that direct attack by trematodes on the reproductive system of molluscs is rare and that the majority of trematodes cause little physical damage to the host's reproductive system. However, in Tomahawk Lagoon, New Zealand, McArthur and Featherston (1976), found that 7 of the 9 trematode species infecting the ovoviviparous, parthenogenetic hydrobiid *Potamopyrgus antipodarum*, invaded the gonad and reduced or suppressed egg production. All of the trematodes developing in *C. badgerensis* invade the gonad or surrounding tissues and, almost certainly, interfere with the reproduction of the host. Healthy adult males store large amounts of sperm in the posterior vas deferens, which becomes creamy pink, and very conspicuous. However, the posterior vas deferens of infected adult males is greatly reduced, and contains little or no sperm. Oocytes can be easily seen in healthy adult females as they pass along the oviduct from the ovary. Oocytes are rarely present, however, in infected adult females. Parasitization of *C. badgerensis* probably results initially in reduction of the reproductive capacity, and, in

chronic infections, eventual castration. Castration may result directly from invasion of the gonad, or indirectly from invasion of the digestive gland. In the latter case, it may be a consequence of deprivation of nutrients supplied by the digestive gland and the blood and also of pressure upon the adjacent gonad.

Marked reduction in the size of the penis occurs in infected males. A similar phenomenon has been observed in other prosobranch snails. Rees (1936), found that greater reduction in the terminal genitalia of *Littorina littorea* occurred in snails infected with parasites which caused direct physical destruction of the gonad, than in those infected with parasites which did indirect damage.

7.1.5 Discussion

Male and female snails were found to occur in equal proportions in the size range A.L. 2.0 - 2.5 mm (Figure 7.2). Snails of this size are young, reproductive adults. In the next size range, A.L. 2.5 - 3.0 mm. there were significantly more male than female snails. There was no significant difference, however, in the abundance of male and female snails with A.L. greater than 3.0 mm. The results indicate that there is a differential mortality of the sexes at different stages in their life-histories. This differential mortality may be related to the high incidence of primary trematode infections in adult snails in Calvert's Lagoon. The burden of trematode parasitism, added to the energy demands of reproduction, may be too great a strain for some members of each sex. 'Susceptible' adult females may be killed more quickly than 'susceptible' adult males.

Numerous studies of trematode infections of snail populations have shown that the incidence of multiple simultaneous infections with different trematode species is low compared to the incidence of single infections. This has been attributed either to the specificity of the miracidial stage, or to the development of immunity subsequent to the establishment of the first infection (Erasmus, 1972). However, consideration of the

rarity of multiple infections means little unless the observed incidence of such infections is compared to that expected by chance. The expected incidence of multiple infections is calculated by multiplication of the proportion of snails in the population infected with each of the trematode species, and the number of snails in the sample. Different workers have found that, depending on the trematode species concerned, the observed incidence of multiple infections may be the same as, greater than, or less than, the expected incidence. Ewers (1960), found that there was an unusually high frequency of double infections with a heterophyid (*Stictodora* sp.) and a schistosome (*Austrobilharzia terrigalensis*) in the estuarine snail *Velacumantis australis*, in an Australian coastal lagoon. He postulated that infection with one of the trematode species rendered it more prone to infection by the other. James (1969), found an unusually high incidence of double and triple trematode infections in *Littorina saxatilis* in Wales. In his calculations, however, he underestimated the expected incidences of multiple infections, by multiplying the incidences of single infections with each species, rather than the overall incidences of each species (i.e. in single and multiple infections). Bourns (1963), found that 4 of the 6 trematode species infecting *Lymnaea stagnalis* in a Canadian marsh, occurred in multiple infections, and 9 of the 10 possible combinations of double and triple associations were found to occur. Two of the combinations occurred less frequently than expected, 2 occurred at the expected frequency, and 5 occurred more frequently than expected. Wright (1971), postulated that prior infection with one trematode may predispose a snail to attack by another, by:

(1) increasing the chemical attractiveness of the snail so that other miracidia are able to respond to it from a greater distance; or

(2) causing some change in the behaviour of the snail so that it is placed at greater risk of infection.

In some cases, unexpectedly low frequencies of multiple trematode infections may be due to interspecific competition between the intramolluscan developmental stages. Lie *et al.* (1965) showed that echinostome rediae actively prey upon the sporocysts of a schistosome, xiphidiocercariae and strigeids, when they occur together in certain lymnaeid snails in Malaysia.

The incidence of multiple infections of *Coxiella badgerensis*, involving the 5 most abundant trematodes, were investigated. All of the 10 possible double associations, and 6 of the 10 possible triple associations occurred. The incidences of 6 of the double associations, and all of the triple associations, were not significantly different from those expected by chance. However, 4 of the double associations occurred significantly less frequently than expected by chance. The existence of a system of acquired immunity in molluscs is still questionable and the limited data on the subject are in conflict (Wright, 1971). Just as behavioural changes of the host snail, related to parasitism, may increase the frequency of certain multiple associations (Wright, 1971), logically, they may also decrease the frequency of associations involving other trematode species. Vertical movement of *Coxiella badgerensis*, away from areas of low oxygen tension, has been observed in Calvert's Lagoon. Infected snails are less able to tolerate anaerobic conditions than healthy snails (Wright, 1971), and hence are more likely to move towards the surface of the lagoon. This behaviour would decrease their chances of further infection with trematodes, such as microphallids, whose eggs hatch only after ingestion by the molluscan host, because trematode eggs would be concentrated on the bed of the lagoon.

Statistical analysis of the incidence of primary trematode infections in *Coxiella badgerensis* showed that there was significant seasonal variation. There was a sequential increase in the overall level of infection, from winter through to autumn. This was probably

due to the increase in average water temperature during this period (Figure 1.4) and consequent acceleration in the rate of development of trematode eggs in the lagoon, and intra-molluscan developmental stages. Although water temperature starts to drop in autumn, the peak of infection in this season, rather than summer, was probably due to the time required for the development of trematodes in the lagoon. Depending on the temperature and trematode species, eggs which are undifferentiated when deposited generally take from 10 days to 3 weeks to mature, and free daughter sporocysts appear in molluscan tissues about 3 weeks after invasion by a miracidium (Erasmus, 1972). Seasonal variation in the overall level of primary trematode infections may also reflect variation in the influx of trematode eggs into the lagoon. Changes in the total bird population at Calvert's Lagoon were observed. These were related mainly to the breeding cycles of the birds, the availability of food, and the weather conditions at the lagoon. The relative abundance of the more common bird species at the lagoon did not vary greatly, and hoary-headed grebes were always the most abundant species. This was probably responsible for the fact that the relative abundance of trematode families infecting *C. badgerensis* did not vary significantly between seasons. In fact, the relative abundance of the more common trematode species was fairly constant throughout the period of study.

McArthur and Featherston (1976), found that primary trematode infections reduced or suppressed egg production in *Potamopyrgus antipodarum* in Tomahawk Lagoon in New Zealand, however they considered that the low incidence of such infections (4%), made it unlikely that parasitism was a factor regulating the snail population. The incidence of primary trematode infections in adult snails at Calvert's Lagoon, however, varies from about 30 to 100%, and parasitism is probably an important factor regulating the snail population. There is a high probability that any snail inhabiting Calvert's Lagoon will be invaded by one or more miracidia, and eventually be castrated. During the last decade, however,

since studies of trematodes at the lagoon began, the snail population in Calvert's Lagoon has been consistently very large. The incidence of primary trematode infections in snails is directly related to the age of the host (Figure 7.7), and although a very large proportion of adult snails are parasitized and eventually castrated, reproduction by the uninfected and recently infected adults, must be sufficient to maintain a large, viable snail population in the lagoon.

7.2 Austrochiltonia australis (Sayce, 1901)
syn. *Austrochiltonia subtenuis* (Sayce, 1902)
(Order Amphipoda; Family Ceinidae; Subfamily Chiltoniinae)

7.2.1 Taxonomic status

The genus *Austrochiltonia*, was erected by Hurley (1959), to include 2 similar Australian species, *A. australis* and *A. subtenuis*. The genus was later rediagnosed, and the 2 species redescribed, by Williams (1962). The morphology, ecology and distribution of the species are very similar. Both have been recorded from a variety of habitats: large mesotrophic lakes, lagoons in coastal sand dunes, small ponds and dams, small sluggish streams and fairly large creeks.

Despite a detailed comparative study of *A. australis* and *A. subtenuis*, Williams (1962), was able to find only 3 characters to distinguish the species:

(a) the presence or absence of a ramus on the third uropod,

(b) the difference in the relative length of the first antenna and the body, and

(c) the difference in the relative length of the flagellum and peduncle of the first antenna .

Williams (1962), stated that "these differences, as well as other morphological characters of a non-specific nature (e.g. gnathopods), exhibit a considerable degree of phenotypic variation".

According to Williams (1962), the absence or presence of a ramus

FIG. 7.11

Crustacean intermediate hosts

A

B

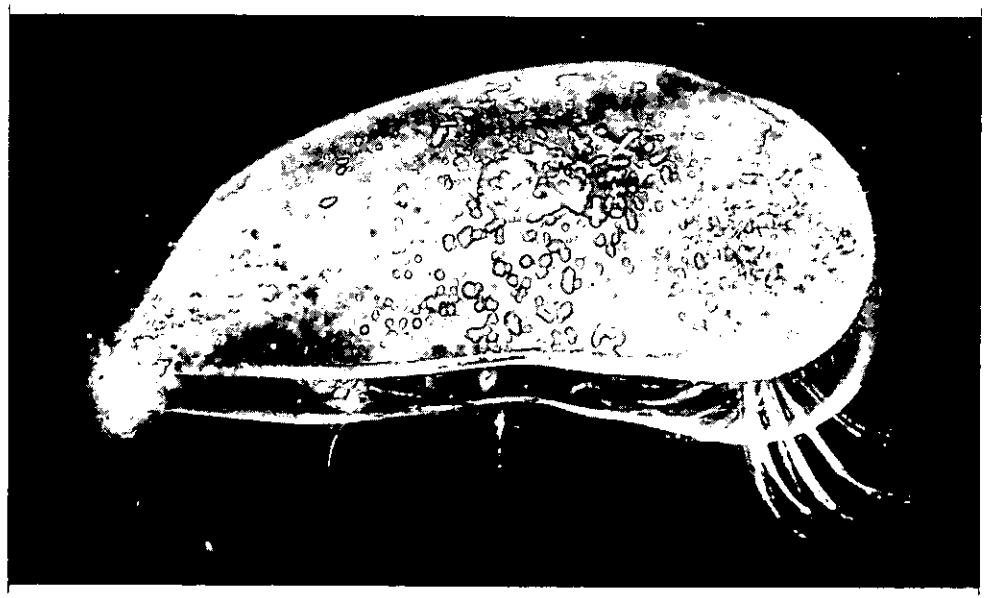


FIGURE 7.11 A, *Austrochiltonia australis*, gravid female; B, *Mytilocypris tasmanica*.

on uropod 3 was quite distinct: in *A. australis*, uropod 3 was 2-segmented, an intersegmental joint between peduncle and ramus being clearly visible and unmistakeable, whilst in *A. subtenuis* uropod 3 was 1-segmented, and although a slight "furrow" may rarely occur on the segment body, a ramus was obviously not present. At one locality (Casterton, Victoria), where both *Austrochiltonia* species occurred, they were collected in more or less equal proportions, and amongst the large number of third uropoda examined, none were found that could be regarded as of intermediate form.

The specific difference in relative length of the first antenna and the body was found by Williams (1962), to be less distinct. Although the difference between the mean of 0.42 for 21 *A. australis* males and 0.36 for 26 *A. subtenuis* males was significant ($P<0.01$), the range of variation was from 0.27 to 0.56 for *A. australis*, and from 0.28 to 0.58 for *A. subtenuis*. There was an indication that in both species, the ratio of the length of the first antenna to the body length altered with changes in body size (Williams, 1962).

Williams (1962), reported that the specific difference in the relative length of the flagellum and peduncle of the first antenna was distinct. He found a very significant difference between the mean of 1.96 for 21 *A. australis* males and 1.25 for 26 *A. subtenuis* males ($P<0.001$). However, a later study of the biology of *A. subtenuis* (Lim, 1964), has shown that the average number of segments added to the flagellum of the first antenna at each moult is variable, 1 or 2 segments being added at each moult up to at least the ninth moult. Hence the relative length of the flagellum and peduncle may vary with age.

In the present study, a large number of third uropoda of adult amphipods in Calvert's Lagoon, were examined to determine which of the *Austrochiltonia* species inhabit the lagoon. It was found, as indicated by Williams (1962), that there was considerable morphological variation in the form of the third uropod. However, almost continuous variation was observed, from third uropoda that were distinctly 2-segmented, to

those which were 1-segmented. In most specimens, an intersegmental joint was distinct, and the ramus was recessed into the peduncle (Figure 7.12C). Other forms varied from those in which the ramus was not recessed into the peduncle, but an intersegmental joint was clearly defined, to those in which a transverse crease occurred in the segment body in the same position as an intersegmental joint, but extended only part of the way across the segment, from a distinct 'notch' (thinning of the skeleton), on one side. In other forms, there was either no evidence of an intersegmental joint, or there was a 'notch' on one side of the segment, in the same relative position as an intersegmental joint, but no transverse crease, (Figure 7.12E). Specimens in which an intersegmental joint was distinct, are referred to here as "australis" type, and the other forms are referred to as "subtenuis" type. Some amphipods were found to have one third uropod distinctly 2-segmented, and the other 1-segmented. These forms are referred to as "australis/subtenuis" type, (Figure 7.12D).

The third uropoda of 104 amphipods, collected at Calvert's Lagoon, were examined: 86.5% were "australis" type, 5.8% were "subtenuis" type and 7.7% were "australis/subtenuis" type. Gravid females, collected at Calvert's Lagoon, were isolated from each other in containers in the laboratory until they had released their young, on average 10 (7 - 22) days later. The third uropoda of the females were then examined. The young of 14 "australis" type females were kept in one container, of 3 "subtenuis" type females in another and of 2 "australis/subtenuis" type females in a third container. The young were maintained in the laboratory, and after 2 to 3 months, when fully grown, the third uropoda of amphipods from each of the containers were examined. The results are shown in Table 7.7.

Females of the "australis" and "australis/subtenuis" types produced only young of the "australis" type. However, females of the "subtenuis" type produced young of all three types.

The morphology of the third uropod has been used to distinguish

FIG. 7.12 Third uropoda of Austrochiltonia

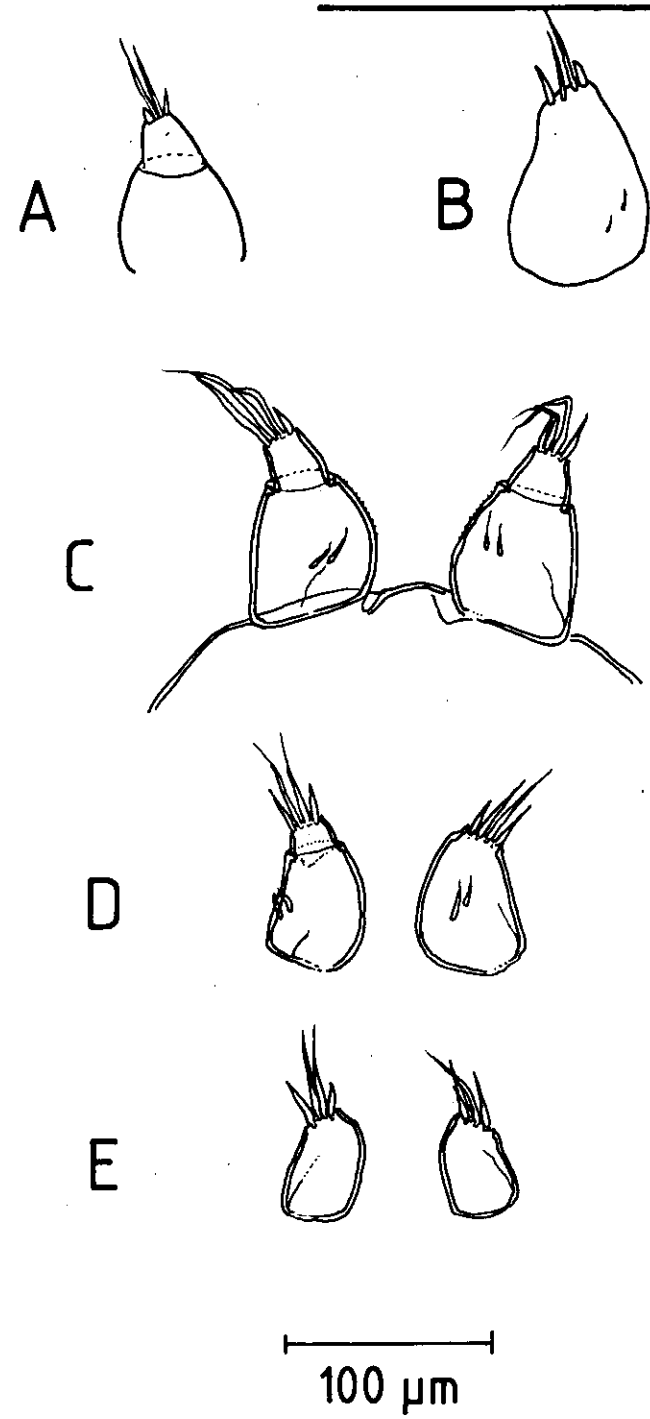


FIGURE 7.12 A, *A. australis*, (Williams, 1962 Figure 3N); B, *A. subtenuis*, (Williams, 1962 Figure 6D); C, "australis" type, Calvert's Lagoon, D, "australis/subtenuis" type, Calvert's Lagoon; E, "subtenuis" type, Calvert's Lagoon.

TABLE 7.7 Morphological variation in the third uropoda of offspring of *Austrochiltonia* females.

Parent type	Number of offspring examined	Offspring type (%) "australis"	"australis/subtenuis"	"subtenuis"
"australis"	55	100	0	0
"australis/ subtenuis"	19	100	0	0
"subtenuis"	55	92.7	5.5	1.8

the 2 species in the genus *Austrochiltonia*. The results of this study indicate that this distinction is not valid, and that *A. subtenuis* should be considered a synonym of *A. australis*.

In Victoria, colouration of the eggs of *A. australis* and *A. subtenuis* are reported to be distinct, with *A. australis* having yellowish eggs and *A. subtenuis* green eggs (Lim and Williams, 1972). However, in Calvert's Lagoon, both "australis" type and "subtenuis" type *Austrochiltonia* usually have green eggs. Colour variation of amphipods in Calvert's Lagoon has been shown to be related to infection by trematode cysts (7.2.3), with infected individuals gradually changing from blue-green to yellow-orange. Parasitism might also be found to influence egg colour.

Only *A. australis* has been recorded from New South Wales, whereas both *A. australis* and *A. subtenuis* have been recorded from Victoria and Tasmania, and only *A. subtenuis* has been recorded from South Australia and West Australia. This distribution may reflect clinal variation in the morphology of *Austrochiltonia*. Lim and Williams (1971), noted that *Austrochiltonia*, which occurs in many temporary water bodies and newly formed farm dams, has efficient dispersal mechanisms, and suspected that birds are involved. In fact, *A. australis* was found on a duck shot at Sandford, near Calvert's Lagoon (Tasmanian Museum records). The morphometric differences found by Williams (1962), in specimens of *A. australis* and *A. subtenuis* from a lagoon at Casterton, Victoria, may be due to the heterogeneity of origin of amphipods in that lagoon. Morphologically

dissimilar amphipods may have been transported to the lagoon from widely separated and distinct habitats.

7.2.2 *Infection*

Amphipods in Calvert's Lagoon are heavily infected by metacercarial cysts of the microphallids, *Levinseniella tasmaniae* and *Maritrema calvertensis*. A sample of 120 amphipods (body length greater than 2 mm), was collected in May 1978. Examination revealed that 91.7% harboured trematode cysts; 54.2% being infected by *L. tasmaniae* and 88.3% by *M. calvertensis*. Cysts of both species occurred in 54.2% of amphipods; 34.2% were infected by *M. calvertensis* alone and 3.3% were infected by *L. tasmaniae* alone.

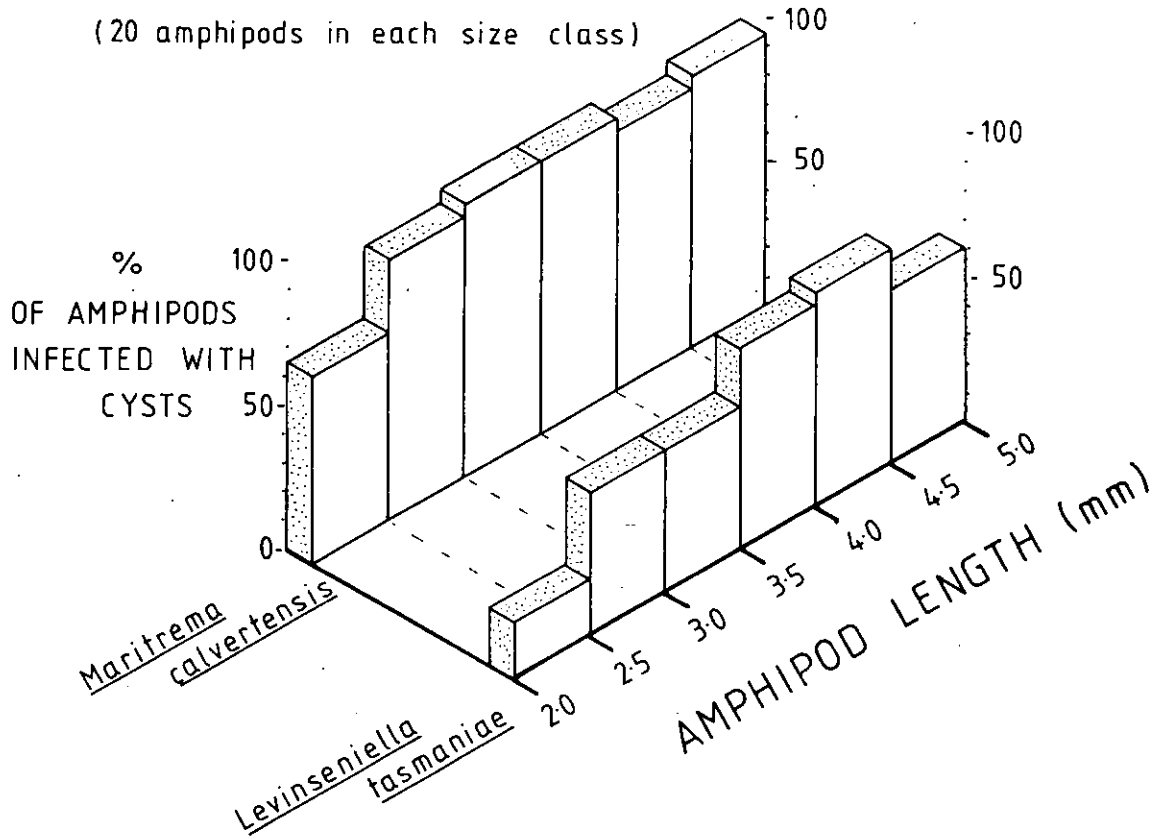
The incidence of double infections in *A. australis* expected by chance alone is $\frac{54.2}{100} \times \frac{88.3}{100} \times 120 = 57.4$, which is not significantly different from that observed (Table 7.8).

In any investigation of infection, consideration must be given to the host population structure. Lim (1964), found that in a population of *Austrochiltonia* at Lake Modewarre, Victoria, the sex-ratio of amphipods longer than 2.5 mm varied during the year, with females always being more abundant than males. He reported that the percentage of males varied from 26 to 47%, with an overall average of 35%. At Calvert's Lagoon, in a sample of 124 amphipods longer than 2.5 mm, collected in May 1978, 38% were males. This sex-ratio differs significantly from a 50:50 ratio ($X_1^2 = 7.03$; $0.001<P<0.01$). Of the 70 amphipods in this sample that were less than 4 mm long, only 29% were males, a highly significant deviation from a 50:50 ratio ($X_1^2 = 12.86$; $P<0.001$), however, exactly 50% of the 54 amphipods from 4 to 6 mm long were males. The amphipods longer than 4 mm were examined for metacercarial cysts, and the data were logarithmically transformed to a normal distribution for statistical analysis. The relationship between sex and infection is shown in Table 7.9.

known (Barrett and Butterworth, 1968; Bethel and Holmes, 1977), however such changes are due to the bright orange colour of the parasite. The cysts of *L. tasmaniae* are colourless, and the orange colour of *Austrochiltonia australis* appears to be due to the accumulation of orange pigments in the host tissues. These pigments may be carotenoids,which have been recorded in many invertebrates, including crustaceans (Cheesman *et al.*, 1967; Czeczuga and Czerpak, 1968). Carotenoids have been implicated in many energy transfer mechanisms in plants and animals and may be involved in the respiration and electron transport systems of invertebrates (Zavras and James, 1979). Zs-Nagy (1971), found that in molluscs, carotenoids substitute molecular oxygen in the metabolism when the animal is in a low-oxygen environment. Zavras and James (1979), suggested that *Littorina littorea* may be adapted to conditions of low-oxygen tension, by utilizing carotenoids obtained from its algal food, and further, that the ability of the snails to accumulate carotenoids may be genetically controlled. Accumulation of carotenoids in *A. australis*, therefore, may be an adaptation to low-oxygen tensions in the body tissues, resulting from infection with the large metacercarial cysts of *L. tasmaniae*. Such an adaptation would be of selective advantage particularly in conditions of low environmental oxygen tension. Corollaries of this hypothesis are that amphipods infected with *L. tasmaniae* would become orange at a rate inversely proportional to the environmental oxygen tension; and directly proportional to environmental temperature, and the number of metacercarial cysts present.

Bethel and Holmes (1973), found that amphipods infected with cystacanths of *Corynosoma constrictum* and *Polymorphus paradoxus* were more likely, than healthy amphipods, to be eaten by their bird predators. They believed that altered evasive behaviour and abnormal responses to light of infected amphipods were the main factors responsible for the increased vulnerability of these animals to predation; however, the abnormal colour of such infected amphipods may also be a factor in their

FIG. 7.13 Relationship between length of Austrochiltonia australis and incidence of infection with cysts of Maritrema calvertensis and Levinseniella tasmaniae.



cysts of each species was greater in larger amphipods. There was a marked decrease, however, in the mean number of cysts of *L. tasmaniae* in amphipods longer than 4.5 mm, relative to the previous size class. This may have been due to the death of older amphipods that were heavily infected by the relatively large cysts of *L. tasmaniae*.

TABLE 7.10 The relationship between the length of amphipod and number of cysts of *Levinseniella tasmaniae* and *Maritrema calvertensis*.

Size range of amphipods (mm)	Number of amphipods	*L. tasmaniae* mean	variance	range	*M. calvertensis* mean	variance	range
2.0 - 2.5	20	0.3	0.4	0 - 2	2.3	8.5	0 - 11
2.5 - 3.0	20	0.9	1.1	0 - 11	4.0	14.5	0 - 14
3.0 - 3.5	20	0.9	1.7	0 - 5	3.9	7.3	0 - 9
3.5 - 4.0	20	2.7	13.6	0 - 15	6.1	38.7	0 - 26
4.0 - 4.5	20	4.2	23.0	0 - 17	8.4	118.7	0 - 40
4.5 - 5.0	20	3.0	13.5	0 - 11	8.7	73.7	0 - 36

The observed relationship between sex-ratio and size of amphipods may be a result of differential mortality of the sexes at different stages of their life-history, and this differential mortality may be related to trematode parasitism. Metacercarial cysts may be more harmful to young males than young females, perhaps because the former have lower food reserves than the latter. The strain of egg production, added to the deleterious effects of parasitism, may cause an increased mortality rate for older females, relative to males of the same age.

7.2.3 Parasitism and host colour

Holmes (1901), recorded colour changes in individual amphipods of the species *Amphithoe longimana*, at Woods Hole, Massachusetts. Individuals were found to change from blue-green to red-brown. The colour changes were produced by variation of 5 factors: "the colour of the chitinous integument, the colour of the blood and tissues, the contents of the alimentary canal, the colour of the sex glands, and the pigment cells" (Holmes, 1901). Lim (1964), reported colour variation in *Austrochiltonia*

in Victoria, from blue-green to yellow, "some with a reddish tinge", however, no colour changes were observed in individual specimens. At Calvert's Lagoon, *Austrochiltonia australis* varies in colour from blue-green to bright orange. Amphipods raised under controlled conditions in the laboratory are uniformly pale green in colour. Every orange amphipod examined has been found to harbour cysts of *Levinseniella tasmaniae*. Many of these amphipods were also infected by *Maritrema calvertensis*, however, some were not.

Some isolated gravid females, kept in the laboratory at ±15°C for 2 weeks (until they released their young), were observed to change colour from green to orange. Eight of these females, averaging 4.8 (3.7 - 5.5) mm long, were dissected. All were infected with cysts of both trematode species. They harboured an average of 7.5 (4 - 11) cysts of *L. tasmaniae* and 13.9 (5 - 30) cysts of *M. calvertensis*.

In a random sample of 127 amphipods (body length greater than 2 mm), collected in May 1978, 55.1% were blue-green or green, 29.1% were orange and 15.7% were intermediate in colour (i.e. orange-green). The size of amphipods of different colour is shown in Table 7.11.

TABLE 7.11 The relationship between length and colour of amphipods collected in May 1978.

Amphipod colour	Number of amphipods	Length (mm) $\bar{x}$ ± 1 S.D.	Range
orange	45	4.2 ± 0.7	3.0 - 5.6
orange-green	20	3.7 ± 0.7	2.6 - 5.8
green	45	3.4 ± 1.1	2.0 - 5.3

Orange amphipods were significantly longer than green amphipods, ($t = 4.12$; d.f. = 88; $P<0.001$), and significantly longer than orange-green amphipods ($t = 2.66$; d.f. = 63; $P = 0.01$). There was no significant difference, however, between the lengths of orange-green and green amphipods ($t = 1.32$; d.f. = 63; $P>0.1$).

Eighty amphipods of different colour, but similar size, were selected from the above sample and examined for metacercarial cysts. The percentage infection of amphipods of different colour, with cysts of each of the trematode species, is shown in Figure 7.14. The number of cysts per amphipod were recorded and the data normalised by logarithmic transformation, for statistical analysis.

TABLE 7.12 The number of metacercarial cysts of *L. tasmaniae* in amphipods of different colour, but similar size.

Amphipod colour	Number of amphipods	Number of cysts $\bar{X}a$	V_{Xa}	$\bar{X}t$	V_Xt	$\bar{X}g$	Confidence limits (about $\bar{X}g$)
orange	30	4.23	10.19	0.64	0.07	3.40	2.57 - 4.45
orange-green	20	4.20	17.64	0.60	0.10	3.03	1.93 - 4.53
green	30	0.97	6.24	0.17	0.08	0.47	0.17 - 0.84

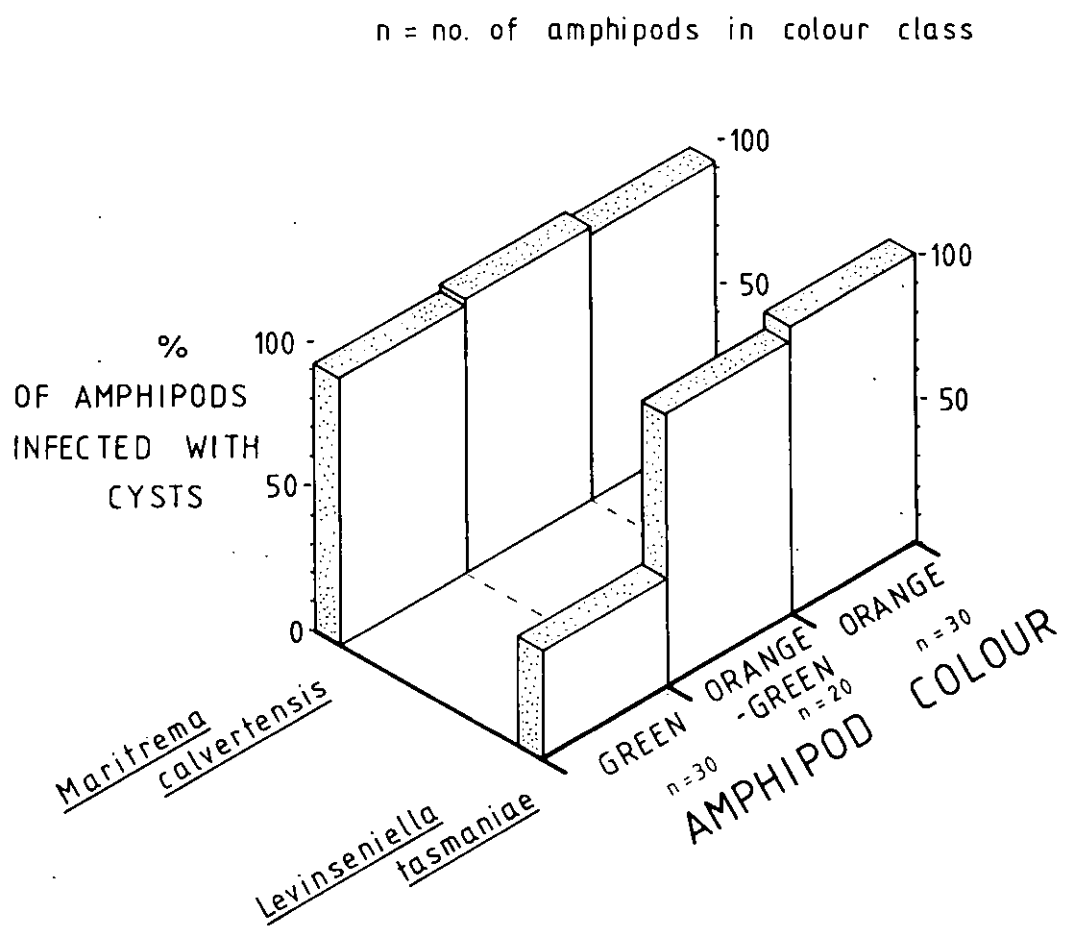
All of the orange amphipods, and 95% of orange-green amphipods were infected by *L. tasmaniae*, whilst only 37% of green amphipods harboured cysts of this trematode. There was no significant difference between the number of cysts of *L. tasmaniae* in orange and orange-green hosts ($t = 0.47$; d.f. = 48; $P>0.1$). There were, however, significant differences between the number of cysts of *L. tasmaniae* in orange and green hosts ($t = 7.00$; d.f. = 58; $P<0.001$) and between orange-green and green hosts ($t = 5.10$; d.f. = 48; $P<0.001$).

TABLE 7.13 The number of metacercarial cysts of *M. calvertensis* in amphipods of different colour, but similar size.

Amphipod colour	Number of amphipods	Number of cysts $\bar{X}a$	V_{Xa}	$\bar{X}t$	V_Xt	$\bar{X}g$	Confidence limits (about $\bar{X}g$)
orange	30	13.80	394.23	0.93	0.21	7.55	4.86 - 11.47
orange-green	20	9.20	114.80	0.82	0.17	5.54	3.29 - 8.96
green	30	5.87	48.60	0.68	0.14	3.81	2.54 - 5.53

The incidence of *M. calvertensis* was very high, from 90 to 95%, in amphipods of each colour. The number of cysts of this species in orange amphipods was significantly greater than in green amphipods

FIG. 7.14 Relationship between colour of Austrochiltonia australis and infection with cysts of Maritrema calvertensis and Levinseniella tasmaniae.



($t = 2.32$; d.f. = 58; $0.02<P<0.05$), however, there was no significant difference between the numbers of cysts in orange and orange-green hosts ($t = 0.93$; d.f. = 48; $P>0.1$), or in orange-green and green hosts ($t = 1.16$; d.f. = 48; $P>0.1$).

A direct relationship exists between the number of trematode cysts in amphipods and amphipod length (Table 7.10), and between length and colour (Table 7.11). However, the 80 amphipods discussed above were selected because of their similar size. The length of these amphipods is shown in Table 7.14.

TABLE 7.14 The length of a sample of amphipods of different colour but similar size, that were examined for metacercarial cysts (ref. Tables 7.12 and 7.13).

Amphipod colour	Number of amphipods	Length (mm) $\bar{x} \pm 1$ S.D.	Range
orange	30	4.2 ± 0.7	3.0 - 5.6
orange-green	20	3.7 ± 0.7	2.6 - 5.8
green	30	3.9 ± 1.0	2.0 - 5.3

In this selected sample, there was no significant difference between the lengths of orange and green amphipods ($t = 1.35$; d.f. = 58; $P>0.1$), or between the lengths of orange-green and green amphipods ($t = 0.83$; d.f. = 48; $P>0.1$). Hence the significant differences in the number of cysts of *L. tasmaniae* in orange and green hosts, and in orange-green and green hosts, cannot be explained by differences in the sizes, (or ages), of the amphipods.

Laboratory-bred amphipods, which were uniformly green in colour, were exposed to infection by cercariae of *L. tasmaniae* and then maintained at 25°C for 28 days, or at 15°C for 57 days (2.3.6). Metacercarial cysts developed at both temperatures, but none of the hosts became orange. Ten laboratory-bred amphipods, experimentally exposed to cercariae of *L. tasmaniae*, were maintained at 25°C for 14 days and then transferred to a laboratory, where they were kept at room temperature for a further 165 days. By this time, the original amphipods had

reproduced and several hundred individuals of various ages were present in the container. All were green, except for 2 which were orange. Dissection of the orange amphipods revealed that both were infected by *L. tasmaniae*, one harbouring 4 cysts and the other 2 cysts. Thirty green adults from the container were dissected, but none were infected. The results are shown in Table 7.15.

TABLE 7.15 The colour and size of 2 laboratory-bred amphipods infected by *L. tasmaniae* and 30 uninfected amphipods maintained under the same conditions.

Amphipod colour	Number of amphipods	Length (mm) x ± 1 S.D.	Number of cysts x ± 1 S.D.
orange	2	4.6 ± 0.2	3.0 ± 1.4
green	30	5.5 ± 0.9	0

The 2 orange amphipods were survivors of the original 10 amphipods that were exposed to infection. The green amphipods consisted mainly of descendants and possibly some survivors of the original 10 animals. All but 4 of the green amphipods in the above sample, 17 of which were females, were larger than the 2 older, infected individuals. The implication is that parasitism by *L. tasmaniae* may inhibit the growth of *Austrochiltonia australis*.

The results of the present study indicate that parasitism, by the relatively large metacercariae of *L. tasmaniae*, can induce a gradual colour change in *Austrochiltonia australis*, from green, to orange-green, to orange. It is not known whether the smaller metacercaria of *M. calvertensis* can also induce this colour change. The colour change occurs over a period of many weeks or months; the period possibly being influenced by environmental factors, such as O_2 tension, temperature and diet.

No previous records of colour variation in crustaceans, related to infection with trematodes, are known by the author. Colour changes in amphipods infected by cystacanths of various acanthocephalans are

known (Barrett and Butterworth, 1968; Bethel and Holmes, 1977), however such changes are due to the bright orange colour of the parasite. The cysts of *L. tasmaniae* are colourless, and the orange colour of *Austrochiltonia australis* appears to be due to the accumulation of orange pigments in the host tissues. These pigments may be carotenoids,which have been recorded in many invertebrates, including crustaceans (Cheesman *et al.*, 1967; Czeczuga and Czerpak, 1968). Carotenoids have been implicated in many energy transfer mechanisms in plants and animals and may be involved in the respiration and electron transport systems of invertebrates (Zavras and James, 1979). Zs-Nagy (1971), found that in molluscs, carotenoids substitute molecular oxygen in the metabolism when the animal is in a low-oxygen environment. Zavras and James (1979), suggested that *Littorina littorea* may be adapted to conditions of low-oxygen tension, by utilizing carotenoids obtained from its algal food, and further, that the ability of the snails to accumulate carotenoids may be genetically controlled. Accumulation of carotenoids in *A. australis*, therefore, may be an adaptation to low-oxygen tensions in the body tissues, resulting from infection with the large metacercarial cysts of *L. tasmaniae*. Such an adaptation would be of selective advantage particularly in conditions of low environmental oxygen tension. Corollaries of this hypothesis are that amphipods infected with *L. tasmaniae* would become orange at a rate inversely proportional to the environmental oxygen tension; and directly proportional to environmental temperature, and the number of metacercarial cysts present.

Bethel and Holmes (1973), found that amphipods infected with cystacanths of *Corynosoma constrictum* and *Polymorphus paradoxus* were more likely, than healthy amphipods, to be eaten by their bird predators. They believed that altered evasive behaviour and abnormal responses to light of infected amphipods were the main factors responsible for the increased vulnerability of these animals to predation; however, the abnormal colour of such infected amphipods may also be a factor in their

increased vulnerability to predation. Barrett and Butterworth (1968) and Bethel and Holmes (1977), believed that the bright orange to red colour of cystacanths of *Polymorphus minutus* and *P. paradoxus* may make infected gammarids more noticeable to definitive hosts.

Isopods infected with cystacanths of *Acanthocephalus* species became light-coloured (Seidenberg, 1973), and showed altered behaviour (Muzzall and Rabalais, 1975). Camp and Huizinga (1979), experimentally demonstrated that light-coloured, hyperactive isopods, *Asellus intermedius*, infected with *Acanthocephalus dirus*, were significantly more vulnerable to predation by their natural fish predator, than uninfected isopods.

It is not known whether amphipods infected with *L. tasmaniae* exhibit any behavioural abnormalities, or whether the orange colour of infected amphipods increases their vulnerability to predation. However, orange amphipods swimming in the dark water of Calvert's Lagoon, are certainly more conspicuous to human observers than blue-green or green amphipods, and they may also be more conspicuous to their bird predators.

7.3 Mytilocypris tasmanica McKenzie, 1966
(Subclass Ostracoda, Order Podocopida, Family Cyprididae)

This large distinctive ostracod (Figure 7.11), was described by McKenzie (1966), from specimens collected at Calvert's Lagoon. It is an active swimmer and is very abundant in the lagoon. *M. tasmanica* serves as intermediate host for *Maritrema calvertensis*, but has never been found to harbour metacercarie of *Levinseniella tasmaniae*.

In a sample of 68 specimens (longer than 2.5 mm), collected in May 1978, 86.7% were infected with metacercarial cysts of *M. calvertensis*. Seventy-five percent of ostracods from 2.5 to 3.0 mm long were infected, 95% of ostracods from 3.0 to 3.5 mm were infected and 90% of ostracods from 3.5 to 4.0 mm were infected.

The proportion of males in different size classes in this sample varied markedly from 0.20 (valve length 2.5 to 3.0 mm), to 0.70 (valve

length 3.0 to 3.5 mm), to 0.67 (valve length 3.5 to 4.0 mm). There were significantly more females than males in the smallest size class (X_1^2 = 7.2, 0.01>P>0.001). Although there were more males than females in each of the other size classes, the differences were not significant at the 5% level. All ostracods in this sample were examined for trematodes and the data was logarithmically transformed for statistical analysis. The relationships between sex, size and infection are shown in Table 7.16.

TABLE 7.16 The relationships between sex, size and infection with *M. calvertensis* cysts, in a sample of 68 ostracods: (a) valve length 2.5 to 3.0 mm, (b) valve length 3.0 to 3.5 mm and (c) valve length 3.5 to 4.0 mm.

Ostracods Sex	No.	Number of metacercarial cysts $\bar{X}a$	Xt	V_{Xt}	Xg	Confidence limits (95%)
(a) male	4	3.3	0.581	0.058	2.8	0.6 - 8.2
female	16	2.2	0.409	0.098	1.6	0.8 - 2.8
(b) male	14	10.2	0.905	0.150	7.0	3.8 - 12.4
female	6	10.3	0.971	0.078	8.4	3.8 - 17.4
(c) male	18	11.9	0.945	0.130	7.8	4.8 - 12.3
female	9	8.4	0.672	0.347	3.7	0.7 - 12.3

There was no significant difference between the infection of males and females in each size class: (a) t = 1.20, P>0.1; (b) t = 0.43, P>0.1; and (c) t = 1.28, P>0.1. The average number of cysts in males was directly related to size. The average number of cysts in females increased in ostracods up to 3.5 mm long, however there was a marked decrease in the average number of cysts in females from 3.5 to 4.0 mm long.

The relationship between sex-ratio and size of ostracods, like that found in a sample of amphipods from the lagoon, may be a result of differential mortality of the sexes at different stages of their life-history. The differential mortality may be related to trematode parasitism, with parasitism being more harmful to young males than young females (that perhaps have greater food reserves), and being more harm-

ful to older females, weakened by the energy demands of egg production, than males of the same age.

7.4 Birds

A variety of birds occur at Calvert's Lagoon throughout the year (Figure 1.6). Some of these birds feed on aquatic invertebrates and ingest metacercarial cysts of trematodes that develop in *Coxiella badgerensis*. Many of them drop faeces, laden with trematode eggs, into the lagoon. Many of the birds are itinerants, however some, namely black swans, hoary-headed grebes and perhaps musk ducks, appear to be long-term residents.

Much information has been published on trematodes infecting waterfowl around the world (Beverley-Burton, 1972; Bezubik, 1956; Keymer *et al.*, 1962; Lapage, 1961; McDonald, 1969a, b; Mahoney and Threlfall, 1978; and Rind, 1974), however records of trematodes from Australian, particularly Tasmanian, waterfowl are fragmentary. The only previous accounts of trematodes from Tasmanian birds are by Smith (1971; and 1974), and Munday and Green (1972). Examination of some birds killed at Calvert's Lagoon during a Zoology Honours project in 1970, revealed that they served as definitive hosts for various trematodes developing in *C. badgerensis* (Smith, 1971). During the present study, birds of other species were killed at the lagoon and examined for parasites.

The gizzard contents of birds killed at Calvert's Lagoon are shown in Table 7.17. The diets of the birds vary, but many of them include snails, amphipods and ostracods. The diets are, in part, reflected by the composition of the trematode fauna of each bird host (Table 7.18). Most of the trematode species found in the birds develop in *C. badgerensis* in Calvert's Lagoon, however the opisthorchiid *Pachytrema* sp., and unidentified echinostomes, were presumably acquired elsewhere, as no opisthorchiid or echinostome developmental stages were identified in *C. badgerensis*. Although the alimentary tract of no musk ducks were

TABLE 7.17 The composition of gizzard contents of birds killed at Calvert's Lagoon

Bird	No.	Gizzard contents*							Date killed
		plants	snail (*Coxiella*)	Amphipod (*A. austral*)	Ostracod (*M.tasman.*)	Caddisfly larvae	Damselfly (Lestids)	Beetle adult	
Black duck	1	+++	-	-	-	-	-	+	27/4/78
	2	+++	-	-	-	-	-	+	27/4/78
Black swan	1	+++	-	-	-	-	-	-	8/8/78
	2	+++	-	-	-	-	-	-	8/8/78
Coot	1	+++	++	-	-	+	-	-	8/8/78
Hoary-headed grebe	1	-	-	+	++	+++	+	-	31/7/78
	2	-	+	++	-	++	-	-	31/7/78
	3	-	+++	+	-	++	+	-	14/8/78
	4	-	-	+	++	+++	+	-	14/8/78
Musk duck**	1	+	+	++	++	++	-	-	27/4/78
Black fronted*** dotterel	1	++	-	+	-	-	-	+	4/8/70
Chestnut teal***	1	++	+	++	-	-	+	+	31/10/70
Hooded dotterel***	1	-	+++	++	-	-	-	-	14/7/70
	2	-	+++	++	-	-	-	-	14/7/70
	3	-	+++	++	-	-	-	-	5/8/70
Red capped dotterel***	1	-	++	+	-	-	-	++	6/10/70

*% gizzard contents (V/V)
+++ = 51 - 100
++ = 11 - 50
+ = 1 - 10

** alimentary tract not examined for parasites
*** birds killed and examined for parasites, during Zoology Honours study, 1970 (Smith, 1971)

TABLE 7.18 The composition of the trematode fauna of birds killed at Calvert's Lagoon.

Bird	No.	Trematode family: Microphallidae			Psilostomatidae			Notocotylidae			Strigeid.	Opisth.	Echino.	Renic.
		M. calvertensis	*L. tasmaniae*	*A. coxiellae*	*P. oxyurus*	*Psilostomum sp.A*	*Psilostomum sp.B*	*P. caecai*	*P. bursae*	?Notocotylid sp.B	*Apatemon gracilis*	*Pachytrema* sp.	Unident. echinostome	Unident. renicolid
Black duck	1	+	+	-	+	-	-	+	+	-	-	-	+	-
	2	-	-	-	-	-	-	-	-	-	+	-	-	-
Black swan	1	-	-	-	+	+	-	+	-	+	-	-	-	-
	2	-	-	-	-	+	+	+	-	+	-	-	-	-
Coot	1	-	-	+	+	+	-	-	-	-	-	-	-	-
Hoary-headed grebe	1	+	+	+	-	+	-	+	-	-	-	-	-	-
	2	+	+	+	-	+	+	+	-	-	-	+	-	-
	3	+	+	+	+	+	+	+	-	-	-	-	-	-
	4	+	+	+	+	-	-	+	-	-	-	-	-	+
Chestnut teal*	1	+	+	-	-	-	-	-	-	-	-	-	-	-
Black fronted* dotterel	1	+	+	-	-	-	-	-	-	-	-	-	-	-
Hooded dotterel*	1	+	+	+	-	+	-	-	-	-	-	-	-	-
	2	+	+	+	-	-	-	-	-	-	-	-	-	-
	3	+	+	+	-	-	-	-	-	-	-	-	-	-
Red capped* dotterel	1	-	+	+	-	-	-	-	-	-	-	-	+	-

+ trematode present
- trematode absent

(* birds killed and examined for parasites, during Zoology Honours study, 1970 (Smith, 1971).

examined for parasites, the gizzard contents of one that was killed at the lagoon, indicates that they feed on aquatic invertebrates and hence probably serve as hosts for some of the trematodes developing in *C. badgerensis*.

Various organs of each bird were examined; however, the only trematodes found outside the alimentary canal were 2 adults of *Pachytrema* sp. in the gall bladder of a hoary-headed grebe, and *Paramonostomum bursae* n.sp. which occurred in the bursa fabricius of one black duck. *Levinseniella tasmaniae* and *Paramonostomum caecai* n.sp. were concentrated in the caeca of various hosts. Although the mesenteric blood vessels of each bird were scrutinized for schistosomes, none were found. This is surprising, as the developmental stages of an avian schistosome were present in *C. badgerensis* throughout the year. It is possible that the adult schistosomes were overlooked, or that they inhabit blood vessels in parts of the body that were not examined (such as the nasal region), or that they infect hosts that were not examined, such as musk ducks.

The distributions of flukes in the alimentary canals of 2 black ducks and 2 hoary-headed grebes are shown in Figures 7.15 and 7.16. The preferred habitats of most species are well defined and correspond closely with those observed in experimentally infected ducklings. There is considerable overlap in the distribution of *Maritrema calvertensis* and *Atriophallophorus coxiellae*, however the former species was always found to extend further anteriorly in the small intestine, than the latter.

Investigation of the trematode fauna of birds at Calvert's Lagoon has shown that the birds serve as hosts for a wide variety of trematodes. The diversity of the trematode fauna, as well as varying between bird species, also varies greatly between different individuals of the same species. For example, 2 black ducks were killed while standing together on the beach of Calvert's Lagoon. One of the birds was found to harbour 6 trematode species, 5 of which develop in *C. badgerensis*, whereas the

FIG. 7.15 Distribution of trematodes in two black ducks that were killed at Calverts Lagoon, April 1978.

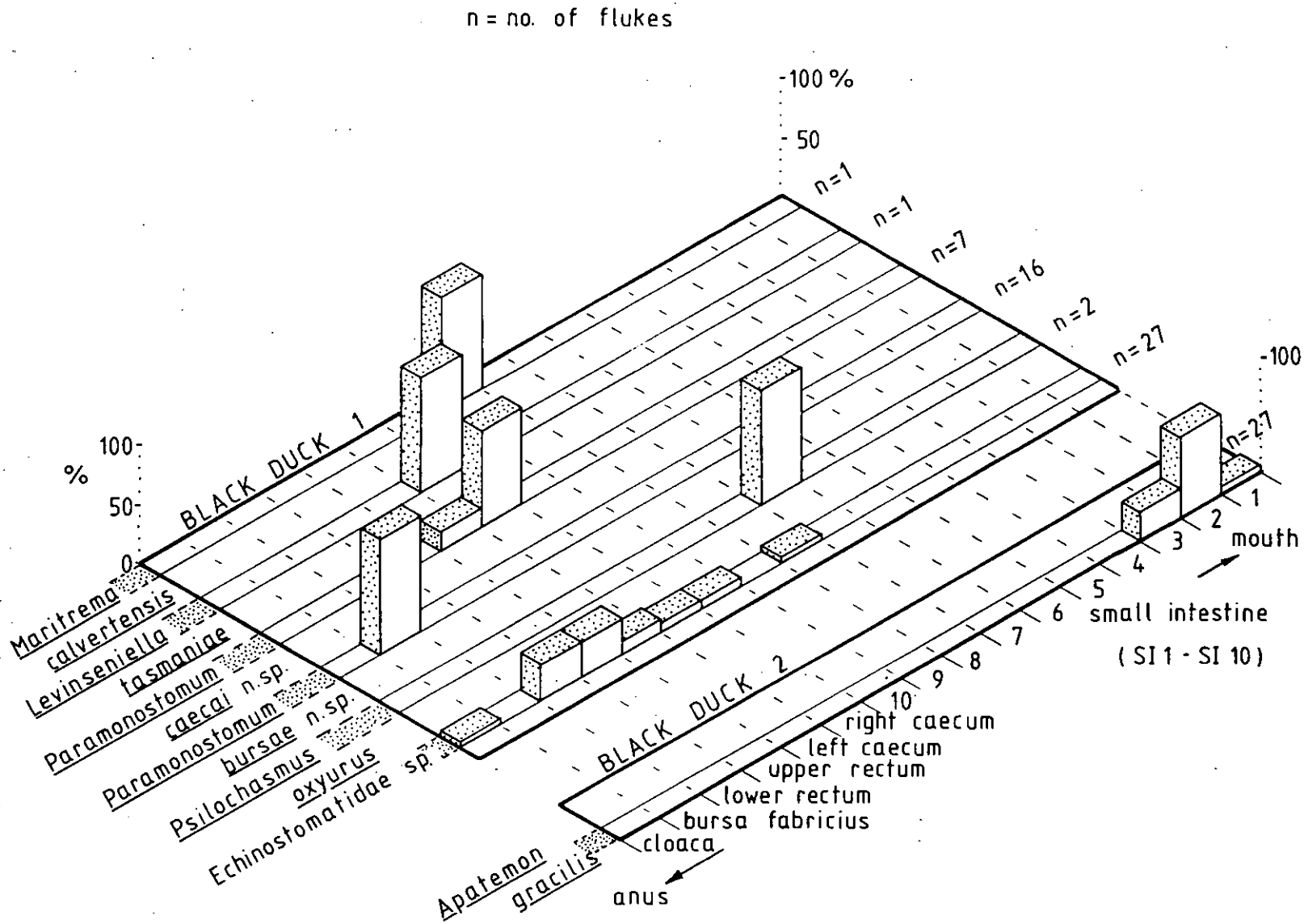
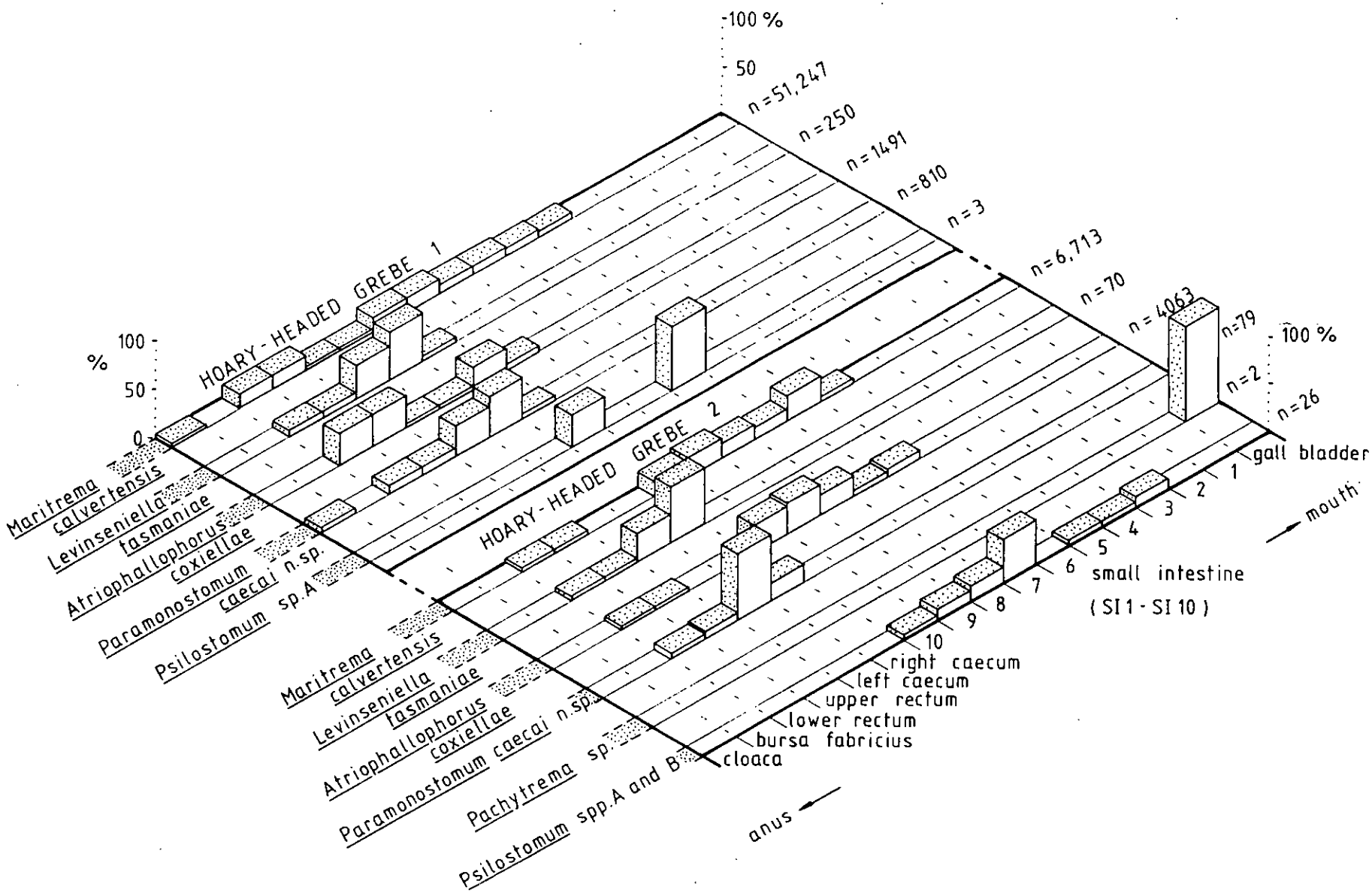


FIG. 7.16 Distribution of trematodes in two hoary-headed grebes that were killed at Calverts Lagoon, July 1978.



other bird, presumably a recent immigrant, harboured only *Apatemon gracilis*. *A. gracilis* is not known to complete its life-cycle at Calvert's Lagoon, but does complete its life-cycle at Lake Crescent, on the Central Plateau, about 100 km NW of Calvert's Lagoon. The maximum number of trematode species in any host, 7, was found in 2 hoary-headed grebes. Of the long-term avian residents of Calvert's Lagoon, the hoary-headed grebes are probably most important in maintaining the high levels of infection that were observed in aquatic invertebrates in the lagoon. They are present in large numbers throughout the year and in contrast to the swans, coots and ducks, feed mainly on invertebrates.

7.5 Salmo trutta (Linnaeus, 1758) (Family Salmonidae)

Calvert's Lagoon was once an excellent brown trout fishery. In 1963, however, during an unusually hot, dry summer, there was a drastic decrease in water level, and as a consequence, greater temperature fluctuations and an increase in the alkalinity. Coincidental with this, the trout in the lagoon died. In November 1970, the Inland Fisheries Commission conducted an experiment with yearling trout in cages; however, all died within 8½ hours in the lagoon. Over the next few years the lagoon level rose and the alkalinity of the water decreased. In September 1975, 85,000 brown trout fry were released into the lagoon, followed by releases of 50,000 and 62,000 brown trout fry in September and October 1976. An investigation was conducted into the diet of the trout and their possible role in the life-cycles of trematodes developing in *Coxiella badgerensis*. During this investigation, a baited wire cage-trap (for small fish), was set in the lagoon; however, none were caught. Brown trout are the only fish that have been recorded in the lagoon.

In June 1978, 5 trout were netted at Calvert's Lagoon and examined for parasites. Age determinations, based on scale growth, indicated

that 3 of the trout were survivors of the 1975 fry liberation, and 2 were survivors of the 1976 fry liberations (Inland Fisheries Commission Newsletter, Vol. 7, (4), pp.4-7, July 1978). All of the fish had grown rapidly in the lagoon. The composition of stomach contents of the fish is shown in Table 7.19.

TABLE 7.19 Composition of the stomach contents of brown trout taken from Calvert's Lagoon, July 1978.

	Brown trout				
	(1)	(2)	(3)	(4)	(5)
Weight (kg)	2.34	1.29	1.08	1.00	0.98
Length (cm)	60	49.5	46.2	47.7	43.5
Sex	M	M	M	F	M
Age (years)	3	3	2	2	2
Stomach contents*					
snail (*C. badgerensis*)	-	-	-	+	-
amphipod (*A. australis*)	++	+	+	+	++
ostracod (*M. tasmanica*)	+	+++	-	+	+++
damsel-fly larvae (Lestidae)	++	+	+++	+	+
caddis-fly larvae (Leptoceridae)	++	+	++	+++	-
aquatic bugs (Notonectidae; Corixidae)	+	+	+	+	-

* % (V/V) of stomach contents
+++ = 51 - 100
++ = 11 - 50
+ = 0 - 10

No helminth infections were found in any of the fish examined. Many microphallid and notocotylid metacercarial cysts were found in the alimentary tracts of the fish, but no excysted trematodes were present. The various other organs examined (eyes, brain, gonads, skeletal muscle, heart, liver and kidney), were also free of infection. At the time of sampling, brown trout did not appear to serve as either intermediate or definitive hosts for any of the trematode species developing in *Coxiella badgerensis*.

Since being introduced into Tasmania as ova from Great Britain

in 1864, brown trout have become widely distributed in rivers and still water-bodies throughout the State. No records of their helminth fauna, however, have been published. In New Zealand, where brown trout were introduced as ova from Tasmanian stocks, 6 trematode species have been recorded as parasites of this fish (Dix, 1968; and Hewitt and Hine, 1973). Three of the species, *Derogenes varicus*, *Lecithocladium seriollelae* and *L. magnacetabulum* were found in the stomachs of sea trout (Dix, 1968). *Coitocaecum anaspidis* inhabits the intestine of young freshwater trout (Macfarlane, 1939), and a small number of metacercarie of *Telogaster opisthorchis* and *Stegodexamene anguillae* were found in the muscles of young freshwater trout (Macfarlane, 1945 and 1952). Of these trematodes, only *Coitocaecum anaspidis* has been recorded in Tasmania. It was described from progenetic metacercariae taken from the mountain shrimp, *Anaspides tasmaniae*, (Hickman, 1934). In a study of the helminth parasites of brown trout in New Zealand, Dix (1968), concluded that there was a reduction in the parasitic fauna compared with that of trout in their native habitat; and that the parasites of introduced trout showed low host specificity.

Although no trematodes were found infecting trout in Calvert's Lagoon, the reintroduction of this fish had a marked effect on the diversity of species developing in *Coxiella badgerensis*. No trematodes that utilize fish as intermediate hosts were recorded during a study in 1970 (Smith, 1971). However, *Heterophyid spp.A*, *B* and *C* (Heterophyidae) and *Renicolid sp.A* (Renicolidae), whose life-cycles may include a fish intermediate host, and *Apatemon gracilis* (Strigeidae), whose life-cycle definitely includes a fish intermediate host, were found developing in *C. badgerensis* during the present study. Eggs of these species were probably dropped into the lagoon by piscivorous birds, such as cormorants and herons, attracted by the presence of the fish. Although no metacercariae were found in the adult trout examined, it is not known whether young trout could act as intermediate hosts for these trematodes.

PART VI

GENERAL DISCUSSION

Chapter 8 COASTAL LAGOONS AS FOCI FOR TREMATODE LIFE-CYCLES

The desirability of an ecological approach to studies of trematode life-histories has been emphasized by many authors (e.g. Cable and Hunninen, 1940; Dogiel, 1962; Wright, 1971 and Erasmus, 1972). Studies of the parasites of various water-bodies by Wesenburg-Lund (1934), Wikgren (1956), Wisniewski (1958), Chubb (1963, 1964), Zdarska (1964) and Styczynska-Jurewicz (1966), have shown that the trematode fauna of an aquatic biocoenosis is related to a number of ecological factors. In a closed, shallow, eutrophic environment like Calvert's Lagoon, that is visited by many birds, a relatively high incidence of trematode infections in the aquatic invertebrates would be expected. The diversity of trematodes is usually proportional to the size of the water body and to the diversity of visiting birds. The molluscan fauna of the water body is an important factor determining the trematode fauna, as trematodes show greatest specificity at the primary host level (Wright, 1960). Molluscs vary markedly in their susceptibility to trematode parasitism. Gastropods are much more important than bivalves in the establishment of digenean life-cycles; some gastropod families (viz. Hydrobiidae, Lymnaeidae, Physidae, Planorbidae and Thiaridae), have been found to be more commonly involved in trematode life-cycles than others (Ewers, 1964); and some snail species harbour a far greater variety of trematodes than others. Most snail species that have been studied harbour fewer than 10 trematode species (Ewers, 1964); however 51 species have been recorded in *Bithynia tentaculata* (Erasmus, 1972), 43 in *Lymnaea natalensis* (Porter, 1938) and 21 in *Stagnicola emarginata* (Cort *et al.*, 1937). No trematodes have been recorded from the ubiquitous *Potomopyrgus jenkinsi*, whereas the apparently closely related *Hydrobia ulvae*, is host for a wide range of trematodes (Wright, 1971). Most studies of the incidence of cercariae

in snails have been concerned with freshwater habitats. It is clear from such studies that different cercarial types are not represented equally, and that in freshwater environments, xiphidiocercariae and furcocercariae are by far the most abundant types. Erasmus (1972), observed that furcocercariae predominate in larger water bodies and xiphidiocercariae are the most abundant cercarial type in smaller aquatic environments.

A chance discovery in 1967 of hundreds of trematode cysts *(Atriophallophorus coxiellae)* in an hydrobiid snail at Calvert's Lagoon, was the stimulus for studies that have revealed this brackish water body to be a focus for the life-cycles of a large and diverse assemblage of trematodes. The snail, *Coxiella badgerensis*, has been found to harbour the developmental stages of 17 species from 7 trematode families. In order of abundance of primary infections, the families were: Microphallidae (3spp.), Schistosomatidae (1sp.), Notocotylidae (4spp.), Renicolidae (2spp.), Psilostomidae (3spp.), Heterophyidae (3spp.) and Strigeidae (1sp.). It was only possible to classify 9 of the species to the generic level and these belonged to 7 genera. About 80% of primary trematode infections of *C. badgerensis* were caused by the 3 microphallid species *Atriophallophorus coxiellae, Levinseniella tasmaniae* and *Maritrema calvertensis*. About 65% of primary infections were by xiphidiocercariae *(L. tasmaniae, M. calvertensis* and *Renicolid sp.A)*, and about 15% were by furcocercariae *(Schistosome sp.A* and *Apatemon gracilis)*. The only other invertebrates serving as trematode hosts in the lagoon were ostracods and amphipods. Ostracods were infected only by *M. calvertensis* and amphipods by both *M. calvertensis* and *L. tasmaniae*. The adults of 9 of the trematodes developing in *C. badgerensis* were found to infect a wide range of birds at Calvert's Lagoon. Two of the trematodes, *Apatemon gracilis* and *Psilochasmus oxyurus*, are cosmopolitan; however, the geographical distributions of the others are unknown.

The life-cycles of some of the 17 trematode species have been demonstrated in the laboratory and the life-cycles of others can be inferred from the available evidence and by comparison with related species. All of the species infecting *C. badgerensis* are believed to be avian trematodes. The life-histories of the 3 microphallid species, 3 psilostomes and 2 of the notocotylids,(*Paramonostomum bursae* n.sp. and *P. caecai* n.sp.), have been demonstrated experimentally and adults of each of these species have been recovered from laboratory ducklings. The adult of *Apatemon gracilis* was found in a duck shot at Calvert's Lagoon. One snail was found to be infected with the sporocysts and cercariae of this species, which utilizes a fish as its second intermediate host. No metacercariae, however,were found in fish in the lagoon. Adults of *A. gracilis* were recovered from laboratory ducklings after the birds had been fed with cysts from naturally infected specimens of the native fish *Galaxias auratus*, caught at Lake Crescent about 100 km NW of Calvert's Lagoon. The cercariae of *Notocotylid spp.A* and *B* encyst on various submerged surfaces in the lagoon, such as plant stems and snail shells, and presumably reach maturity in birds after being accidentally ingested. *Schistosome sp.A*, like all avian blood flukes, would infect its definitive host by direct cercarial penetration of the skin, or exposed membranes (such as nasal membranes). The life-cycles of heterophyids with free-swimming cercariae, such as *Heterophyid spp.A, B* and *C*, usually involve encystment in a fish intermediate host; however, some freshwater species encyst in amphibians. The few known life-cycles of renicolids involve development in marine or freshwater gastropods and encystment in either the same gastropod species (Werding, 1969), a bivalve (Stunkard, 1964; Werding, 1969), or a fish (Wright, 1956; Pearson, 1979). *Renicolid sp.A* has a free-swimming cercaria, however *Renicolid sp.B* encysts in its primary intermediate host. The trematodes that utilize fish as their second intermediate hosts are unlikely to be able to complete their life-cycles at Calvert's Lagoon, where introduced brown trout, that appeared to

be free of any trematode infections, are the only fish.

Rankin (1940), found that in the marine snail *Nasa obsoleta*, the incidence of the microphallid *Gynaecotyla nassicola* (adults of which are harboured by migratory birds), varied seasonally with a peak in spring. Etges (1953), studied the life-histories of the microphallids *Maritrema obstipum* and *Levinseniella amnicolae*, and found that in the freshwater snail *Amnicola pilsbryi* the incidence of primary infections with these species reached a peak in late summer and autumn. He indicated that the periodicity of these infections was due to the migratory behaviour of the birds which served as definitive hosts. At Calvert's Lagoon, the level of primary trematode infections in adult snails was relatively high throughout the present study. In samples collected at Site 1 from April 1976 to September 1978, it varied from 27 to 93%, at an average of 66%. The incidence of primary infections in 12 monthly samples of snails collected at Site 1 from July 1977 to June 1978, varied significantly between seasons, with a gradual increase from winter through to autumn. This variation is believed to have been mainly due to changes in the water temperature, which increased from winter through to a peak in summer. The incidence of primary infections may also have been influenced by variation in the total number of birds at the lagoon. Changes in the number and diversity of birds were mainly related to their breeding cycles, the availability of food and the weather conditions at the lagoon. The incidence of primary infections did not vary significantly between the 4 sampling sites around the lagoon and the relative abundance of the trematode families represented in the primary infections, did not vary significantly between seasons. The Microphallidae was consistently the most abundant trematode family and *Maritrema calvertensis* was consistently the most abundant microphallid. The uniformity observed in the composition of the trematode fauna of Calvert's Lagoon reflected the relative uniformity in the composition of the avian fauna. The most abundant bird, the hoary-headed grebe, is sedentary and the other birds

are either sedentary or nomadic within Tasmania (Thomas, 1979). Of the birds that may feed on aquatic animals or plants in Calvert's Lagoon, only the anatids, white-faced heron and great cormorant, are known to move occasionally between Tasmania and the Australian mainland. Dogiel (1962) classified parasites that were picked up and lost by migratory birds in the course of their travels, as "migration parasites". He reported that microphallids were found to be the main "migration parasites" in sandpipers (Limicolae), migrating along coastal routes from the Northern Hemisphere. Some of these birds migrate as far south as Tasmania. Although no migratory birds were recorded at Calvert's Lagoon during the present study, many migratory species, such as sandpipers, turnstones and curlews, inhabit the nearby coast (Thomas, 1979). Such birds may occasionally visit the lagoon and thus act as dispersal agents for trematodes developing in *Coxiella badgerensis*.

Some long-term changes were noticed in the trematode fauna of Calvert's Lagoon. Samples of adult snails were only collected at Site 1 in 6 months of 1976, 8 months of 1977 and 7 months of 1978 (Figure 7.6); however, the average incidence of primary trematode infections in these samples increased from 43 (27 - 78)% in 1976 to 64 (40 - 87)% in 1977, to 84 (67 - 94)% in 1978. The apparent general increase in the incidence of primary infections in *Coxiella badgerensis* over this 3 year period coincided with an overall decrease in the level of the lagoon, and may have been caused by the higher temperatures, increased temperature fluctuations and greater density of invertebrate hosts, that would accompany such a change in the volume of the lagoon. Eleven more trematode species were found during the present study than during one conducted at Calvert's Lagoon in 1970 (Smith, 1971). This increase in diversity of the trematode fauna appears to be related to the reintroduction of trout into the lagoon in 1975. No trematodes that utilize fish as intermediate hosts were recorded in 1970; however, during the present study, 4 species (*Heterophyid spp.A, B* and *C* and *Renicolid sp.A*)

were found that may have a fish intermediate host in their life-cycles, and *Apatemon gracilis* was found, that is known to utilize fish as second intermediate hosts. These trematodes were probably introduced by piscivorous birds such as cormorants and herons, attracted by the presence of trout in the lagoon.

Seasonal variation in the incidence of secondary infections in adult snails, amphipods and ostracods , was not analysed statistically, however, the incidence in these hosts seemed to be high throughout the year. Etges (1953), found that the incidence of 2 microphallid species in their second intermediate host *Asellus communis*, was highest in late summer and autumn. Bridgman (1969) also found that the incidence and intensity of infections with metacercarial cysts of *Microphallus choanophallus*, in the freshwater shrimp *Macrobrachium ohione*, were highest in late summer and autumn.

The incidence and intensity of infection with parasites that are long-lived and relatively benign, generally increase with the age of the host (Dogiel, 1962). The incidence of primary and secondary infections in snails at Calvert's Lagoon, were directly related to the size and presumably the age, of the host. Similarly, the incidence and intensity of infection with microphallid cysts in the crustaceans in the lagoon also showed an increase with host size. Sex-ratios in snails, amphipods and ostracods were found to be related to size, possibly as a result of differential mortality due to parasitism, at different stages in the life-histories of the sexes. In each of the invertebrate host species, a sudden decrease occurred in the proportion of young, adult females, possibly as a consequence of the combined strain of egg production and the burden of trematode parasites. Restricted reproductive capacity and eventual parasitic castration, result from most, if not all, primary infections in *C. badgerensis*. The high incidence of these infections presumably makes them a limiting factor on the snail population at the lagoon. It is not known how trematode parasitism affects reproduction

of the amphipod and ostracod hosts; however, the large numbers of cysts found in some individuals must interfere with the physiology of these crustaceans and presumably limit their reproductive capacity.

Some changes in the external appearance and behaviour of infected snails and amphipods were noticed. Some snails in the population were found to have very fragile shells and an unusually high proportion of these were infected by the microphallid *Atriophallophorus coxiellae*. There was a marked reduction in the size of the penis of infected male snails, probably as a result of destruction of the testis by trematode developmental stages. Under conditions of low oxygen tension, snails were found to move towards the water surface and sometimes emerge. A significantly high proportion of these emergent snails were found to be infected by *A. coxiellae*. It is postulated that a general upward movement of heavily infected snails may occur in the lagoon as a response to low oxygen tension in the parasitized host tissues. A relationship between amphipod colour and infection with cysts of *Levinseniella tasmaniae* was found, which is believed to be the first record of a link between crustacean colour and parasitism by a trematode. All uninfected amphipods were green and all orange amphipods were found to harbour the large cysts of *L. tasmaniae*. It is suggested that the bright orange colouration may be due to carotenoid pigments in the tissues of *Austrochiltonia australis* and that accumulation of these pigments may be an adaptation to low oxygen tensions in host tissues, caused by cysts of *L. tasmaniae*. Carotenoids have been shown to substitute molecular oxygen in the metabolism of molluscs in a low-oxygen environment (Zs-Nagy, 1971), and they may play a similar role in *A. australis*.

Considerable attention has been given in the literature to the incidence of simultaneous primary infections of snails with different trematode species. Early field observations indicated that multiple infections were rare (Erasmus, 1972); however, later studies have shown that the incidence of multiple infections may be less than, the same as,

or greater than, that expected by chance, depending on the trematode species involved (James, 1969; Wright, 1971). The incidences of 16 out of 20 possible double and triple associations between the 5 most abundant trematodes at Calver's Lagoon, did not differ significantly from the incidences expected by chance alone. The incidences of 4 double associations, however, were significantly less than that expected. It is suggested that altered behaviour, such as movement towards the lagoon surface by snails with certain trematode infections, may decrease the probability of such snails acquiring further trematode infections.

Simultaneous infection of a snail by 3 microphallid species has not been previously recorded. This phenomenon, which occurs at Calvert's Lagoon, provided an opportunity to compare the life-history and biology of 3 microphallids utilizing the same hosts in the same habitat. The intramolluscan stages of *Atriophallophorus coxiellae* were readily distinguished from those of *Maritrema calvertensis* and *Levinseniella tasmaniae*, after the development of cercariae, because the cercaria of *A. coxiellae* is a rudimentary, "blastocercaria", which soon encysts within the daughter sporocyst, whereas those of *M. calvertensis* and *L. tasmaniae* are typical, free-swimming microphallid xiphidiocercariae. At first indistinguishable, the xiphidiocercariae of these 2 species were found to be distinguished by many morphological, behavioural and ecological characteristics. There was a distinct periodicity in the patterns of emergence of each cercaria from the host snail. However, the peak of emergence of *L. tasmaniae* was during the day, whereas emergence of *M. calvertensis* occurred mainly after dark, in the late evening. After swimming freely for a period, the cercaria of *L. tasmaniae* attached itself by a mucoid 'cyst', almost anywhere on the surface of an amphipod, and then bored directly through the host's exoskeleton. The smaller cercaria of *M. calvertensis* was able to swim freely for about twice as long as that of *L. tasmaniae*, and then invaded its crustacean host through the limbs or ventral surface. In amphipods, this was usually near the base of the pleopods. After alight-

ing on the host, the cercaria of *M. calvertensis* invaded through the intersegmental membrane of the nearest joint. Although both microphallids were infective to amphipods under laboratory conditions, only *M. calvertensis* was infective to ostracods. Growth and development of each species in their mutual crustacean host were markedly different. *L. tasmaniae* grew at a faster rate and encysted after a longer period of time than *M. calvertensis*. The growth and development of these microphallids in their poikilothermic second intermediate host were directly related to environmental temperature. Development of both species was arrested at 5°C, but, after as long as 4 weeks at this temperature, development continued normally when the temperature was elevated. All 3 microphallid species were found in the alimentary tracts of hoary-headed grebes at Calvert's Lagoon. There was some overlap in their distributions, however *L. tasmaniae* lived almost exclusively in the intestinal caeca, whereas *M. calvertensis* and *A. coxiellae* inhabited the lower small intestine, with the range of *M. calvertensis* extending further anteriorly than that of *A. coxiellae*. Some hoary-headed grebes harboured thousands of the tiny flukes, but showed no apparent ill-effects. Each of the microphallids commenced egg production within a few hours of being ingested by the bird host. The maximum recorded longevity of these microphallids in laboratory ducklings was 2 days for *L. tasmaniae*, 12 days for *A. coxiellae* and 19 days for *M. calvertensis*. It is believed that the longevities of *A. coxiellae* and *M. calvertensis* are somewhat reduced under natural conditions.

Sogandares-Bernal and Lumsden (1964), suggested that short-lived ovigerous microphallid adults may be passed from natural hosts in much the same way as tapeworm proglottids. Bridgman (1969) showed that whole microphallid adults can be infective to snails when he infected the snail *Lyrodes parvula* with *Microphallus choanophallus* by casting whole gravid flukes into aquaria containing snails. In the family Microphallidae, there appear to be evolutionary trends towards short-term infection of

definitive hosts, rapid egg-production by adult flukes,and reduction of the life-cycle to 2 hosts (e.g. *A. coxiellae*). Each of these trends increase the probability of large numbers of microphallid eggs being quickly returned to the aquatic habitat of their intermediate hosts (Deblock, 1971, 1977). Such a life-history strategy may be an adaptation to the migratory or nomadic behaviour of many of the bird hosts of microphallids.

A preliminary investigation was made by the author, of the trematode faunas of other Tasmanian coastal lagoons, to determine the likely distribution of trematodes found at Calvert's Lagoon. Samples of snails from 9 coastal lagoons were examined for trematodes (Table 8.1). The locations of these lagoons are shown in Figure 1.1. Six of the water bodies, all brackish or saline, were inhabited by *Coxiella badgerensis*. Trematodes occurring at Calvert's Lagoon were found in the same molluscan host at Sloping Lagoon in the SE of Tasmania, and at 4 lagoons in the Cape Portland area in the NE; none of which open to the sea. No trematodes were found in *C. badgerensis* at Troyheleener Lagoon, which is intermittently open to the sea. Specimens of *Physastra gibbosa* and *Rivisessor gunni*, from 2 freshwater lagoons, were infected by trematodes not recorded at Calvert's Lagoon. Only one infection was found in these snails that may have been caused by a trematode occurring at Calvert's Lagoon. The viscera of one specimen of *R. gunni* from Big Punchbowl was packed with about 120 cysts, (cyst diameter 114 (106 - 122)μ, average thickness 8μ), resembling those of *Atriophallophorus coxiellae*, however the metacercariae were not excysted *in vitro* and so an accurate identification was impossible.

The results of this brief survey indicate that the trematodes occurring at Calvert's Lagoon are widely distributed along the eastern seaboard of Tasmania, developing in landlocked, brackish lagoons, inhabited by *C. badgerensis*. In the south-east of the Australian mainland, *C. striata*, which may be conspecific with *C. badgerensis*, (Smith and Kershaw, 1979), is widespread in brackish and saline inland waters.

TABLE 8.1 Trematode infections in snails of some coastal lagoons that (a) never open to the sea and (b) open periodically to the sea.

	(a) freshwater					brackish					(b) saline	
Lagoon (No.)	30		38		47	19	41	18	22	21	35	
Snail host	Pg	Rg	Pg	Rg	Pg	Cb	Cb	Cb	Cb	Cb	Cb	Pn
No. of snails	30	30	10	10	20	20	30	10	5	5	30	30
Collection date	6/77		7/77		7/78	3/78	2/78	3/78	11/78	11/77	6/77	
The trematodes found at Calvert's Lagoon:												
Microphallidae												
M. calvertensis	-	-	-	-	-	++	++	-	-	-	-	-
L. tasmaniae	-	-	-	-	-	++	++	-	-	-	-	-
A. coxiellae (met.)*	-	?+	-	-	-	++	+	+	-	-	-	-
Psilostomidae												
Psilostomum sp.A (met.)	-	-	-	-	-	++	++	++	+++	+++	-	-
Psilostomum sp.B } (met.)	-	-	-	-	-	++	++	+++	-	-	-	-
P. oxyurus }												
Schistosomatidae												
Schistosome sp.A	-	-	-	-	-	-	++	-	-	-	-	-
Notocotylidae												
P. bursae n.sp.	-	-	-	-	-	-	+	-	-	-	-	-
P. caecai n.sp.	-	-	-	-	-	-	+	-	-	-	-	-
Notocotylid sp.A	-	-	-	-	-	-	++	-	-	-	-	-
Notocotylid sp.B	-	-	-	-	-	-	-	-	-	-	-	-
Heterophyidae												
Heterophyid sp.A	-	-	-	-	-	-	-	-	-	-	-	-
Heterophyid sp.B	-	-	-	-	-	-	-	-	-	-	-	-
Heterophyid sp.C	-	-	-	-	-	+	-	-	-	-	-	-
Renicolidae												
Renicolid sp.A	-	-	-	-	-	-	+	+	-	-	-	-
Renicolid sp.B	-	-	-	-	-	-	-	-	-	-	-	-
Strigeidae	-	-	-	-	-	-	-	-	-	-	-	-
Apatemon gracilis	-	-	-	-	-	-	-	-	-	-	-	-
Other trematodes												
Allocreadiidae												
?Coitocaecum anaspidis (met.)	-	++	-	-	-	-	-	-	-	-	-	-
Echinostomatidae (met.)	-	+	-	-	-	-	-	-	-	-	-	-
Schistosomatidae												
avian schistosome	+	-	-	-	-	-	-	-	-	-	-	-
Fasciolidae	-	-	+	-	-	-	-	-	-	-	-	-

Key to snails

Cb = *Coxiella badgerensis*; Pg = *Physastra gibbosa*
Pn = *Potamopyrgus niger*; Rg = *Rivisessor gunni*

Key to lagoons

30 = Big Punchbowl; 38 = Guards; 47 = Gibbs; 19 = N of Tregaron;
41 = Sloping; 18 = NE of Tregaron; 22 = nr Little Musselroe;
21 = S of Tregaron; 35 = Troyheleener.

% infection in sample

1 - 10 = +
11 - 50 = ++
51 - 100 = +++

(* 'met.' = metacercarial cysts)

Many of these lakes and lagoons are inhabited by the amphipod *Austrochiltonia australis*, and by birds found at Calvert's Lagoon. They may also serve as foci for the life-cycles of trematodes recorded at Calvert's Lagoon.

The present study of the trematode fauna of a brackish coastal lagoon in Tasmania has shown that coastal lagoons may serve as foci for the life-cycles of a wide diversity of avian trematodes throughout the year. Each lagoon has a characteristic trematode fauna that is determined by a number of ecological factors, such as the number and diversity of birds, the identity and availability of invertebrate hosts, the frequency of marine invasions, the salinity range, and the flora. The most important of these factors is the identity of the molluscan inhabitants of the lagoon. At Calvert's Lagoon, developmental stages of trematodes were found to be integral components of the biocoenosis, inter-relating with their hosts and their physical environment. This lagoon acts as a continuous reservoir of parasites. Itinerant birds serve as dispersal agents: acquiring trematode burdens at the lagoon, transporting adult flukes and releasing their eggs into other water bodies in Tasmania, and possibly on the Australian mainland, and overseas. The life-histories of a number of trematodes found at Calvert's Lagoon remain to be elucidated, viz. the avian blood fluke *Schistosome sp.A*, the kidney flukes *Renicolid spp.A* and *B*, *Heterophyid spp.A*, *B* and *C*, and *Notocotylid spp.A* and *B*. These and many other parasitological problems raised by the present study, warrant further investigation. It is hoped that this study will stimulate future research into the trematode faunas of Calvert's Lagoon and other aquatic habitats, and contribute to a greater appreciation and understanding of the trematodes of Tasmanian birds.

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(Note: The above references are presented in the style recommended by the journal *Parasitology*.)

APPENDIX 1

A record of *Maritrema oocysta* (Lebour, 1907) (Trematoda:Microphallidae), in the white-faced heron at Lake Crescent, Tasmania.

INTRODUCTION

The intramolluscan stages of *Maritrema oocysta* have been recorded in Europe by several authors. *Cercaria oocysta* Lebour, 1907, which encysts in the primary intermediate host, was first described from specimens infecting *Paludestrina stagnalis* (syn. of *Hydrobia ulvae)*, from Northumberland, Great Britain. Rothschild (1937), recorded the flame-cell formula of the cercaria and metacercaria. An account of the intramolluscan developmental stages infecting *Hydrobia ulvae* on the Normandy coast was presented by Deblock (1975a).

The adult, first described by Nicoll (1907), under the name *Maritrema humile*, from the digestive tract of *Tringa totanus*, was re-described by Leonov (1958), and Deblock and Capron (1960). Rothschild experimentally demonstrated that *M. humile* is the adult of *Cercaria oocysta* (Rothschild, 1942; Rothschild and Clay, 1952, p.204). Gravid adults conforming to the redescription of *M. humile* by Deblock and Capron (1960), have been found in a mammal (the water rat),and birds (the darter and the little grebe), in Queensland (Deblock and Pearson, 1968b). In June 1979, 2 white-faced herons were shot at Lake Crescent, Tasmania, and both were found to harbour large numbers of a minute microphallid. Without more information on the life-history of this trematode, it is tentatively identified as *Maritrema oocysta*.

Family: MICROPHALLIDAE Travassos, 1920

Sub-family: MARITREMATINAE Nicoll, 1907

Genus: MARITREMA Nicoll, 1907

MARITREMA OOCYSTA (LEBOUR, 1907) ROTHSCHILD, 1942

Synonyms (acc. to Deblock, 1975a): *M. humile* Nicoll, 1907

Pseudomaritrema innae
Leonov, 1958
Cercaria A Rothschild,
1936

ADULT (Figure 1)

The anatomy and morphology of this fluke is characteristic of the genus *Maritrema*, and conforms more or less to the description of *M. humile* by Nicoll (1907), and to the redescription of *M. humile* by Deblock and Capron (1960). The dimensions of ovigerous specimens are presented in Table 1.

Description

Body dorsoventrally flattened, oval to pyriform. Small quincuncially arranged spines cover body. Oral sucker subterminal-ventral, ventral sucker near middle of body. O.S.:V.S. ratio = 0.96. Pharynx small, weakly developed; prepharynx usually longer than oesophagus. Caeca diverge obtusely, extending almost to lateral body wall, anterior to, frequently overlapping cirrus pouch. Cirrus pouch thick, J-shaped; C.P.L.:B.L. ratio = 0.53. Oval to triangular ovary sub-median to dextral. Symmetrical testes posterolateral to ventral sucker, anterior borders at level of posterior border of ovary. Uterus not extending forward of ventral sucker, containing up to 85 relatively large eggs. Vitellaria arranged in 2 semi-circular brackets, around posterior half of body. Vitelline ducts arise near anterior border of each testis, extend postero-medially, uniting to form vitelline reservoir posterior to ovary. Excretory bladder conspicuous, Y-shaped. Flame-cell formula not determined.

FIG. 1 Maritrema oocysta

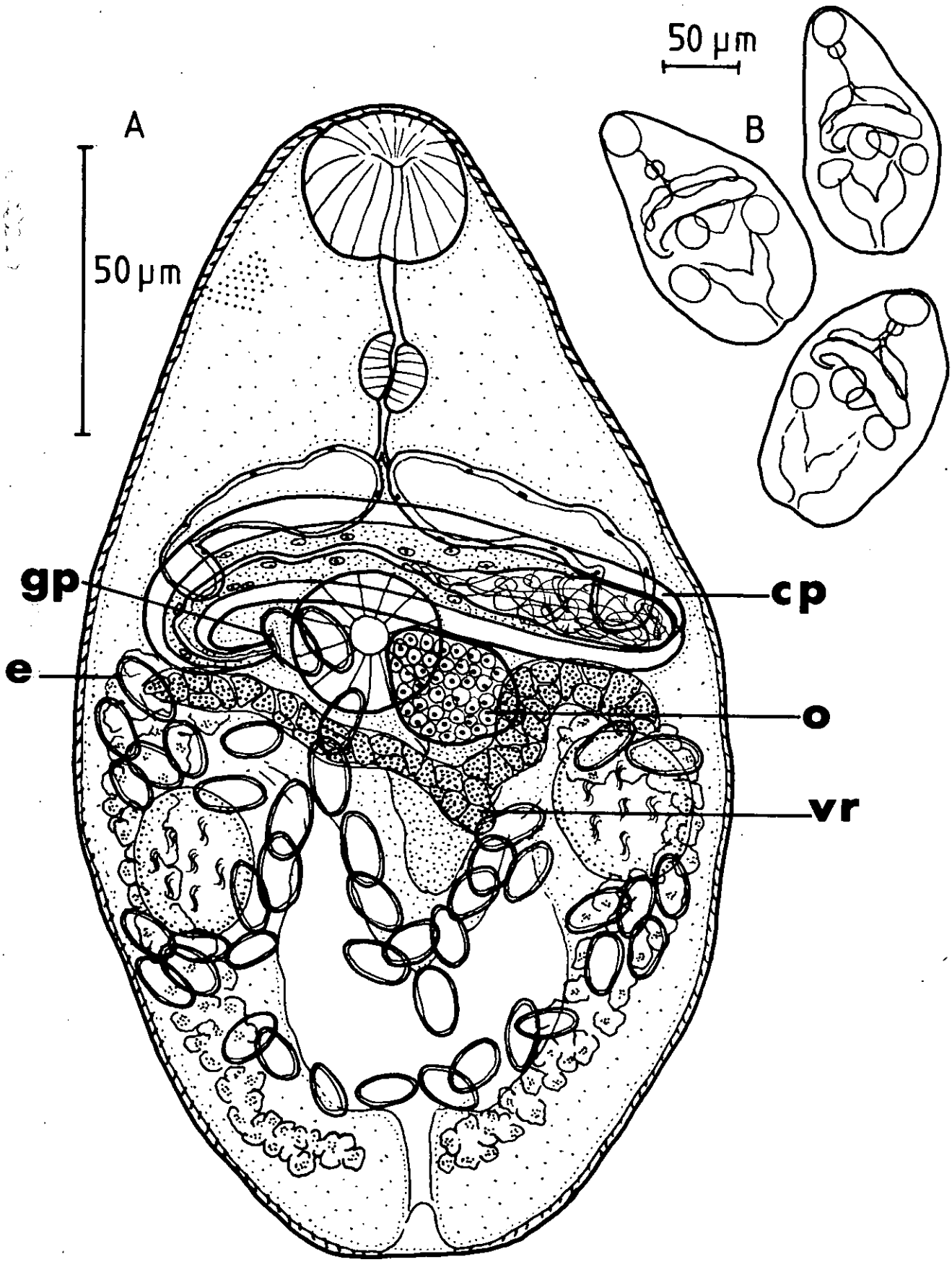


FIGURE 1 A, gravid adult, dorsal view; B, three gravid adults, dorsal view, showing slight variation in body shape and distribution of organs. (cp: cirrus pouch; e: egg; gp: genital pore; o: ovary; vr: vitelline reservoir.)

Host: *Ardea novaehollandiae* Latham

Geographical location: Lake Crescent

Date of collection: 27/6/79 (coll. by R.B. Mawbey, S.J. Smith)

Habitat: Small intestine

Material: Over 50 flukes, live and fixed, stained and unstained. Gravid adults deposited at the Tasmanian Museum: K890 and K891.

Habitat

The distribution of flukes in the 2 white-faced herons is shown in Figure 2. They were found throughout most of the length of the digestive tract, but were concentrated in the middle to upper regions of the small intestine in Heron No. 1, and in the lower small intestine of Heron No. 2. The specimens in the latter bird, which was dissected 26 hours after death, were more likely to have been displaced from their *in vivo* habitats than those in Heron No. 1, which was dissected 5 hours after death. The wide distribution of flukes in both birds was probably due to the birds being infected frequently as they fed on the intermediate host species in Lake Crescent.

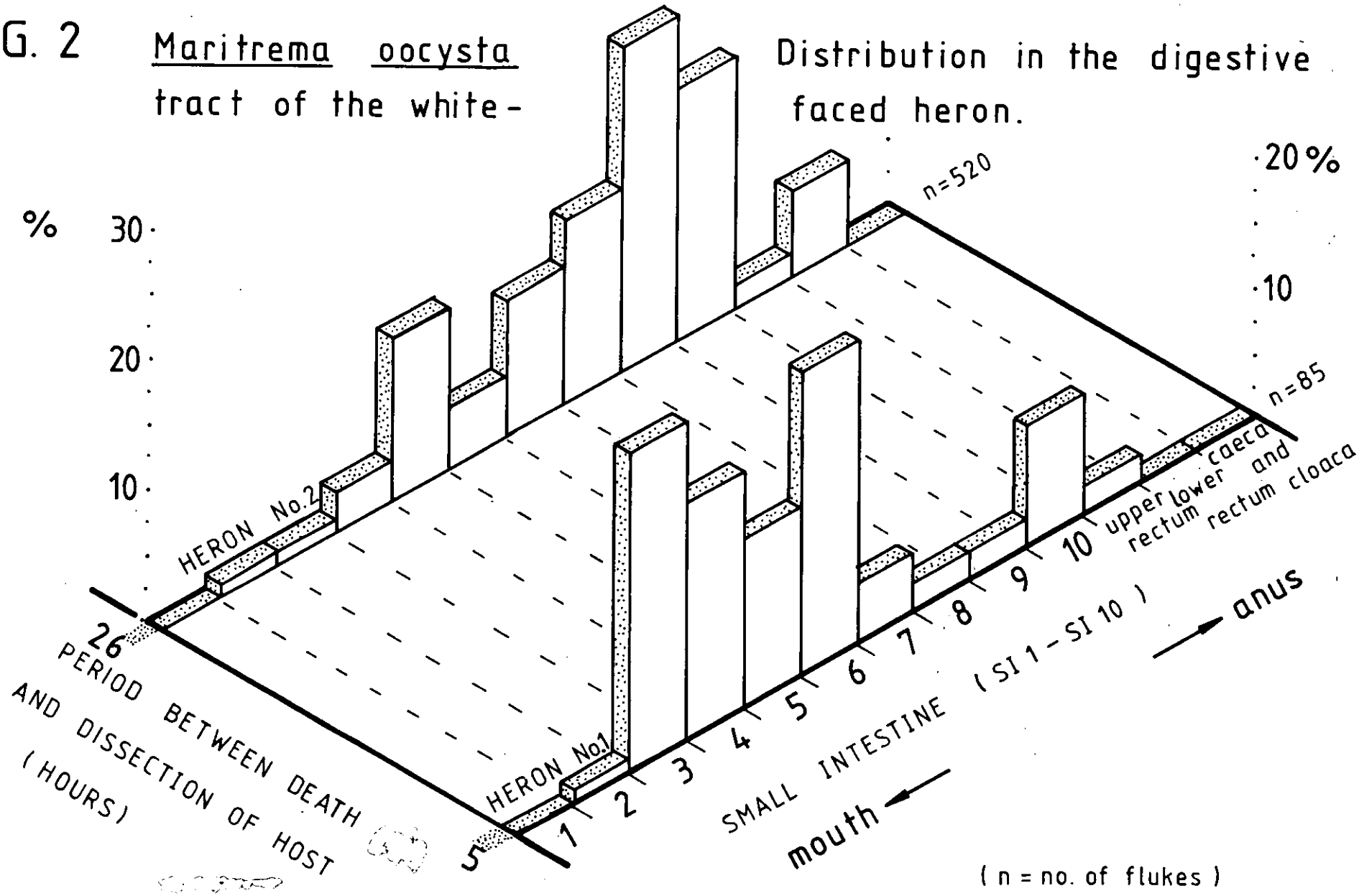
Host diet

The approximate composition (%V/V) of the recognizable food in the stomach-gizzard region of the host birds is shown in Table 2. Fish, *Galaxias auratus*, that had been swallowed whole, comprised most of the food in both birds, and various aquatic invertebrates, and some plant matter made up the remainder.

DISCUSSION

This diminutive fluke is similar in morphology and anatomy to adults of *Maritrema oocysta* recovered from the redshank in Europe (Nicoll, 1907; Deblock and Capron, 1960). Dimensions of ovigerous specimens from the redshank are shown, for comparison, in Table 1.

FIG. 2 Maritrema oocysta Distribution in the digestive tract of the white-faced heron.



(n = no. of flukes)

TABLE 1 *Maritrema oocysta*. Dimensions of ovigerous adults: (a) from the white-faced heron, Lake Crescent; (b) from the water rat, Queensland; (c) from the redshank, France (Deblock and Capron, 1960) and (d) from the redshank, England (Nicoll, 1907).

	(a)	(b)	(c)	(d)
Sample size	12	10	7 / 3	-
Body length	198 (175 - 213)	243 (224 - 270)	228 / 340	(280-400)
Body width	111 (95 - 124)	124 (106 - 133)	109 / 174	(120-160)
Oral sucker length	27 (23 - 30)	25 (23 - 27)	22 / 28	(25-31)
Oral length width	28 (27 - 30)	25 (23 - 27)	20 / 28	-
Ventral sucker length	29 (27 - 30)	33 (29 - 36)	23 / 28	(30-34)
Ventral sucker width	28 (27 - 30)	33 (27 - 34)	23 / 28	-
Prepharynx length	14 (11 - 15)	12 (2 - 21)	13.5/ 42	19
Pharynx length	14 (11 - 15)	16 (13 - 17)	15 / 17	19
Pharynx width	12 (11 - 15)	12 (11 - 13)	12.5/ 17	10
Oesophagus length	8 (6 - 11)	16 (10 - 23)	16.5/ 26	(40-50)
L. caecum length	46 (42 - 53)	46 (42 - 65)	50 / 70	-
R. caecum length	50 (40 - 53)	50 (38 - 61)	50 / 70	-
Cirrus pouch length	105 (95 - 114)	111 (91 - 129)	105 / 98.5	93
Cirrus pouch width	23 (21 - 26)	26 (21 - 30)	22 / 31	32
Cirrus pouch thickness	5.5 (4.8 - 7.2)	6.2 (4.8 - 6.7)	- / -	-
Seminal vesicle length	49 (38 - 61)	62 (46 - 76)	55 / 73	-
Seminal vesicle width	12 (11 - 13)	14 (8 - 21)	12 / 20	-
Ovary length	25 (17 - 30)	31 (25 - 36)	22 / 50	-
Ovary width	19 (15 - 21)	28 (21 - 38)	22 / 27	-
L. testis length	25 (23 - 30)	36 (30 - 42)	19 / 34	-
L. testis width	22 (19 - 23)	29 (27 - 30)	20 / 40	-
R. testis length	25 (21 - 29)	36 (30 - 42)	20 / 32	-
R. testis width	21 (19 - 23)	30 (27 - 34)	21 / 37	-
No. eggs in uterus	61 (32 - 85)	129 (82 - 180)	(59-95)	-
Roundness (B.W./B.L.)	0.56	0.51	0.48/0.51	-
O.S.(l+w)/V.S.(l+w)	0.96	0.76	0.91/1.00	-
C.P.L./B.L.	0.53	0.46	0.46/0.29	-
Dimensions of eggs:				
Sample size	20	20	-	-
Egg length	18 (16 - 20)	16 (14 - 17)	18 / 16	(16-18)
Egg width	9 (8 - 10)	8 (7 - 10)	9.5 / 9	(8-11)

TABLE 2 The approximate composition of the food in the stomach-gizzard region of white-faced herons shot at Lake Crescent.

Food	Heron No. 1 % V/V	Heron No. 2 % V/V
FISH. *Galaxias auratus*	90	90
TRICHOPTERA. (Caddis larvae)		
Hydropsychidae	5	5
Helicopsychidae	0.5	-
COLEOPTERA.		
Dytiscidae (elytra)	0.5	2
MOLLUSCA. (bivalve)		
Pisidium sp.	0.5	-
(gastropod)		
Rivisessor gunni	0.5	-
AMPHIPODA. *Austrochiltonia* sp.	0.5	-
DECAPODA. *Paratya tasmaniensis*	0.5	-
ISOPODA. Phreatoicidae	-	1
Plant matter.	2	2

A microphallid species infecting the water rat in Queensland has been identified as *M. oocysta* (Deblock and Pearson, 1968b). A sample of this species was kindly sent to the author by Dr. Pearson, University of Queensland, and dimensions of 10 ovigerous adults from this sample are also shown in Table 1.

The body of the trematode infecting the white-faced heron is markedly smaller than those in the redshank and water rat. The oral sucker of the former is relatively large and the pharynx relatively small and oesophagus short. Details of the reproductive system, however, are similar in worms from each host. In particular, the cirrus pouch in all worms is relatively thick-walled, about 1/3 to 1/2 of body length, and more or less J-shaped. All of these flukes are similar to *M. calvertensis*, but are readily distinguished by the different body shape and the relative size, thickness and shape of the cirrus pouch. *M. calvertensis* has a rounder body and its cirrus pouch is relatively longer (about 2/3 body length), and thinner, and shaped like an inverted V.

The life-cycle of *M. oocysta* in Europe is discussed in Section 2.2.8. In Western Europe it has a reduced life-cycle, with metacercarial cysts

being formed in its brackishwater primary intermediate host, the hydrobiid snail *Hydrobia ulvae*. A small hydrobiid, *Rivisessor gunni*, which inhabits Lake Crescent, had been eaten by one of the infected herons. It is interesting to note that a specimen of this snail species, collected during the present study at Big Punchbowl Lagoon on the east coast of Tasmania, was found to be infected by a large number of small trematode cysts (Table 8.1). The cysts, however, were round, whereas those of *M. oocysta* are oval (Deblock, 1975a).

APPENDIX 2

Descriptions of two strigeoid trematodes, *Apatemon (Apatemon) gracilis* (Rudolphi, 1819) and *Diplostomum (Dolichorchis) galaxiae* n.sp., which encyst in the freshwater fish, *Galaxias auratus* Johnston, in Lake Crescent, Tasmania, and notes on their life-histories.

INTRODUCTION

Galaxias auratus Johnston is a small freshwater fish, endemic to Lakes Sorell and Crescent in Tasmania. It harbours, among other helminths, the encysted stages of 2 strigeoid trematodes. *Apatemon (Apatemon) gracilis* (Rudolphi, 1819), encysts in the body cavity, orbit and occasionally in the vitreous humour of the eye, forming thick-walled white cysts. *Diplostomum (Dolichorchis) galaxiae* n.sp. encysts in the musculature, causing visible black spots throughout the body and head (Figure 1).

The metacercariae of both species can be excysted *in vitro* and identified to the family level. Cysts from naturally infected *G. auratus* were fed to domestic ducklings, *Anas platyrhynchos*, and gravid adults of both species were later recovered. A black duck, *Anas superciliosa*, killed at Calvert's Lagoon, was found to be infected with *Apatemon gracilis*, and white-faced herons, *Ardea novaehollandiae*, feeding on *G. auratus* at Lake Crescent, were found to harbour hundreds of adults of *D. galaxiae* n.sp.

Apatemon (Australapatemon) intermedius (Johnston, 1904) Dubois, 1937 is the only member of this genus previously recorded in Australia. In South Australia, it encysts in freshwater leeches and matures in the black swan (Johnston and Angel, 1951; Dubois and Pearson, 1965). Four species of *Diplostomum* have previously been recorded in Austalia: *D. (Adenodiplostomum) triangulare* (Johnston, 1904); *D. (Diplostomum) murrayense* (Johnston and Cleland, 1938); *D. (D). amygdalum* Dubois and Pearson, 1965 and *D. (Dolichorchis) auriculosum* Dubois and Pearson, 1967. These

FIG. 1 *Galaxias auratus* the fish intermediate host of *Apatemon gracilis* and *Diplostomum galaxiae* n.sp.

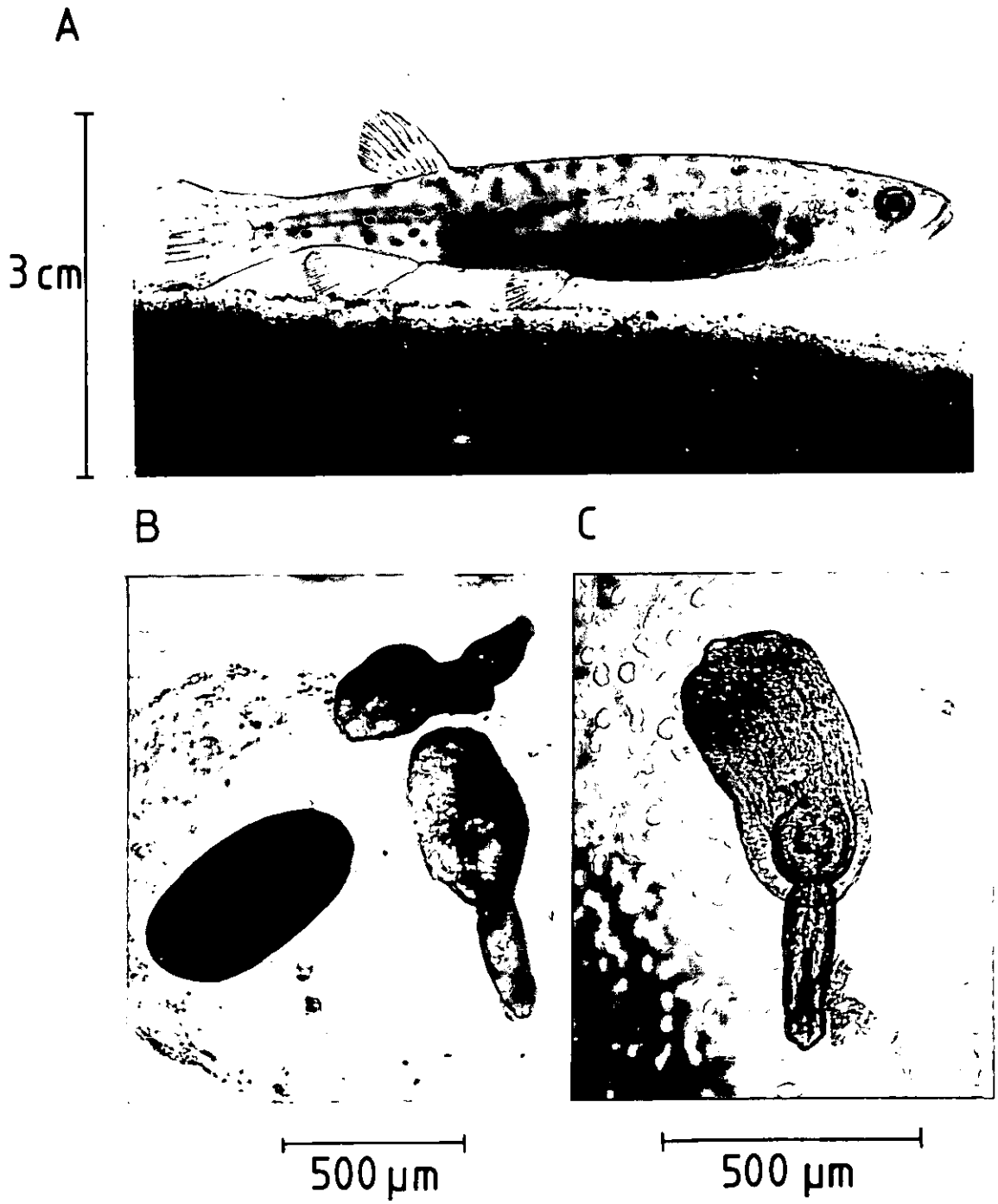


FIGURE 1 A, photograph of live infected fish, cysts of *D. galaxiae* n.sp. visible as black spots in translucent musculature of host; B, inverted microscope photograph of *D. galaxiae* n.sp. cyst in excystment solution at 39°C, two excysted metacercariae creeping on transparent remains of 'outer cyst', black oval 'inner cyst' remains intact; C, live excysted metacercaria of *D. galaxiae* at 39°C, ventral view. (Photograph A courtesy of Mr. J. Lim.)

Diplostomum species infect piscivorous birds of the families Alcedinidae, Anhingidae, Ardeidae and Laridae.

Superfamily: STRIGEOIDAE Railliet, 1919

Family: STRIGEIDAE Railliet, 1919

Genus: APATEMON Szidat, 1928

APATEMON (APATEMON) GRACILIS (RUDOLPHI, 1819) SZIDAT, 1928

ADULT (Figure 2)

The dimensions of ovigerous adults from a naturally infected black duck and experimentally infected laboratory ducklings, are presented in Table 1.

Body strongly flexed dorsally at junction of fore and hind-body. Cup-shaped forebody separated by deep constriction from larger, arcuate hind-body. Oral sucker round, mouth subterminal, prepharynx absent; short oesophagus bifurcates immediately posterior to small, round pharynx; narrow caeca (with orange-brown contents conspicuous in live worms from black duck), extend to posterior of body. Ventral sucker more or less round. Discrete, ovoid gland at base of foliaceous holdfast organ. Large interconnected paranephridial canals prominent throughout body; excretory pore terminal. Reproductive organs confined to hind-body. Testes lobed, contiguous, tandem, posterior to ovary. Seminal vesicle coiled between posterior testis and genital cone, narrows distally, joining terminal part of uterus to form hermaphrodite duct. Ovary oval, contiguous to anterior testis. Oviduct passes dorsally, from posterodorsal border of ovary, gives rise to Laurer's canal, which opens dorsal to anterior testis. Ciliated ootype overlies dorsal lobe of anterior testis. Vitellaria confined to hind-body, ventral and overlapping gonads. Vitelline reservoir dorsal, inter-testicular. Uterus loops anteroventrally from ootype, extends along ventral border of testes,

TABLE 1 *Apatemon gracilis.* Dimensions of: (a) metacercariae, excysted *in vitro* after 2 hours at 41°C; (b), (c) and (d) adults recovered from experimentally infected ducklings after different periods of infection; and (e) adults from a naturally infected black duck.

Infection period (days)	(a) –	(b) 3,19	(c) 4,21	(d) 8,1	(e) –
Sample size	4	5	6	5	3
Body length	590 (550 - 665)	957 (847 - 1028)	1335 (1225 - 1452)	1182 (1074 - 1300)	1542 (1315 - 1845)
Fore-body depth	264 (255 - 274)	355 (331 - 391)	397 (369 - 437)	360 (350 - 380)	510 (469 - 529)
Fore-body length (FBL)	416 (393 - 469)	429 (348 - 499)	544 (499 - 590)	423 (393 - 469)	579 (469 - 741)
Fore-body width	314 (300 - 327)	467 (433 - 502)	-	-	-
Hind-body depth	114 (-)	312 (304 - 319)	402 (369 - 452)	354 (296 - 380)	479 (423 - 514)
Hind-body length (HBL)	174 (166 - 197)	514 (454 - 559)	791 (650 - 907)	759 (680 - 832)	912 (786 - 1104)
Hind-body width	129 (125 - 133)	346 (342 - 350)	-	-	-
Oral sucker depth	72 (68 - 76)	87 (84 - 91)	103 (95 - 110)	85 (76 - 91)	86 (76 - 95)
Oral sucker length	78 (76 - 80)	94 (87 - 99)	103 (95 - 114)	95 (84 - 114)	108 (99 - 118)
Oral sucker width	80 (76 - 84)	93 (91 - 95)	-	-	-
Ventral sucker depth	91 (87 - 95)	129 (114 - 141)	146 (122 - 167)	144 (129 - 156)	139 (114 - 156)
Ventral sucker length	90 (84 - 99)	120 (106 - 133)	127 (122 - 137)	129 (122 - 133)	155 (137 - 167)
Ventral sucker width	95 -	108 (99 - 118)	-	-	-
Pharynx depth	34 -	53 -	64 (61 - 72)	64 (53 - 68)	34 -
Pharynx length	34 -	60 (53 - 65)	57 (49 - 61)	62 (57 - 65)	46 -
Pharynx width	32 (30 - 34)	44 (42 - 46)	-	-	-
Holdfast organ depth	55 (49 - 61)	76 (53 - 99)	81 (61 - 95)	84 (76 - 95)	92 (84 - 106)
Holdfast organ length	43 (34 - 46)	68 (65 - 72)	63 (46 - 76)	43 (34 - 53)	65 (53 - 72)
Holdfast organ width	46 -	-	-	-	-
Ovary depth	-	-	123 (103 - 144)	-	143 (122 - 175)
Ovary length			89 (84 - 95)	-	81 (72 - 125)
Anterior testis depth	-	-	208 (175 - 266)	-	274 (217 - 346)
Anterior testis length	-	-	158 (114 - 220)	-	252 (213 - 304)
Posterior testis depth	-	-	244 (213 - 281)	-	275 (171 - 388)
Posterior testis length	-	-	234 (220 - 255)	-	282 (163 - 418)
O.S.(l+w)/V.S.(l+w)	0.85	0.82	0.75	0.66	0.66
FBL/HBL	2.39	0.83	0.69	0.56	0.63
Eggs in uterus	-	+	+	+	+

FIG. 2 Apatemon gracilis

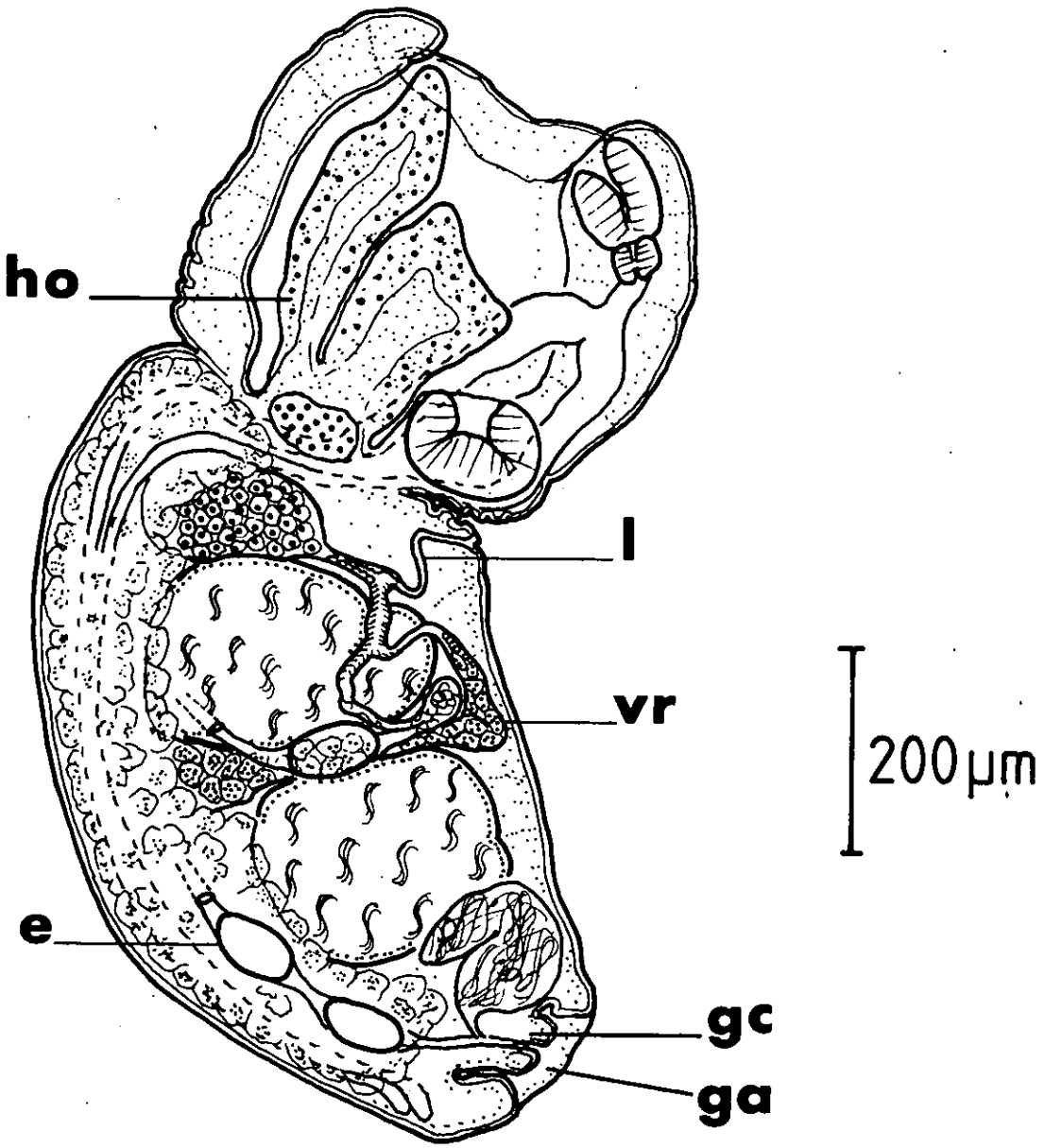


FIGURE 2 Gravid adult, after 4,21 days in experimentally infected duckling. (e: egg; ga: genital atrium; gc: genital cone; h: holdfast organ; l: Laurer's canal; vr: vitelline reservoir.)

joins seminal vesicle posterior to posterior testis. Hermaphrodite duct opens through protrusible genital cone, within subterminal-dorsal copulatory bursa (= genital atrium). Uterus contains up to 14 eggs.

Hosts: *Anas platyrhynchos* L. (experimental), *A. superciliosa* Gmelin

Geographical location: Lake Crescent (fish intermediate host); Calvert's Lagoon (black duck)

Date of collection: fish - 2/4/78, 26/6/78 (coll. R. White, R.B. Mawbey); black duck - 27/4/78 (coll. R.B. Mawbey, S.J. Smith)

Habitat: Upper small intestine

Material: Tasmanian Museum: adults from black duck, Calvert's Lagoon, K887; adults from laboratory duckling K888; excysted metacercariae, K889.

Relationships

Adults recovered from a naturally infected black duck and experimentally infected laboratory ducklings fell within the range of previous descriptions of *Apatemon (Apatemon) gracilis* (Dubois, 1951; Beverley-Burton, 1961; Ricci and Carrescia, 1961; and Vojtek, 1964). This species is noted for its morphological variability and Dubois (1953), suggested that it be divided into 10 sub-species. Beverley-Burton (1961), however, observed that specimens taken from the same host can be assigned to several of these sub-species, and hence considered it preferable to regard this cosmopitan species with a wide host range, as polytypic, rather than as a collection of sub-species.

A. (Apatemon) gracilis and *A. (Australapatemon) minor* Yamaguti, 1933, have frequently been considered conspecific (Dubois, 1968), and it is often impossible to determine which species is referred to when *A. gracilis* is reported, unless life-history studies have been made.

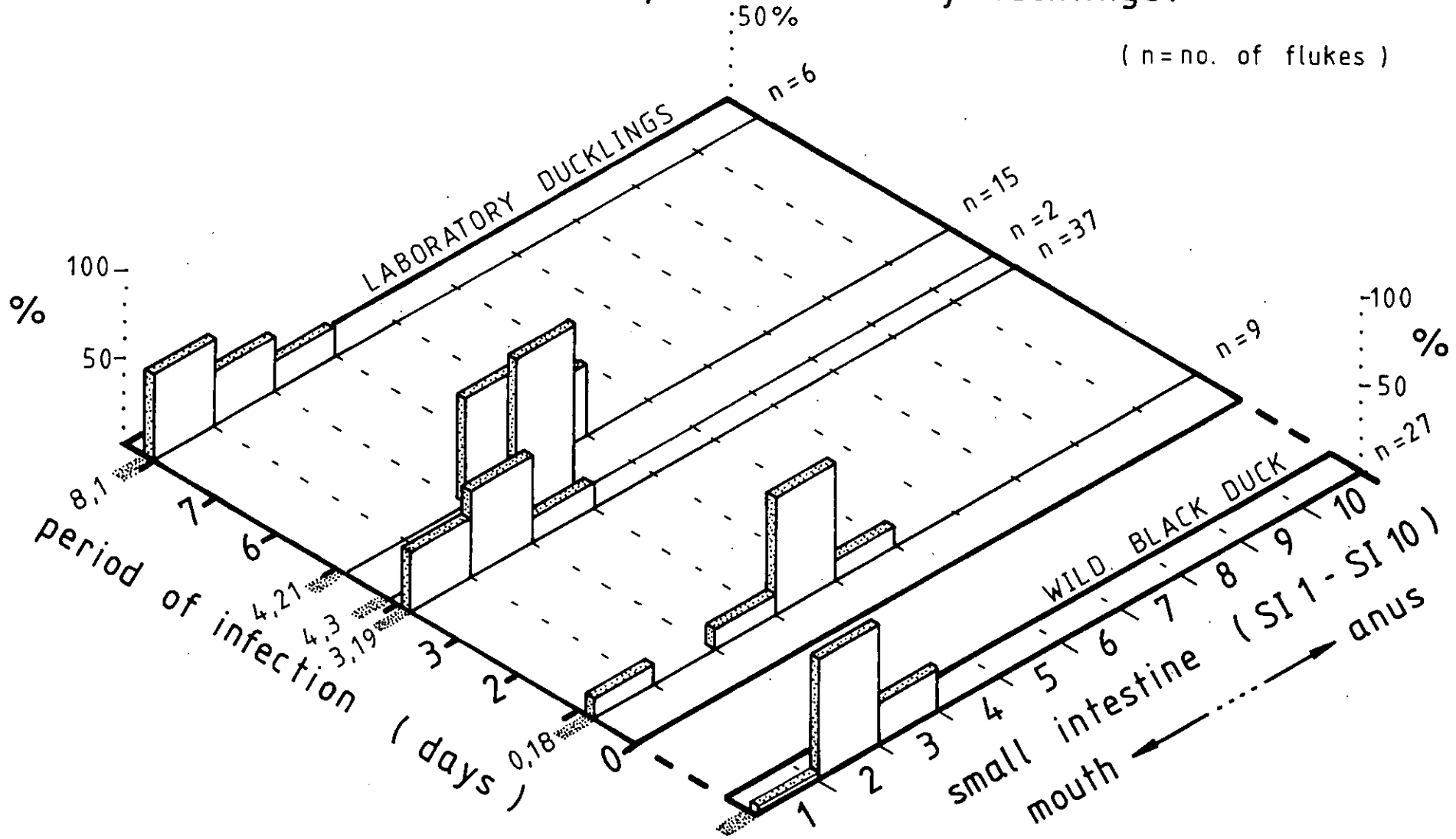
The former species utilizes fish as intermediate hosts and the latter, leeches (Blair, 1976). The present material clearly belongs to the former species.

Biology

Domestic ducklings, raised under controlled conditions, were fed metacercarial cysts taken from naturally infected *Galaxias auratus*. The birds were dissected at intervals from 0,18 to 30,19 days. Seventy-one percent (5/7), of the ducklings were infected, with from 2 to 37 flukes. The percentage of metacercariae that were recovered as adult flukes varied from 8 to 93, and the maximum longevity recorded was 8,1 days. The flukes were concentrated in SI4 after 0,18 days, SI2 after 3,19 days, SI3 after 4,21 days and SI2 after 8,1 days. A black duck killed at Calvert's Lagoon contained 27 adults of this species, concentrated in SI2. The distribution of adults in naturally and experimentally infected hosts is shown in Figure 3.

Excysted metacercariae are very immature and genital primordia barely discernible. After 0,18 days in a laboratory duckling little growth or development had occurred; however, growth proceeded rapidly over the next few days. As the reproductive organs developed, the hind-body grew from being a stumpy appendage to being larger than the fore-body (Figure 4). After 3,19 days the FBL/HBL ratio had decreased from 2.39 for excysted metacercariae, to 0.83. At this stage the adults were mature and producing eggs. The hind-body continued to grow after egg production had commenced and after 8,1 days the FBL/HBL ratio was 0.66. The number of uterine eggs was not directly related to the age of ovigerous adults (Table 2). A maximum of 14 eggs was found in flukes after 4,3 and 4,21 days; however, after 8,1 days the maximum number of eggs in any fluke was only 5. The

FIG. 3 Apatemon gracilis. Distribution in the digestive tracts of a wild black duck, and laboratory ducklings.



maximum number of eggs found in flukes infecting the black duck was 7.

TABLE 2 *Apatemon gracilis*. The number of uterine eggs in adults: (a) from experimentally infected ducklings and (b) from the black duck.

Infection period (days)	No. flukes	No. of uterine eggs Mean	Range
(a) 3, 19	30	0.5	(0 - 1)
4, 3	2	11.0	(8 - 14)
4, 21	11	3.5	(0 - 14)
8, 1	5	2.8	(1 - 5)
(b) -	11	2.0	(0 - 7)

EGG (Figure 4)

The broadly oval egg is operculate and densely packed with granular vitelline cells. Colourless when formed, the egg-shell is tanned golden as it passes through the uterus. A clear spherical body, about 19μ diameter, underlies the operculum, and may be homologous to the 'viscous cushion' in the egg of *Fasciola hepatica* (Wilson, 1968), and play a similar role in hatching.

The dimensions of live and fixed eggs in flukes from laboratory ducklings and fixed eggs in flukes from a wild black duck, are shown in Table 3. There was little difference in the size of fixed eggs from different hosts, however, fixed eggs were significantly smaller than live eggs.

FIG. 4 Apatemon gracilis

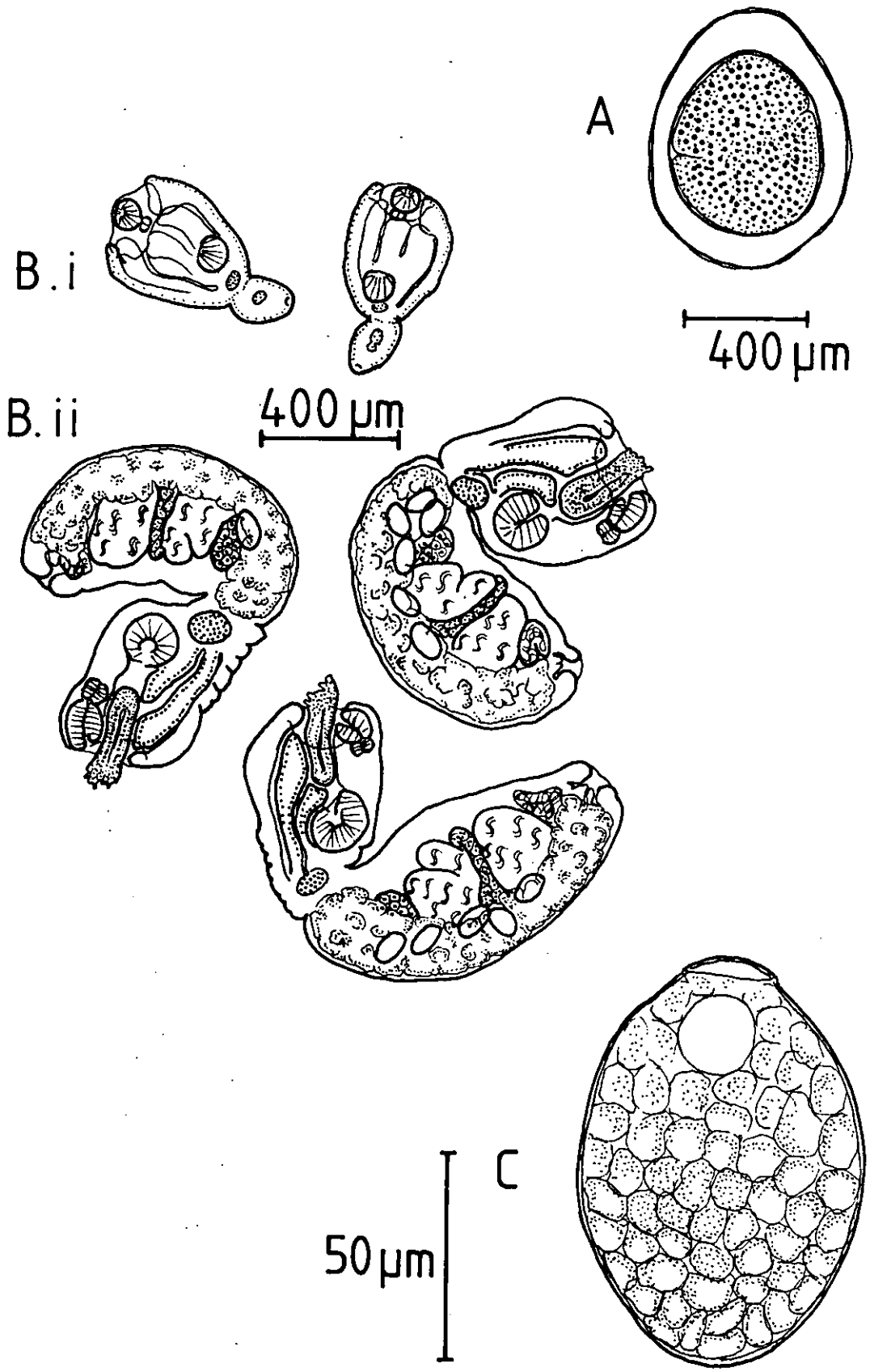


FIGURE 4 A, metacercarial cyst; B.i, excysted metacercariae after 2 hours at 41°C, and B.ii, gravid adults after 8,1 days in laboratory duckling (B.i and B.ii drawn to same scale); C, egg deposited in laboratory duckling.

TABLE 3 *Apatemon gracilis*. Dimensions of live and fixed eggs in flukes from laboratory ducklings, and fixed eggs in flukes from a wild black duck.

Host	Live/ fixed	No. eggs	Length	Width
Duckling 1	Live	6	103 (95 - 106)	71 (68 - 72)
Duckling 1	Fixed	6	99 (95 - 103)	63 (61 - 68)
Duckling 2	Fixed	9	99 (95 - 106)	61 (57 - 67)
Black duck	Fixed	6	97 (87 - 103)	59 (53 - 68)

METACERCARIA

Metacercarial cyst (Figure 4)

The thick-walled, white, oval to pyriform cyst, most frequently occurs in the body cavity of *Galaxias auratus*, particularly in connective tissue adjacent to the intestine. It is also frequently associated with the eye, either in the vitreous humour, or just outside the eyeball, near the optic nerve. The cyst sometimes occurs between the peritoneum and muscle. Ten out of 14 fish collected in June 1978 and June 1979, from the Clyde River where it enters Lake Crescent, were infected with *Apatemon gracilis*. The average number of cysts per infected fish was 5.5 (1-17) and the average size of the fish was 6.4 (4.8 - 9.9) cms. Eighty percent of these cysts were in or next to the body cavity, 18% were behind the eyeball next to the optic nerve and 2% were in the vitreous humour. The resilient cyst wall is translucent and homogeneous, varying in thickness up to about 220μ. A thin outer membrane, presumably of host origin, sometimes connects adjacent cysts. The densely packed contents of the cyst are opaque. Dimensions of 10 cysts from the body cavity and 2 cysts from the vitreous humour of the eye, are shown in Table 4. There is no marked difference in size or morphology of the cysts from different locations in the fish host.

TABLE 4 *Apatemon gracilis*. Dimensions of live metacercarial cysts, dissected from naturally infected *Galaxias auratus*; from the brook stickleback, *Eucalia inconstans*, in North Dakota, U.S.A. (Hoffman, 1959), and from the rainbow trout *Salmo gairdneri*, in Scotland (Blair, 1976).

Host fish	No. cysts	External dimensions Length	External dimensions Width	Internal dimensions Length	Internal dimensions Width
G. auratus (body)	10	886 (816-968)	718 (650-756)	587 (423-650)	493 (348-552)
G. auratus (eye)	2	877 (847-907)	665 (650-680)	544 (529-559)	529 (484-575)
E. inconstans	-	1000	600	507	444
S. gairdneri	10	605 (542-660)	396 (356-426)	503 (465-542)	293 (279-310)

Excystment

In vitro excystment of metacercariae occurred at 41°C after the following treatment: immersion in 2% pepsin plus 10% 0.1N HCl in Hank's saline for 10 mins.; followed by brief washing in Hank's saline; then into 0.02M sodium dithionite in Hank's saline for 15 mins.; followed by further brief washing; and finally incubation in 0.5% pancreatin plus 0.2% sodium taurocholate in Hank's saline for 2 hours. This technique is similar to that used by Blair (1976) to excyst *A. gracilis*.

Excysted metacercaria (Figure 4)

The dimensions of some excysted metacercariae are presented in Table 1. The fore-body is cup-shaped and relatively larger than in the adult worm. The oral and ventral suckers, pharynx and holdfast organ are well-developed in the fore-body, but genital primordia are barely discernible in the rudimentary hind-body. Body fluids and lipid droplets were seen moving through large paranephridial canals, throughout the body of live worms.

Family: DIPLOSTOMIDAE Poirier, 1886

Tribe: DIPLOSTOMINI Dubois, 1936

Genus: DIPLOSTOMUM Von Nordmann, 1832

DIPLOSTOMUM (DOLICHORCHIS) GALAXIAE N.SP.

ADULT (Figure 5)

The description of the adult of this new *Diplostomum* species is based on gravid flukes recovered from experimentally infected laboratory ducklings and naturally infected white-faced herons. The species is named after the endemic Tasmanian fish, *Galaxias auratus*, that serves as its second intermediate host. Dimensions of the holotype and other ovigerous and non-ovigerous adults, from naturally infected white-faced herons, are shown in Tables 5 and 6.

Fore-body elongate, more or less rectangular, anterior border trilobate, posterior and posterolateral borders recurved ventrally. Hind-body conical, dorsally flexed, distinct from fore-body. Tegumental spines on fore-body diminish in size posteriorly; hind-body aspinous. Tegumental gland cells distributed over anterior half of fore-body. Oral sucker oval, situated on prominent anterior protuberance, bordered by well-developed crescentic lappets. Transversely oval oral sucker, and mobile mushroom-shaped holdfast organ protrude into concavity of fore-body. Anastomosing network of large, fluid-filled paranephridial canals extend throughout body. Excretory pore terminal. Mouth subterminal ventral; pre-pharynx absent; narrow inconspicuous caeca diverge immediately posterior to small oval pharynx, extend into hind-body. Bipartite gland at base of large, holdfast organ. Anterior testis dextral or sinistral, round to wedge-shaped. Posterior testis bilobed, elongate lobes joined by narrow antero-dorsal commissure. Voluminous, coiled seminal vesicle lies between lobes of posterior testis, narrowing posteriorly to form ejaculatory duct, which joins uterus. Oval sub-median ovary situated anteriorly in hind-body. Oviduct extends posterolaterally to ootype, surrounded by Mehlis' gland, opposite posterior half of anterior testis. Laurer's canal opens dorsally, at level of anterior testis. Seminal receptacle lateral

FIG. 5 Diplostomum galaxiae n.sp.

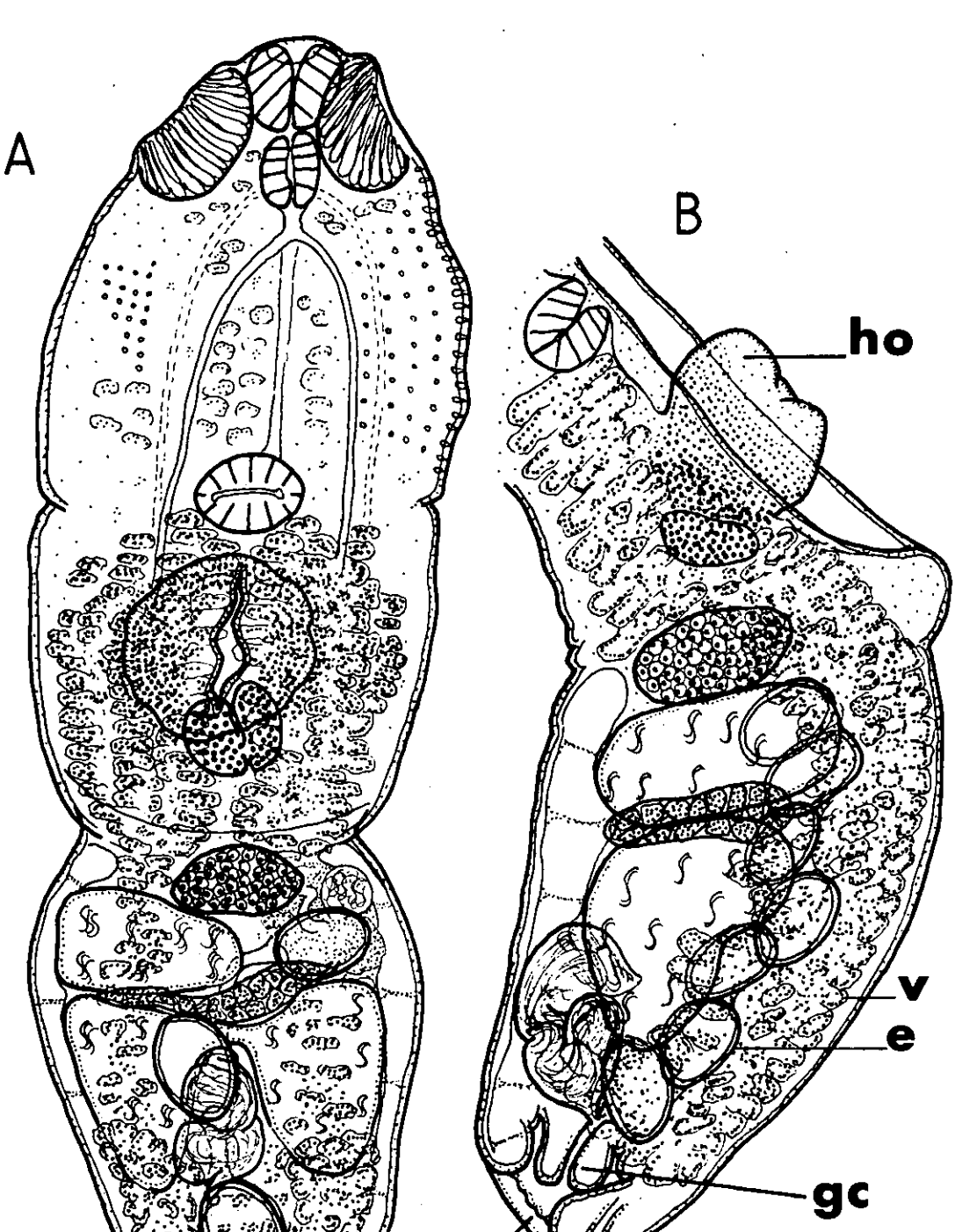


FIGURE 5 A, gravid adult, sinistral anterior testis, from white-faced heron, dorsal view; B, gravid adult, dextral anterior testis, from white-faced heron, lateral view of hind-body. (e: egg; ep: excretory pore; ga: genital atrium; gc: genital cone; ho: holdfast organ; v: vitellaria.)

to ovary. Vitellaria occupy posterior half of fore-body, extending to posterior border of ventral sucker; mainly ventral, lateral distribution in hind-body. Vitelline reservoir inter-testicular. Uterus loops antero-ventrally, then extends posteriorly, ventral to seminal vesicle, uniting with ejaculatory duct. Short hermaphrodite duct traverses small, protrusible, genital cone, within subterminal, dorsal genital atrium. Up to 11 eggs in uterus.

The location of the anterior testis of this species is variable. In one host, a white-faced heron, 58% of 92 mature adults (i.e. with vitellaria producing phenolic egg-shell precursors), had a sinistral anterior testis and the remaining 42% had a dextral anterior testis. The position of the testis is always clearly sinistral or dextral - there are no intermediates. No distinct morphological differences could be found between the 'sinistral' and 'dextral' types (Tables 5 and 6). However, only 30% of the 'sinistral' adults were ovigerous, compared with 54% of the 'dextral' adults; and there was a slight difference in the number of uterine eggs in each type: 1.9 (1 - 6) in 'sinistral' adults, and 3.1 (1 - 11) in 'dextral' adults. The fecundity of this species may be related to the postion of the anterior testis.

The translocation of the anterior testis is a remarkable phenomenon, not previously recorded in this genus; however, it is considered to be due to intra-specific variation and not sufficient to warrant specific separation of the 'sinistral' and 'dextral' types.

Hosts: *Anas platyrhynchos* L. (experimental); *Ardea novaehollandiae* Latham

Geographical location: Lake Crescent

Date of collection: fish intermediate host - 2/4/78, 26/6/78 (coll. R. white, R.B. Mawbey); white-faced herons - 27/6/79 (coll. R.B. Mawbey, S.J. Smith)

TABLE 5 *Diplostomum galaxiae* n.sp. Dimensions of the holotype and other ovigerous adults, of 'sinistral' and 'dextral' types, from the white-faced heron.

	'Sinistral'		'Dextral'		Holotype
Sample size	11		14		1 ('sinistral')
Body length	957	(892 - 1058)	1002	(832 - 1074)	937
Fore-body depth	133	(121 - 166)	141	(106 - ±97)	-
Fore-body length (FBL)	549	(484 - 665)	517	(393 - 635)	575
Fore-body width	342	(302 - 401)	369	(287 - 438)	318
Hind-body depth	269	(242 - 302)	266	(227 - 287)	-
Hind-body length (HBL)	437	(348 - 499)	467	(378 - 499)	378
Hind-body width	289	(272 - 302)	296	(204 - 333)	280
Oral sucker depth	49	(46 - 43)	64	(53 - 76)	-
Oral sucker length	65	(57 - 76)	64	(57 - 68)	65
Oral sucker width	63	(61 - 67)	71	(59 - 84)	65
Ventral sucker depth	-		53	-	-
Ventral sucker length	63	(59 - 68)	62	(57 - 65)	53
Ventral sucker width	73	(67 - 80)	70	(61 - 80)	70
Pharynx length	49	(46 - 57)	48	(46 - 49)	46
Pharynx width	36	(34 - 38)	34	-	38
Left lappet length	87	(76 - 91)	81	(76 - 84)	91
Left lappet width	46	(42 - 53)	36	(27 - 49)	53
Right lappet length	87	(76 - 95)	81	(68 - 99)	76
Right lappet width	49	(46 - 53)	40	(34 - 49)	53
Holdfast organ depth	70	(68 - 72)	72	(68 - 76)	-
Holdfast organ length	136	(110 - 160)	154	(133 - 179)	141
Holdfast organ width	136	(114 - 148)	-		141
Ovary depth	-		95	-	-
Ovary length	68	(61 - 76)	65	(42 - 84)	61
Ovary width	90	(80 - 95)	85	(65 - 106)	91
Anterior testis depth	163	-	176	(141 - 194)	-
Anterior testis length	91	(80 - 114)	102	(78 - 144)	84
Anterior testis width	121	(87 - 141)	130	(84 - 198)	125
Posterior testis:					
Left lobe depth	137	-	144	(122 - 167)	-
Left lobe length	141	(114 - 198)	156	(110 - 213)	118
Left lobe width	98	(76 - 114)	78	(61 - 95)	103
Right lobe depth	123	(110 - 133)	143	(125 - 167)	-
Right lobe length	158	(114 - 205)	164	(110 - 213)	133
Right lobe width	96	(80 - 110)	97	(68 - 125)	87
O.S.(l+w)/V.S.(l+w)	0.94		1.02		1.06
FBL/HBL	1.26		1.11		1.52
No. uterine eggs	1.90	(1 - 6)	3.14	(1 - 11)	2

TABLE 6 *Diplostomum galaxiae* n.sp. Dimensions of mature (i.e. vitellaria producing phenolic egg-shell precursors), non-ovigerous adults of 'sinistral' and 'dextral' types, from the white-faced heron.

	'Sinistral'	'Dextral'
Sample size	17	14
Body length	889 (771 - 1058)	983 (847 - 1210)
Fore-body depth	106 (91 - 121)	132 (121 - 151)
Fore-body length (FBL)	496 (423 - 635)	581 (469 - 711)
Fore-body width	351 (318 - 408)	363 (302 - 408)
Hind-body depth	234 (227 - 257)	259 (242 - 272)
Hind-body length (HBL)	396 (333 - 484)	429 (348 - 544)
Hind-body width	292 (272 - 333)	292 (272 - 318)
Oral sucker depth	60 (49 - 76)	66 (57 - 70)
Oral sucker length	63 (49 - 68)	65 (49 - 72)
Oral sucker width	70 (61 - 76)	67 (61 - 70)
Ventral sucker depth	61 -	70 (65 - 76)
Ventral sucker length	62 (53 - 68)	63 (57 - 72)
Ventral sucker width	76 (68 - 84)	72 (61 - 84)
Pharynx length	51 (42 - 57)	53 -
Pharynx width	38 (30 - 46)	35 (32 - 38)
Left lappet length	81 (72 - 99)	84 (72 - 91)
Left lappet width	40 (30 - 53)	39 (34 - 46)
Right lappet length	80 (68 - 95)	81 (76 - 87)
Right lappet width	41 (30 - 57)	39 (30 - 46)
Holdfast organ depth	75 (68 - 84)	81 (68 - 91)
Holdfast organ length	125 (114 - 133)	138 (114 - 167)
Holdfast organ width	137 (129 - 148)	130 (114 - 156)
Ovary depth	76 -	-
Ovary length	76 (68 - 91)	66 (65 - 68)
Ovary width	89 (76 - 106)	82 (80 - 84)
Anterior testis depth	148 -	103 (91 - 114)
Anterior testis length	100 (80 - 114)	109 (95 - 118)
Anterior testis width	117 (95 - 171)	108 (103 - 114)
Posterior testis:		
Left lobe depth	122 -	115 (95 - 137)
Left lobe length	162 (148 - 190)	163 (137 - 190)
Left lobe width	106 (95 - 118)	103 -
Right lobe depth	109 (95 - 114)	108 (99 - 114)
Right lobe length	164 (129 - 190)	126 (103 - 152)
Right lobe width	101 (87 - 118)	99 -
O.S.(l+w)/V.S.(l+w)	0.96	0.98
FBL/HBL	1.25	1.35

Habitat: Upper small intestine

Type material: Tasmanian Museum: holotype K884, ringed (gravid adult); paratypes K885 (adults) and K886 (excysted metacercariae)

Relationships

This species has the characteristics of the subgenus *Dolichorchis* Dubois, 1961, i.e. asymmetrical anterior testis, elongated lobes of the posterior testis joined by a narrow dorsal commissure and the presence of a genital cone. Eight species of this subgenus have previously been described, all of which reach maturity in piscivorous birds; however, no intramolluscan developmental stages have yet been found. The metacercarial stage has only been described for *D. heronei* Srivastava, 1954 (syn. *D. ketupanensis* sensu Ganapati and Rao, 1962 nec Vidyarthi, 1937, according to Williams, 1967), *D. leonensis* Williams, 1967 and *D. tregenna* Nazmi, 1932. The metacercariae of *D. heronei* and *D. leonensis* encyst in the musculature of freshwater fish in India and Sierra Leone respectively; however, according to Khalil (1963), the metacercaria of *D. tregenna* remains unencysted in the brain of *Clarias*, a fish of the River Nile. The only other previous record of the metacercaria of a *Diplostomum* species encysting in fish is the metacercaria, *Diplostomulum pigmentata* Singh, 1956, which encysts in the muscles of freshwater cyprinoid fish in the Allahabad region of India, where *D. heronei* and *D. ketupanensis* Vidyarthi, 1937, have been recorded.

D. galaxiae n.sp. most closely resembles *D. heronei* and *D. ketupanensis*, in general morphology, distribution of vitellaria, shape and size of anterior testis and relative size of lappets. In all 3 species, vitellaria are extensively distributed in the posterior part of the fore-body, extending anteriorly as far as the ventral sucker, whereas in *D. marahoueense* Baer, 1957, and *D. auriculosum* Dubois and Pearson, 1967, vitellaria are restricted in the fore-body, to a narrow zone around the holdfast organ, and do not extend anteriorly as far as the oral sucker.

In *D. buteii* Vidyarthi, 1937, *D. tregenna* and *D. leonensis*, vitellaria are distributed anteriorly well beyond the ventral sucker nearly to the pharynx. The distribution of vitellaria in *D. duboisi* Anantaraman and Balasubramaniam, 1953 is similar to *D. galaxiae* n.sp., however the anterior testis of the former species extends the width of the hind-body, as in *D. buteii* and *D. leonensis*. Dubois (1968), considered that *D. duboisi* is a synonym of *D. buteii*.

D. ketupanensis Vidyarthi, 1937, is very similar in morphology to *D. heronei;* however, the body and organs of the former are about 2 to 3 times as large as the latter; and whereas uterine eggs are absent in the former, at least 4 may occur in the uterus of the latter. Williams (1967), considered that *D. ketupanensis* sensu Ganapati and Rao, 1962, but not *D. ketupanensis* Vidyarthi, 1937, is a synonym of *D. heronei*, and the present author concurs. *D. galaxiae* n.sp. is significantly smaller than *D. ketupanensis* Vidyarthi, 1937, has a more elongate fore-body, and contains up to 11 uterine eggs. The anterior testis is variable in the former and sinistral in the latter.

D. heronei and *D. galaxiae* n.sp. are similar in size, however the former has a flat, round fore-body, whereas that of *D. galaxiae* n.sp. is elongate and very concave. The anterior testis of *D. heronei* is dextral and the posterior testis is relatively longer than that of *D. galaxiae* n.sp. The excysted metacercaria of *D. heronei* is markedly smaller and has a relatively rounder fore-body and larger hind-body than that of *D. galaxiae* n.sp. The discovery of the intramolluscan developmental stages of these species would elucidate their relationship, however it is presently considered that the differences in morphology of adults and metacercariae and the different intermediate and definitive hosts, warrant their specific separation.

A key to the species of the subgenus *Dolichorchis* is given below.

1A Anterior testis L-shaped; extends width of hind-body.....2

1B Anterior testis not L-shaped; lateral in anterior hind-body.....4

2A Vitellaria distributed anteriorly beyond ventral sucker.....3

2B Vitellaria not distributed anteriorly beyond ventral sucker.

Intestinal parasite of the Indian pariah kite, in S.E. India.....

D. duboisi Anantaraman and Balasubramaniam, 1953

3A Posterior testis H- or ⋀- shaped.

Intestinal parasite of the Indian pariah kite, in S.E. India; and *Buteo rufinus rufinus*, in N. India.....*D. buteii* Vidyarthi, 1937

Syn.: *Bolbophorus orientalis* Vidyarthi, 1938

3B Posterior testis V-shaped.

Metacercariae encyst in muscles of freshwater fish in Sierre Leone. Adults mature experimentally in cattle egret and chickens.....

D. leonensis Williams, 1967

4A Vitellaria not distributed anteriorly beyond ventral sucker.....5

4B Vitellaria distributed anteriorly beyond ventral sucker.

Diplostomulum unencysted in brain of freshwater fish in River Nile. Adult intestinal parasite of Egyptian kite, Cairo, Egypt.

D. tregenna Nazmi, 1932

5A Vitellaria restricted to region of holdfast organ; anterior distribution not reaching ventral sucker.....6

5B Vitellaria distributed widely in posterior hind-body; anterior distribution reaching ventral sucker.....7

6A Ventral sucker larger than oral sucker. Lappets projecting anterior to oral sucker.

Intestinal parasite of darter, in Queensland, Australia.....

D. auriculosum Dubois and Pearson, 1967

6B Oral sucker larger than ventral sucker. Lappets not projecting anterior to oral sucker.

Parasite of upper small intestine of fishing owl, Ivory Coast.....

D. marahoueense Baer, 1957

7A Small flukes, body length less than 1.50 mm.....8

7B Large flukes, body length more than 1.50 mm.

Intestinal parasite of Northern brown fishing owl, in N. India....

D. ketupanensis Vidyarthi, 1937

8A Fore-body flat, round; anterior testis dextral.

Metacercaria encysts in muscles of freshwater fish, Andhra Pradesh. Adult intestinal parasite of grey pond heron in N. and E. India.....*D. heronei* Srivastava, 1954

Syn.: *D. ketupanensis* sensu Ganapati and Rao, 1962, nec Vidyarthi, 1937.

8B Fore-body elongate, ± rectangular, very concave; anterior testis variable.

Metacercaria encysts in muscles of freshwater fish, Lake Crescent. Adult parasite in upper small intestine of white-faced heron, Tasmania, Australia.....*D. galaxiae* n.sp.

Biology

Laboratory ducklings, fed cysts from naturally infected *Galaxias auratus*, were sacrificed at periods ranging from 0,10 to 30,19 days. Fifty percent (3/6) of the ducklings became infected, however each infected bird yielded only one fluke. The average infectivity rate of metacercariae in these ducklings was only about 1 in 100. Although ducklings are not very susceptible hosts for *D. galaxiae* n.sp., 2 flukes did grow to maturity. Excysted metacercariae are about ½ adult size, and genital primordia are discernible in the relatively short, stumpy hind-body. As growth occurs, the reproductive system develops and the hind-body increases

in size relative to the fore-body. One small, immature fluke was found in SI7, 10 hours after infection of a duckling (Figure 6). After 4,3 days, one specimen was living in SI1, and had grown to adult size and form. It was sexually mature, with vitellaria producing phenolic egg-shell precursors, and sperm filled the seminal vesicle, but no uterine eggs were present. After 12,4 days, one specimen was living in SI1, and it contained 1 uterine egg.

The distribution of adults in 2 white-faced herons killed while feeding on galaxiids at Lake Crescent, are shown in Figure 6. They occurred from SI1 to SI6, but were concentrated in SI2 and SI3. The composition of the gut contents of these birds are shown in Appendix 1, Table 2.

EGG (Figure 7)

The broadly oval egg has a small round operculum and is densely packed with granular vitelline cells. The egg-shell is relatively thin, clear when formed, but becomes tanned yellow. A clear spere, possibly a "viscous cushion", about 24μ diameter, underlies the operculum. The dimensions of live eggs taken from the lumen of the upper small intestine of infected herons, and dimensions of fixed uterine eggs of 'sinistral' and 'dextral' adults from the same hosts are shown in Table 7.

TABLE 7 *Diplostomum galaxiae* n.sp. Dimensions of live and fixed eggs produced by adults in naturally infected white-faced herons.

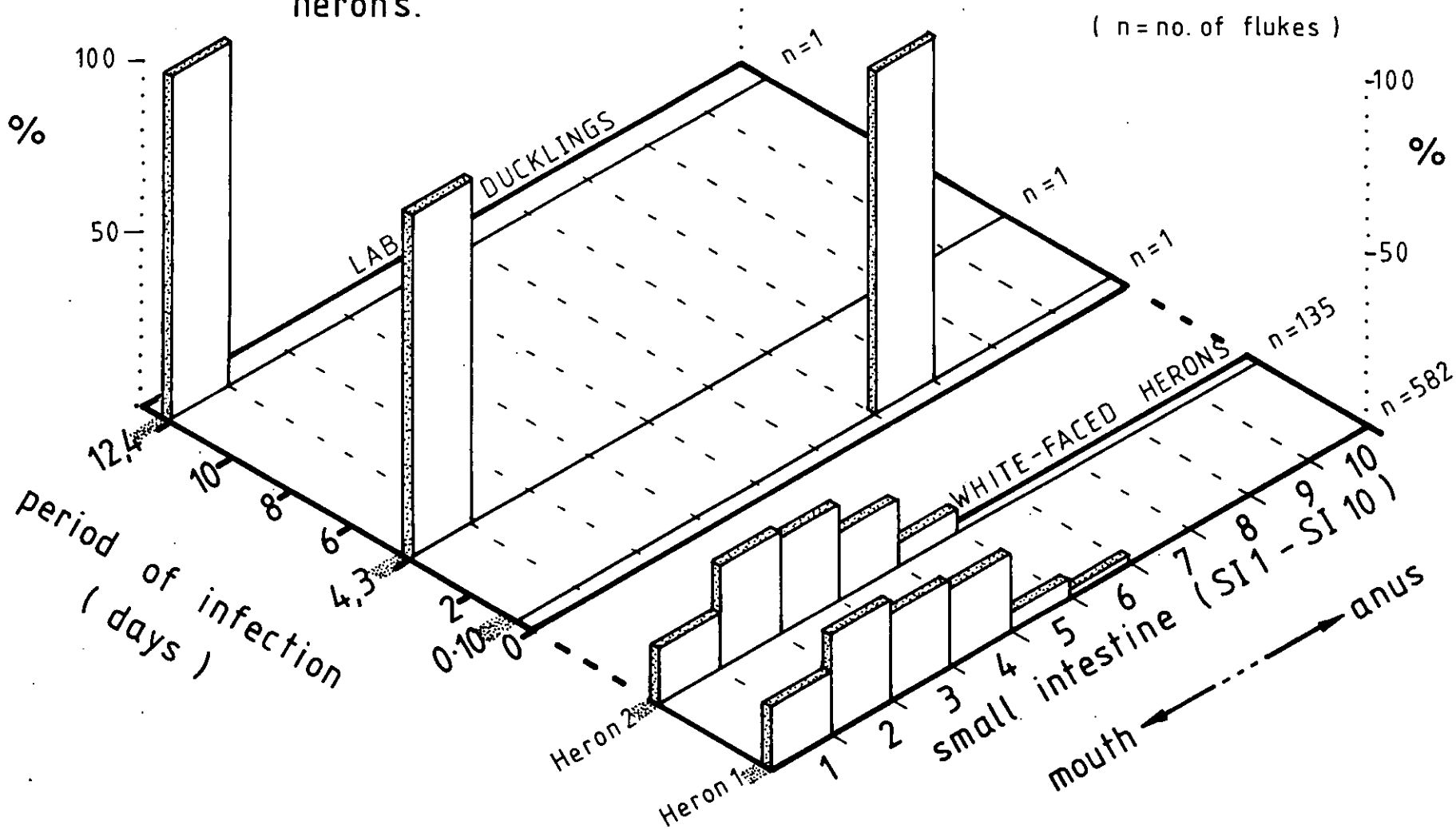
Type	Live/ fixed	No. eggs	Length	Width
Mixed	Live	15	95 (89 - 101)	65 (63 - 67)
'Sinistral'	Fixed	10	98 (91 - 103)	59 (53 - 65)
'Dextral'	Fixed	12	97 (87 - 99)	60 (53 - 65)

METACERCARIA

Metacercarial cyst (Figures 1 and 7)

The entire black cyst is composed of a thin, but resilient, transparent

FIG. 6 Diplostomum galaxiae n.sp. Distribution in digestive tracts of laboratory ducklings, and wild, white-faced herons.



inner layer of parasite origin, and a thick cellular outer layer of host origin. The 'inner cyst' which separates readily from the 'outer cyst', is oval, and encloses a curled metacercaria, partially surrounded by densely packed, coarse lipid droplets. The outermost layer of the 'outer cyst', or host reaction coat, consists of black pigmented melanocytes, which makes even deep-seated cysts conspicuous in the translucent body of *Galaxias auratus*. Cysts are distributed widely in the musculature of the body and head, causing "black spot", or "black grub" disease (Figure 1). The dimensions of the cyst are shown in Table 8.

TABLE 8 *Diplostomum galaxiae* n.sp. Dimensions of live metacercarial cysts, dissected from naturally infected *Galaxias auratus*.

	No.	Length	Width
External dimensions 'outer cyst'	10	1125 (1043 - 1210)	866 (771 - 937)
External dimensions 'inner cyst'	10	629 (575 - 643)	354 (333 - 386)

All of 14 fish collected in June 1978 and June 1979, from the Clyde River where it enters Lake Crescent, were infected with *Diplostomum galaxiae* n.sp. The average number of cysts per fish was 42.7 (10 - 104), and the average size of the fish was 6.4 (4.8 - 9.9) cms.

Excystment

In vitro excystment occurs readily at 41°C, after exposure to various solutions of digestive enzymes in different sequences (including the treatment described previously for *Apatemon gracilis)*. The most effective treatment is that used by Mitchell *et al.*, (1978), to excyst *Cotylurus erraticus:* incubation in a solution of 0.5% pancreatin and 0.2% sodium taurocholate in Hank's saline for 2 or 3 hours. During excystment, the cellular host capsule is dissolved away, freeing the thin, oval, 'inner cyst'. The metacercaria becomes active, revolving and stretching rapidly, until eventually escaping from the cyst.

FIG. 7 Diplostomum galaxiae n.sp.

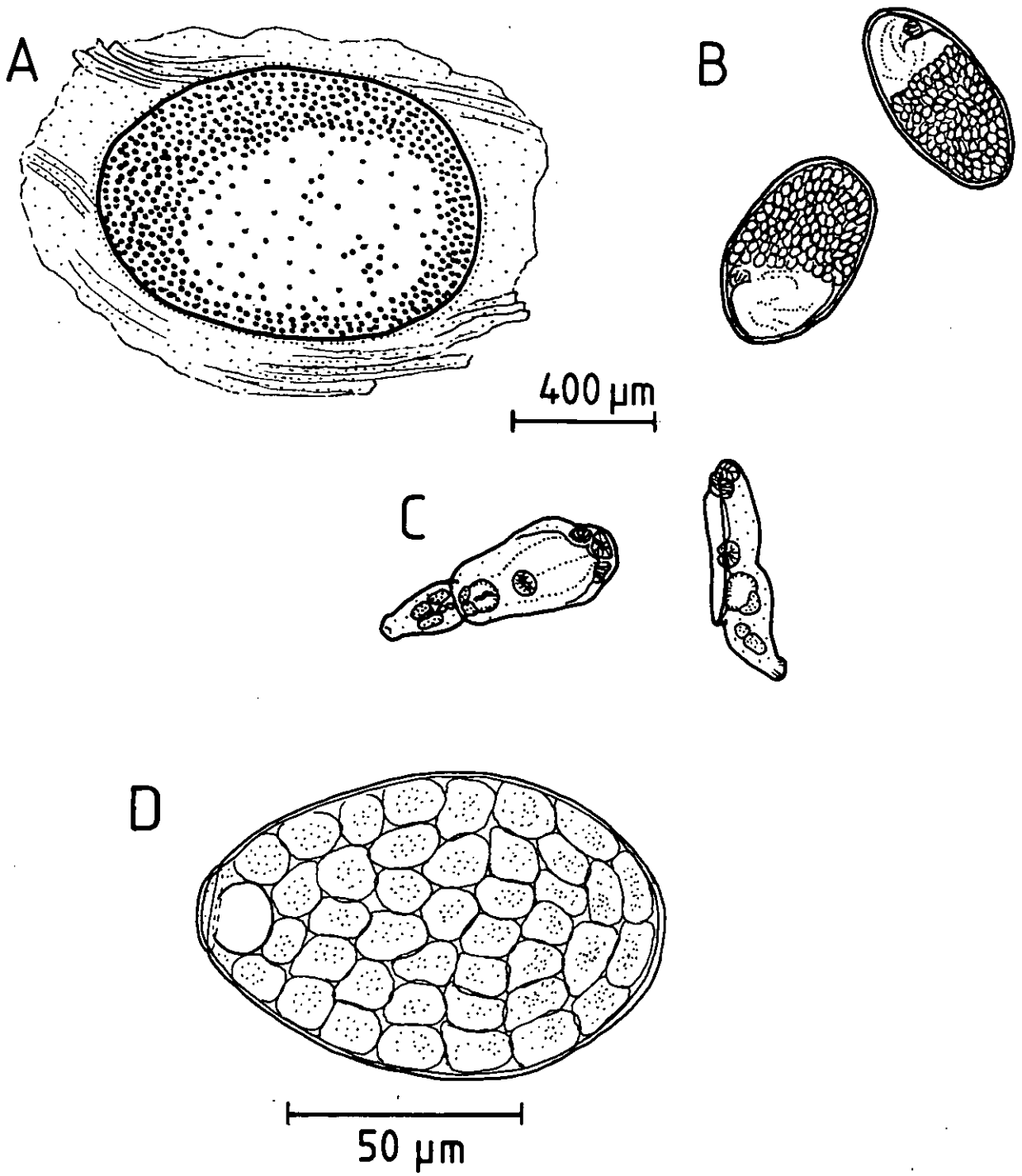


FIGURE 7 A, black cyst embedded in muscle of fish host; B, oval 'inner cyst' removed from thick cellular 'outer cyst'; C, excysted metacercariae after 3 hours at 41°C, (A, B, and C drawn to same scale); D, egg.

Excysted metacercaria (Figures 1 and 7)

The size and development of excysted metacercariae varies greatly (Table 9). The fore-body is relatively large and elongate and the hind-body, a rudimentary stump. Gonadal primordia are visible in advanced metacercariae, and 'dextral' and 'sinistral' types discernible. When 39 metacercariae, dissected from fish collected in June 1979, were excysted, 51% were mature enough to distinguish the position of the anterior testis: 55% of these were 'dextral' and 45% were 'sinistral'. In advanced metacercariae, the oral sucker, pharynx and caeca are formed, lappets and holdfast organs are well-developed and the bilobed gland at the base of the holdfast organ is conspicuous. The fore-body tegument is spinous, spines diminishing in size posteriorly, and the hind-body tegument is aspinous. Large paranephridial canals, through which body fluids and small lipid droplets move, anastomose throughout the body. The genital pore is slightly dorsal to the terminal excretory pore.

DISCUSSION

Neither of the 2 strigeoid species encysting in *Galaxias auratus* at Lake Crescent, have previously been recorded in Australia. *Apatemon (Apatemon) gracilis* and *Diplostomum (Dolichorchis) galaxiae* n.sp. encyst in different tissues of the fish host and naturally infect different definitive hosts. Domestic ducklings are much more susceptible to experimental infection by *A. gracilis* than by *D. galaxiae* n.sp. This is not surprising, as anatids are the main definitive hosts for *A. gracilis* around the world, whereas there are no previous records of a species in the subgenus *Dolichorchis* infecting an anatid. A black duck, *Anas superciliosa*, shot at Calvert's Lagoon 100 kms from Lake Crescent, was found to harbour *A. gracilis*, but not *D. galaxiae* n.sp., and white-faced herons, shot at Lake Crescent, harboured only *D. galaxiae* n.sp., despite the fact that about 70% of the fish being eaten by the herons contained cysts of *A. gracilis*.

TABLE 9 *Diplostomum galaxiae* n.sp. Dimensions of metacercariae excysted *in vitro* after 3 hours at 41°C: (a) too immature to distinguish anterior testis; (b) 'sinistral' type; (c) 'dextral' type.

	(a)	(b)	(c)
Sample size	11	7	13
Body length	401 (355 - 461)	691 (582 - 816)	665 (575 - 756)
Fore-body length	319 (287 - 363)	513 (438 - 605)	479 (393 - 559)
Fore-body width	176 (156 - 228)	213 (194 - 232)	218 (194 - 247)
Hind-body length	82 (68 - 98)	183 (144 - 227)	186 (151 - 204)
Hind-body width	68 (57 - 72)	123 (106 - 137)	123 (114 - 148)
Oral sucker length	46 (40 - 49)	54 (51 - 57)	55 (46 - 61)
Oral sucker width	45 (42 - 46)	49 (46 - 53)	52 (42 - 65)
Ventral sucker length	40 (38 - 46)	42 (38 - 46)	45 (38 - 51)
Ventral sucker width	44 (42 - 48)	47 (42 - 51)	52 (42 - 63)
Pharynx length	29 (25 - 34)	33 (30 - 40)	36 (30 - 42)
Pharynx width	21 (19 - 23)	25 (23 - 29)	25 (23 - 29)
Left lappet length	49 (46 - 49)	70 (61 - 72)	70 (61 - 72)
Left lappet width	31 (27 - 34)	38 (34 - 42)	41 (36 - 42)
Right lappet length	46 (42 - 49)	69 (57 - 76)	71 (61 - 80)
Right lappet width	30 (27 - 34)	38 (30 - 44)	43 (34 - 61)
Holdfast organ length	69 (68 - 72)	104 (99 - 114)	107 (91 - 114)
Holdfast organ width	74 (53 - 76)	81 (76 - 87)	81 (72 - 91)
Ovary length	-	22 (19 - 23)	27 -
Ovary width	-	24 (23 - 25)	23 -
Anterior testis length	-	38 (34 - 46)	39 (34 - 46)
Anterior testis width	-	30 (23 - 38)	34 (27 - 42)
Posterior testis:			
Left lobe length	-	41 (34 - 49)	47 (38 - 53)
Left lobe width	-	27 (23 - 30)	29 (23 - 34)
Right lobe length	-	42 (34 - 49)	43 (34 - 49)
Right lobe width	-	28 (27 - 30)	31 (27 - 38)
O.S.(l+w)/V.S.(l+w)	1.08	1.16	1.10
FBL/HBL	3.89	2.80	2.58

The cercariae and metacercariae of *A. gracilis* are known to occur in Iceland and Scotland (Blair, 1976); Wales (Crocombe, 1959); Central Europe (Vojtek, 1964); Japan (Yamaguti, 1933); and North America (Hoffman, 1959; Lester, 1974). In each case ducklings and chicks served as experimental hosts. The genera *Ancylastrum* and *Lymnaea* serve as molluscan hosts and the fish intermediate hosts include members of the families Cobitidae, Cottidae, Eleotridae, Gasterosteidae, Gobiidae and Salmonidae. Cysts from these fish intermediate hosts are very similar in morphology, but vary in size. The sizes of cysts from fish in Tasmania, U.S.A. and Scotland, were shown in Table 4. It is noteworthy that the external cyst dimensions vary much more between host

species than do the internal cyst dimensions, the thickness of the cyst wall apparently being related to the identity of the fish host.

The molluscan hosts of these strigeoid species at Lake Crescent are not known, however *Potamopyrgus* spp. (Timms, 1978), *Physastra gibbosa* (B. Smith, pers. comm.) and *Rivisessor gunni*, inhabit the lake and an unidentified furcocercaria is known to develop in *P. gibbosa*. At Calvert's Lagoon, trematode developmental stages which conform to those of *A. gracilis* (Blair, 1976), have been found in *Coxiella badgerensis* on one occasion.

The discovery of *A. gracilis* and *D. galaxiae* n.sp. in Tasmania could be a reason for concern for local trout fishing and trout farming interests. It is not known how specific *D. galaxiae* n.sp. is at the secondary intermediate host level, however *A. gracilis* infects both rainbow and brown trout in Scotland. Experimentally infected trout of both species were found to have cysts of *A. gracilis* concentrated around the head and a small percentage within the eye (Blair, 1976). Further studies are needed to determine the extent of these parasites in Tasmania and whether their intermediate host range extends to introduced fish species.

APPENDIX 3

An account of four microphallid trematodes (*Gynaecotyla hickmani* n.sp., *Gynaecotyla macrocotylata* n.sp., *Maritrema eroliae* Yamaguti, 1939 and *Microphallus paragrapsi* n.sp.), infecting the estuarine crab *Paragrapsus gaimardii* (M.Edw.) at Bruny Island, Tasmania.

INTRODUCTION

A population of the estuarine crab *Paragrapsus gaimardii* (M. Edw.) at Great Bay, Bruny Island, Tasmania, is infected with metacercarial cysts of four microphallid species: *Gynaecotyla hickmani* n.sp., *Gynaecotyla macrocotylata* n.sp., *Maritrema eroliae* Yamaguti, 1939 and *Microphallus paragrapsi* n.sp. (Microphallidae Travassos, 1920).

The Tasmanian vertebrate and molluscan hosts of these trematodes have not been discovered. Metacercariae of all of the species existed *in vitro* when exposed to digestive enzymes at 40°C. Eggs were produced *in vitro* by sexually advanced metacercariae of each species, within 2 days of incubation in various culture media at that temperature (Chapter 6). Immature adults of *Microphallus paragrapsi* n.sp. and a single ovigerous *M. eroliae* adult were recovered from experimentally infected ducklings.

Super subfamily GYNAECOTYLIDI (Yamaguti, 1939)

Subfamily GYNAECOTYLINAE (Yamaguti, 1939)

Tribe GYNAECOTYLINI (Yamaguti, 1939)

Genus GYNAECOTYLA Yamaguti, 1939

GYNAECOTYLA HICKMANI N.SP.

Dimensions of the holotype and other relatively mature, non-ovigerous, unflattened excysted metacercariae, are shown in Table 1. The adult is illustrated in Figure 1 and is described below from ovigerous and mature, non-ovigerous, specimens obtained by *in vitro* culture of excysted metacercariae. Dimensions of unflattened ovigerous adults are not available.

FIG. 1 Gynaecotyla hickmani n.sp.

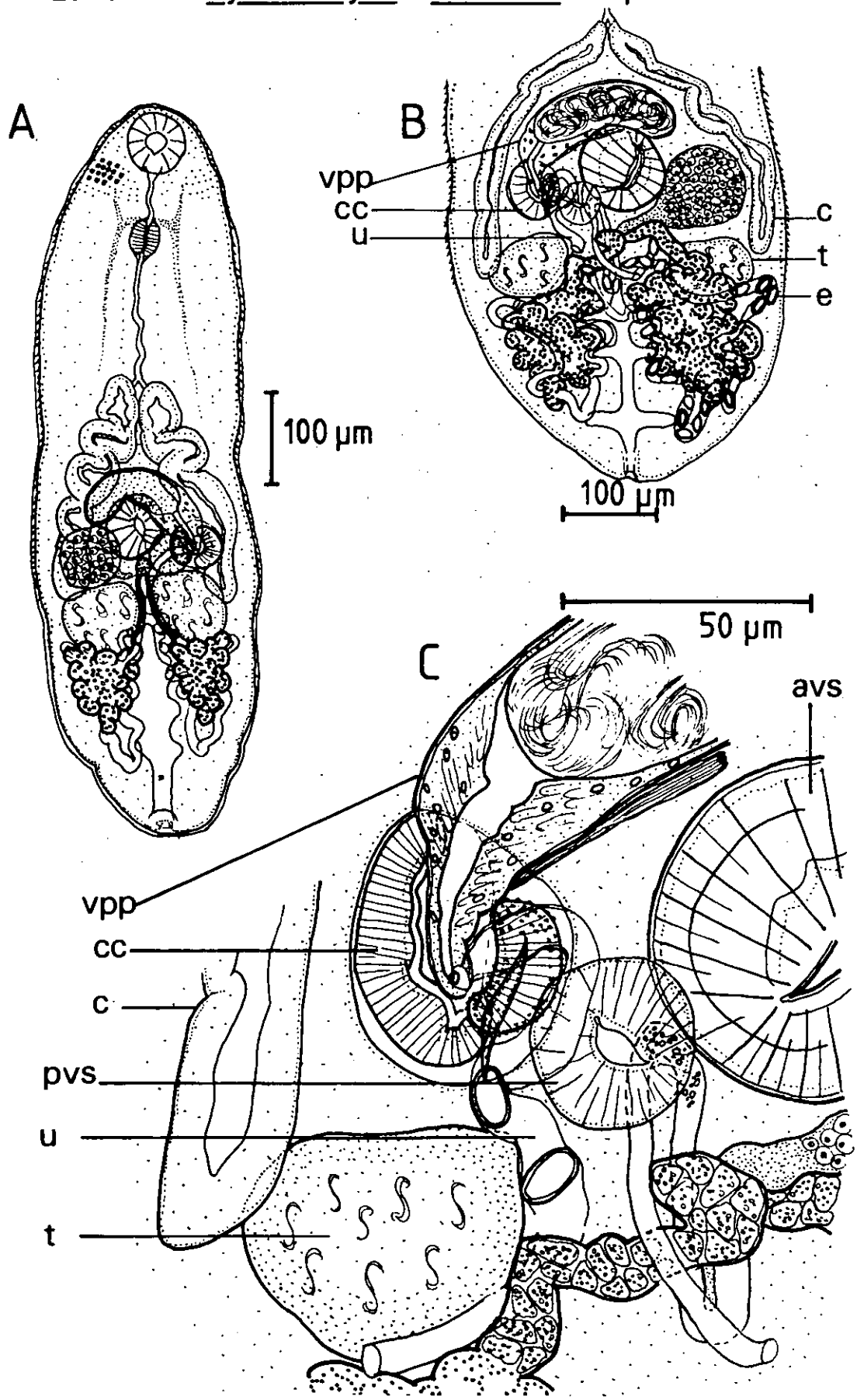


FIGURE 1 A, mature excysted metacercaria after 12 hours at 41°C, holotype, dorsal view; B, gravid adult, slightly flattened, ventral view; C, detail of cornucotyle region, ventral view. (avs: antiporal ventral sucker; e: egg; c: caecum; cc: cornucotyle; pvs: poral ventral sucker; u: uterus; vpp: vesiculoprostatic pouch; t: testis.)

ADULT

Body spatulate, dorsoventrally flattened, maximum width usually at level of gonads. Quincuncially arranged tegumental spines extend to level of testes, diminishing in size posteriorly. Tegumental glands distributed over anterior body. Oral sucker round, mouth subterminal ventral. Prepharynx about ½ length of oesophagus. Pharynx oval, oesophagus bifurcates in anterior ¼ of body. Caeca relatively short, terminating at or before mid-level of testes. Antiporal ventral sucker much larger, more muscular than weakly developed poral ventral sucker. Testes posterolateral, oval symmetrical. Vesiculoprostatic pouch arcuate, between caeca, partly dorsal to antiporal ventral sucker. Large sausage-shaped seminal vesicle occupies proximal 7/8ths of pouch; short pars prostatica opens through ejaculatory orifice, between fleshy lobes of cornucotyle.* Cornucotyle intermediate in size between ventral suckers, smaller than oral sucker. External and internal lobes of cornucotyle situated side by side, in same plane. Round sinistral ovary lies between antiporal ventral sucker, sinistral testis, sinistral caecum. Short oviduct passes posteromedially to ootype, posterior to ventral suckers. Uterus forms initial anterior loop, then forms loops posterior to testes. Metraterm enters genital atrium dorsally, opening adjacent to ejaculatory orifice. Vitelline glands clustered in distinct post-testicular groups. Large vitelline ducts arise in centre of each group, pass anteromedially to small vitelline reservoir, ventral to ootype. Numerous eggs contained in uterus, measuring 20 (17 - 23) × 10 (8 - 11)μ, (fixed, flattened). Flame-cell formula not determined. Excretory vesicle typical of genus, resembling Cross of Lorraine.

* The cornucotyle (= copulatory apparatus within the genital atrium, (Deblock, 1974b)), consists of a large smooth outer lobe, 40 (38 - 42) × 14 (11 - 15)μ, and smaller inner lobe, 30 (27 - 34) × 21 (19 - 23)μ, its surface studded with conical protuberances. A thin sclerotinized layer lines the inner surface of the external lobe. A narrow bundle of muscle fibres extends from the mid-posterior margin of the vesiculoprostatic pouch to the base of the cornucotyle.

TABLE 1 *Gynaecotyla hickmani* n.sp. Dimensions of metacercariae excysted *in vitro*: (a) after about 3 hours at 41°C and (b) the holotype after 12 hours at 41°C. All specimens relatively mature, with active vitellaria, but no eggs.

	(a)	(b)
Sample size	20	1
Body length (BL)	735 (582 - 839)	703
Body width	220 (190 - 255)	217
Oral sucker length (OS)	54 (48 - 61)	54
Oral sucker width	54 (48 - 61)	48
Prepharynx length	43 (23 - 57)	38
Oesophagus length	156 (118 - 190)	129
Pharynx length	38 (34 - 42)	34
Pharynx width	29 (25 - 32)	27
Left caecum length	264 (220 - 304)	232
Right caecum length	264 (224 - 304)	234
Antiporal ventral sucker length (AVS)	69 (61 - 84)	68
Antiporal ventral sucker width	65 (53 - 80)	65
Poral ventral sucker length (PVS)	43 (38 - 48)	42
Poral ventral sucker width	36 (32 - 40)	40
Cornucotyle length (C)	44 (38 - 49)	42
Vesiculoprostatic pouch length (VPL)	122 (106 - 148)	137
Vesiculoprostatic pouch width	35 (30 - 40)	34
Ovary length (O)	60 (49 - 67)	72
Ovary width	53 (46 - 61)	63
Left testis length	85 (63 - 99)	89
Left testis width	66 (53 - 84)	74
Right testis length	81 (68 - 91)	91
Right testis width	63 (42 - 80)	68
VPL:BL ratio	0.17	0.19
Relative size (l+w) of organs:	AVS>O≈OS>C>PVS	AVS≈O>OS>C>PVS

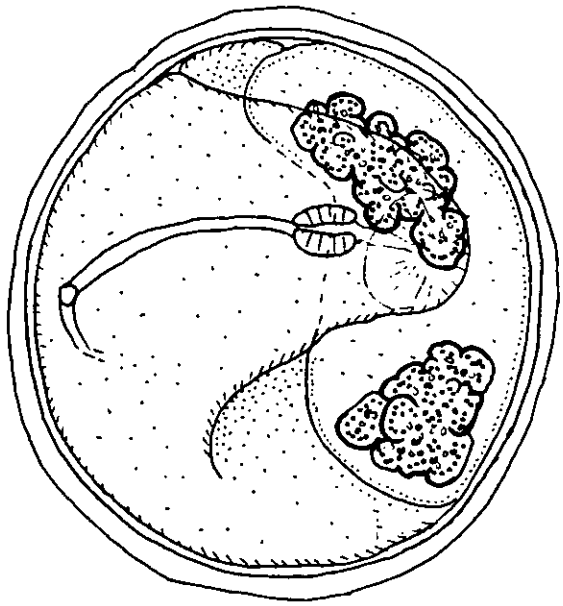
METACERCARIAL CYST (Figure 2)

The large round cyst occurs mainly in the green gland, but also in the body cavity of *Paragrapsus gaimardii*, either free or loosely bound by connective tissue. The cyst wall, about 28μ thick, is composed of a wide translucent outer layer, often yellowish, and a narrow, clear inner layer. Dimensions of 20 cysts, their identity confirmed by *in vitro* excystment, are shown in Table 2.

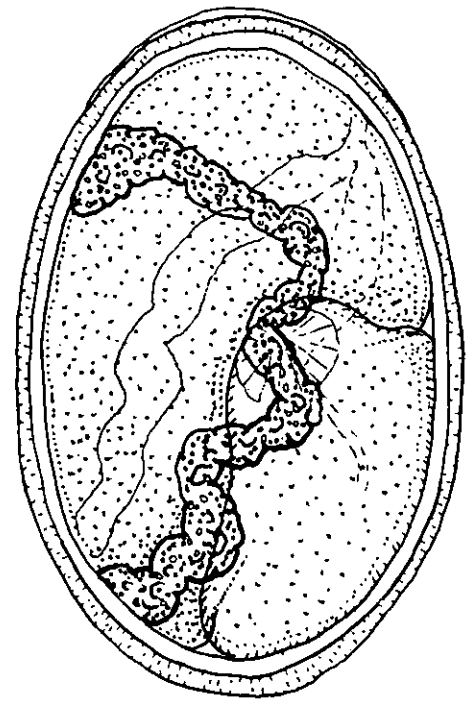
TABLE 2 *Gynaecotyla hickmani* n.sp. Dimensions of live metacercarial cysts dissected from naturally infected *Paragrapsus gaimardii* (n = 20).

External dimensions	Length	416 (378 - 514)
	Width	396 (348 - 499)
Internal dimensions	Length	359 (334 - 384)
	Width	342 (315 - 365)

FIG. 2 TREMATODE CYSTS INFECTING PARAGRAPSUS GAIMARDII

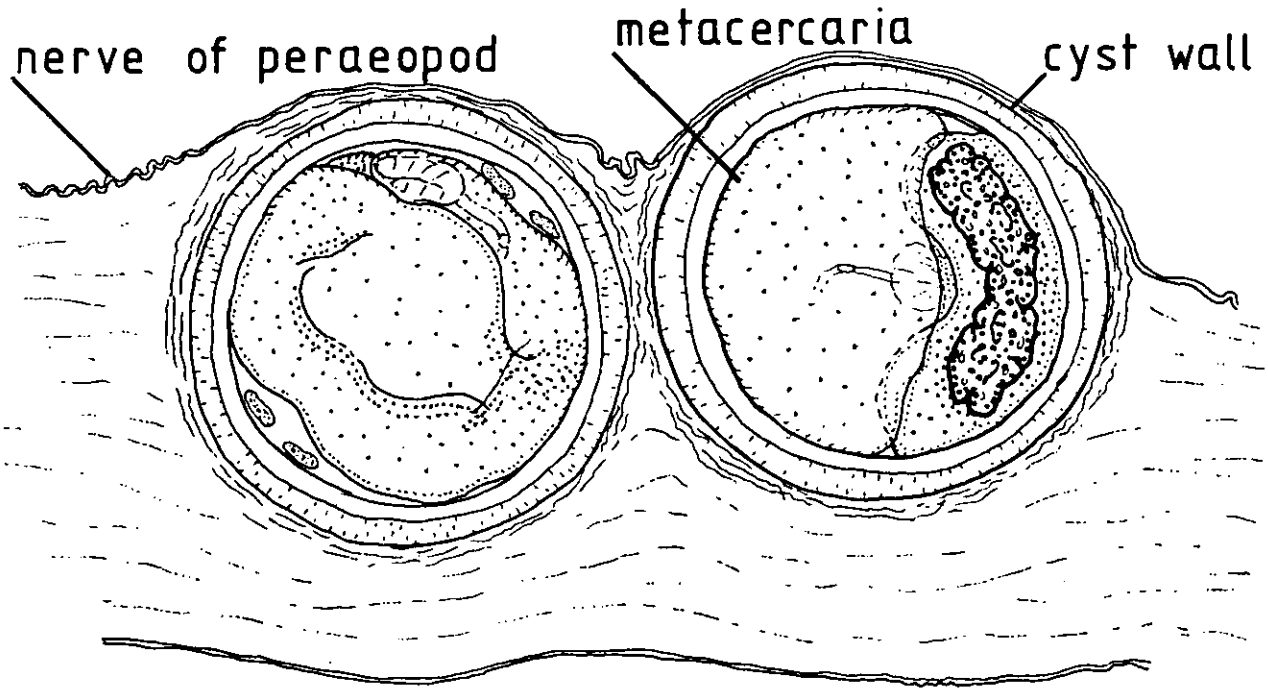


Gynaecotyla hickmani n.sp.



Maritrema eroliae

200 µm



Microphallus paragrapsi n.sp.

In vitro excystment occurs at 41°C when incubated in either 1% trypsin, or 0.5% pancreatin solutions, in Hank's saline. Metacercariae excyst at varying stages of maturity, from those in which the sex organs are not fully developed, to those in which gametogenesis and vitellogenesis are advanced.

Crustacean intermediate host: *Paragrapsus gaimardii* (M. Edw.)

Geographical location: Great Bay, Bruny Island, Tasmania.

Site of encystment: Green gland; general body cavity

Type material: Tasmanian Museum - K895, holotype, mature excysted metacercaria (ringed); K896, paratypes, gravid adults (flattened), K897 and K898, paratypes, mature excysted metacercariae.

Relationships

This new species of *Gynaecotyla* is named after Dr. J.L. Hickman, who discovered its metacercarial cyst with that of *Microphallus paragrapsi* n.sp., in the crab *P. gaimardii*, at Great Bay. Using the key to species of the genus *Gynaecotyla*, proposed by Deblock (1974b), *G. hickmani* n.sp. keys out with *G. brisbanensis* Deblock and Pearson, 1968, which infects shore birds in Queensland and *G. bridgmani* Deblock, 1974b, a parasite of charadriiform birds in Japan. Although similar in shape and general anatomy, it is distinctly larger than both of those species and differs from them in several respects. In *G. bridgmani*, conspicuous sclerotinized pieces, arranged in a Y-shape, occur in the cornucotyle; and the vesiculoprostatic pouch is relatively large (VPL:BL from 0.2 to 0.25). In *G. hickmani* n.sp., no sclerotinized pieces occur in the cornucotyle and the vesiculoprostatic pouch is relatively small (VPL:BL $\leq$0.2). The form and ornamentation of the cornucotyle of *G. brisbanensis* are similar to those of *G. hickmani* n.sp., however, the thin, longitudinal sclerotinized sheet in the middle of the cornucotyle of *G. brisbanensis*, is not present in the cornucotyle of *G. hickmani* n.sp. In *G. brisbanensis*,

the arc of the vesiculoprostatic pouch is not subtended by bundles of muscle fibres and the diameter of the oral sucker is greater than or equal to the diameter of the antiporal ventral sucker; whereas in *G. hickmani* n.sp., the vesiculoprostatic pouch is subtended by a narrow band of muscle fibres, extending from the pouch to the cornucotyle and the antiporal ventral sucker is much larger than the oral sucker.

GYNAECOTYLA MACROCOTYLATA N.SP.

A minority of the *Gynaecotyla* metacercariae that excysted *in vitro* from large, round cysts taken from the body cavity of *Paragrapsus gaimardii*, were obviously different from *Gynaecotyla hickmani* n.sp. and were found to belong to another new species, *Gynaecotyla macrocotylata* n.sp. *G. macrocotylata* n.sp. differs from *G. hickmani* n.sp. in many respects. The more obvious differences are that in *G. macrocotylata* n.sp., the cornucotyle is larger than the oral and ventral suckers, the 2 ventral suckers are similar in muscular development, the poral ventral sucker is similar in size to the oral sucker, the cornucotyle contains 3 large sclerotinized pieces,and the caeca are relatively long, usually terminating posterior to the testes.

The adult, illustrated in Figure 3, is described below, from specimens that were excysted and cultured *in vitro* at 41°C. Dimensions of the gravid holotype and some relatively mature, non-ovigerous, excysted metacercariae, are shown in Table 3.

ADULT

Body elongate spatulate. Tegumental spines diminish posteriorly, extending to level of testes. Tegumental gland cells distributed over anterior body. Oral sucker round, mouth subterminal ventral. Prepharynx about ¼ length of oesophagus, pharynx oval; oesophagus bifurcates in anterior ⅓ of body. Caeca long, convoluted, usually terminating posterior to testes. Both ventral suckers well developed,

FIG. 3 *Gynaecotyla macrocotylata* n.sp.

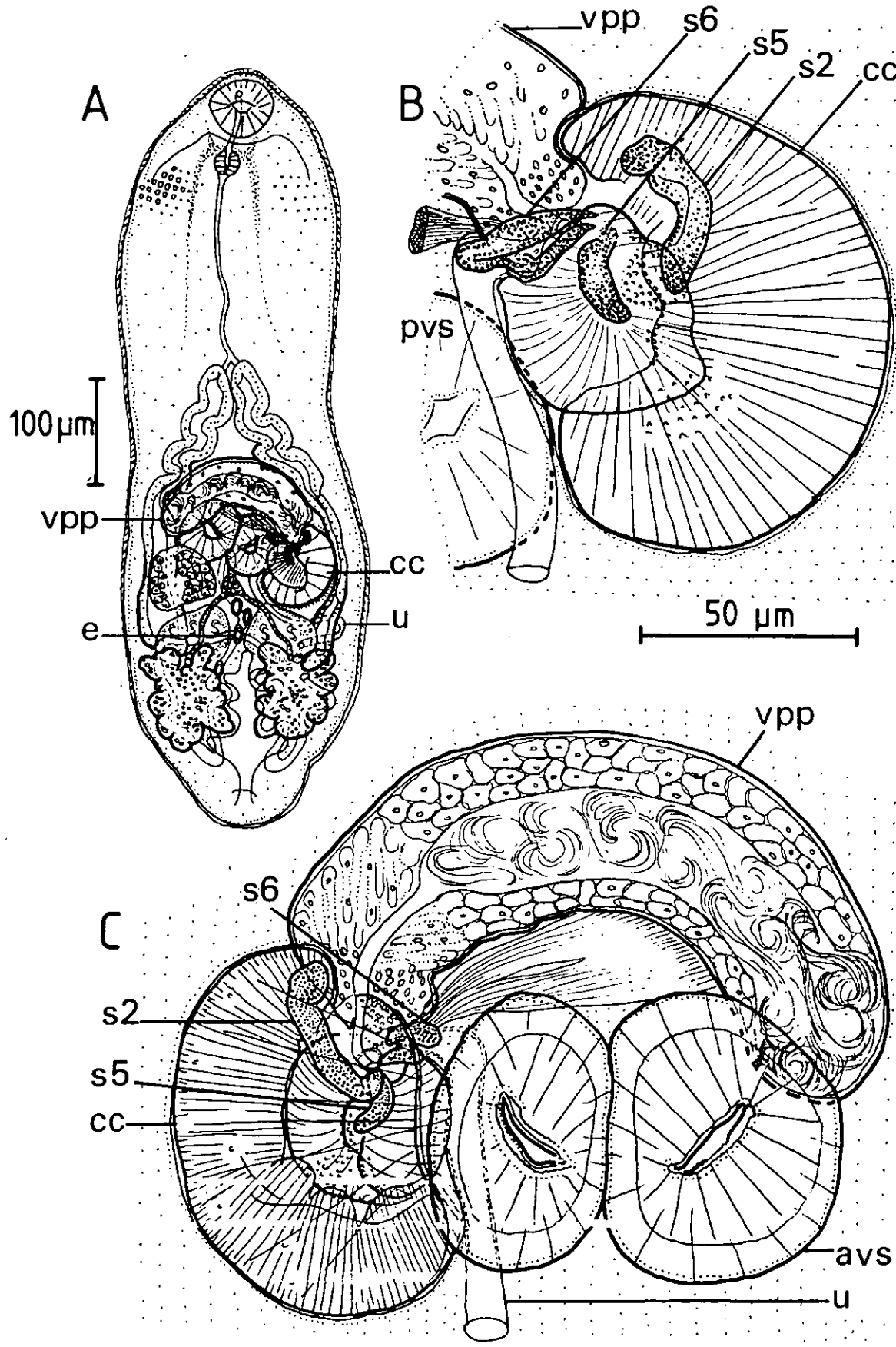


FIGURE 3 A, gravid adult, holotype, dorsal view; B, detail of cornucotyle, flattened, dorsal view; C, vesiculoprostatic pouch and ventral suckers, ventral view. (avs: antiporal ventral sucker; cc: cornucotyle; e: egg; pvs: poral ventral sucker; s2, s5 and s6, sclerotinized pieces in cornucotyle; u: uterus; vpp: vesiculoprostatic pouch.)

antiporal ventral sucker slightly larger than poral ventral sucker. Testes posterolateral, oval, symmetrical. Vesiculoprostatic pouch arcuate, partly dorsal to ventral suckers, between caeca. Spatulate seminal vesicle occupies proximal 2/3rds of pouch; pars prostatica opens through ejaculatory orifice, into genital atrium. Cornucotyle larger than oral and ventral suckers. External lobe largely ventral, enveloping smaller internal lobe. Three sclerotinized pieces in anterior part of cornucotyle. Round, sinistral ovary situated between sinistral testis and proximal end of vesiculoprostatic pouch, partly dorsal to antiporal ventral sucker. Oviduct passes posteromedially to median ootype. Uterus forms loops posterior to testes, then passes anteriad to genital atrium. Metraterm enters atrium dorsally, opening near ejaculatory orifice. Vitelline glands clustered in two distinct post-testicular bunches. Vitelline ducts pass anteriad, joining to form small round median vitelline reservoir. Eggs numerous, measure 17 (16 - 19) × 12 (11 - 13)μ, (fixed, flattened). Flame-cell formula not determined. Excretory vesicle typical of genus.

The external lobe of the cornucotyle measures about 91 × 48μ and the internal lobe about 47 × 42μ. The surfaces of the lobes are smooth except for scattered small pointed projections (Figure 3). According to the scheme of Deblock (1974b,p.323), for describing the sclerotinized pieces of the cornucotyle: No.2 is elongate and bent obliquely in the middle, about 32 × 10μ; No. 5 is rod shaped, about 20 × 7μ; and No. 6 is forked, about 17 × 8μ. A wide bundle of well developed muscles extends from piece No. 6 to the posterior wall of the vesiculoprostatic pouch.

METACERCARIAL CYST

The cyst is round and large and occurs mainly in the green gland, but also in the general body cavity of *Paragrapsus gaimardii*. To date it has not been distinguished from that of *G. hickmani* n.sp. In January

TABLE 3 *Gynaecotyla macrocotylata* n.sp. Dimensions of: (a) mature, non-ovigerous, excysted metacercariae, after about 3 hours *in vitro* at 41°C and (b) the holotype, a gravid fluke, after about 12 hours *in vitro* at 41°C.

	(a)	(b)
Sample size	12	1
Body length (BL)	779 (665 - 937)	703
Body width	238 (224 - 258)	227
Oral sucker length (OS)	55 (49 - 61)	48
Oral sucker width	56 (53 - 61)	53
Prepharynx length	44 (34 - 57)	38
Oesophagus length	201 (160 - 228)	160
Pharynx length	40 (36 - 44)	36
Pharynx width	30 (27 - 32)	27
Left caecum length	323 (289 - 372)	285
Right caecum length	329 (296 - 380)	308
Antiporal ventral sucker length (AVS)	68 (61 - 74)	68
Antiporal ventral sucker width	59 (53 - 68)	57
Poral ventral sucker length (PVS)	63 (57 - 68)	61
Poral ventral sucker width	46 (42 - 51)	48
Cornucotyle length (C)	78 (65 - 95)	84
Cornucotyle width	71 (68 - 72)	72
Vesiculoprostatic pouch length (VPL)	166 (156 - 182)	160
Vesiculoprastatic pouch width	45 (42 - 49)	46
Ovary length (O)	65 (57 - 72)	70
Ovary width	57 (53 - 61)	61
Left testis length	70 (65 - 80)	68
Left testis width	57 (51 - 65)	53
Right testis length	66 (61 - 72)	65
Right testis width	58 (49 - 65)	53
VPL:BL ratio	0.21	0.23
Relative size (l+w) of organs:	C>AVS≈O>PVS≈OS	C>AVS≈O>PVS≈OS

1980, about 50 large, round metacercarial cysts from the body cavity of *P. gaimardii* were measured and then exposed to digestive enzymes *in vitro*; however, all of the metacercarie that excysted were *G. hickmani* n.sp. In December 1979, similar unmeasured cysts were exposed to digestive enzymes *in vitro* and the ratio of the 2 *Gynaecotyla* species in 100 excysted metacercariae was 85 *G. hickmani* n.sp. to 15 *G. macrocotylata* n.sp. The metacercaria of *G. macrocotylata* n.sp. excysts at varying stages of maturity, the more advanced specimens having active vitellaria, producing phenolic egg-shell precursors.

Crustacean intermediate host: *Paragrapsus gaimardii* (M. Edw.)

Geographical location: Great Bay, Bruny Island, Tasmania

Site of encystment: Green gland, general body cavity

Type material: Tasmanian Museum - K899, holotype, gravid adult (ringed); K900, paratypes, gravid adults (flattened); K901 and 902, paratypes, mature excysted metacercariae.

Relationships

According to the key to species of *Gynaecotyla* presented by Deblock (1974b), *G. macrocotylata* n.sp. most closely resembles the large form of *G. longiintestinata* (syn. *G. gallica*). In the latter species, however, the oral sucker is larger than the poral ventral sucker and larger than or equal to the antiporal ventral sucker. The form and arrangement of sclerotinized pieces in the cornucotyle, which are consistent within *Gynaecotyla* species, differ markedly between *G. macrocotylata* n.sp. and *G. longiintestinata*, e.g. in the latter species No. 6 is not branched and No. 2 is absent, whereas in the former, No. 6 is forked and No. 2 is well developed.

The name *G. macrocotylata* n.sp. is proposed for this new species as the cornucotyle is massive and both ventral suckers are large and muscular.

Super subfamily MARITREMATIDI (Nicoll, 1907)

Subfamily MARITREMATINAE Nicoll, 1907

Tribe MARITREMATINI (Nicoll, 1907)

Genus MARITREMA Nicoll, 1907

MARITREMA EROLIAE YAMAGUTI, 1939

Syn. (according to Deblock, 1975b): *M. kitanensis* Shibue, 1953

M. magnicirrus Belopolskaia, 1952

M. urayensis Ogata, 1951

The adult, illustrated in Figure 4, is described from ovigerous specimens cultured *in vitro*. Dimensions of unflattened, ovigerous adults are not available, however they appear to be the same size as newly excysted metacercariae and dimensions of the latter are shown in Table 4.

ADULT

Body elongate pyriform to triangular. Small tegumental spines cover body except at posterior extremity. Tegumental gland cells anterior to cirrus pouch. Oral sucker round. Prepharynx about ½ length of oesophagus; pharynx oval; oesophageal bifurcation about 1/3rd body length from anterior end. Caeca diverge acutely, extend to midlevel of metraterm. Ventral sucker round, larger than oral sucker, located in posterior ½ of body. Symmetrical, oval testes situated posterolaterally. Massive J-shaped cirrus pouch, about 2.5μ thick. Flexure of pouch antero-dorsal to ventral sucker. Seminal vesicle large, clavate, in proximal ½ of dextral limb of cirrus pouch. Distal end of seminal vesicle tapers to short, narrow folded seminal canal which widens to voluminous, convoluted pars prostatica. Large prostate gland cells granular, extensive within cirrus pouch. Invaginated spiny cirrus leads from level of anterior seminal vesicle to sinistral genital atrium, proximal part densely lined by simple sharp spines, increasing in size distally from 5 × 2 to 10 × 3μ; distal part lined by large, flattened, thorn-like spines, variable in size 12 (6 - 19) × 7 (5 - 10)μ. Evaginated cirrus, directed anteriorly, about 150μ long (fixed, flattened), with small simple spines covering distal part, increasing in size to several rows of thorn-like spines near base of cirrus. Aspinous 'heel' at base of evaginated cirrus; crown of spines around genital aperture. Triangular ovary multilobed (usually three main lobes) median, dorsal to ventral sucker, bounded anteriorly by cirrus pouch, —

FIG. 4 Maritrema eroliae

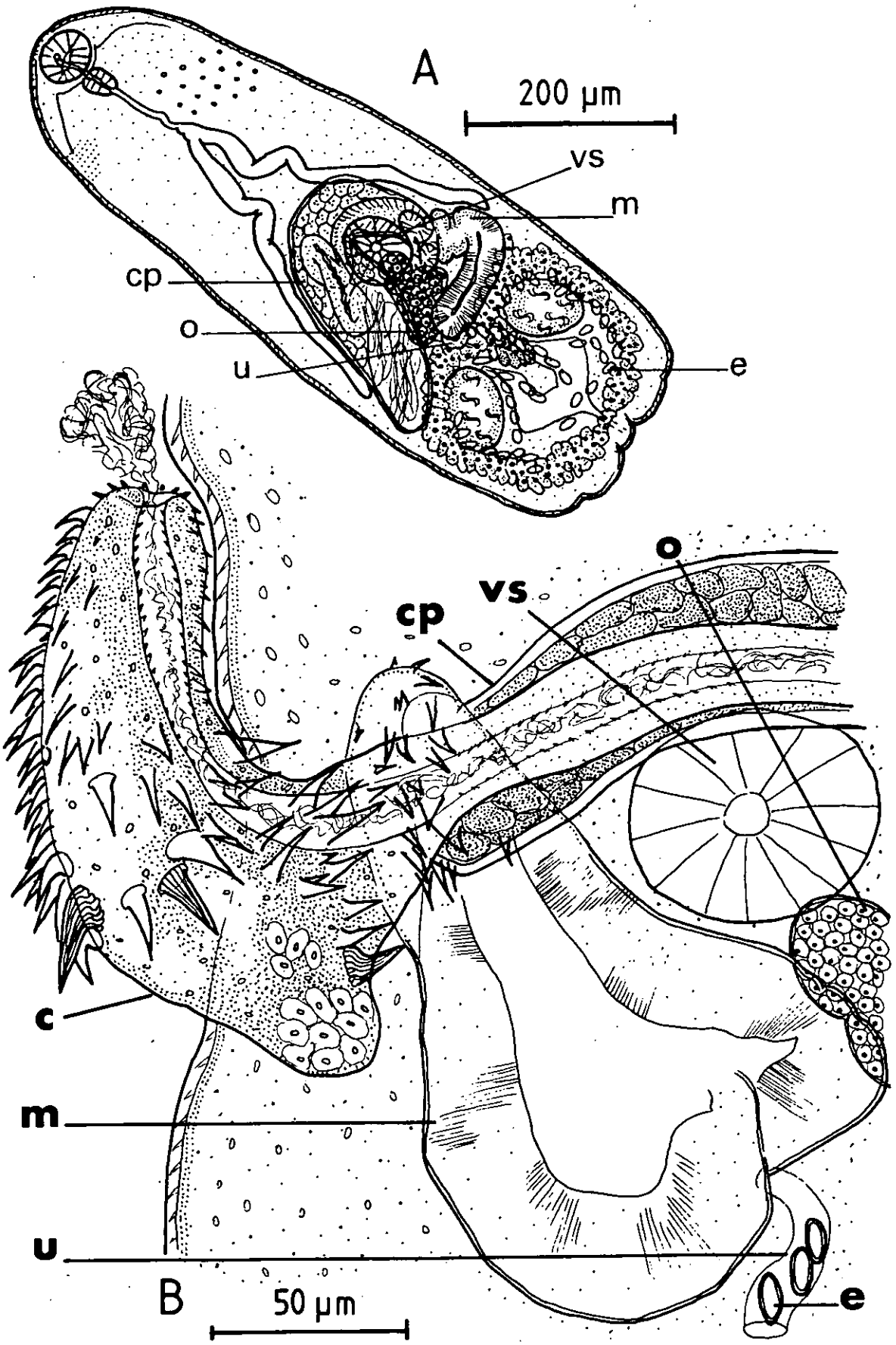


FIGURE 4 A, gravid adult, ventral view; B, everted cirrus of gravid adult, flattened, dorsal view. (c: cirrus; cp: cirrus pouch; e: egg; m: metraterm; o:ovary; vs: ventral sucker; u: uterus.)

TABLE 4 *Maritrema eroliae*. Dimensions of metacercariae excysted *in vitro* after about 3 hours at 41°C. All metacercariae relatively mature, with active vitellaria (n = 15).

Body length (B.L.)	683 (620 - 794)
Body width (B.W.)	258 (217 - 293)
Oral sucker length (O.S.)	47 (40 - 53)
Oral sucker width	51 (46 - 57)
Prepharynx length	40 (30 - 53)
Oesophagus length	86 (65 - 133)
Pharynx length	28 (23 - 30)
Pharynx width	24 (21 - 27)
Left caecum length	292 (251 - 342)
Right caecum length	307 (274 - 357)
Ventral sucker length (V.S.)	64 (61 - 70)
Ventral sucker width	61 (57 - 67)
Cirrus pouch length (C.P.L.)	350 (323 - 380)
Cirrus pouch width	82 (68 - 91)
Seminal vesicle length	141 (114 - 156)
Seminal vesicle width	59 (49 - 68)
Metraterm length	117 (103 - 137)
Metraterm width	62 (46 - 80)
Ovary length	108 (87 - 125)
Ovary width	59 (46 - 76)
Left testis length	71 (65 - 76)
Left testis width	52 (42 - 57)
Right testis length	76 (65 - 87)
Right testis width	53 (46 - 61)
Roundness, B.L./B.W.	2.65
O.S. (l+w)/V.S.(l+w)	0.78
C.P.L./B.L.	0.51

posteriorly by metraterm. Oviduct leads posteriorly to median ootype, surrounded by well-developed Mehlis' gland. Uterus loops around each testis, mainly lying within vitelline ring; enters vast, muscular metraterm medially, posterior to ovary. Metraterm lining about 13μ thick, traversed by radial fissures widening towards lumen. Eggs numerous, oval 18 (16 - 19) × 9 (8 - 10)μ. Vitelline glands form post-ovarian ring, closed posteriorly, open near ootype. Short transverse vitelline ducts unite medially forming short longitudinal vitelline reservoir. Flame-cell formula not determined. Excretory vesicle Y-shaped, arms extending to anterior margins of testes.

METACERCARIAL CYST (Figure 2)

The cyst is oval and quite large, dimensions are presented in Table 5.

The cyst wall is composed of a clear, uniform inner layer, about 10μ thick, overlain by a darker layer, about 12μ thick, which is traversed by fine, radial striations, or fissures. A thin outer membrane, about 1μ wide, envelops the whole cyst. The cyst occurs free, or lightly bound by connective tissue, within the body cavity of *Paragrapsus gaimardii*.

Excystment occurs *in vitro* at 41°C when incubated in 0.5% pancreatin, or 0.5% trypsin and 0.05% sodium taurocholate, in Hank's saline. Metacercariae reach an advanced state of sexual development, and although they excyst at varying degrees of maturity, more advanced specimens have phenolic egg-shell precursors in the vitellaria and commence egg production within a few hours at 41°C.

TABLE 5 *Maritrema eroliae*. Dimensions of live metacercarial cysts, dissected from *Paragrapsus gaimardii* (n = 20).

External diameter	Length	458 (423 - 484)
	Width	304 (289 - 331)
Internal diameter	Length	402 (370 - 423)
	Width	254 (239 - 274)

Crustacean intermediate host: *Paragrapsus gaimardii* (M. Edw.)

Geographical location: Great Bay, Bruny Island, Tasmania

Site of encystment: Body cavity

Material: Tasmanian Museum - K892 (flattened, gravid adult, cirrus everted), K893 (gravid adults), K894 (non-ovigerous, excysted metacercariae).

Relationships

The anatomy of this microphallid, with very large cirrus pouch, spiny cirrus, vast metraterm and median ovary, is characteristic of a distinctive and homogeneous group of *Maritrema* species: *M. eroliae* Yamaguti, 1939, *M. echinocirrata* Leonov, 1958, *M. patulus* Coil, 1955 and *M. misenensis* (Palombi, 1940) Prevot, Bartoli and Deblock, 1976.

The evaginated cirrus of the latter species is free of spines, except for a zone around the base, mainly proximal to a distinct 'heel'. *M. patulus* is morphologically identical to *M. eroliae* and when its life-cycle is discovered it may fall into synonymy with *M. eroliae*, (Prevot *et al.*, 1976). *M. echinocirrata* differs from *M. eroliae* only in the absence of the large, thorn-like spines near the base of the evaginated cirrus. The Tasmanian flukes conform to the range of variation described for *M. eroliae*,(Deblock, 1975b).

The metacercaria of *M. eroliae* has previously been found encysting in various decapod crustaceans in Japan (Ogata, 1951; Shibue, 1953 and Bridgman *et al.*, 1972). The oval cyst varies greatly in size and thickness, in relation to the identity of the second intermediate host. The cysts found in Tasmanian crabs are intermediate in size between those infecting *Macrophthalmus dilatatus*, 520 - 690 × 360 - 450μ (Ogata, 1951 and Bridgman *et al.*, 1972), and those infecting *Neocaridina denticulata*, 280 - 320 × 240 - 270μ (Shibue, 1953) and *Scopimer* spp., 310 - 360 × 210 - 240μ(Ogata, 1951). The adult of *M. eroliae* has been found in birds inhabiting the eastern border of the Pacific Ocean (Deblock, 1975b), and in Australia, has been recorded in the Mongolian dotterel in Queensland, and in a fish, the leatherjacket, from the River Derwent, Tasmania (Last,1975).

Super subfamily: MICROPHALLIDI (Ward, 1901)

Subfamily: MICROPHALLINAE (Ward, 1901)

Tribe: MICROPHALLINI (Ward, 1901)

Subtribe: MICROPHALLINA (Ward, 1901)

Genus: MICROPHALLUS (Ward, 1901)

MICROPHALLUS PARAGRAPSI N.SP.

The adult, illustrated in Figures 5 and 6, is described below, from ovigerous,and mature non-ovigerous,specimens . Dimensions of unflattened, ovigerous specimens are not available; however, gravid adults appear to be the same size as relatively mature excysted metacercariae, dimensions of which are shown in Table 6. Dimensions of very immature excysted

metacercariae are shown in the same Table. The holotype and other non-ovigerous adults recovered from an experimentally infected duckling 17 hours after infection, were intermediate in size between immature and mature excysted metacercariae (Table 9).

ADULT

Body varies from pyriform to elongate spatulate, frequently with distinct waist just anterior to seminal vesicle, causing dumb-bell appearance. Lateral body margins of worms cultured *in vitro* generally folded ventrally. Quincuncially arranged tegumental spines diminish in size posteriorly; extending to waist level. Tegumental gland cells distributed over anterior body. Transversely oval to round oral sucker, mouth subterminal ventral. Short pre-pharynx about 1/20th length of oesophagus. Oesophageal bifurcation in posterior ½ of body. Caeca relatively short, terminating at midlevel of ventral sucker. Round ventral sucker smaller than oral sucker. Testes oval, equal, posterolateral. No cirrus pouch; seminal vesicle oval, partly dorsal to ventral sucker. Short seminal canal leads posteriorly to expanded pars prostatica, about 6μ diameter, at base of male papilla. Prostate gland cells located between seminal vesicle and male papilla, clustered around seminal canal; secreting through cluster of undetermined number of small ducts, into pars prostatica. Retracted male papilla coiled or folded within genital atrium, measures 34 (30 - 38) × 29 (27 - 30)μ. Everted male papilla, tubular, not lobed, directed posteromedially; length 66 (53 - 76)μ, width at base 26 (23 - 30)μ, width at tip 22 (19 - 25)μ. Ejaculatory canal, about 4μ diameter, more or less axial within male papilla. Oval ovary, contiguous to dextral caecum and testis, dextral and partly dorsal to ventral sucker. Oviduct passes posteromedially to bulbous seminal receptacle, from which Laurer's duct leads to dorsal

FIG. 5 Microphallus paragrapsi n.sp.

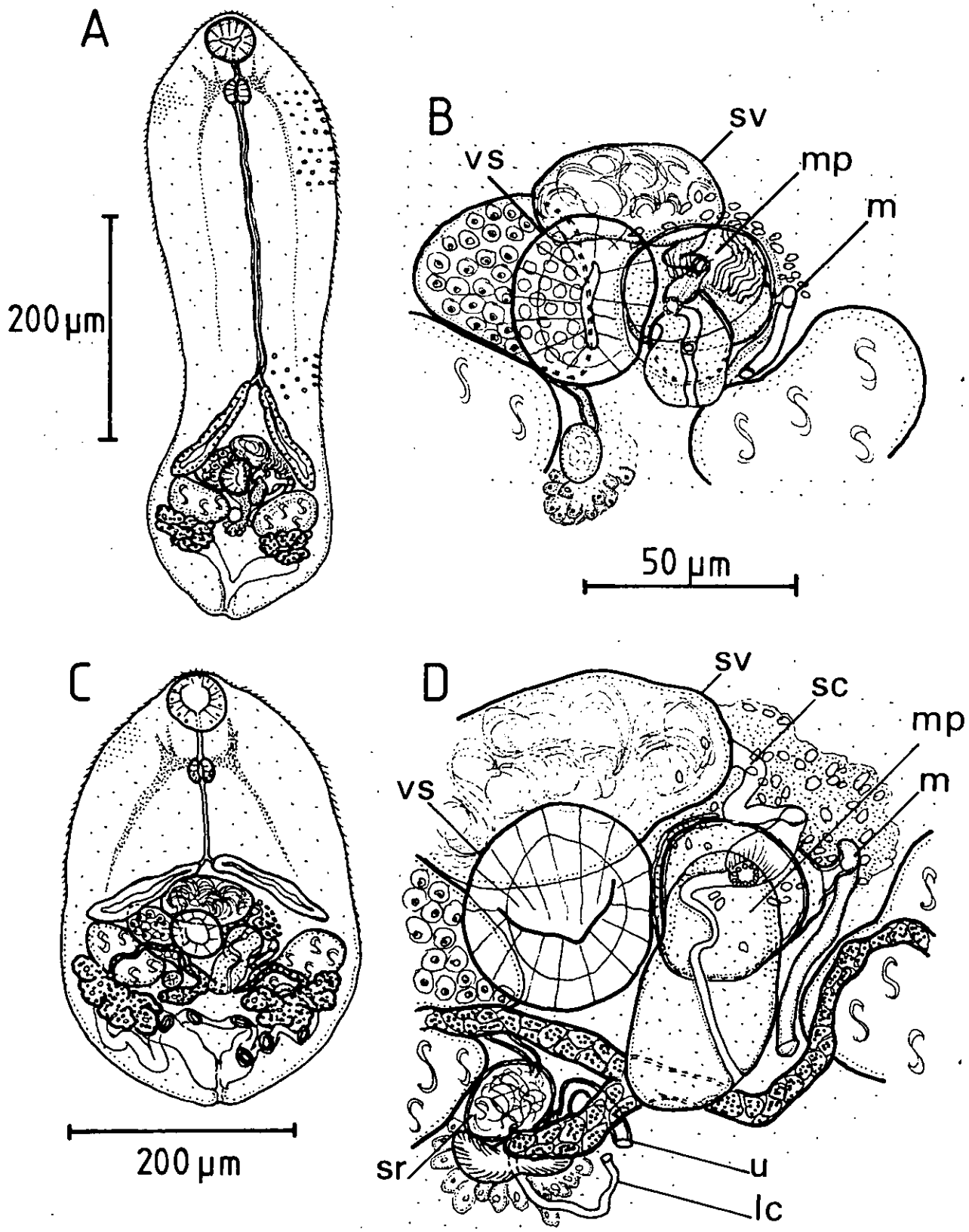


FIGURE 5 A, non-ovigerous adult, holotype, after 17 hours in laboratory duckling, ventral view; B, detail of ventral sucker region of holotype; C, gravid adult, cultured *in vitro* at 41°C, flattened, ventral view; D, detail of ventral sucker region of adult shown in C. (lc: Laurer's canal; m: metraterm; mp: male papilla; sc: seminal canal; sr: seminal receptacle; sv: seminal vesicle; u: uterus; vs: ventral sucker.)

surface. Ootype located between testes, posterior to ventral sucker. Uterus forms post-testicular loops, overlapping each testis. Metraterm enters left side of genital atrium. Vitelline gland cells clustered in compact bunches posterior to each testis. Vitelline ducts pass anteromediad, joining to form elongate vitelline reservoir, posterior to ventral sucker. Oval eggs, numerous 19 (17 - 21) × 10 (8 - 13)μ, (fixed flattened). Flame-cell formula not determined. Excretory vesicle V-shaped, limbs extending to testes.

TABLE 6 *Microphallus paragrapsi* n.sp. Dimensions of metacercariae excysted *in vitro* after about 3 hours at 41°C: (a) smaller, less mature specimens; (b) larger, more mature specimens, some with active vitellaria.

Sample size	20	20
Body length (B.L.)	355 (287 - 469)	579 (484 - 665)
Body width (B.W.)	156 (137 - 177)	184 (156 - 209)
Oral sucker length (O.S.)	38 (34 - 40)	40 (34 - 46)
Oral sucker width	40 (36 - 42)	44 (42 - 49)
Prepharynx length	4 (4 - 49)	12 (4 - 27)
Oesophagus length	115 (87 - 144)	275 (220 - 319)
Pharynx length	22 (17 - 23)	25 (19 - 27)
Pharynx width	18 (17 - 19)	21 (19 - 25)
Left caecum length	88 (76 - 99)	128 (110 - 156)
Right caecum length	87 (72 - 99)	126 (103 - 137)
Ventral sucker length (V.S.)	35 (30 - 38)	40 (34 - 46)
Ventral sucker width	31 (23 - 36)	40 (34 - 44)
Seminal vesicle length	-	39 (34 - 42)
Seminal vesicle width	-	27 (23 - 36)
Ovary length	31 (23 - 34)	41 (38 - 46)
Ovary width	25 (17 - 30)	34 (30 - 38)
Left testis length	45 (34 - 53)	59 (57 - 61)
Left testis width	38 (29 - 46)	51 (38 - 57)
Right testis length	50 (34 - 61)	61 (53 - 68)
Right testis width	35 (27 - 42)	46 (42 - 51)
B.L./B.W.	2.28	3.15
O.S.(l+w)/V.S.(l+w)	1.18	1.05

METACERCARIAL CYST (Figure 2)

The cyst is round, colourless and quite thick-walled. Its dimensions are shown in Table 7. The uniformly thick cyst wall, about 36 (27 - 44)μ wide, is composed of 2 layers: a clear, homogeneous inner

FIG. 6 Microphallus paragrapsi n.sp.

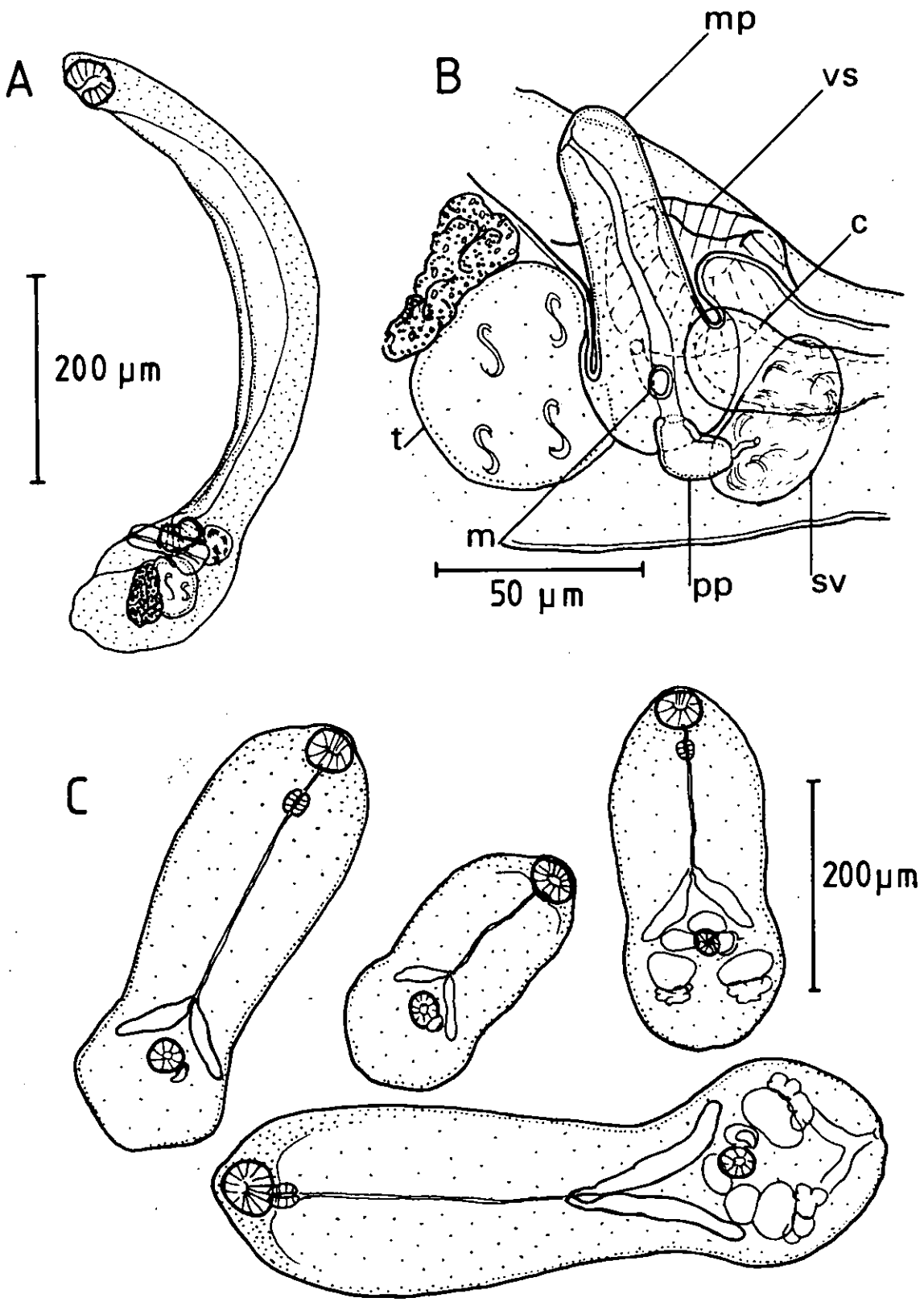


FIGURE 6 A, excysted metacercaria after 12 hours at 41°C, lateral view; B, detail of everted male papilla of metacercaria shown in A, lateral view; C, excysted metacercariae after 12 hours at 41°C, showing variation in form and size. (c: caecum; m: metraterm; mp: male papilla; pp: pars prostatica; sv: seminal vesicle; t: testis; vs: ventral sucker.)

layer, 14 (10 - 19)μ and a darker, outer layer with fine radial striae 22 (17 - 27)μ. The cyst occurs within the large nerves innervating the legs and claw muscles of the crab host, particularly near the coxae. No behavioural changes related to this infection, have been noticed in the crab host.

TABLE 7 *Microphallus paragrapsi* n.sp. Dimensions of live metacercarial cysts dissected from *Paragrapsus gaimardii* (n = 20).

External dimensions	Length	319 (300 - 353)
	Width	310 (285 - 331)
Internal dimensions	Length	246 (228 - 266)
	Width	239 (224 - 251)

Metacercariae excysted *in vitro* at 41°C when incubated in 0.5% pancreatin in Hank's saline. There was great variation in the time required for excystment in this enzyme solution. The time was directly related to the size and maturity of excysted metacercariae; smaller, less mature metacercariae, possibly with incompletely formed cyst walls, excysted sooner than larger, more mature specimens (Table 8).

TABLE 8 *Microphallus paragrapsi* n.sp. Size of metacercariae excysted *in vitro* at 41°C: (a) after 1½ hours and (b) from 1½ to 12 hours.

	n	length	width
(a)	20	351 (249 - 507)	154 (125 - 175)
(b)	20	479 (333 - 665)	185 (144 - 213)

Crustacean intermediate host: *Paragrapsus gaimardii* (M.Edw.)

Geographical location: Great Bay, Bruny Island, Tasmania

Site of encystment: Nerves of legs and claws

Type material: Tasmanian Museum - K903, holotype, adult (ringed); K903, paratypes, adults (not ringed); K904, paratypes, flattened, gravid adults (ringed); K905 and K906, paratypes, excysted metacercariae.

TABLE 9 *Microphallus paragrapsi* n.sp. Dimensions of the holotype (a) and other non-ovigerous adults (b), recovered from an experimentally infected duckling 17 hours after infection.

	(a)	(b)
Sample size	1	9
Body length (B.L.)	544	460 (325 - 544)
Body width (B.W.)	171	159 (137 - 179)
Oral sucker length (O.S.)	42	38 (34 - 40)
Oral sucker width	44	41 (36 - 44)
Prepharynx length	11	14 (8 - 23)
Oesophagus length	243	199 (110 - 258)
Pharynx length	27	22 (19 - 25)
Pharynx width	19	19 (17 - 23)
Left caecum length	114	95 (72 - 110)
Right caecum length	114	98 (80 - 110)
Ventral sucker length (V.S.)	40	39 (34 - 42)
Ventral sucker width	34	34 (30 - 38)
Ovary length	38	40 (36 - 46)
Ovary width	34	34 (27 - 38)
Left testis length	57	55 (46 - 65)
Left testis width	38	40 (30 - 46)
Right testis length	55	54 (46 - 61)
Right testis width	46	46 (38 - 49)
B.L./B.W.	3.18	2.89
O.S.(l+w)/V.S.(l+w)	1.16	1.08

Relationships

The species of *Microphallus* encysting in the nerves of the crab *Paragrapsus gaimardii* in Tasmania is considered to be new and is named after its second intermediate host. Only one other microphallid species is known to encyst in the nerves of the limbs of its intermediate host: *Microphallus pachygrapsi* Deblock and Prevot, 1968 encysts in the crab *Pachygrapsus marmoratus* on the Mediterranean coast of France. *Microphallus paragrapsi* n.sp. and *M. pachygrapsi* are similar in size, but differ in the following respects: the former has round metacercarial cysts, O.S.:V.S. ratio greater than 1, and male papilla length greater than V.S. diameter; and the latter has oval cysts, O.S.:V.S. ratio less than 1, and male papilla length less than V.S. diameter. According to a key to species of *Microphallus* presented by Deblock (1971), the species most similar to *M. paragrapsi* n.sp. is *M. minutus*, a tiny fluke discovered in water rats captured on the banks of the Murray River at Tailem Bend,

South Australia (Johnston, 1948), and rediscovered and redescribed by Deblock and Pearson (1969) in the same host, captured near Brisbane, Queensland. The adults of *M. paragrapsi* n.sp. and *M. minutus* are very similar, however the average size of gravid *M. minutus* adults 290 × 160µ (Deblock and Pearson, 1969), is slightly less than the average size of the more immature excysted metacercariae of *M. paragrapsi* n.sp., 355 × 156µ, and markedly less than the average size of mature excysted metacercariae of *M. paragrapsi* n.sp., 579 × 184µ. The male papilla of *M. minutus*, 53 × 27µ, is smaller and less elongate than that of *M. paragrapsi* n.sp., 66 × 26µ. In relation to body size, the oral and ventral suckers of *M. minutus* are much larger than those of *M. paragrapsi* n.sp. On the basis of these differences and in the absence of further life-history information, *M. paragrapsi* n.sp. and *M. minutus* are considered to be distinct, but closely related species. Four other species of *Microphallus* have been recorded in Australia (Deblock and Pearson, 1969), however, all are readily distinguished from *M. paragrapsi* n.sp. by details of the reproductive system: *M. minus* has a large, robust male papilla of the 'papillorobustus' type; *M. papillornatus* has an ornamented male papilla; *M. vaginosus* has an unusual, large metraterm, with thick, folded walls and *Microphallus* sp. has a voluminous, ovoid papilla, about the same size as the ventral sucker.

DISCUSSION

The incidence of infection of *Paragrapsus gaimardii* with microphallid trematodes has not been determined, however the impression gained from dissection of many crabs from Great Bay, at irregular intervals over several years, is that almost all adult crabs, of both sexes, are infected by *Gynaecotyla* spp., *Maritrema eroliae* and *Microphallus paragrapsi* n.sp., throughout the year. Two large male crabs dissected in July 1977 were infected by an average of 197 (118 - 275) metacercarial cysts of *Gynaecotyla* spp., 195 (90 - 300) of *M. paragrapsi* n.sp and 14 (2 - 26)

of *Maritrema eroliae*. These crabs also contained an average of 19 (15 - 23) large, unencysted immature metacercariae of *Gynaecotyla* spp.. These specimens had an oral sucker and relatively large, conspicuous excretory bladder and some individuals had rudimentary digestive and reproductive systems. Delayed encystment is known in the life-cycles of a number of *Gynaecotyla* species, and in *G. adunca* and *G. longiintestinata*, a small percentage of metacercariae mature and produce eggs without encysting (Deblock, 1977). The cysts of the 2 *Gynaecotyla* species infecting *P. gaimardii* have not been distinguished, however, the ratio of the 2 species in 100 metacercariae excysted *in vitro* from cysts dissected in December 1979, was about 6 *G. hickmani* n.sp. to 1 *G. macrocotylata* n.sp.

The molluscan hosts of these 4 microphallid species are unknown. In the littoral zone at Great Bay, however, numerous gastropod species occur, of which *Zeacumantis diemenensis*, *Bembicium auratum*, *Littorina unifasciata* and *Austrocochlea* spp., are very abundant.

The Mongolian dotterel harbours *Maritrema eroliae* in Queensland and is an infrequent visitor to Tasmanian shores (Thomas, 1979). An estuarine fish, the leatherjacket, caught in the Derwent River in 1975, was found to harbour a single immature adult of *M. eroliae* in its intestine (Last, 1975); however, eggs are unlikely to be produced in this host as experimental evidence indicates that a temperature of about 40°C is required for oviproduction in *M. eroliae*. Attempts to experimentally infect 8 domestic ducklings with hundreds of cysts of the 4 microphallid species found in *P. gaimardii*, were not very successful. No ducklings were infected by the 2 *Gynaecotyla* species; 3 ducklings, dissected 17, 18 and 26 hours after being fed with cysts, were infected with 16, 1 and 1 immature adults of *Microphallus paragrapsi* n.sp., respectively; and one duckling, dissected after 17 hours, was infected by one gravid adult of *Maritrema eroliae*, that contained 52 uterine eggs.

The discovery and identification of 4 microphallid species concurrently infecting a single species of crab in Tasmania presents many challenging

problems. The site of encystment of *Microphallus paragrapsi* n.sp. is unusual and presumably of selective value to the parasite. It may increase its chances of transmission by somehow making its intermediate host more vulnerable to predation. Investigation of the effect of the cysts on the function of the nerves and the behaviour of *P. gaimardii*, may explain why 2 closely related microphallids, *M. paragrapsi* n.sp. in Tasmania and *M. pachygrapsi* in France, invade and encyst in the nerves of their crustacean hosts. The life-histories of none of the 4 microphallids infecting *P. gaimardii* are known. Morphological, behavioural and ecological characteristics, that were found to distinguish the developmental stages of microphallids infecting *Coxiella badgerensis* at Calvert's Lagoon, may prove useful in elucidating the life-cycles of the trematodes infecting *P. gaimardii* at Great Bay.