# TAXONOMIC AND BIOLOGICAL STUDIES OF PROTOZOAN PARASITES IN TASMANIAN MARINE FISHES

Xiao-qun Su BSc (Shaanxi Normal University), MSc (Academia Sinica)

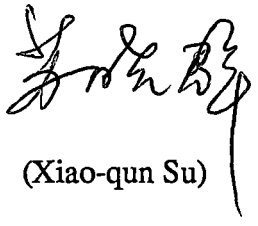
Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy in Zoology at the University of Tasmania, Hobart

July 1993

## STATEMENTS

I hereby declare that this thesis does not contain any material which has been accepted for the award of any other degree or diploma in any tertiary institution, and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Names are assigned in this thesis to 12 new species. It is fully recognised that a thesis has no standing as a publication for the naming of new taxa as defined in the *International Code for Zoological Nomenclature*. The names given here are those to be used when formal descriptions are submitted to scientific journals for formal publication.



(Xiao-qun Su)

## ABSTRACT

Twelve species of marine fish were sampled over a 22 month period from Dru Point, North-West Bay, south-east of Hobart, Tasmania, Australia, to investigate the protozoan parasites present on the external surface and in the internal organs of fish. A total of 16 species of protozoan parasites belonging to 11 genera (in addition to the collective group *Microsporidium),* 10 families and 4 phyla were obtained. Among these, 12 new species are described. Two new Australian records and two unidentified forms are reported.

Detailed ultrastructural studies were carried out for *Zschokkella leptatherinae* sp. nov., a myxosporean from the hepatic ducts of the atherinid fish, *Leptatherina presbyteroides*. The development of *Z. leptatherinae* can be described by four stages: pansporoblast formation, capsulogenesis, valvogenisis and sporoplasm maturation. The formation of the early pansporoblast of *Z. leptatherinae* is by the union of two generative cells. Two spores are produced from one pansporoblast at the end of sporogenesis.

The surface topography and the adhesive discs of *Paratrichodina tasmaniensis* sp. nov. from the gills of two species of atherinid fish *Leptatherina presbyteroides, Atherinosoma microstoma* and *Trichodina nesogobii* from the gills of gobio, *Nesogobius* sp.1 were examined using scanning electron microscopy. Both ciliates have two ciliary bands around the body surface. The adoral ciliary spiral turns 150-200$^0$ in *P. tasmaniensis,* while it is about 370-400$^0$ in *T. nesogobii.* The aboral ciliary band consists of three distinct sets of cilia in both species; however the length of the cilia, as well as the development of the septum which separates each set of cilia, are different. The adhesive disc of *T. nesogobii* shows a similar structure as observed by the light microscope. An ellipsoidal foramen is visible clearly in the denticle of *P. tasmaniensis* which corresponds to a small dot in silver impregnated specimens.

Ecological studies were conducted on *Zschokkella leptatherinae* and *Paratrichodina tasmaniensis* and their two host species, *Atherinosoma microstoma* and *Leptatherina presbyteroides*. The frequency distributions, the seasonal variations of parasite infection as well as the relationships between the parasites and the host fishes were investigated. The frequency distribution of *Z. leptatherinae* in both fish species and *P. tasmaniensis* in *L. presbyteroide* fit the log-normal distribution model. The infection of *Z. leptatherinae* showed no significantly seasonal variation, while a pronounced seasonal variation was revealed in the infestation of *P. tasmaniensis*. The prevalence and intensity of infestation were high in summer and spring, low in winter and autumn in both *Atherinosoma*

*microstoma* and *Leptatherina presbyteroides*. Infection of *Z. leptatherinae* occurred more frequently in larger hosts in both fish species. For *P. tasmaniensis*, the variation of infestation in different size group was closely related to the seasonal variations of infection. There were no significant differences in the prevalence of infection of both parasite species between the male and female samples of *A. microstoma* and *L. presbyteroides*.

The interspecific associations between six species of protozoan parasites and the spatial distributions of *Paratrichodina tasmaniensis* on the gills of host fish were also analysed. A positive association was found between *P. tasmaniensis* and *Trichodina australis* in *A. microstoma*. A negative association existed between *T. australis* and *Zschokkella leptatherinae, Trichodina* sp. and *Z. leptatherinae* in *A. microstoma; P. tasmaniensis* and *Z. leptatherinae, Clausophrya branchialis* and *Z. leptatherinae* in *L. presbyteroides*. A preliminary investigation revealed that *P. tasmaniensis* has a significantly high affinity for the left gill arches in both *A. microstoma* and *L. presbyteroides*.

Both *Zschokkella leptatherinae* and *Paratrichodina tasmaniensis* are pathogenic agents of atherinid fishes. The liver of *L. presbyteroides* infected by *Z. leptatherinae* shows clear histopathological change. Proliferation, enlargement and thickening of the hepatic ducts are observed. The epithelial cells of infected fish are lower than the normal cells. The parasites also provoke blockage of the hepatic ducts and atrophy of the neighbouring liver parenchyma. In more advanced infection, over 85% of the liver tissue is replaced by the plasmodia; and necrosis occurs finally.

The gills of *Atherinosoma microstoma* infested by *Paratrichodina tasmaniensis* show sloughing of the epithelial cells of secondary lamellae, hyperplasia of interlamellar and secondary lamellar epithelial cells and fusion of the adjacent secondary lamellae. Using the transmission electron microscope, hypertrophy of the epithelial cells of secondary lamellar is observed. The pillar cells are irregular and the basement membrane is thinner than those in uninfested fishes. The pattern of surface ridges is often disrupted.

## ACKNOWLEDGMENTS

I am deeply indebted to my supervisor, Dr. R.W.G. White, for his invaluable guidance and helpful suggestions throughout the study.

I would like to thank Mr. Robert Tennent, Mrs Ireien Jocabs (Anatomy), Mr. Wieslaw Jablonski (Central Science Laboratory) and Dr. David Thomas for their assistance in preparing samples and using the transmission and scanning electron microscopes.

Thanks are also due to Dr. Jennifer Gorst (Plant Science) for providing access to the interference light microscope; Dr. Tony Reese (CSIRO Fisheries) and Mr. Jerry Lim for their help and guidance with the photographic work.

I gratefully acknowledge Prof. D. M. Stoddart and many other members of the Zoology Department staff. In particular, Dr. Leon Barmuta for his help in the preparation of data for computer analysis; Barry Rumbold, Alan Dumphy and Wayne Kelly for their assistance when required; Sam Thalmann, Reid Armstrong, Ron Mawbey and John Moverley for their assistance in the field.

I wish to express my appreciation to Dr. J. L. Hickman for his unceasing encouragement and interest in the study.

My sincere thanks are also due to Mr. Laurie Zambon (ELICOS Centre) and Miss Debbi Ploughman (Biomedical Library) for correcting and improving my English in the initial drafts; Mrs Maeve Parker for doing proof reading of this thesis.

Thanks are also extended to my father, sisters and brother for their moral support throughout the study.

Final, and special thanks, are due to my husband, Hai-lin Li for his patience, support and encouragement.

This study was supported by a University of Tasmania Merle Weaver Postgraduate Scholarship.

# TABLE OF CONTENTS

# CHAPTER 1 - GENERAL INTRODUCTION

Protozoans are common parasites of both freshwater and marine fishes. To date, approximately 2420 species in six phyla of the protozoa group have been recorded infecting fish; more than 800 are found in marine fish (Lom, 1986; Lom and Dykova, 1992). Many protozoans are serious pathogens and several are major pests in freshwater or marine aquaculture and in the breeding of ornamental fishes. The diseases they caused have significant economic consequences, including direct death and loss of efficiency in the food conversion process. Best known protozoan induced syndromes include the whirling disease caused by *Myxobolus cerabralis* in young freshwater salmonoids which occurs throughout the world; it induces a tail chasing movement, permanent spinal column deformities, and frequently leads to death of the host (Hoffman, 1990). Whitespot disease caused by *Ichthyophthirius multifiliis* is another common disease in freshwater fish; it provokes respiratory obstruction and osmoregulatory perturbation during excystment of trophozoites. Mortality of host fish is induced when large numbers of parasites occur (Hines and Spira, 1973).

In marine fishes, the bodonid flagellate *Ichthyobodo,* the ciliates *Cryptocaryon, Brooklynella, Petalonema* and trichodinids are common and perhaps the most serious pathogens (Lom, 1986). Myxosporeans localizing in the skeletal muscles are also destructive parasites. *Unicapsula muscularis* (Davis, 1924), *Unicapsula seriolae* (Lester, 1982) and several *Kudoa* species (Lom, 1986; Langdon, 1991) cause either extensive necrosis or softening and liquefaction ("milk flesh") of muscles in their hosts rendering their meat unsightly and unpalatable. Protozoan parasites invading other organs of marine fishes also cause serious problems (Chen and Power, 1972; McVicar, 1975).

Increasing awareness of the role which fish-infecting protozoans play in freshwater or marine aquaculture has, in recent years, led to numerous publications on various aspects of their biology. In particular, Lom (1986) reviewed the biology of protozoan parasites of marine fishes. Lom and Dykova (1992) have recently published a major monograph entitled *"Prototoan Parasites of Fishes"* summarising the essential data on taxonomy, morphology, biology and pathogenicity of all protozoan groups encountered in fishes.

However, in Australia, little is known of the taxonomy and morphology of fish-infecting protozoans, and virtually nothing is available on their ecology. Although infections by several protozoan parasites have been reported previously from cultured fishes in Tasmania (Langdon and Humphrey, 1985; Langdon, 1988; Langdon, 1990b), only few works investigated in detail the pathologenicity of *Paramoeba* sp. in rainbow trout and Atlantic salmon in marine fish farms in Tasmania (Roubal *et al.*, 1989; Munday *et al.*, 1990, 1993).

The aim of this study is first to study the taxonomy and morphology of protozoan parasites occurring in some Tasmanian marine fishes. The examinations, identifications and descriptions of 16 parasite species, including 12 new species, comprises the first section of the thesis. The developmental cycle of an endoparasite was revealed by transmission electron microscopy; scanning electron microscopy was used to examine the surface topography and adhesive discs of two trichodinids. The second aim of this study is to investigate the ecology of two protozoan parasites which were found frequently in atherinid fishes. Over 16 month field study was conducted in a small coastal bay in order to obtain basic data of their frequency distributions, seasonal variations, the relationships between these parasites and their two host fish species, and the spatial distributions as well as the pathogenicity of parasites. The interspecific associations of six parasite species occurring in atherinid fishes on a basis of 22 month sampling period were also studied.

# CHAPTER 2 - LITERATURE REVIEW

## 2.1 Taxonomy

### 2.1.1 Microsporidia

The Microsporidia is a group of highly specialized intracellular parasites found in a great diversity of animals including almost all groups of invertebrates and low vertebrates. Although microsporidians as a rule do not cause encephalitis in mammals, they are best known as serious parasites of arthropods and fishes (Canning, 1977).

The first microsporidian species was found in Lepidoptera by Naegeli in 1857. Man's attention had been focused on these parasites because of *pebrine* or silk worm disease caused by *Nosema bombycis* Nageli, 1857. Thirty years later, Moniez (1887) reported the first microsporidia, *Glugea anomala* (Moniez, 1987) Gurley, 1893 from a teleost fish, *Gasterosteus aculeatus*. New species of microsporidia and new host records have been added regularly since that time. A checklist published by Poisson (1953) includes 40 species of previously reported fish-infecting microsporidia.

By 1977, over 94 microsporidian species had been recorded from fish (Sprague, 1977a). More recently, mainly due to the extensive use of electron microscopy, the study of microsporida has developed rapidly. Since 1977, at least four new genera, namely, *Tetramicra* Matthews and Matthews, 1980; *Loma* Morrison and Sprague, 1981; *Microgemma* Ralphs and Matthews, 1986; and *Microfilum* Faye, Toguebaye and Bouix, 1991; and 26 new species have been reported (Lom and Dykova, 1992).

Fourteen genera, in addition to the collective group Microsporidium, are now known to infect fish tissues (Canning, 1990). Most of these genera are specific for fish. All fish-infecting species have spores with an extremely large posterior vacuole, unmatched by microsporidia from other hosts (Lom and Dykova, 1992).

Microsporidia has been recognised as a distinct group within the Protozoa since 1882 when Balbiani suggested the order Microsporidia to accommodate *Nosema bombycis* and a few related organisms. The first simple classification system of Microsporidia was proposed by Thelohan (1892). Under the name Glugeidae, Microsporidia was included wrongly in the order Myxosporidia. Two genera, *Glugea* Thelohan, 1891; which was confused with previous *Nosema* Naeheli, 1857 and *Thelohania* Henneguy, 1892 were distinguished in the family Glugeidae. This view was supported by Gurley (1893) and Labbe (1899). Gurley coined a new name, Cryptocystes to replace "Microsporidies" proposed by Balbiani (1882). In attempt to classify and differentiate between

microsporidians at the generic level, Gurley (1893) distinguished between those species which produce isolated spores and those which sporulate within a pansporoblast membrane. He created the genus *Pleistophora* Gurley, 1893 for species within the latter type, which produce a variable but large number of spores always more than eight within a subpersistent pansporoblast membrane. Labbe (1899) followed Thelohan and Gurley in treating Microsporidia within the order Myxosporidia. However, he restored the ordinal name proposed by Balbiani. Like Thelohan and Gurley, Labbe confused *Nosema* and *Glugea*, and used the old name *Nosema* and the family Nosematidae replacing *Glugea* and the family Glugeidae respectively.

Perez (1905) made a significant contribution to the classification of Microsporidia by distinguishing, for the first time, the genera *Nosome* and *Glugea*. Stempell (1909) was the first to follow Balbiani in treating Microsporidia as a distinct order from Myxosporidia. In his new classification system, the Glugeidae and Nosematidae were, for the first time, distinguished as distinct families. Stempell also created the family Pleistophoridae. Furthermore, Stempell used a wider variety of characters in his classification than had any previous authors.

Leger and Hesse (1922) proposed a modification of Stempell's classification. Their system was "based upon the form of spores and which, therefore, fundamentally different from any previous ones" (Kudo, 1924). The families Nosematidae and Pleistophoridae were merged to form a single family under the name of Glugeidae as organisms in both families had "spores pyriform". Concurrently, two suborders and three new families were added; all were "based upon the form of spores". Kudo (1924) adopted the classification by Leger and Hesse "with slight modifications", which consisted of transposing the order of the two main categories and substituting the name Nosematidae for the Glugeidae. This modified system remained as the main form of microsporidian classification until early 1960's when members of this group were re-evaluated using the electron microscopy. Wenyon (1926) also adopted the classification of Leger and Hesse but improved it by recognising both Glugeidae and Nosematidae. However, his changes were generally ignored by later authors.

The only significant improvements ever made in the classification of Leger and Hesse were those that tended to destroy it. Weiser (1961) abolished the family Coccosporidae by transferring the type species of the type genus to the *Nosema* (cited by Sprague, 1977b). Vavra (1968) demonstrated that one of the major characters which was used in distinguishing suborders in the classification of Leger and Hesse (1922) was a misconception resulting from previously limited observations; therefore, he proposed the

abolition of the suborders Monocnidea and Dicnidea. Meanwhile, the order Microsporidia was raised to class status by Corliss and Levine (1963), still in the subphylum Cnidospora. Lom and Vavra (1962), Vavra (1966), and Lom and Corliss (1967) concluded that Microsporidia are unrelated to Myxosporidia. This led Sprague (1970) to recognise both groups as subphyla within the Protozoa.

Tuzet *et al.* (1971) abandoned entirely the system proposed by Leger and Hesse (1922). Unfortunately, when suggesting some fundamental changes, they stopped far short of making as complete and accurate a revision as they might have made on the basis of the data available to them. On the positive side, Tuzet *et al.* (1971) established the suborders Pansporoblastina and Apansporoblastina, which employed a physiological concept in delimiting taxa. Emphasising the uniqueness of the Microsporidia, Sprague (1977b) and Weiser (1977) proposed elevation of it to the phylum, and Sprague (1977b) introduced the term Microspora. The taxonomic system of Protozoa was revised by the Society of Protozoologists and the phylum status of microsporidians was retained (Levine *et al.*, 1980).

The spore shape was used as the main distinguishing character in the classification of Microsporidia proposed by Leger and Hesse (1922). However, its declining use in taxonomy is shown by the failure today of many authors even to described spore shape of new species. It is generally accepted now that the diagnostic characters are the fine structures of spores and the developmental stages (Lom and Dykova, 1992). The classification system proposed by Sprague (1977b) and Weiser (1977) used the ultrastructural characters defining genera and families. The system proposed by Sprague (1977b) recognised 47 genera, that of Weiser (1977) recognised 38 genera.

Since 1977, Sprague's classification has been widely used in the taxonomy of Microsporidia. Along with the development of study in these parasites, more new data have been added. In his recent publication, Canning (1990) summarized all the new taxa in the phylum Microspora.

Serological, immunological and biochenical techniques have been applied to the taxonomy of the Microsporidia (Larsson, 1986). To date, these techniques have not solved any of the major problems of microsporidian taxonomy and, as indicated by Vavra *et al.* (1981), they cannot yet be applied usefully to genera or higher taxa and may serve only to separate morphologically similar organisms .

2.1.2 Myxosporea

The Myxosporea is a group of organisms which are almost exclusively parasitic in fish. They have occasionally been found in amphibians (Theoforides *et al.* 1981, Sarkar, 1982). Coelozoic species infect cavities, mainly of the gall bladder and urinary tract, while histozoic species live in tissue where the plasmodia are encased by a layer of host fibroblasts and appear as cyst-like formations.

The first mention of a myxosporean occurs in the work of Jurine (1825), who discovered the cysts in the musculature of freshwater fish. However, the author did not detect the parasite nor ever understand which group of organisms he discovered; he only observed the disease caused by these organisms. In 1838, Mayer (cited by Shulman, 1966) detected the spores of myxosporean and Muller (1843) termed them "psorosperms". Butschli (1881a, 1881b, 1881c) was the first to correctly understand myxosporeans and gave them the name Myxosporidia.

A major contribution to the study of myxosporean taxonomy was made by Auerbach (1910b, c), who described a large number of new species. In 1919, Kudo published a monograph on all the myxosporean species discovered to that time and presented diverse information on those species. This work led to a more intensive study of myxosporeans. By 1933, research on myxosporeans was conducted extensively in the world and an up-to-date list was published by Kudo (1933).

Since 1933, many more new species of Myxosporea have been reported. In his monograph, Shulman (1966) described several hundreds species of Myxosporea from the former USSR. This was a significant contribution to the systematics of Myxosporea. To date, at least 46 genera and about 1200 species of Myxosporea have been recorded from fish (Lom and Noble, 1984; Lom, 1987).

Eleven new genera and around 50 new species of Myxosporea have been reported since 1984. These genera are *Spirosuturia* Chen and Hsieh, 1984; *Triangula* Chen and Hsieh, 1984; *Laterocaudata* Chen and Hsieh, 1984; *Septemcapsula* Hsieh and Chen, 1984; *Neothelohanellus* Das and Haldar, 1986; *Paramyxoproteus* Wierzbicka, 1986; *Neobipteria* Kovaleva, Gaevskaya and Krasin, 1986; *Meglitschia* Kovaleva, 1988; *Tetrauronema* Wu, Wang and Jiang, 1988; *Noblea* Kovaleva, 1989; and *Hennegoides* Lom, Tonguthai and Dykova, 1991 (Lom and Dykova, 1992).

Although the Myxosporea was discovered at the beginning of 19th century, the systematic position of Myxosporea was not understood until much later. Butschli

(1881a, 1881b, 1881c) named this group as Myxosporidia and placed it within the Class Sporozoa. Schaudinn (1899, 1900) divided Sporozoa into two subclasses: Telosporidia and Neosporidia; Myxosporidia was placed in the latter.

Thelohan (1889, 1895) made valuable contributions to the classification of the Myxosporidia. He was the first to classify the order on the basis of the features of the spore. In his system, the Myxosporidia was divided into three families and seven genera. Most genera established by Thelohan have been retained to date. Unfortunately, he also included the Microsporidia wrongly in the order Myxosporidia. Gurley (1893, 1894) and Labbe (1899) attempted to modify Thelohan's system. However, in both their systems, the Microsporidia was retained in the Myxosporidia.

In contrast, Doflein (1899) treated the Myxosporidia and Microsporidia as separate orders and he was the first to introduce the term Cnidosporidia for a group drawn from these two orders. He distinguished the Myxosporidia into two legions based on the number of pansporoblasts: Disporea (1 family, 2 genera) and Polysporea (3 families, 8 genera). However, it was subsequently realized that the number of spores formed during the vegetative stages was not a reliable character. Therefore, Doflein's system gained little favour.

Auerbach (1910a) suggested the inclusion of the Actinomyxidia in the Cnidosporidia. The latter was separated from Sporozoa as a subclass under the class Amoebosporidia by Hartmann in 1923 (cited by Shulman, 1966).

Kudo (1920) proposed a new system on the basis of the shape of spores. He divided the Myxosporidia into three suborders: Eurysporea (1 family, 5 genera), Sphaerosporea (2 families, 2 genera) and Platysporea (3 families, 8 genera). After 13 years, he perfected this system by introducing new data (Kudo, 1933). Tripathi (1948a) proposed a division of the Myxosporidia into the suborders based on the positions of polar capsules inside the spores. In his system, the Myxosporidia was distinguished into two suborders, six families and 21 genera. This system rectified the major mistakes in Kudo's classification by emphasising the role of polar capsules, a feature not used by Kudo. Tripathi's system became widely accepted by protozoologists.

Although Poisson (1953) raised the Microsporidia, Myxosporidia and Actinomyxidia to the rank of classes, many authors, represented by Jirovec *et al.* (1953) and Shulaman (1990) accepted the elevation of Cnidosporidia to the class and the retention of

Myxosporidia and Actinomyxidia as subclasses (Jirovec *et al.*, 1953). Shulman (1966) also presented a new diagnosis to the Myxosporidia.

Meglitsch created his own system for the Myxosporidia in 1960. Many less important and unrelated features were used in this system. The only new and positive feature was the inclusion of the number of spore valves. He also abolished many positive features of the systems of Kudo and Tripathi. To emphasise the absence of logic and unity of concept in the system proposed by Meglitsch, Shulman (1966) established a system based on the spore morphology. This system was widely adopted and has survived for about 20 years.

The systematic position of the Cnidosporidia was considered by several authors. Lom and Vavra (1962) raised it to a subphylum, which included one class assigned for the Myxosporidia and the Actinomyxidia and another class for the Microsporidia. Levine (1962), Corliss (1962) (cited by Shulman, 1966) and Poisson (1953) suggested the elevation of Cnidosporidia to a separate phylum. In a new classification of the Protozoa, Levein *et al.* (1980) placed the class Myxosporea together with the class Actinosporea in an independent phylum, Myxozoa.

With more new data of myxosporeans, the controversial features inherent in Shulman's system have been become more obvious. Lom and Noble (1984) established a new revised classification of the class Myxosporea. This classification continues to be exclusively spore-based, like the systems of Kudo, Tripathi and Shulman. Two orders, 16 families and 46 genera were distinguished within the class Myxosporea in this system.

### 2.1.3 Trichodinidae

The members of the family Trichodinidae are generally found as ectoparasites or ectocommensals of freshwater and marine fishes. A few species have also been described from amphibians, coelenterates, molluscs and crustaceans.

The first species of *Trichodina* Ehrenberg, 1838 *(Trichodina pediculus* Ehrenberg, 1838) was described from freshwater *Hydra* by Ehrenberg (1838). Fish-infecting trichodinids were discovered much later. Wallengren (1897) was the first to report a trichodinid, *Trichodina domerguei* Wallengren, 1897 from freshwater fish. Since then, many new species and new host fish records have been published. Tripathi (1948b) listed 31 previously reported *Trichodina* species, of which, 21 from freshwater fishes, 10 from marine fishes.

Until the middle 20th century, the description of trichodinids was mainly based upon the observations of live specimens. The validity of many of these species is now questioned (Van As and Basson, 1987). A turning point in the taxonomy of trichodinids came in 1958 when Lom proposed the use of the silver impregnation technique and uniform specific characteristics in the taxonomy of trichodinids. The morphology of the adhesive basal disks, as indicated by Lom (1959, 1961), "is not only most important for the specific diagnosis of these similarly shaped ciliates, but in many cases it is the only feature facilitating their identification or differentiation."

The works of Raabe (1958, 1959), Lom (1960, 1961, 1962, 1963), Lom and Laird (1969), Lom (1970a), Lom and Haldar (1976, 1977) Basson *et al.* (1983), Basson and Van As (1987, 1989) and Van As and Basson (1989, 1992) contributed significantly to the taxonomy of trichodinids. A checklist including 58 previously described trichodinids occurring in marine and euryhaline fish, was published by Lom and Laird (1969). To date, seven of nine genera and more then 150 species within the family Trichodinidae have been recorded from fish (Basson and Van As, 1989; 1991), .

In an early attempt at the systematics of the Peritricha-Mobilia by Fabre-Domergue (1888), *Trichodina* was allocated to the family Urceolariidae. Faure-Fremiet (1943), in his system for the Urceolariidae, divided the genus *Trichodina* into two subgenera: *Trichodina* and *Cyclochaeta* Jackson, 1875. The skeletal ring in species of the first group only have hooks but no cirri; while in the second group, species have a crown of cirri. Mueller (1938) established a new genus, *Vauchomia* Mueller, 1938; for the endoparasitic urceolarids which have a system of 'myonemes' and in which the oral groove makes more than two turns. However, the validity of this genus was questioned by Tripathi (1948b). The genus *Trichodinella* Sramek-Husek, 1953 was created by Raabe (1950). The distinguishable feature of this genus is that the oral spiral is only about 180° around.

A new system for the Urceolariidae proposed by Lom (1958), divided the genus *Trichodina* into three subgenera: *Trichodina, Vauchomia,* and *Cyclochaeta.* Lom (1959) divided four subgenera within the genus *Trichodinella,* namely, *Trichodinella, Foliella* Lom, 1959, *Tripartiella* Lom, 1959; and *Dogielina* Raabe, 1959. Lom's system gained wide support as illustrating efficiently the similarities and differences of species within the family Urceolariidae. Raabe (1963) suggested a new system in which he distinguished two subfamilies within the family Urceolariidae: Urceolariinae and Trichodinidae. The latter included seven fish-infecting genera *(Trichodinella, Dipartiella* Stein, 1961; *Tripartiella, Trichodina, Poljanskina* Raabe, 1963; *Vauchomia,*

*Paravauchomia* Raabe, 1963 and one mollusca-infecting genus, *Semitrichodina*. Kazubski, 1958. However, the genus *Poljanskina* was not accepted by Evdokimova *et al.* (1969). Lom and Haldar (1976) also suggested that *Poljanskina* is not valid. Concurrently with the work of Raabe (1963), Lom (1963) developed a further system for the urceolariid ciliates of the *Trichodinella*-group from one he established in 1959. Two genera were distinguished within this group: *Trichodinella*, which was divided further into three subgenera: *Trichodinella*, *Foliella* and *Dipartiella* and *Tripartiella* which was divided into subgenera *Tripartiella* and *Paratrichodina* Lom, 1963; the latter was a new taxon.

Corliss (1979) created the phylum Ciliphora. The family Trichodinidae, and other four families, were included within the suborder Mobilina Kahl, 1933. Seven genera were grouped within this family, i.e. *Dipartiella, Paratrichodina, Semitrichodina, Trichodina, Trichodinella, Tripartiella* and *Vauchomia.*

Two new genera have been established since 1977. *Trichodoxa* Sirgel, 1983 from snails (Sirgel, 1983) and *Hemitrichodina* Basson and Van As, 1989 from freshwater fishes (Basson and Van As, 1989). Therefore, the family Trichodinidae now includes nine genera, seven of which are found in fish. The differential diagnosis of these genera were presented by Basson and Van As (1989).

## 2.2 Morphology

### 2.2.1 Microsporidia

Until 1951, all species of microsporidia were identified and described following examination by light microscopy. Weissenberg (1911), Leger and Hesse (1922), Kudo (1924), Chatton and Courri (1923) used spore dimensions, host and site of infection as the main criteria in their classification systems. Such evidence was found to be inconclusive by Weiser and Colozzi (1972) when cross infections between different hosts showed variability in spore dimensions. It is now known that the genera and species of microsporidia can only be defined accurately using ultrastructural characters (Vavra 1968, Loubes and Maurand, 1975; Larsson, 1986; Faye and Togyebate, 1990).

Electron microscopy has been used widely in the study of fish-infecting microsporidians since the early 1960s. The first report using this technique was published by Lom and Vavra (1963a) who studied the ultrastructure of several types of microsporidian spores including *Pleistophora hyphessobryconis* Schaperclaus, 1941, a parasite of several freshwater fish species. The authors found that the structure to be essentially the same in all species studied. Sprague and Vernick (1966) investigated the developmental stages of

*Glugea* sp. in stickleback, *Apeltes quadracus*. Lom and Corliss (1967) made a detailed study on the ultrastructure of developmental stages and spores of *Pleistophora hyphessobryconis*. Sprague and Vernick (1968) described the ultrastructure of developmental stages and xenoma of *Glugea weissenbergi* Sprague and Vernick, 1968 from the stickleback, *Apeltes quadracus* and Erickson *et al.* (1968) described the everted polar filaments of the same species. Over the last 25 years, the ultrastructures of microsporidians have been reported regularly. For *Pleistophora*, the detailed ultrastructure of the developmental stages has been investigated in four species, namely, *P. littoralis* Canning and Nicholas, 1980 (Canning and Nicholas, 1980), *P. typicalis* Gurley, 1893 (Canning and Nicholas, 1980), *P. hippoglossoideos* Bosanquet, 1910 (Morrison *et al.*, 1984), *P. senegalensis* Faye and Toguebaye, 1990 (Faye and Toguebaye, 1990). Three other species, *Pleistophora* sp., *P. cepedianae* Putz, Hoffman and Dunbar, 1965 and *P. mirandellae* Vaney and Conte, 1901 have also been studied briefly with the electron microscopy (Canning *et al.*, 1979; Price, 1983; Maurand *et al.*, 1988).

For *Glugea*, the ultrastructure of the developmental stages and xenoma have been studied in *G. atherinae* Berrebi, 1979 (Berrebi, 1979), *G. truttae* Loubes, Maurand and Walzer, 1981 (Loubes *et al.*, 1981), *G. anomala* (Canning *et al.*, 1982) and *G. pimephales* Morrison, Hoffman and Sprague, 1985 (Morrison *et al.*, 1985). In the study of *G. anomala*, Canning *et al.*, (1982) found that the *Glugea* is virtually a polysporous and pansporoblastic genus. This discovery cause transfer of *Glugea* from the Apansporoblastina to Pansporoblastina, which had been retained since 1924 (Kudo, 1924). Other studies of the ultrastructure of *Glugea* included Weidner (1976) on the wall of the xenoma induced by *G. stephani* Hagenmuller, 1899 and Lom *et al.* (1979) on the structure of the xenoma wall and periphera zone of the xenoma of *G. anomala.*

Since the genus *Loma* Morrison and Sprague, 1981 was established in 1981 (Morrison and Sprague, 1981a), six species have been reported. The electron microscope was used to study the xenoma and the development of all these six species (Morrison and Sprague, 1981b, c; Morrison and Sprague, 1983; Loubes *et al.*, 1984; Bekhti and Bouix, 1985). The genus *Loma* was first reported as a member of the Apansporoblastina (Morrison and Sprague, 1981a). Later, Morrison and Marryatt (1986) suggested that it should be treated as a pansporoblastic genus since the parasites develop within a sporophorous vesicle of parasite origin. Several other species which have been studied with electron microscopy include *Spraguea lophil* Doflein, 1898 (Loubes, *et al.*, 1979); *Tetramicra brevifilum* Matthews and Matthews, 1980 (Matthews and Matthews, 1980); *Microsporidium rhabdophilia* Modin, 1981 (Modin, 1981); M. *zhanjiangensis* Hua and Zhang, 1988

(Hua and Zhang, 1988); *M. arthuri* Lom, Dykova and Shaharom, 1990 (Lom *et al.*, 1990); *Microgemma hepaticus* Ralphs and Matthews, 1986 (Ralphs and Matthews, 1986), *Enterocytozoon salmonis* Chilmonczyk, Cox and Hedrick, 1991 (Chilmonczyk *et al.*, 1991), and *Microfilum lutjani* Faye, Toguebaye and Bouix, 1991 (Faye *et al.*, 1991).

In comparison to the large number of studies which have utilized transmission electron microscopy, few have used scanning electron microscopy (Lom and Weiser, 1972; Larrson 1986). In a first, preliminary study, Lom and Weiser (1972) indicated that the use of a SEM may supply additional features for species differentiation.

2.2.2 Myxosporea

Myxosporeans have two different formations in their life cycle: plasmodia and spores. The former are the vegetative (trophic) stages of the parasites. The majority of early morphological studies were conducted by the light microscope. The structure of plasmodia was studied by a number of authors. It was shown that the cytoplasm of plasmodia can be divided into two parts: ectoplasm and endoplasm (Shulman, 1966). There are two types of nuclei within the endoplasm: large vegetative nuclei which carry out only trophic function and reproduce asexually and small generative nuclei which give rise to the pansporoblast in which spores formed (Noble, 1943, 1944). Lipid and glycogen droplets were also detected in the endoplasm (Petrushevskii, 1932).

Spores of the Myxosporea have a multicellular structure which comprise valves, polar capsules, and sporoplasm. Many papers concerning the morphology of spores examined by light microscopy have been published, e.g. Thelohan (1889); Butschli (1881b, c); Kudo (1921); Lom and Vavra (1963b); Lom (1964).

An important landmark in the history of the morphological study on myxosporea occurred in 1960 when Grasse introduced the electron microscope to a study of the vegetative stages of *Sphaeromyxa* Thelohan, 1892 Since then, the ultrastructure of plasmodia has been extensively studied. The plasmodia is limited by a single unit membrane, which is coated by amorphous material (Lom and de Puytorac, 1965b), granular material (Desser *et al.*, 1983) or a dense, fuzzy material (Desser and Paterson, 1978a; Davis and Sienkowski, 1988). The surface of plasmodia is smooth or thrown into palisade-like folds or rugose external surface. Numerous pinocytotic channels extended from the inner membrane into the cytoplasm (Lom and de Puytorac, 1965b; Desser and Paterson, 1978a; Desser *et al.*, 1983; Davis and Sienkowski (1988). Lom and de Puytorac (1965a, 1965b) revealed that the structure of the plasmodia varies

greatly between the histozoic and coelozoic myxosporeans although the sporogenesis of these parasites are similar. The surface of histozoic plasmodia is smooth, while in coelozoic species, the surface is projected into various types of pseudopodial projections. The pinocytotic vesicles are found in both histozoic and coelozoic plasmodia. Comparing the ultrastructure of plasmodium wall of two histozoic species, *Henneguya exilis* Kudo, 1929 and *Myxobolus (Myxosoma) funduli* Kudo, 1918 with that of other myxosporeans, Current *et al.* (1979) indicated that the plasmodia of histozoic myxosporeans have a single or double outer unit membrane which forms pinocytotic canals extending into the parasite ectoplasm. In contrast, plasmodia of coelozoic myxosporeans have a single outer unit membrane which forms numerous microvilli-like projections extending into the host fluids. The coelozoic plasmodia of *Myxidium giardi* Cepede, 1906 and its attachment to the epithelium of the urinary bladder were studied by Paperna *et al.* (1987) using scanning and transmission electron microscopy. The results showed a few cup-like pinocytotic depressions and a dense endoplasmic reticulum located in the peripheral cytoplasm of the plasmodia. Consistently, Uspenskaya (1969), Lom (1969a), and Grasse and Lavette (1978) also recorded the pinocytotic systems in coelozoic plasmodia of other myxosporeans. Paperna *et al.* (1987) concluded that the differences in the fine structure of plasmodia between histozoic and coelozoic myxosporeans exist in the structure of their pellicle and the pattern of the pinocytotic system.

The ultrastructure of the plasmodium wall differs not only among the histozoic and coelozoic myxosporeans, but also from one species to another in the same genus (Current and Janovy, 1976). Even in a single species, the fine structure may be different between the clinical types. Interlamellar and intralamellar plasmodia of *Henneguya exilli,* reported by Current and Janovy (1978), differ in the structure of plasmodium wall, surface coat and in the relationship with the host cell. The interlamellar and subdermal plasmodia of *Myxobolus exiguus* Thelohan, 1895 also show different surface structures (Pulsford and Matthews, 1982).

Uspenskaya (1982) found that the mode of nutrition in myxosporeans is closely connected with the surface fine structure and the shape and dimension of the plasmodium. Three types of nutrition have been reported in myxosporeans, including extracellular digestion of food by means of enzymes secreted by the trophozoite, contact or membrane digestion followed by an active transport and/or pinocytosis, and phagocytosis followed by an intracellular digestion inside food vacuole. These nutritional types may occur in various combinations in different myxosporean species.

Cheissin *et al.* (1961) published the first observation on the ultrastructure of polar capsules in *Myxobolus uniporus* Fujita, 1927 and *M. carassii* Klokacheva, 1914. Their results demonstrated that the polar filament is coiled within the capsule. The structure of polar filaments was also investigated by Lom and his colleague (Lom, 1964; Lom and Vavra, 1963a; 1965). They demonstrated that the polar filament is a single, hollow tube and the discharge channel of the filament is distally plugged with electron dense material.

The fine structures and morphogenesis of myxosporeans have been studied in various species: *Henneguya pinnae* Schubert, 1967 (Schubert, 1968), *Sphaeromyxa* sp. (Lom, 1969a), *Myxidium zealandicum* Komourdjian *et al.*, 1977 (Hulbert *et al.*, 1977), *Henneguya exilis* (Current and Janovy, 1977), *Myxobolus* sp. (Desser and Paterson, 1978a), *H. adiposa* Minchew, 1977 (Current, 1979), *Myxobolus funduli* (Current *et al.*, 1979), *Ceratomyxa shasta* Noble, 1950 (Yamamoto and Sanders, 1979), *Sphaerospora renicola* Dykova and Lom, 1982 (Lom *et al.*, 1982), *Myxobolus exiguus* (Pulsford and Matthews, 1982), *Thelohanellus nikolskii* Akhmerov, 1955 (Desser *et al.*, 1983), *S. angulata* Fujita, 1912 and *Sphaerospora carassii* Kodo, 1919 (Desser *et al.*, 1983), *S. tincae* Plehn, 1925 and *S. galinae* Evlanov, 1981 (Lom, Korting and Dykova, 1985), *Kudoa paniformis* Kabata and Whitaker, 1981 (Stehr, 1986), *Kudoa lunata* Lom, Dykova and Lhotakova, 1983 (Lom and Dykova, 1988), *Zschokkella russelli* Tripathi, 1948 (Davis and Sienkowski, 1988), *Myxidium giardi* (Azevedo *et al.*, 1989), *S. ictaluri* Hedrick, McDowell and Groff, 1990 (Hedrick *et al.*, 1990), and *S. dicentrarchi* Ariadna and Pilar, 1992 (Ariadna and Pilar, 1992). The fine structure of mature spores of *Unicapsula pflugfelderi* Schubert, Sprague and Reinboth, 1975 and *Myxobolus hendricksoni* Mitchell, Seymour and Gamble, 1985 have also been investigated by Schubert *et al.* (1975) and Mitchell *et al.* (1985).

In addition to transmission electron microscopy, scanning electron microscopy has also been used in investigations of the plasmodia (Paperna *et al.*, 1987) and spores of myxosporidians (Lom and Hoffman, 1971; Komourdjian *et al.*, 1977; Desser and Paterson, 1978b; Hine, 1979, 1980; Inoue and Hoshina, 1983; Davies, 1985; Mitchell *et al.*, 1985; Azevedo *et al.*, 1989). Recently, the freeze fracture technique was used to study the plasma membrane of myxosporidian valve cells by Desportes-Livage and Nicolas (1990).

2.2.3 Trichodinidae

So far, more than 150 species of trichodinids have been reported from the world (Basson and Van As, 1991). Most studies of these organisms have based on the light microscopy and only a few species have been studied by the electron microscope. The first species of

trichodinids to be studied by the transmission electron microscope were two endoparasitic species, *Trichodina urinicola* Fulton, 1923 from the urinary bladder of *Rana esculenta,* and *Trichodinopsis paradoxa,* from the intestine of *Cyclostoma elegans* (Faure-Fremiet, *et al.*, 1956 a, b; Favard *et al.*, 1963).

Lom (1973) studied the adhesive disc of *Trichodinella epizootica* Raabe, 1950 from the gills of *Perca fluviatilis* using scanning and transmission electron microscopy. He found that the attachment of parasite to the host's surface is the result of an elaborate coordination of denticles, radial pins, spikes of the border membrane and aboral ciliary wreaths. The denticles of trichodinids were also examined using SEM for *Trichodina lucioperca* (cited by Lom and Dykova, 1992), *Trichodina heterodentata* Duncan, 1977 and other trichodinids (Van As and Basson, 1987; 1989). Van As and Basson (1989) developed a method of removing the soft material which often obscures the shape of the denticles when observed using the scanning electron microscope.

The surface topography of trichodinids, including the morphology of the ciliary bands as well as the structure of adhesive discs, have been investigated with scanning electron microscopy in three species: *Trichodina oviducti* Poljansky, 1951 from the copulatory sac of female thorny rays, *Raja radiata* (Khan *et al.*, 1974); *T. truttae* Mueller, 1937 from the skin of cultured juvenile Pacific salmon, *Oncorhynchus* spp, steelhead trout *Salmo gairdneri* and *Oncorhynchus keta,* (Arthur and Margolis, 1984; Urawa and Arthur, 1991) and *T. japonica* Imai, Miyazaki and Nomura, 1991 from the gill of cultured Japanese eel, *Anguilla japonica* (Imai *et al.*, 1991). Imai *et al.* (1991) suggested that the species of trichodinid ciliates may be identified by the the scanning electron microscopy since the denticles and radial pins are clearly shown under the the scanning electron microscopy.

Hausmann and Hausmann (1981a, b) conducted two detailed electron transmission microscopic investigations on the ultrastructure of the locomotor fringe, oral apparatus and adhesive disc of *Trichodina pediculus* Ehrenberg, 1838 from freshwater medusa, *Craspedacusta sowerbii.*

## 2.3 Transmission and developmental cycles

### 2.3.1 Microsporidia

The life cycle of microsporidia is generally monoxenous. Transmission normally occurs when spores, contaminating the environment or the diet, are ingested by a new host (Canning, 1977). However, the alternation of host in the life cycle has also been

reported. Becnel (1992) discovered that *Amblyospora californica* can be transmitted horizontally by feeding meiospores from larvae of *Culex tarsalis* (definitive host) to the copepod *Macrocyclops albidus* (a new intermediate host), and, conversely, by feeding spores produced in adult copepods to larval mosquitoes. The existence of an intermediate host has not been shown for fish-infecting (cited by Lom and Dykova, 1992). For these microsporidians, only the transmission of infection by intramuscular or intraperitoneal injection of mature spores into a new host was successful (cited by Lom and Dykova, 1992). The life cycle of microsporidia can be divided into two phases: merogony and sporogony. The sporoplasm is injected into the primary site of infection, usually in the gut epithelium via the extruded polar filament. From there, the infection may extend to other tissues and organs (Ishihara, 1968; Weidner, 1972). The reorganisation of cytoplasm may take place within the sporoplasm on contact with the host's cells (Weidner, 1972). By vegetative reproduction, the sporoplasm becomes to a meront. A period of rapid multiplication (merogony) follows during which the parasites increase in number by both multiple and binary fission. Meronts may divide immediately after nuclear division, or karyokinesis may produce additional nuclei without cytokinesis. The later plasmodium may divide by plasmotomy into daughter plasmodia, or may segment into uninucleate meronts again. The meronts may be encased by a thick amorphous wall, e.g., in *Pleistophora* (Canning and Nicholas, 1980; Faye and Toguebaye, 1990), or may be in direct contact with the host cytoplasm, e.g., in *Nosema* (Cali, 1971), *Spraguea* Vavra and Sprague, 1976 (Loubes *et al.*, 1979), *Microgemma* (Ralphs and Matthews, 1986) and *Microfilum* (Faye *et al.*, 1991).

Sporogony is the developmental sequence leading to production of spores. The sporont differs from the meront in having a layer of amorphous material secreted externally to its outer cell membrane (Canning and Nicholas, 1980; Ralphs and Matthews, 1986; Faye and Toguebaye, 1990). Sporont nuclei may divide once or several times. It has been suggested by Sprague (1977) that the first division is meiotic and proceeding divisions are mitotic. Two types of sporont, giving rise to two kinds of spores, have been recorded from *Pleistophora typicalis* (Canning and Nicholas, 1980), *P. littoralis* (Canning *et al.*, 1979), *P. priacanthusis* Hua and Dong, 1983 (Hua and Dong, 1983), and *P. mirandellae* (Maurand *et al.*, 1988). The sporont may divide into two sporoblasts as in, for example, *Nosema* (Cali, 1971), *Ichthyosporidium* Caullery and Mesnil, 1905 (Sprague, 1965), and *Jirovecia* Weiser, 1977 (Lom and Dykova, 1992). In other genera, there are several divisions of the sporogonial nucleus before cytoplasmic separation, which produce various sized plasmodial sporont. The multinucleate sporont produces

several sporoblasts either directly, as in *Thelohania* Henneguy, 1892 (Gassoumer and Ellis, 1973), or it may segment by plasmotomy into smaller plasmodia eventually producing numerous sporoblasts as in *Pleistophora* (Canning and Nicholas, 1980). In *Glugea,* the multinucleate sporont produces many uninucleate intermediate stages called sporoblast mother cells; these divide by binary fission. Therefore, the sporogony is polysporoblastic (Canning *et al.*, 1982). However, Loubes *et al.* (1981) considered only the last division of the sporont in *Glugea truttae* as sporogony.

In some genera (*Nosema, Spraguea, Microgemma,* and *Microfilum*), sporogonic stages continue to lie directly in the host cytoplasm. In most genera, however, a special envelope separates the parasite and host cytoplasm; this envelope may develope from the amorphous layer on the surface of the sporont into a thick persistent wall as in *Pleistophora* (Canning and Nicholas, 1980; Faye and Toguebaye, 1990) or may split off from the sporont cell membrane at the beginning of sporogony to become a thin fragile membrane as in *Glugea* (Canning *et al.*, 1982). The term "sporophorous vesicle" (pansporoblast) is defined for this parasite-origine envelope. In another situation, the envelope is produced by the cytoplasm of host cell as in *Tetramicra* (Matthews and Matthews, 1980). The term of "parasitophorous vacuole" is used to described the host-origin envelope.

The sporoblast is considerably larger than the spore and has dense cytoplasm which produces future organelles. The origin of the polar filament and its associated organelles have been investigated by a few authors (Sprague and Verinck, 1966; Sprague *et al.*, 1968; Sprague and Vernick, 1969; Szollosi, 1971). It is now understood that the polar filament and its associated organelles derives from the golgi apparatus (Sprague and Vernick, 1969; Szollosi, 1971). Except for the spore dimorphism in some *Pleistophora* species, mature spores of most microsporidian species are of uniform shape and size. A complete dimorphism involving the whole life cycle occurs in *Sprague lophii.*

2.3.2 Myxosporea

About 70 papers have been published on the life cycles of myxosporeans in the fish hosts. However, a total understands of the life cycles of these organisms has still not been obtained. As early as 1895, Thelohan reported the hatching of myxosporean spores in the digestive tract of fish, but most attempts at experimental infection by orally administered spores have failed.The few successes, most involving *Myxobolus (Myxosoma) cerebralis* use spores aged in aquatic muds a few months (Uspenskaya,

1955, 1984; Halliday 1976; Yunchis, 1981). However, in *Ceratomyxa shasta*, even the method of using aged spores failed (cited by Lom, 1987).

It was not until 1984 that the direct mode of transmission of myxosporeans was challenged. Wolf and Markiw (1984) discovered the necessity of an oligochaete intermediate host in the life cycle of *Myxobolus cerebralis*. It was found that the "maturation" of spores in the aquatic substrates is actually resulted from the ingestion of spores by tubificid worms *(Tubifex tubifex)* which inhabit mud at the substrates (Wolf and Markiw, 1984; Wolf *et al.*, 1986). The ingested spores further develop as a species of the genus of*Triactinomyxon,* a representative of the other of the two classes of the phylum Myxozoa, the Actinosporea.*Triactinomyxon* completes its own developmental cycles inside the oligochaete, producing spores with long caudal appendages which after being ingested by the salmonid host initiate *M. cerebralis* infection. Such a life cycle is unparalleled among the protists: two different sexual processes, two sporogenesis resulting in two different kinds of resistant spores. However, to date this finding has been confirmed only by El-Matbouli and Hoffmann (1989) in *M. cerebralis* and *M. cotti* El-Matbouli and Hoffman, 1987 and Hedricks in *M. pavlovskii* Akhmerov, 1954 (cited by Lom and Dykova, 1992).

In the model of *Myxobolus-Triactinomyxon* transformation, upon contact with the host fish, an amoeboid sporoplasm escapes from the *Triactinomyxon* spore and penetrates the epithelium of the skin or fins, from where it moves into the deeper tissue layer (Markiw, 1989). When direct transmission occurs, the spore is thought to be ingested by the fish and it then hatches in the digestive tract of the host. The amoebula is believed to reach directly the final site of infection. Before or after this happens, two haploid nuclei fuse to form a synkaryon or two uninucleate sporoplasm fuse to form a zygote. The nucleus divides until many generative cells and vegetative nuclei appear (Lom, 1987, Garden, 1992; Lom and Dykova, 1992).

The formation of the early pansporoblast was first detected with transmission electron microscopy by Lom and de Puytorac (1965b). It was found that the union of two generative cells gives rise to the pansporoblast. This binucleate origin has been demonstrated by several authors and has now become widely acceptable (Schubert, 1968; Current and Janovy, 1977; Dykova *et al*, 1987; Lom, 1987). The generative cell may have two different structural types as in *Henneguya adiposa* and *Myxobolus exiguus* (Current, 1979; Pulsford and Matthews, 1982). In such cases, the binucleate

pansporoblast is formed by one each of the structural types of generative cells (Current, 1979). Occasionally, three types of generative cells were found with one having only an excretory function (Grasse and Lavette, 1978). Generally, two generative cells unite, one becoming the pericyte which envelops the inner one which becomes the sporont and finally produces two spores. A special association of generative cells was reported in *Myxidium rhodei* Leger, 1905 by Dykova *et al.* (1987).

An opposite opinion about the formation of early pansporoblast is reported by Hulbert *et al.* (1977) on the basis of their investigation on *Myxidium zealandicum.* They found that the binucleate pansporoblast is formed by the mitotic division of a uninucleate generative cell.

The sporogenesis and mature spores of the Myxosporea have been studied in various species using transmission electron microscopy. Species studied include *Henneguya pinnae* (Schubert, 1968), *Sphaeromyxa* sp. (Lom, 1969a), *Myxidium zealandicum* (Hulbert *et al.*, 1977), *Henneguya exilis* (Current and Janovy; 1977), *H. adiposa* (Current, 1979), *Myxobolus* sp. (Desser and Paterson, 1978a), *Myxobolus funduli* (Current *et al.*, 1979), *Myxobolus exiguus* (Pulsford and Matthews, 1982), *Thelohanellus nikolskii* (Desser *et al.*, 1983), *Zschokkella russelli* (Davies and Sienkowski, 1988), *Myxidium giardi* (Azevedo *et al.*, 1989). Usually, the sporont within the enveloping cell divides until 10 cells are present; these then differentiate and form into two groups. Each group comprises two capsulogenic cells, one binucleate sporoplasm, and two valvogenic cells, which eventually gives rise to a mature spore, with two polar capsules and an infective sporoplasm, surrounded by a shell composed of two valves. A meiosis is involved in this process to bring the spores to a haploid state.

Sporogenesis of the genera *Sphaerospora* Thelohan, 1892; *Ceratomyxa* Thelohan, 1892; and *Kudoa* Meglitsch, 1947 is different from that of the other myxosporeans. Spores in these genera are produced by the direct division of the generative cell without the formation of a pansporoblast (Yamamoto and Sanders, 1979; Hamilton, 1980; Lom *et al.*, 1982; Lom *et al.*, 1985; Stehr, 1986; Lom and Dykova, 1988).

Although the early stage of sporogenesis is similar, the late developmental stages of sporogenesis are different among *Sphaerospora, Ceratomyxa* and *Kudoa.* The differences even exist between the species of *Sphaerospora.* In *Ceratomyxa shasta,* the sporoblast forms 12 nucleate cells that give rise to two groups of six cells and result in

the formation of two spores (Yamamoto and Sanders, 1979), while sporogenesis of *Kudoa paniformis* and *K. lunata* is monosporous (Stehr, 1986; Lom and Dykova, 1988). Two uninucleate sporoplasm are found in *C. shasta* and in two *Kudoa* species. Sporogenesis in most *Sphaerospora* species are disporous (Lom, *et al*, 1982; Desser *et al*, 1983; Lom *et al.*, 1985; Ariadna and Pilar, 1992); however, monosporous development has been reported in *S. carassii* (Desser *et al.*, 1983), *S. galinae* (Lom *et al.*, 1985), and *S. ictaluri* (Hedrick *et al.*, 1990). Mature spores of *S. angulata, S. carassii* (Desser *et al.*, 1983), and *S. dicentrarchi* (Ariadna and Pilar, 1992) have a binucleate sporoplasm, while two uninucleate sporoplasms have been detected in *S. renicola* (Lom *et al.*, 1982), *S. tincae, S. galinae* (Lom *et al.*, 1985), and *S. elegans* Thelohan, 1892 (Feist *et al.*, 1991).

In addition to the sporogonic stages in the primary sites of the host, some myxosporeans also have an extrasporogonic phase in their life cycle. *Sphaerospora renicola* is a common parasite in the renal tubules of common carp and other freshwater fishes. The extrasporogonic stages of this form have been found in the blood (Csaba, 1976; Lom *et al.*, 1983) and swimbladder (Molnar and Kovacs-Gayer, 1986). The extrasporogonic stages of *S. elegans* and *Myxidium lieberkuehni* Buetschli, 1882 and the presporogonic stages of *Myxobolus cerebralis* have also been reported in the eye and kidney (cited by Lom and Dykova, 1992). These stages may represent a proliferative phase of myxosporeans to ensure the sporogonic cycle proceeds and to spread the infection throughout the fish organs.

### 2.3.3 Trichodinidae

Unlike that of the Microsporida and Myxosporea, the life cycle of Trichodinidae is simple. Transmission normally occurs when ciliates contaminating the environment are encountered by a new host (Van As and Basson, 1987). The species occurring in the urinary tract may escape in the urine of their hosts and are transmitted via water at spawning time (Mueller, 1938); the species parasitizing the oviduct may be transmitted venereally (Khan, 1972).

Reproduction is normally by means of binary fission, and under certain conditions, conjugation also occurs (Padnos and Nigrelli, 1942; Van As and Basson, 1987). During the binary fission, the macronucleus undergoes a series of morphological changes. The micronucleus divides mitotically to form two micronuclei. The body shape of the ciliate also changes. A constriction appears first in the adhesive disc, and then in the denticulate

ring. The macronucleus and the whole body then divide into two daughter individuals (Padnos and Nigrelli, 1942; Tripathi, 1948b; Feng, 1985). The adhesive disc separates into two semi-circles which then form two smaller discs in daughter individuals. The number of denticles in the daughter individual is reduced to half the original number; this is then instated by the formation of the full number of new denticles on the periphery of the old ring. The latter is then resorbed. New radial pins arise, each between a pair of the old ones (Padnos and Nigrelli, 1942; Tripathi, 1948b; Feng, 1985; Van As and Basson, 1989; Lom and Dykova, 1992).

The vestibulum persists in one of the daughter individuals while a new one develops in the other (Tripathi, 1948b). The oral cilia, gullet and contractile vacuole are found absorbed during the division of *Urceolaria mitra* Siebold and *T. steinii* Clarapede and Lachmann, 1858 (Peshkowsky, 1923), while they are retained in *T. spheroides* Padnos and Nigrelli, 1942 and *T. branchicola* Tripathi, 1948 (Padnos and Nigrelli, 1942; Tripathi, 1948b). Feng (1985) observed that the oral spiral and the contractile vacuole also divide into two parts during the division of *T. nobillis* Chen, 1963.

The processes of conjugation has been described by Peshkowsky (1923) for *Trichodina steinii*, and Padnos and Nigrelli (1942) for *T. spheroides*. Before conjugation, two ciliates come into close contact. They fuse in their oral regions and establish a cytoplasmic bridge between each other. The macronuclus disintegrates, while the micronucleus undergoes meiotic divisions resulting in four haploid nuclei. Three of them are reabsorbed, while the fourth divides once more to produce two nuclei, a stationary and a migratory pronucleus. The migratory pronucleus finds its way to the partner's stationary nucleus to fuse with it, forming a diploid synkaryon. After that, both partners separate, and each produces four daughter individuals. The macronucleus arises from one of the products of the division of the synkaryon (Lom and Dykova, 1992). Reorganisation of the denticulate ring occurs during the conjugation and the number of denticles in the new rings becomes the same as the number in the old ones (Padnos and Nigrelli, 1942).

## 2.4 Diseases caused by myxosporeans, microsporidians and trichodinids and pathology of fish hosts

### 2.4.1 Microsporidian diseases and pathology of fish hosts

*Pleistophora anguillarum* Hoshina, 1951 is a causative agent of the well known and harmful "beko" disease of Japanese eel, *Anguilla japonica*. It parasitizes mainly in the

skeletal muscle of fish and causes softening, dilation and necrosis of the muscle, resulting in various degrees of body curvature. In the advanced infection, muscle fibres almost disappear, and the connective fibres proliferate and fill in these sites, leading to the formation of infective foci (T'sui and Wang, 1988).

*Pleistophora ovariae* Summerfelt, 1964 causes an economically damaging disease in the golden shiner, *Notemigonus crysoleucas*. The infected oocytes of fish become heavily infiltrated with microsporidian and the infection can be passed on to the next host generation. Severe infection significantly affects the fecundity of fish (Summerfelt and Warner, 1970). *Loma salmonae* Putz, Hoffman and Dunbar, 1965 infects gills of freshwater salmonids; the xenomas of this microsporidian induce the epithelial hyperplasia with fusion and distortion of gill filaments. Heavy losses of salmonids due to *L. salmonae* infection have been reported (cited by Roberts, 1989).

*Glugea hertwigi* Weissenberg, 1911 has long been known to infect acutely many freshwater and marine populations of the American smelt, *Osmerus mordax*. The cysts of parasites are embedded in the intestine wall and cause the occlusions of the intestines of fish. This induces the destruction of intestinal tissues and the starvation of hosts. It also causes the degeneration of sexual organs, growth retardation, a decline in fertility, and mortality (Chen and Power, 1972).

There are also several other serious microsporidian diseases in marine fish. *Glugea anomala* invades the connective tissue of European sticklebacks, *Gasterosteus aculeatus* and *Pungitius pungitius*, and induces the hypertrophy of connective-tissue cells, resulting in thick-walled cysts up to 4 mm in diameter (Dogiel *et al.* 1958). The infection also causes the deformation of the body and mortality of host fish (Kudo, 1924; Dogiel *et al.*, 1958). *Glugea stephani* infects the gut wall of several species of marine flatfish, the epithelial layer frequently disappears and the intestine becomes flaccid and apparently non-functional. Heavily infected fishes lose weight rapidly and die (McVicar, 1975). *Pleistophora macrozoarcides* Nigrelli, 1946 induces complete hyalinization and destruction of muscle in ocean pout, *Macrozoarces americanus*. *Pleistophora gadi* Polyanski, 1955 infection results in cysts of 5-8 mm in diameter in cod muscle, which can kill wild cod fingerlings (cited by Roberts, 1989).

Other species inflicting heavy damage are *Glugea atherinae* in *Atherina boyeri*, (Berrebi and Bouix, 1978), *Loma branchialis* Morrison and Sprague, 1981 infecting gills of gadid

fish (cited by Lom, 1986), and *Tetramicra brevifilum* infecting musculature of turbot, *Scophtalmus maximus* (Matthews and Matthews, 1980).

2.4.2 Myxosporean diseases and pathology of fish hosts

Myxosporeoses are as varied as they are numerous. Both sporogonic and extrasporogonic stages of the myxosporean life cycle are known to cause disease, and often they are fatal. The best known example is the whirling disease caused by *Myxobolus cerabralis* Hofer, 1903 in young salmonids which occurs throughout the world, especially in Europe and America (Hoffman, 1990). Another common disease of salmonids occurring in Europe and North America is the proliferative kidney disease (PKD), which is caused by the extrasporogonic stages of *Sphaerospora* (Clifton-Hadley *et al*, 1984). *Ceratomyxa shasta* is also a serious pathogen for juvenile salmonids in western USA. These diseases kill the fish hosts and cause large scale losses on fish farms as well as in wild fishes. For young cyprinids, renal sphaerosporosis caused by *Sphaerospora renicola*, swimbladder inflammation (SBI) caused by the second extrasporogonic cycle of the same species, and kidney enlargement disease (KED) induced by *Hoferellus (Mitraspora) cyprini* Doflein, 1898 are economically important diseases in Europe and Japen (Ahmed, 1974; Dykova and Lom, 1982, 1988; Csaba *et al.*, 1984).

Diseases caused by myxosporeans are common in marine fishes as well as in freshwater fishes. The myxosporeans in the skeletal muscles are the most destructive parasites. *Unicapsula muscularis* Davis, 1924 invades the muscle of halibut, *Hippoglossus stenolepis* and causes the extensive necrosis of flesh-wormy disease in halibut (Davis, 1924). Several species of *Kudoa* Meglitsch, 1947, *Hexacapsula* Ari and Matsumoto, 1953 and *Unicapsula seriolae* Lester, 1982 cause a softening and liquefaction of the muscles (milky flesh) in their hosts, rendering their meat unsightly and unpalatable (Rhode, 1976; Kabata and Whitaker, 1981; Lester, 1982; Patashnik *et al.*, 1982; Konagaya, 1983; Lom, 1986; Langdon, 1991). In mariculture, *Kudoa amaniensis* Egusa and Nakajima, 1980 destroys skeletal muscles of yellow-tails, *Seriola quinqueradiata* in Japan, and a species of *Hexacapsula* inflicts lethal brain infections on cultured seabass (Lom, 1986).

The pathology of fish caused by myxosporeans has been extensively studied. Gills of fish are frequently invaded by myxosporeans. The infected gills show deformation or atrophy in the secondary lamellae; the epithelial cells of the secondary lamellae become

distended or hypertrophied (Dykova and Lom, 1978; Kalavati and Narasimhamurti, 1985; Hedrick, Groff and Mcdowell, 1990). Moderate to severe hyperplasia is also a common phenomenon in the gill infections; this often induces the fusion of the secondary lamellae, resulting in a drastic reduction in the respiratory surface (Obiekezie *et al.*, 1988; Hedrick *et al.*, 1990). The blood vessels in infected gills are dilatated or deformed (Dykova and Lom, 1978; Kovacs-Grayer and Molnar, 1984). In severe infections, haemorrhage, anaemia, and necrosis occur, and finally, death is induced (Roberts, 1989; Hedrick *et al.*, 1990).

Histozoic myxosporeans in the kidney often causes pathological changes in their hosts. *Myxidium giardi* induces the rupture and degenerative changes of the lining epithelium of renal tubules in European eel, *Anguilla anguilla.* Parasites also displace part or entire glomerular tufts and capillaries (Ventura and Paperna, 1985). *Sphaerospora colomani* Baska, 1990 causes severe damage to the tubular epithelium of glomeruli in *Acipenser ruthenus* (Baska, 1990). Kidneys of *Sparus aurata* infected by *Sphaerospora* sp. display completely destroyed glomeruli, enlarged Bowman's capsule and obliteration or displacement of the capillary tuft (Sitja-Bobadilla and Alvarez-Pellitero, 1990).

Only a few species of coelozoic myxosporeans are considered to be truly pathogenic. *Myxidium folium* Bond, 1938 from *Fundulus heteroclitus* produces the distension and blockage of hepatic ducts, atrophy of tubular epithelium as well as connective tissue reaction around the infected tubules (Bond, 1938). *Myxidium oviforme* Parisi, 1912 from the gall bladder and hepatic ducts of Atlantic salmon, *Salmo salar*, causes intense inflammation of the liver. The gall bladder becomes hypertrophied and obstructed by the large plasmodia which penetrate the bile ducts and cause biliary hyperplasia. Eventually, particularly in maturing freshwater salmon, the liver breaks down and disintegrates (Walliker, 1968a). The hepatic ducts of *Ciliata mustela* infected with *Zschokkella russelli* show the proliferation, enlargement and thickening of hepatic ducts, lowering of the duct epithelium and pericholangits (Davies, 1985). Similar pathological changes have also been observed in bullhead, *Cottus gobio* infected by *Z. nova* Klokacheva, 1914 However, the mature spores of *Z. nova* also invade the liver parenchyma and cause necrosis (Bucher *et al.*, 1992). *Zschokkella icterica* Diamant and Paperna, 1992 invades the hepatic ducts of rabbitfish, *Siganus luridus* and induces more severe pathological changes including the blockage of the hepatic ducts by the parasite; this lead to cholestasis and duct breakdown, massive hepatic necrosis, ascites and jaundice (Diamant and Paperna, 1992). *Chloromyxum cyprini* Fujita, 1927 occurring in the gall bladder and

hepatic parenchyma of cyprinid fishes, provokes massive necrosis of the hepatic parenchyma (Lom and Dykova, 1981).

Infected bile also undergoes the chemical changes as indicated by a yellow colour and changes in the density and viscosity. In a heavily infected gall bladder, the bile often becomes so viscous that it does not flow. The gall bladder contents are frequently of a white, cheese-like consistency (Davies, 1985; Roberts, 1989).

2.4.3 Diseases caused by trichodinids and pathology of fish hosts

Trichodinids usually occur on the gills and the skin of freshwater and marine fishes. Under normal conditions, they are found in small numbers and cause little or no pathology to their hosts. But when poor environmental conditions prevail, trichodinid populations increase rapidly and this may cause the epithelial damage with subsequent secondary invasions by bacteria and fungi. The larvae and fry of fish are especially vulnerable, and severe mortality can occur (Lom, 1973; Van As and Basson, 1987).

Injury to the host fish is achieved by the border membrane. This membrane can cut its sharp edge into the tissue of host fish and pull the cells into the vaulted disc to form a cup-like sucker (Lom, 1973). In some cases, trichodinids even feed on the epithelial cells and erythrocytes of the hosts (Gordon, 1962; Lom, 1973).

*Trichodina truttae,* a skin parasite of freshwater fish, causes severe trichodinosis in cultured freshwater salmonids (Khan, 1991; Takeda *et al*, 1969; Urawa and Arthur, 1991; Urawa, 1992). The fish infested with this ciliate show signs of listlessness, erratic swimming and inappetence. The infestation also induces excessive mucus secretion; epithelial sloughing and lesions permit the entry of opportunistic bacteria which can eventually cause ulcers and death in both adult and fry (Khan, 1991; Urawa and Arthur, 1991). In other trichodinid infections, white patches or whitish film consisting of disintegrating epithelial cells and mucus cover the whole body (Lom, 1973). The skin may also shows the hyperplasia of epithelial layers (Hines and Spira, 1974).

In the gill tissue, heavy invasions by trichodinids are obvious by mucus hypersecretion, disintegration of the epithelium and haemorrhages (Lom, 1973). The proliferation and subsequent degeneration of the chloride cells have been recorded in the infestations of *Trichodina nigra* Lom, 1961 and *Trichodina mutabilis* Kazubski and Migala, 1968

(Ivanova, 1970). For marine fishes, a serious problem is caused by gill trichodinids. In puffers *(Spheroides maculatus), Trichodina spheroidesi* and *T. halli* feed on the red blood cells of the host fish and destroy completely the gill epithelium, leaving large denuded areas among the filaments. The death of the host occurs under such conditions (Padnos and Nigrelli, 1942). The gills of lingcod, *Ophiodon elongatus* infested with *Trichodina* sp. show haemorrhaging of gill capillaries and ingestion of gill epithelium by the ciliate. This ciliate may also induce excessive mortality of lingcod (Gordon, 1962). Trichodinids have also been reported to cause losses and diseases in marine fish farms; some are even fatal (Grabda, 1991). The growth of farmed turbot, *Scophthalmus maximus* was found to be affected by *Trichodina* sp. (Sanmartin Duran *et al.*, 1991). The infested turbot were always underweight compared to the parasite-free controls. Pearse (1972) reported that the mortality of one-year-old hatchery-reared plaice, *Pleuronectes platessa* occur due to a mixed infestation of a trichodinid ciliate and a monogenean trematode.

## 2.5 Seasonal variations of infection of protozoan parasite

Parasitic infections are highly dependent on a number of factors in the external environment and on the mode of life of the hosts. Previous investigations on the infection of protozoan parasites have demonstrated the presence of the seasonal variation in the infection of many protozoan parasites. Pellifero *et al.* (1979) reported that the level of infection of *Myxobolus amurensis* Akhmerov, 1960 in cyprinids increases in early spring and summer and stabilizes of lower levels in autumn and winter. Foott and Hedrick (1986) observed that the prevalence of infective stage of proliferative kidney disease in rainbow trout, *Salmo gairdneri* rises to the peak value in summer. El-Tantawy (1989) found that the prevalence of infection of myxosporeans in freshwater fishes increases in summer and rapidly decreases in autumn and winter. Accoring to Willomitzer (1980), the prevalence and intensity of infestations of *Trichodina* sp. and *Ichthyophthirius multifiliis* Fouqet, 1876 on grasscarp (*Ctenopharyngodon idella)* shows the highest values in the period between August and October in Czechoslovakia. The summer season is also the favourable for the growth of *Glugea hertwigi* in young-of-the-year *Osmerus eperlanus mordax* (Haley, 1954; Delisle, 1969).

Among the environmental factors, temperature has a pronounced influence on the seasonal biology of most poikilotherms (Kennedy and Hine, 1969; Lom and Laird, 1969; Rawson, 1976). Walkey (1967) indicated that "since fish are poikilothermic animals, temperature changes in the external environment are transmitted to the parasite

fauna". High temperature in the summer is generally considered to be favourable for the growth of parasites (Haley, 1954; Delisle, 1969; Olson, 1976, 1978; Takvorian and Cali, 1984). The experiments of Schaperclaus (cited by Shulman, 1966) and Udey *et al.* (1975) demonstrated that a rise in the temperature results in an increase of developmental rates of *Ceratomyxa shasta* and *Myxobolus cerebralis*. Established infections of *C. shasta* proceed at a slower than normal rate at temperatures below 10° C (Schafer, 1968). The experiment of Solangi *et al.* (1982) revealed that the low temperatures inhibit all the developmental stages of *Eimeria funduli* Duszynski, Solangi and Overstreet, 1979.

Except for temperature, other factors may also affect the seasonal variation of parasite infection. Some protozoan parasites show a winter-peaked infection. Noble (1957) reported that the infections of myxosporeans are low in summer (19%) and high in winter (40%). Bauer *et al.* (1973) found *Chloromyxum truttae* Leger, 1906 appearing during late autumn and early winter. Cone (1979) observed that a maximum of 34% of perch, *Perca flavescens* collected from Megaquadaric Lake, Canada, during winter were infected with myxosporeans and infection was absent in summer. The perch, collected from Green Bay, USA, showed 36% infection from March to May and no infection in August (Guilford, 1963). Lom (1979b) found that infections of *Henneguya psorospermica* Thelohan, 1895 in *Perca fluviatilis* in central Europe occurred only during cold months with peak infections from January to March. The same trend was also observed in the infections of *Myxobolus dujardini* Thelohan, 1892 (cited by Mitchell, 1977), *Trypanosoma platessae* Lebailly, 1904 (Cottrell, 1977), *Myxobolus funduli* (Knight *et al.*, 1977, 1978) and *Hennguya waltairensis* Narasimhamurti and Kalavati, 1972 (Narasimhamurti and Kalavati, 1984). This winter-peaked infection was considered to be caused by the dominant effect of the immuno-hormonal mechanism (Cottrell, 1977). This is regarded as an important factor stimulating the development of parasites (Shulman, 1979). It has been well document that low temperatures inhibit the immune mechanism in low vertebrates (Avtalion, 1981).

The abundance of intermediate hosts, and the migration and feeding habits of fish, also affect the seasonality of parasite infection. In an investigation on the infection of *Cryptobia salmositica* Katz, 1951 in Pacific salmon *Oncorhynchus* spp., Bower and Margolis (1984) demonstrated that the seasonal variations in the prevalence and intensity of parasites relate to the seasonal return to freshwater of adult salmon in late summer and autumn, and the concomitant increase in the abundance of leech vectors. Similarly, van den Broek (1979b) observed that the immigration of young uninfected fish into estuaries

has reduced the frequency of *Cryptocotyle lingua*. Noble (1957) indicated that an active predatory habit, combined with a taste for a wide variety of food, appears to predispose fish to heavy parasitic infection. A sluggish bottom-feeder with little tendency toward migration, and a limited taste for food variety, tends to acquire relatively few kinds and numbers of parasites.

Reda (1988) suggested that the fish parasites can be distinguished into three groups with respect to the water temperature. Winter species are forms which cause a high level of infection of host fish in winter and early spring and a low level in summer. Summer species cause a high level of infection in summer and a low level in cool seasons of the year; the minimum infection occurs in autumn and winter. In the third group are species which are unrelated to water temperature; the prevalence and intensity of infection of these forms are maintained at the same level throughout the year.

Some protozoan parasites vary irregularly with respect to the seasons. These species include *Myxobolus exiguus* from *Abramis brama* (Reda, 1988); *Myxobolus muelleri* Buetschli, 1882 from *Mylocheilus caurinus, Richardsonius balteatus* and *Catostomus macrocheilus* (Mitchell, 1989); *Haemogragarina bigenmina* Laveran and Mesnil, 1901 from *Blennius pholis* (Eiras and Davies, 1991); *Myxobolus pseudodispar* Gorbunova, 1936 and *Zschokkella nova* from *Rutilus rutilus* (Brummer-Korvenkontio *et al.*, 1991); and *Trichodina* sp. from *Gasterosteus aculeatus* (Chappell, 1969a).

Two or three peaks in a year have also been detected in the infection of protozoan parasites. According to Bond (1939), the infection of *Myxidium folium* in the bile ducts and hepatic ducts shows two peaks in the months of February and November; and *Myxobolus funduli* on the gills shows two peaks in the spring and summer months and one peak in the winter month.

One parasite species may show different infection patterns within the different host species or different organs. Burreson and Zwerner (1982) reported that the infection of a haemoflagellate, *Trypanoplasma bullocki* Strout 1965, is constant throughout the year in *Trinectes maculatus*, while in summer flounder, *Paralichthys dentatus*, the infection is only observed during winter. *Myxobolus (Myxosoma) subtecalis* Bond, 1938, when it parasitizes the brain or kidney, does not fluctuate with the seasons. However, seasonal variation occurs when it infects fins (Bond, 1939). This was explained by the fact that

the release of spores only occurs after the death or decay of the host fish when the parasites are in brain or kidney.

### 2.6 Relationships between parasites and fish hosts

The infection of fish by parasites is a result of complex interactions between the parasite and its host. The size and age of fish is one of the important factors affecting the level of infection. In general, parasites increase in abundance with the size and age of host fish (Dogiel, 1958; Noble, 1960). Cone and Anderson (1977) reported a positive relationship between the infection of *Myxobolus osburni* Herrick, 1936, *M. uvuliferis* Cone and Anderson, 1977 and the size of the host fish, *Lepomis gibbosus*. Lucky (1978) found that the infection of *Myxobolus pavlosvkii* Akhmerov, 1954 increases with the ages of *Hypophthalmichthys molitrix* and *Aristichthys nobilils*. The same trends have also been reported in the infection of a haemoflagellate, *Cryptobia bullocki* Strout, 1965 in smooth flounder, *Liopsetta putnami* (Burn, 1980) and *Myxobolus muelleri* Buetschli, 1882 in *Rutilus rutilus* (Brummer-Korvenkontio *et al.*, 1991). The increase of parasite infection with the size of host fish has been reported by Okada *et al.* (1981) for myxosporean infection in *Peruvian hake,* Janovy and Hardin (1987) for *Myxobolus funduli* in *Fundulus zebrinus* and Arthur *et al.* (1982) for *Pleistophora* sp. in *Theragra chalcogramma*. The explanation for the positive relationship between the parasite infection and the age and size of fish, according to Noble (1957), is the intensive feeding habits of adult fish during the growing stages, including feeding on intermediate hosts, and an accumulation of parasites. In addition to the increase in the prevalence and intensity of infection of one parasite species with the age of fish, the number of parasite species also increases with the age of the hosts (Gorbunova, 1936; Rawson, 1976).

In some cases, however, the infection of protozoan parasites decreases with the age and size of host fish. This may result from an increasing immune response with the age of fish (Stubbs, 1985). Chen and Power (1972) noticed that the prevalence and intensity of *Glugea hertwigi* infection decreases with the increasing length and age of the American smelt, *Osmerus mordax*. Joy *et al.* (1978) observed the same variation in the infection of *Unicauda* sp. in *Campostoma anomalus*. *Myxidium giardi* from *Anguilla anguilla* and *Myxobolus pseudodispar* and *Myxidium rhodei* from *Rutilus rutilus* also display a declining infection level with the fish age (Copland, 1981; Brummer-Korvenkontio *et al.*, 1991).

The independence of the parasite infection with the age of fish was reported by Burn (1980) for *Glugea stephani* in smooth flounder, *Liopsetta putnami*. This parasite also varies in the prevalence and intensity independently of the size of *Pseudopleuronectes americanus* (Takvorian and Cali, 1984).

The level of parasite infection may also be related to the sex of fish. It is generally found that female fishes are less heavily infected with parasites than males (Thomas, 1964b; Joy *et al.*, 1978). This may be due to the presence of oestrogen in female fish (Thomas, 1964b). Thomas (1964b) also demonstrated that this trend tends to be reversed when oestrogen levels are lower during the breeding season in the study on helminth burdens of brown trout, *Salmo trutta* .

The sexual difference in the parasite infection may only exist in some stages of the life of host fish. This was revealed by Pickering and Christie (1980) for the ciliates *Ichthyophthirius* sp. and *Scyphidia* sp. from *Salmo trutta* . The authors found that the prevalence and intensity of the parasite infestation is significantly greater in the mature male fishes compared with the females. However, this difference was only observed during the spawning season and could not be demonstrated in immature fish.

Two possible causes have been suggested for the temporary sexual differences in parasite infection. Some workers think that it may be related to some differences in behaviour and distribution of mature fish within the habitat, which increases their exposure to parasites (Pickering and Christie, 1980). Others believe that mature fish, particularly the mature males, are in some way attracting the motile infective stages of certain parasites. Kearn (1967) has demonstrated that some metazoan ectoparasites locate their hosts by means of chemoreception.

In many cases, however, the parasite infection apperas to be independent of the sex of the host fish. Price (1982) observed that the level of prevalence of *Pleistophora cepedianae* is similar in mature male and mature female gizzard shad, *Dorosoma cepedianum*. Arthur *et al.* (1982) reported a similar phenomenon in the infection of *Pleistophora* sp. from the marine fish, *Theragra chalcogramma*. *Myxidium carinae* Alvarez-Pellitero, 1983 from *Barbus barbus bocagei* and *M. macrocapsulare* Auerbach, 1910 from *Leuciscus cephalus cabeda* also showed the same trends (Alvarez-Pellitero *et al.*, 1983).

Janovy and Hardin (1987) concluded that there are three types of relationships between host sex and parasite infection: one in which the infection is independent of sex, one in which the sex-infection differences are probably of physiological origin, e.g., based on the mucus, colour, or hormonal factors, and one in which the sex-infection differences are likely to be ecological. The infection of endoparasites may simply reflect the differences in the feeding habits related to the sex and gonadal maturation, as the infective stages of many endoparasites are ingested with the food (Pickering and Christie, 1980).

## 2.7 Frequency distributions of parasites

The nature of the parasite distribution is of considerable importance, as it contributes to the determination of both the stability of the systems and the numerical levels of host and parasite abundance (McCallum, 1982). Fisher (1941) was the first to describe quantitatively the distribution of ticks on sheep with a negative binomial model fully describing the data.

Williams (1964) obtained a good fit with the log-series distribution model when he considered a large number of cases of parasitic infection and he indicated that this distribution is more applicable than the negative binomial distribution model. However, Crofton (1971) considered Williams's methods to be less sensitive than the negative binomial model. He found that helminth populations could be adequately described by the negative binomial distribution. Since then, several metazoan parasites have been described by the negative binomial distribution (Kennedy, 1968; Kennedy and Hine, 1969; Pennycuick, 1971a; Anderson, 1974). The distribution of at least three protozoan populations *(Myxobolus funduli* from *Fundulus kanse, Trichodina* sp. from *Fundulus kansae,* and *Ichthyophthirius multifililis* from *Poecilia latipinna)* fit well with the negative binomial model (Knight *et al,* 1977, 1980; Adams, 1980; McCallum, 1982).

Parasite populations where the variance are less than means can be accurately described by the Poisson distribution. The distributions of only a few fish parasites fit well to this model, i.e. a cestode, *Proteocephalus filicollis* from *Gasterosteus aculeatus* (Hopkins, 1959).

The log-series distribution has been described as a limiting form of the negative binomial distribution, in which the uninfected hosts were excluded (Crofton, 1971). When the number of parasites is small, coupled with a small $k$ value, the log-series distribution

often provides a better fit to the observed data than the negative binomial (Admas, 1980). Adams (1980) found that the log-series fits a few populations of parasite including *Trichodina* sp., *Myxobolus funduli* and *Scyphidia* sp. although the negative binomial distribution gave a better fit to the observed data.

The frequency distribution of parasites has also been described by another model, the log-normal distribution model. This model gives a large assemblage of the parasite. Their relative abundance is most likely a product of the interplay of many more or less independent factors which multiplicative by compounded. This distribution model has been used extensively by ecologists. For parasites, malarial parasites in human blood, some other human diseases, and fish-infecting cestode, *Schistocephalus solidus,* have been described adequately by this distribution model (Williams, 1964).

## 2.8 Microhabitats of parasites

The studies on the microhabitats of parasites have been mostly concerned with the distribution of various metazoan parasites on the gills of host fish (Hanek and Fernando, 1978). Generally, parasites appear to be more prevalent on gill arches 2 and 3 (Wootten, 1974; Hanek and Fernando, 1978; Adams, 1986). This may be due to the larger surface area available and the large volume of respiratory current flowing over the second and third pair of gills (Wootten, 1974; Paling, 1968). The degree of infestation is considered to be directly related to the volume of ventilation and the pattern of current flowing over the gills (Hughes and Morgan, 1973) .

Some gill parasites show a preference for gill arch 1 (van den Broek, 1979a; Ramasamy, 1985). An explanation for this distribution preference, according to van den Broek (1979a), is that the interaction of a number of factors may be responsible for the final distribution of parasites.

In addition to the preference for gill arches of parasites, some metazoan parasites also show significant differences in the infestations between the right and left gills. Wootten (1974) reported that the infestation of *Dactylogyrus amphibothrium* has a high prevalence on right gills of *Gymnocephalus cernua* rather than left gills. A similar situation was also observed in the infestation of *Diplozoon paradoxum* on *Abramis brama* by Wiles (1968).

Only a few studies regarding the microhabitat of parasitic protozoans on fish gills have been conducted. The limited studies reveal no significant difference in the prevalence and

intensity of infestation between gill arches and between left and right gills although there is a trend that gill arches 2 and 3 show somewhat higher infestation than arches 1 and 4 (Knight, 1977, 1980; Adams, 1980, 1986).

### 2.9 Studies of fish-infecting protozoa in Australia

In 1909, Johnston and Cleland (1909) detected the "ring bodies" in the red blood cells of leather-jacket, *Monacanthus* sp. from New South Walson, which as indicated by Mackerras and Mackerras (1925), may possibly have been a protozoan. One year later, Johnston and Cleland (1910) reported two new species, *Trypanosoma anguillicola* Johnston and Cleland, 1910 and *T. bancrofti* Johnston and Cleland, 1910 from the blood of freshwater fishes in New South Wales and Queensland respectively, and one myxosporean species, *Chloromyxum* sp. from the muscle of marine fish in West Australia.

Several studies of fish-infecting protozoa in Australia have been conducted since these early reports were published. Twenty new species were reported from freshwater and marine fishes by 1961 (Johnston and Bancroft, 1918; Mackerras and Mackerras, 1925, 1961; Woolcock, 1936). In the early 1980's, Beumer *et al.* (1982) published a checklist including all the fish parasites recorded from Australia and its adjacent Antarctic Territories. Twenty three protozoan species were listed. Subsequent taxonomic investigations on fish-infecting protozoa have been contributed by Lester (1982), Langdon (1987, 1990a), Molnar and Rhode (1988a, 1988b), Burreson (1989), Moser *et al.* (1989), Kent and Moser (1990). More recently, five new species were reported from marine and estuarine fishes and a new checklist was published by Lom *et al.* (Lom, Rohde and Dykova, 1992; 1993).

Few studies have been carried out on the life cycle and pathogenicity of fish-infecting protozoans in Australia. The life cycle of *Ichthyophthirius multifiliis* and *Chloromyxum thyrsites* Gilchrist, 1924 were investigated by Butcher (1943) and Willis (1949) respectively. Although a few early works concerning the diseases caused by protozoan parasites have been recorded in Australia, e.g., in freshwater fishes, the white spot disease (Roughley, 1932; Butcher, 1941; 1947), chilodonellasis (Ashburner and Ehl, 1973) and coccidiosis (Ashburner, 1975) and in marine fishes, the "milky flesh" disease (Willis, 1949; Rohde, 1976; Lester, 1979), there has been no detailed investigation on the pathogenicity of protozoan parasites until 1985 when Langdon *et al.* studied the pathology of bony bream, *Nematalosa erebi* and other native fishes in the Finke River

infested by *Chilodonella hexasticha* Kiernik, 1909. Munday (1985) discovered an amoebic gill disease of sea-caged salmonids in Tasmanian waters caused by *Paramoeba* sp. The pathological changes of fish gills have been described by Roubal *et al.* (1989) and Munday *et al.* (1990). The pathology of nervous systems in both freshwater, and marine fishes and the spinal curvature of freshwater fishes associated with myxosporean species were investigated by Langdon (1987, 1990a) and Rothwell and Langdon (1990). Langdon (1991) also studied the pathology of "milky flesh" in marine fish, *Coryphaena hippurus* due to the infection of *Kudoa thyrsites* Gilchrist, 1924. The pathology of freshwater fish, *Maccullochella peeli* infected by *Goussia lomi* Molnar and Rohde, 1988 was examined by Philbey and Ingram (1991).

In recent years, there has been an increased interest in aquaculture and fish diseases in Australia, and the proceedings of five workshops and two fish diseases courses have been published. Langdon and Humphrey (1985) compiled a checklist of the diseases of Australian fish and shellfish. Callinan (1988), Owens *et al.* (1988) and Langdon (1988, 1990b) reviewed the diseases of Australian native fishes, of tropical marine-culture fish, of introduced Australian fish as well as diseases of Australian finfish. Two monographs published by Rohde (1976, 1990) reviewed marine parasitology in Australia. Rowland and Ingram (1991) reviewed the diseases caused by the ectoparasites and fungi on four freshwater fish species in New South Wales.

In conclusion, 63 species of fish-infecting protozoans have been previously reported from Australia. Twenty three of these are from freshwater fishes and 40 of these are from marine and estuarine fishes. Most studies conducted in Australia concern the taxonomy of protozoan parasites, while a few investigations on the pathogenicity have also been carried out.

# CHAPTER 3 - TAXONOMY OF PROTOZOAN PARASITES IN TASMANIAN MARINE FISHES

## 3.1 Introduction

Since Johnston and Cleland (1910) first reported *Trypanosoma anguillicola*, *T. bancrofti* and *Chloromyxum* sp. from freshwater fishes in Queensland, 43 genera, 63 species and 27 unidentified forms of protozoan parasites have been reported from Australian in addition to the collective group *Microsporidium* Balbiani, 1884. Of these protozoans, 23 species are from freshwater fishes (Johnston and Bancroft, 1918; Mackerras and Mackerras, 1961; Beumer *et al.*, 1982; Langdon and Humphrey, 1985; Callinan, 1988; Langdon, 1987; Molnar and Rohde, 1988b; Langdon, 1988, 1990a, 1990b; Rowland and Ingram, 1991) and 40 species are from marine and estuarine fishes (Mackerras and Mackerras 1925, 1961, Lester, 1982; Beumer *et al.*, 1982; Langdon and Humphrey, 1985; Owens *et al.*, 1988; Calllinan, 1988; Molnar and Rhode, 1988a; Burreson, 1989; Moser *et al.*, 1989; Langdon, 1988, 1990a, 1990b; Kent and Moser, 1990; Langdon, 1991; Lom *et al.*, 1992; 1993). A checklist of these parasites is given in Table 3.1.

In comparison with other countries, studies of fish-infecting protozoans in Australia are relatively scarce. This chapter provides new information on protozoan parasites from Tasmanian marine fishes. It presents information on 16 species including 12 new species, two new Australian record species and two unidentified forms belong to 11 genera in addition to the collective group *Microsporidium*, 10 families, 4 classes and 4 phyla. This study, combined with previous reports, brings the total number of fish-infecting protozoa known in Australia to 54 genera and 77 species (excluding unidentified forms). The systematics and synopses of infection of these 16 species are provided. The descriptions of the detailed structure and figures of them are enclosed. The systematics of these parasites follow the classification proposed by Levine *et al.* (1980) and incorporating the system created by Lom and Noble (1984) for the Myxosporea, and that given by Canning (1990) for the Microsporidia, and Basson and Van As (1989) for the Trichodinidae.

## 3.2 Material and methods

### 3.2.1 Sampling area

All fishes for this study were collected from the seagrass meadows at Dru Point, North-West Bay, situated in south-eastern Tasmania, 20 km south of Hobart (Fig. 3.1). North-West Bay is 5 km wide along its north-east to south-west axis, and is 7 km in length

**Table 3.1** Fish-infecting protozoans reported from Australia.

| Protozoa | Host species | Location | Habitat | Distribution | Source |
|---|---|---|---|---|---|
| **Sarcomastigophora** | | | | | |
| *Amyloodinium ocellatum,* Brown, 1931 | Most species | Gills, skin | Marine | All states | 14 |
| *Piscinoodinium (Oodinium) pilularis* (Schaperclaus, 1954) Lom, 1981 | Most species | Gills, skin | Freshwater | All states | 14 |
| *Crepidoodinium australe* Lom *et al.,* 1993 | *Sillago ciliata* | Gills | Estuarine | NSW | 18 |
| *Trypanoplasm parmae* Mackerras & Mackerras, 1925 | *Parma microlepis* | Blood | Marine | NSW | 19, 20 |
| *Cryptobia* sp. | Many species | Gills | Freshwater | All states | 10, 25 |
| *Trypanosoma anguillicola* Johnston & Cleland, 1910 | *Anguilla reinhardtii, A. mauritana* | Blood | Freshwater | NSW, Qld. | 5, 7, 20 |
| *T. aulopi* Mackerras & Mackerras, 1925 | *Aulopus purpurissatus, Latropiscus purpurissatus* | Blood | Marine | NSW | 19, 20 |
| *T. bancrofti* Johnston & Cleland, 1910 | *Tandanus tandanus* | Blood | Freshwater | Qld. | 5, 7, 20 |
| *T. carchariasi* Laveran, 1908 | *Carcharias* sp., *Odontaspis* sp. | Blood | Marine | NSW | 20 |
| *T. gargantua* Laird, 1951 | *Raja nasuta, Hemiscyllium ocellatum* | Blood | Marine | Qld. | 20 |
| *T. mackerrasi* Burreson, 1989 | *Hemiscyllium ocellatum* | Blood | Marine | Qld. | 3 |

Cont. Table 3.1

| | | | | | |
|---|---|---|---|---|---|
| *Trypanosoma pulchra* Mackerras & Mackerras, 1925 | *Ellerkeldia annulata, Gilbertia semicincta, Parma microlepis* | Blood | Marine | NSW | 19, 20 |
| *T. taeniurae* Burreson, 1989 | *Taeniura lymma* | Blood | Marine | Qld. | 3 |
| *Ichthyobodo (Costia) necator* Henneguy, 1883 | Mainly salmonids, *Carassius auratus*, others | Skin, gills | Freshwater | Tas., Vic., NSW | 12, 14, 29 |
| *Hexamita* sp. | *Symphysodon* spp. | Intestine | Marine | All states | 13 |
| *Paramoeba* sp. | *Salmo salar, Salmo gairdneri* | Gills | Marine | Tas. | 12, 24 |
| *Thecamoeba* sp. | Salmonids | Gills | Marine | Tas., others | 14 |
| **Apicomplexa** | | | | | |
| *Eimeria cheilodactyli* Molnar & Rohde, 1988 | *Cheilodactylus fuscus* | Intestine | Marine | NSW | 21 |
| *E. dykovae* Molnar & Rohde, 1988 | *Cheilodactylus fuscus* | Intestine | Marine | NSW | 21 |
| *E. sillaginis* Molnar & Rohde, 1988 | *Sillago ciliata* | Intestine | Marine | NSW | 21 |
| *E. ciliatae* Molnar & Rohde, 1988 | *Sillago ciliata* | Intestine | Marine | NSW | 21 |
| *E. pleurostici* Molnar & Rohde, 1988 | *Sphaeroides pleurosticus* | Intestine | Marine | NSW | 21 |
| *E. philypnodoni* Molnar & Rohde, 1988 | *Philypnodon grandiceps* | Intestine | Freshwater | NSW | 22 |
| *E. ashburneri* Molnar & Rohde, 1988 | *Macquaria ambigua* | Intestine, pyloric caeca | Freshwater | NSW | 22 |
| *Eimeria* sp. | *Macquaria australasica* | Intenstine | Freshwater | Vic. | 1 |
| *Epieimeria anguillae* Leger & Hollande, 1922 | *Anguilla reinhardti, A. australis* | Intestine, pyloric caeca | Freshwater | Vic. | 22 |

Cont. Table 3.1

| | | | | | |
|---|---|---|---|---|---|
| *Cryptosporidium* sp. | angelfish | Intestine | Marine | ##### | 4 |
| *Goussia arrawarra* Molnar & Rohde, 1988 | *Sillago ciliata* | Intestine | Marine | NSW | 21 |
| *G. microcanthi* Molnar & Rohde, 1988 | *Microcanthus strigatus* | Intestine | Marine | NSW | 21 |
| *G. lomi* Molnar & Rohde, 1988 | *Maccullochella peeli* | Intestine | Freshwater | NSW | 22, 26 |
| *G. langdoni* Molnar & Rohde, 1988 | *Macquaria ambigua* | Intestine, pyloric caeca | Freshwater | NSW | 22 |
| *G. callinani* Molnar & Rohde, 1988 | *Hypseleotris compressa* | Intestine | Freshwater | NSW | 22 |
| *Goussia* sp. Molnar & Rohde, 1988a | *Rhabdosargus sarba* | Liver | Marine | NSW | 21 |
| *Goussia* sp. Molnar & Rohde, 1988b | *Anguilla reinhardti*, *A. australis* | Gut | Freshwater | Vic. | 22 |
| *Haemogregarina aulopi* Mackerras & Mackerras, 1925 | *Aulopus purpurissatus*, *Parma microlepis*, *Latropiscus purpurissatus* | Blood | Marine | NSW | 19, 20 |
| *H. carchariasi* Laveran, 1908 | *Carcharias* sp., *Odontaspis* sp. | Blood | Marine | NSW | 20 |
| *H. gilbertiae* Mackerras & Mackerras, 1925 | *Gilbertia semicincta*, *G. annulata* | Blood | Marine | NSW | 19, 20 |
| *H. hemiscyllii* Mackerras & Mackerras, 1961 | *Hemiscyllium ocellatum* | Blood | Marine | Qld. | 3, 20 |
| *H. parmae* Mackerras & Mackerras, 1925 | *Parma microlepis* | Blood | Marine | NSW | 19, 20 |

Cont. Table 3.1

| | | | | | |
|---|---|---|---|---|---|
| *H. tetraodontis* Mackerras & Mackerras, 1961 | *Tetraodon hispidus, Arothron hispidus* | Blood | Marine | Qld. | 3, 20 |
| *H. bigemind* Laveran & Mesnil, 1921 | *Ecsenius bicolor* | Blood | Marine | Qld. | 3 |
| *Haemohormidium* sp. | *Pomacentrus melanochir* | Blood | Marine | Qld. | 3 |
| **Microsporidia** | | | | | |
| *Pleistopora sciaenae* Johnston & Bancroft, 1918 | *Johnius belangeri* | Ovary | Freshwater | Qld. | 6 |
| *Microsporidium* sp. | Many species | #### | Freshwater | All states | 14 |
| **Myxosporea** | | | | | |
| *Myxidium therapon* Johnston & Bancroft, 1918 | *Hepharstus carbo, Scortum hillii* | Gall bladder | Freshwater | Qld. | 6 |
| *M. sphaericum* Thelohan, 1895 | *Istiblennius meleagris, Salarais fasciatus, Petroscrites* sp. | Gall bladder | Marine | Qld. | 23 |
| *Zschokkella heronensis* Moser *et al.*, 1989 | *Chaetodon plebeius, Choerodon venustus* | Gall bladder | Marine | Qld. | 23 |
| *Coccomyxa meridiei* Lom *et al.*, 1992 | *Herklosichthys castelnaui* | Gall bladder | Marine | NSW | 17 |
| *C. tenuiparies* Lom *et al.*, 1992 | *H.whiteleggii* | Gall bladder | Marine | NSW | 17 |
| *Ortholinea alata* Kent & Moser, 1990 | *Chaetodon rainfordi* | Kidney | Marine | Qld. | 8 |
| *O. australis* Lom *et al.*, 1992 | *Acanthopagrus australis, Rhabdosargus sarba* | Hepatic ducts, gall bladder | Marine | NSW | 17 |

Cont. Table 3.1

| | | | | | |
|---|---|---|---|---|---|
| *Triangula percae* Langdon, 1987 | *Perca fluviatilis* | Brain | Freshwater | Vic., NSW | 9 |
| *Sinuolinea lesteri* Moser *et al.*, 1989 | *Hemiscyllium ocellatum* | Urinary bladder, ureter | Marine | Qld. | 23 |
| *Ceratomyxa sprenti* Moser *et al.*, 1989 | *Chaetodeon aureofasciatus*, *Chaetodon rainfordi*, *Lutjanus amabilis*, *Platycephalus bosschei* | Gall bladder | Marine | Qld. | 23 |
| *C. rohdei* Moser *et al.*, 1989 | *Petroscrites fallax* | Gall bladder | Marine | Qld. | 23 |
| *Ceratomyxa* sp. | *Hembalistes chrysopterus*, *Labropsis australis*, *Labroides* sp., *Ostracion cubicus*, *Epinephelus quoyanus* | #### | Marine | Qld. | 23 |
| *Leptotheca beveridgei* Moser *et al.*, 1989 | *Scorpaenopsis gibbosui* | Gall bladder | Marine | Qld. | 23 |
| *Leptotheca* sp. | *Terapon jarbua* | #### | Marine | Qld. | 23 |
| *Sphaerospora mayi* Moser *et al.*, 1989 | *Atherinomous capricornensis* | Urinary bladder | Marine | Qld. | 23 |
| *Sphaerospora* spp. | *Cyprinus carpio* | Kidney | Freshwater | NSW, Vic. | 10 |
| *Mitraspora (Hoferellus) cyprini* Fujita, 1912 | *Cyprinus carpio*, *Carassius auratus* | Kidney | Freshwater | Tas., Vic., WA | 10, 14 |
| *Chloromyxum pristiophori* Woolcock, 1935 | *Pristiophorus cirratus* | Gall bladder | Marine | #### | 2 |

Cont. Table 3.1

| | | | | | |
|---|---|---|---|---|---|
| *C. noblei* Moser *et al.*, 1989 | *Taeniura lymna, Hemiscyllium ocellatum* | Gall bladder | Marine | Qld. | 23 |
| *Myxobolus (Myxosoma ) ogilbyi* Johnston & Bancroft, 1918 | *Macquaria ambigua* | Gills | Freshwater | Qld. | 6 |
| *M. gadopsii* Langdon, 1990 | *Gadopsis marmoratus* | Maninx, other connective tissue | Freshwater | Vic. | 11 |
| *M. galaxii* Langdon, 1990 | *Galaxias olidus* | Spinal cord | Freshwater | Vic. | 11 |
| *M. plectroplites* Johnston & Bancroft, 1918 | *Macquaria ambigua* | Gall bladder | Freshwater | Qld. | 6 |
| *Myxobolus* sp. Moser, Kent & Dennis, 1989 | *Crenimugal labiosus, Mugil cephalus* | #### | Marine | Qld. | 23 |
| *Myxobolus* sp. Rothwell & Langdon, 1990 | *Platycephalus bassensis* | Brain | Marine | NSW | 28 |
| *Henneguya australis* Johnston & Bancroft, 1918 | *Macquaria ambigua* | Gills | Freshwater | Qld. | 6 |
| *H. gracilis* Johnston & Bancroft, 1918 | *Scortum hillii* | Gills | Freshwater | Qld. | 6 |
| *Henneguya* sp. | *Nematalosa elongata* | Gills | Freshwater | Qld. | 6 |
| *Unicaposula seriolae* Lester, 1982 | *Seriola lalandi* | Muscles | Marine | Qld. | 16 |
| *Kudoa ciliatae* Lom *et al.*, 1992 | *Silago ciliata* | Muscle of intestine | Marine | NSW | 17 |
| *Kudoa* sp. Rohde, 1976 | *Seriola grandis* | Muscles | Marine | Qld. | 27 |
| *Kudoa* sp. Langdon, 1990a | *Lates calcarifer* | Brain | Marine | Qld. | 11 |
| *Kudoa* sp. Langdon, 1990b | *Thunnus maccoyii* | Muscles, nerves | Marine | WA | 11 |

Cont. Table 3.1

| | | | | | |
|---|---|---|---|---|---|
| *Kudoa (Chloromyxum) thyrsites* Gichrist, 1924 | *Coryphaena hippurus, Sardinops neopilchardus, Leionura atun, Thyrsites atun* | Muscles | Marine | WA, NSW, Vic., Tas. | 2, 13 |
| **Ciliophora** | | | | | |
| *Chilodonella cyprini* Moroff, 1902 | Most species | Gills, skin | Freshwater | All states | 12 |
| *C. hexasticha* Kiernik, 1909 | Most species | Gills, skin | Freshwater | All states | 12, 15, 29 |
| *Brooklynella* sp. | dhufish | Gills | Marine | #### | 12 |
| *Ichthyophthirius multifiliis* Fouquet, 1876 | Most species | Skin, gills | Freshwater | All states | 10, 12, 14, 29 |
| *Cryptocaryon irritans* Brown, 1951 | Most species | Skin, gills | Marine | All states | 12, 14, 23, 29 |
| *Tetrahymena* sp. | *Bidyanus bidyanus* | Skin, gills | Freshwater | NSW | 29 |
| *Trichodina* spp. | Most species | Gills, skin | Freshwater, marine | All states | 12, 14, 29 |
| *Trichodinella* spp. | Most species | Gills, skin | Marine | All states | 12, 14 |
| *Apiosoma (Glossatell)* sp. | *Hypseleotris klunzingeri* | Skin | Freshwater | NSW | 29 |
| *Epistylis* sp. | Salmondis | Skin | Freshwater | #### | 12 |
| *Vorticella* sp. | Salmondis | Skin | Freshwater | #### | 12 |
| *Zoothamnium* sp. | Salmondis | Skin | Freshwater | #### | 12 |

Source: 1. Ashburner, 1975; 2. Beumer *et al.*, 1982; 3. Burreson, 1989; 4. Callinan, 1988; 5. Johnston, 1916; 6.Johnston and Bancroft, 1918; 7. Johnston and Cleland, 1910; 8. Kent and Moser, 1990; 9. Langdon, 1987; 10. Langdon, 1988; 11. Langdon, 1990a; 12. Langdon, 1990b; 13. Langdon, 1991; 14. Langdon and Humphrey, 1985; 15. Langdon *et al.*, 1985; 16. Lester, 1982; 17. Lom *et al.*, 1992; 18. Lom *et al.*, 1993; 19. Mackerras and Mackerras, 1925; 20. Mackerras and Mackerras, 1961; 21. Molnar and Rohde, 1988a; 22. Molnar and Rohde, 1988b; 23. Moser *et al.*, 1989; 24. Munday *et al.*, 1990; 25. Owens *et al.*, 1988; 26. Philbey and Ingram, 1991; 27. Rohde, 1976; 28. Rothwell and Langdon, 1990; 29. Rowland and Ingram, 1991. #### no information.

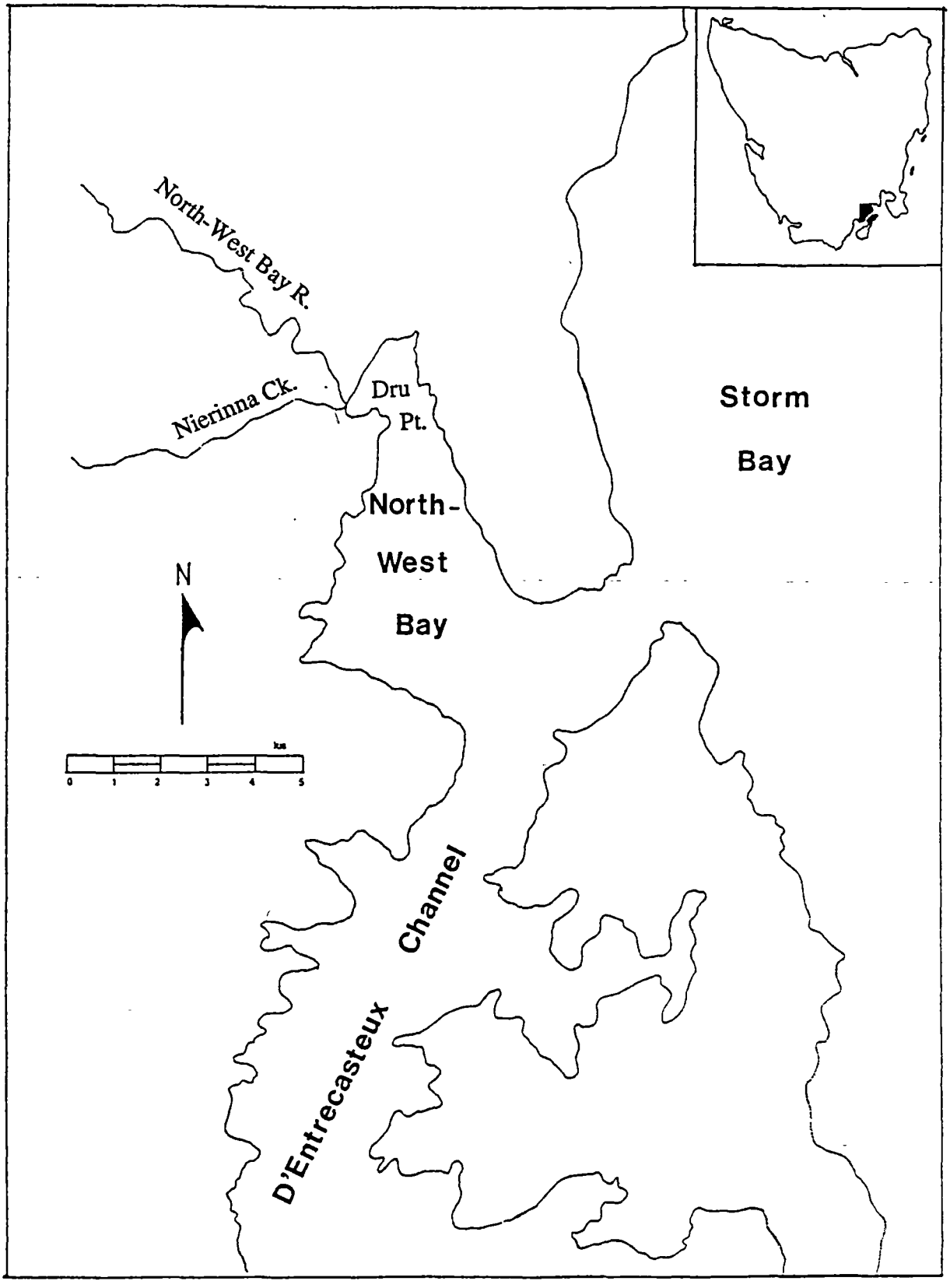



**Fig. 3.1. Map of North-West Bay and the D'Entrecasteux Channel showing the sampling area.**

along a north-west to south-east axis. North-West Bay opens south-easterly into the D'Entrecasteaux Channel. In the top north-west corner, North-West Bay River and Nierinna Creek flow into North-West Bay; they combine to form an estuarine system, which often approaches freshwater conditions during wet winters, while its southern end is completely euhaline (marine) all of the year (Walker, 1979).

The seagrass meadows at Dru Point extend out about 50-100 m from the highest water mark. There are three species of seagrass present in the area: *Heterozostera tasmaniensis* (Martens ex Aschers.) den Hartog, *Zostera muelleri* Irmisch ex Aschers., and *Halophila ovalis* (R. Br.) Hook. f. (Mills, 1991). Various algae are also found (Walker, 1979).

3.2.2 Sampling methods

Twenty two fish collections were made from January 1990 to June 1992. Fish were sampled with a 50 m long, 2 m drop seine (mesh 12 mm knot to knot ). The net was set in a tight arc at a depth of over 1m and then hauled on to the beach. Fish were transferred alive to the laboratory where they were kept in aquaria at 10° C for up to 7 days. A total of 1385 fishes belonging to 12 species were captured over 22 months study period (Table 3. 2).

The detection of the protozoan parasites started with the examination of appearance of gross pathological changes, e.g. cyst-like structures caused by myxosporeans and microsporidians. A sample of blood was taken from the heart using a thin Pasteur pipette introduced through the ventral body wall. Fish were then killed by severing the vertebral columns just behind the skull. Scrapings of mucus from the gills, skin and fins were obtained with the cleaned fine forceps. A small quantity of filtered sea water was added and the mucus was then spread on a cleaned slide. To examine the endoparasites, pieces of tissue about 2 mm in diameter from the stomach, intestine, liver, spleen, gall bladder, kidney, uretas, swimbladder and brain were excised. Ringer's solution was added and the tissue was then compressed between a coverslip on a slide.

3.2.3 Identification of species

To identify protozoan parasites correctly, proper methods of study are necessary. Myxosporean and microsporidian spores were studied in the fresh, unpreserved state (except for the study of the development of *Glugea atherinae).* Stained preparations of some myxosporeans and microsporidians were also made by Heidenhain's iron hematoxylin and Giemsa methods to supplement fresh preparations. Trichodinids were

treated with Klein's silver impregnation technique to reveal the details of the adhesive disc. Ellobiophryids are studied using both living and stained specimens. Heidenhain's iron hematoxylin method was used for ellobiophryids and the nuclei of all ciliates. The samples were examined under a Zeiss Standard Universal microscope equipped with phase contrast and 1600 x oil immersion lens, a Zeiss Axiovert 35 microscope with 1000 x differential interference contrast lens or a Zeiss Axioplan microscope with a special x 40 objective.

For trichodinids, the scanning electron microscope (SEM) was also used to provide additional data. The methods for this technique will be described in Chapter 4.

For histological studies of the development of *Glugea atherinae*, a few pieces of guts and mesentery up to 2-4 mm in diameter from five fishes were fixed with Bouin's solution. They were then embedded in conventional paraffin wax. Material was sectioned at 6-8 μm, and stained with hematoxylin and eosin (H & E).

3.2.4 Preservation of specimens

Methods of preservation varied with the specimen. For myxosporeans and microsporidians, three methods were used in the present study. Fresh fish organs or tissue infected with cysts, xenomas or spores were immersed into 5% formalin; this proved to be an effective means of preserving myxosporeans and microsporidians. Cysts or xenomas were squashed or pieces of infected tissue were spread with fine forceps to make a smear on a slide. They were then mounted in 10% glyceric ethanol. This method can preserve spores for a few months. Smears were made on coverslips. They were then fixed with Schaudinn's fluid for 10-20 min and rinsed with 50% ethanol for 10-20 min. Finally, the smears were preserved in 70% ethanol and stained later with Heidenhain's iron hematoxylin. For the Giemsa method, smears have to be dried in the air.

For ciliates, two methods were used. Scrapings of mucus containing ciliates were spread on a slide and dried in the air. Klein's silver impregnation technique was used later for studying the denticles of trichodinids. The dried material was first impregnated with 2% aqueous solution of silver nitrate for approximate 7 min. Smears were then washed in distilled water, followed by 20 min exposure to ultra-violet light or sunlight. Smears were also made on a coverslip and fixed with Schaudinn's fluid. The latter procedure is the same as for myxosporeans and microsporidians. For all the stained preparations, permanent mounts were made using Canada balsam or Depex.

3.2.5 Description and illustration of species

Descriptive terms used in the present study are defined in Lom (1958) and Lom and Dykova (1992). In the description of *Trichodina* species, the uniform specific characteristics system proposed by Lom (1958) was followed. Detailed descriptions of the denticles were presented in accordance with the method proposed by Van and Basson (1989). The ecological terms used in this thesis follow the definition given by Margolis *et al.* (1982): *Prevalence* is the number of individuals of a host species infected with a particular parasite species divided by number of hosts examined. *Intensity* is the number of individuals of a particular parasite species in each infected host in a sample. *Mean intensity* is total number of individuals of a particular parasite species in a sample of a host species divided by the number of infected individuals of the host species in the sample.

All measurements were made on 50 specimens and given in micrometers (μm) unless indicated in the text. Minimum and maximum values were given, followed in parentheses by the arithmetic mean. Figures were drawn with the aid of a camera lucida. Photomicrographs were taken using a low speed ILFORD FP4 film. Holotype slides are deposited in the Tasmanian Museum and Art Gallery (TMAG), Argyle Street, Hobart, Tasmania, Australia 7000.

## 3.3 Results

Sixteen species including one new genus and 12 new species, two new Australian records and two unidentified forms were reported in this study. Of these, one belongs to the Sarcomastigophora, two belong to the Microspora, seven belong to the Myxozoa, and six belong to the Ciliophora. The systematics of these parasites are given below.

Phylum Sarcomastigophora Honigberg and Balamuth, 1963
Class Zoomastigophorea Calkins, 1909
Order Kinetoplastida Honigberg, 1963
Family Bodonidae Butschli, 1887
Genus *Cryptobia* Leidy,1846
1. *Cryptobia* sp.
Phylum Microspora Sprague, 1977
Class Microspore Delphy, 1963
Order Microsporida Balbiani , 1882
The collective group *Microsporidium* Balbiani, 1884
2. *Microsporidium hepaticum* sp. nov.
Family Glugeidae Thelohan, 1892

Genus *Glugea* Thelohan, 1891

3. *Glugea atherinae* Berrebi, 1979

Phylum Myxozoa Grasse, 1970 emend.

Class Myxosporea Butschli, 1881

Order Bivalvulida Shulman, 1959

Suborder Sphaeromyxina Lom and Noble, 1984

Family Sphaeromyxidae Lom and Noble , 1984

Genus *Sphaeromyxa* Thelohan, 1892

4. *Sphaeromyxa nesogobii* sp. nov.

Suborder Variisporina Lom and Noble, 1984

Family Myxidiidae Thelohan, 1892

Genus *Zschokkella* Auerbach, 1910

5. *Zschokkella leptatherinae* sp. nov.

6. *Zschokkella macrocapsula* sp. nov.

Family Ortholineidae Lom and Noble, 1984

Genus *Ortholinea* Shulman, 1962

7. *Ortholinea striateculus* sp. nov.

Family Ceratomyxidae Doflein, 1899

Genus *Ceratomyxa* Thelohan, 1892

8. *Ceratomyxa arripica* sp. nov

Family Sphaeosporidae Davis, 1917

Genus *Sphaerospora* Thelohan, 1892

9. *Sphaerospora aldrichettae* sp. nov.

Suborder Platysporea Kudo, 1919 emend. Shulman, 1959

Family Myxobolidae Thelohan, 1892

Genus *Myxobolus* Butschli, 1882

10. *Myxobolus aldrichetti* sp. nov.

Phylum Ciliphora Doflein, 1901

Class Oligohymenophorea de Puytorac *et al.*, 1974

Subclass Peritrichia Stein, 1859

Order Peritrichida Stein, 1859

Suborder Mobilina Kahl, 1933

Family Trichodinadae Raabe, 1959

Genus *Paratrichodina* Lom, 1963

11. *Paratrichodina tasmaniensis* sp. nov.

Genus *Trichodina* Ehrenberg, 1831

12. *Trichodina australis* sp. nov.

13. *T. nesogobii* sp. nov.

14. *T. jadranica* Rabbe, 1958
15. *Trichodina* sp.

Suborder Sessilina Kahl, 1933

Family Ellobiophryidae Chatton and Lwoff, 1929

Genus *Clausophrya* Naidenova and Zaika, 1969

16. *Clausophrya branchialis* sp. nov.

These 16 species of parasites were obtained from 12 species of coastal fishes. The number of fish examined and the prevalence of parasites in different fish species are recorded in Table 3.2.

### 3.3.1 Phylum Microspora

*Diagnosis:* Unicellular spores, containing one uninucleate or binucleate sporoplasm; extrusion apparatus always with polar tube and polar cap; without mitochondria; obligatory intracellular parasites in nearly all major animal groups.

3.3.1.1 Collective group *Microsporidium* Balbiani, 1884

This is an assemblage of identifiable species of which the generic positions are uncertain and treated as a generic group for taxonomic convenience.

*Microsporidium hepaticum* sp. nov. (Fig. 3.2, Pl. 3.1A-F)

Hosts: *Rhombosolea tapirina, Acanthaluteres spilomelanurus, Meuschenia freycineti* and *M. australis.*

Location: Liver and gall bladder.

Date: October 1990 to May 1992.

Type material: Holotype no. TMAG-K1308, from *R. tapirina,* is deposited in the TMAG; paratype no. 940006, from *A. spilemelanurus,* is in the collection of author.

Type host: *Rhombosolea tapirina.*

Tens to hundreds of xenomas are scattered as white opaque spots in the liver of host fish; occasionally they are found in the gall bladder. The measurement of xenoma varies with the host species. In *R. tapirina,* they are small rounded or ellipsoid nodules, ranging from 50 to 500 μm. In *A. spilomelanurus, Meuschenia freycineti* and *M. australis,* they are ellipsoidal in shape and measure from 200 μm to 1mm. The xenoma wall is relatively thin, about 1.6-4.7 μm. The sporophorous vesicle membrane is not present (Pl. 3.1A-D).

**Table 3.2** Synopsis of infection of protozoan parasites on their host fishes.

| Host | Number examined | Number infected | Locations | Parasite |
|---|---|---|---|---|
| *Atherinosoma microstoma* | 514 | 84 | HD, GB | *Zschokkella leptatherinae* |
| | | 2 | GB | *Zschokkella macrocapsula* |
| | | 21 | CT | *Glugea atherinae* |
| | | 140 | Gills | *Trichodina australis* |
| | | 46 | Gills | *Trichodina* sp. |
| | | 191 | Gills | *Paratrichodina tasmaniensis* |
| | | 101 | Gills | *Clausophrya branchialis* |
| | | 6 | Skin, gills | *Cryptobia* sp. |
| *Leptatherina presbyteroides* | 589 | 188 | HD, GB | *Zschokkella leptatherinae* |
| | | 3 | GB | *Zschokkella macrocapsula* |
| | | 2 | Ureta | *Ortholinea striateculus* |
| | | 9 | CT | *Glugea atherinae* |
| | | 76 | Gills | *Trichodina australis* |
| | | 12 | Gills | *Trichodina* sp. |
| | | 136 | Gills | *Paratrichodina tasmaniensis* |
| | | 38 | Gill | *Clausophrya branchialis* |
| | | 7 | Skin, gills | *Cryptobia* sp. |
| *Kestratherina esox* | 64 | 4 | HD, GB | *Zschokkella leptatherinae* |
| | | 1 | CT | *Glugea atherinae* |
| | | 1 | Gills | *Trichodina australis* |
| | | 2 | Gills | *Trichodina* sp. |
| | | 13 | Gills | *Paratrichodina tasmaniensis* |
| | | 8 | Gills | *Clausophrya branchialis* |
| | | 2 | Skin, gills | *Cryptobia* sp. |
| *Kestratherina hepsetoides* | 5 | 2 | HD, GB | *Zschokkella leptatherinae* |
| | | 1 | CT | *Glugea atherinae* |
| | | 3 | Gills | *Paratrichodina tasmaniensis* |
| | | 4 | Gills | *Clausophrya branchialis* |
| *Kestratherina brevirostris* | 116 | 6 | HD, GB | *Zschokkella leptatherinae* |
| | | 1 | CT | *Glugea atherinae* |
| | | 3 | Gills | *Paratrichodina tasmaniensis* |
| | | 4 | Skin, Gills | *Cryptobia* sp. |
| *Arripis trutta* | 8 | 2 | GB | *Ceratomyxa arripica* |

Cont. Table 3.2

| | | | | |
|---|---|---|---|---|
| *Aldrichetta forsteri* | 11 | 3 | GB | *Sphaerospora aldrichettae* |
| | | 1 | Gills | *Myxobolus aldrichetti* |
| *Rhombosolea tapirina* | 29 | 23 | L, GB | *Microsporidium hepaticum* |
| *Acanthaluteres spilomelanurus* | 19 | 13 | L, GB | *Microsporidium hepaticum* |
| *Meuschenia freycineti* | 6 | 3 | L, GB | *Microsporidium hepaticum* |
| *Meuschenia australis* | 6 | 4 | L, GB | *Microsporidium hepaticum* |
| *Nesogobius* sp.1 | 23 | 1 | GB | *Sphaeromyxa nesogobii* |
| | | 21 | Gills | *Trichodina jadranica* |
| | | 19 | Gills | *Trichodina nesogobii* |
| Total | 1385 | | | 16 |

* HD = hepatic ducts, GB = gall bladder, L = liver, CT = connective tissue.

**Table 3.3** The biomentrical features of *Microsporidium hepaticum* in different fish species (minimum and maximum values were followed by the arithmetic mean in parentheses).

| | Hosts: *Rhombosolea tapirina* | *Acanthaluteres spilomelanurus* *Meuschenia australis* *Meuschenia freycineti* |
|---|---|---|
| Length of spore | 4.2-5.0 (4.6) µm | 4.3-4.9 (4.8) µm |
| Width of spore | 2.1-2.9 (2.6) µm | 2.5-3.1 (2.7) µm |
| Diameter of posterior vacuole | 1.4-1.8 (1.6) µm | 1.4-1.6 (1.5 ) µm |
| Coils of polar filament | 6-10 (9) | 7-9 (8) |
| Length of polar filament | 34.8-115.3 (53.2) µm | 47.1-108.5 (62.1) µm |
| Diameter of xenoma | round: 50-500 (435) µm | |
| | ellipsoid: 100-450 (320) x 130-300 (223) µm | ellipsoid: 250-1000 (610) µm x 200-480 (350) µm |

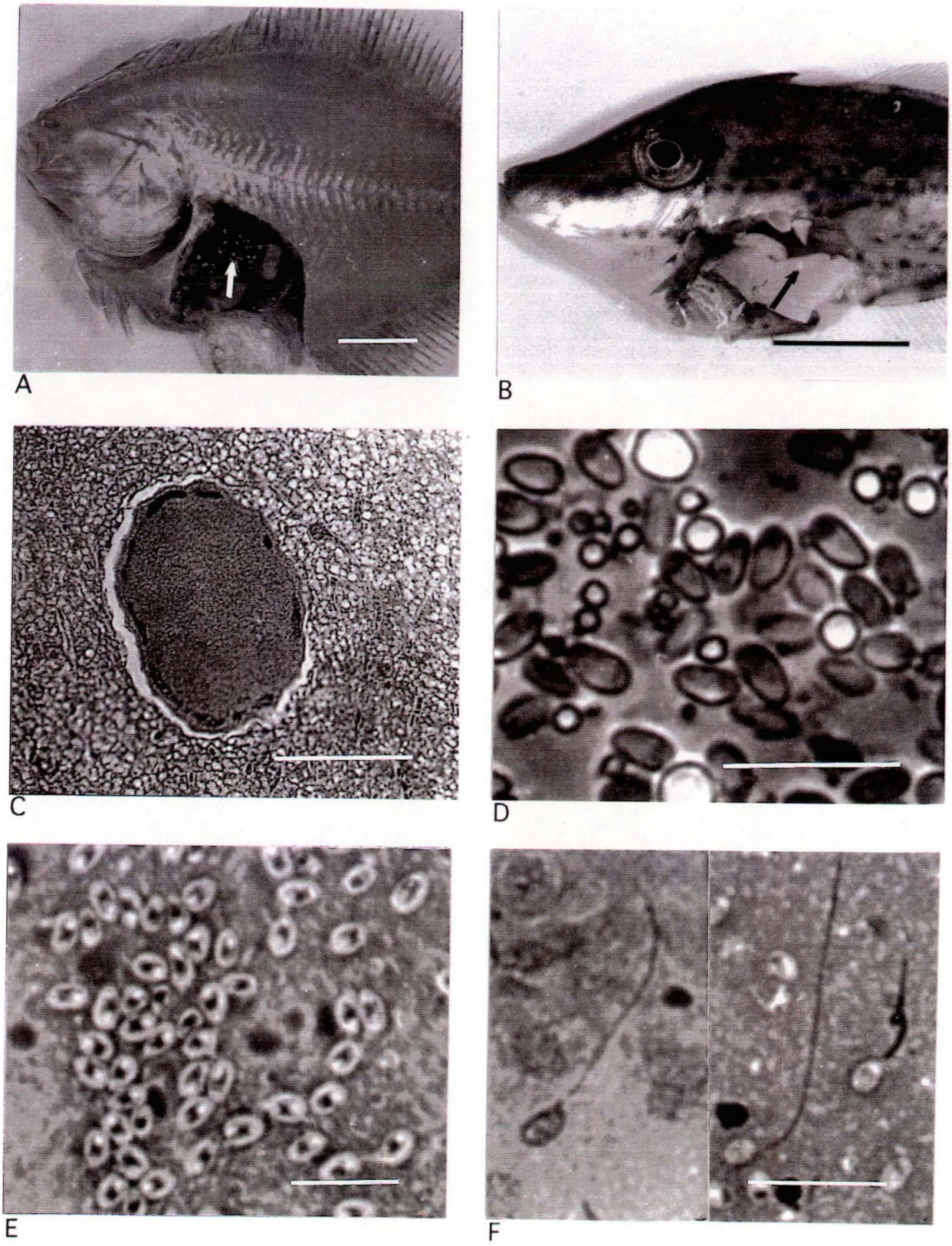



Plate 3.1. Xenomas and spores of *Microsporidium hepaticum*. A. Xenomas in the liver of *Rhombosolea tapirina*. B. Xenomas in the liver of *Acanthaluteres spilomelanurus*. C. Tissue section of *Acanthaluteres spilomelanurus* showing xenoma. D. Fresh spores. E. H & E stained spores. F. Giemsa stained spores. Bars = 1 cm in A, B; 50 μm in C and 10 μm in D to F.

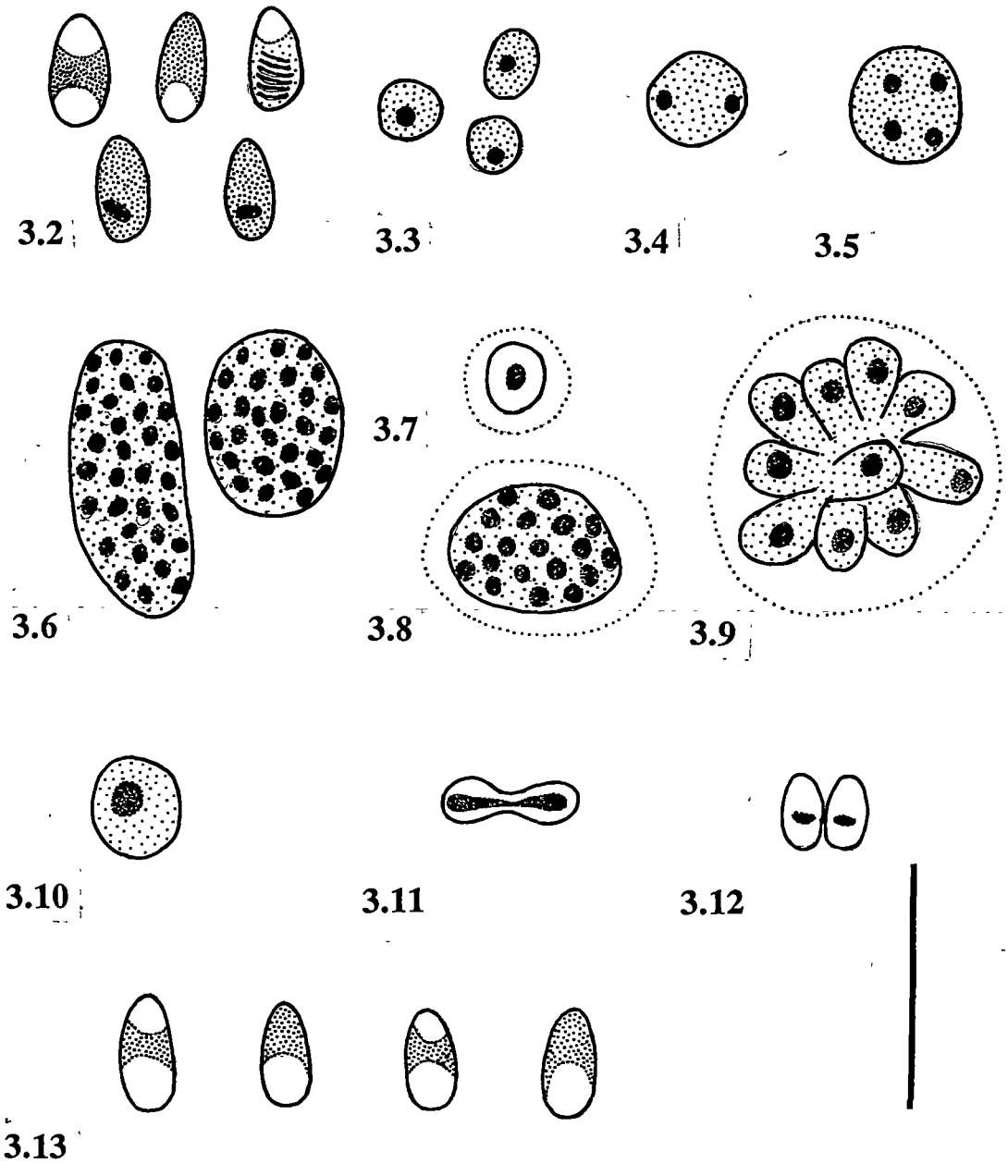



Fig. 3.2 Spores of *Microsporidium hepaticum*. 3.3-3.13. Developmental cycle of *Glugea atherinae*. 3.3. Uninucleate meronts. 3.4. Binucleate meront. 3.5. Tetranucleate meront. 3.6. Multinucleate meronts. 3.7. Sporont. 3.8. Sporogonial plasmodium. 3.9. Sporogonial plasmodium segmenting into sporoblast mother cells. 3.10. Sporoblast mother cell. 3.11. Division of sporoblast mother cell. 3.12. Stained spores. 3.13. Fresh spores. Bar = 10 µm for all Figures.

The spores are ovoid or pyriform and the surface is smooth (Fig. 3.2, Pl. 3.1D). The anterior end is somewhat narrow, while the posterior end is blunt and rounded. A conspicuous vacuole occupies about 1/3 to 1/4 of the posterior end of the spore. After staining with Giemsa, it appears to have only one nucleus in a posterior position of the spores (Fig. 3.2). A polarplast is situated at the anterior end which can be seen clearly in the preparations stained by Heidenhain's iron hematoxylin (Pl. 3.1 E). The sporoplasm forms a girdle-like ring located just anterior to the posterior vacuole (Pl. 3.1 E). The polar filament runs an oblique course backward from the anterior end of the spore and forms a coil in the peripheral region of the cytoplasm. The number of coils is not easy to count but in some spores there are indications of 6 to 10 coils (Fig. 3.2). When extruded, the length of the polar filament measures 34.8-115.3 μm (Pl. 3.1F). The measurements of *Microsporidium hepaticum* are given in Table 3.3.

*Remarks*

The determination of species and genera of microsporidia is mainly based on the spore morphology. Most diagnostic characters can only be obtained with the aid of the transmission electron microscope. The study of the fine structure of xenoma and developmental stages of *Microsporidium hepaticum* are present in Chapter 4. Therefore, the comparison between this species and other previously recorded species is given in that chapter.

3.3.1.2 Genus *Glugea* Thelohan, 1891

*Diagnosis:* Sporulates within the sporophorous vesicle membrane. Meronts have a surface coat which is completly encased by a cisterna of host cell endoplasmic reticulum. Sporogonial plasmodium divides into many sporoblast mother cells, each of which gives rise to two sporoblasts.

*Glugea atherinae* Berrebi, 1979 (Figs. 3.3-3.13, Pl. 3.2 A, B)

Hosts: *Atherinosoma microstoma, Leptatherina presbyteroides, Kestratherina brevirostris* and *K. esox*.

Location: Connective tissues of gut wall, body cavity and mesentery.

Date: January 1990 to January 1991.

Specimens: Slide no. TMAG-K1309 is deposited in the TMAG and slide no. 300071 is in the collection of author.

The infection of this microsporidian is lighter than that of *Microsporidium hepaticum*. One to 16 xenomas were found in infected fish.

*Xenomas*: Round or ovoid xenomas are about 0.5-1.5 mm in diameter, white and opaque. They are found in the connective tissues of the gut wall, body cavity and mesentery of the host fishes. The xenomas are usually enclosed in a capsule, which appears to be composed of hyalinized connective tissue. The thickness of the xenoma wall is up to 3 μm (Pl. 3.2A).

*Developmental stages*: The early initial meront is spherical, about 2.5 μm in diameter or ellipsoidal measuring 3 x 2 μm, with an eccentric nucleus (Fig. 3.3). As the nucleus divides, the mononucleate meront develops into a binucleate meront (Fig. 3.4) and then tetranucleate and multinucleate meronts (Figs. 3.5, 3.6). When nuclei number up to about 60, the meront begins to divide and ultimately produces uninucleate daughter cells. The sporogonic stage is indicated by the formation of a sporophorous vesicle around the sporont (Fig. 3.7). The single nucleate sporont divides to produce a multinucleate, elongate plasmodium (Fig. 3.8). The latter cleaves by radial segmentation into many uninucleate sporoblast mother cells, which are spherical, 3.2 μm in diameter (Figs. 3.9, 3.10). Each mother cell divides into two sporoblasts (Fig. 3.11), which then mature into spores (Fig. 3.12).

*Spores*: Fresh mature spores are ovoid with broad posterior ends (Fig. 3.13, Pl. 3.2B). A large vacuole occupies about 1/3 to 1/2 of the posterior end of the spore. The polarplast occupies the anterior end and is not distinct. A single nucleus, which can only be observed in the stained samples (Fig. 3.12), is situated in a slightly posterior position. The polar filament is uniformly thin, and runs an oblique course backward from the anterior end of the spore and forms a coil. The number of the coil is not clear. The measurements of spores have a length of 3.5-4.8 (4.2) μm and a width of 2.2-2.6 (2.4) μm.

*Remarks*

Berrebi (1978) first reported *Glugea atherinae* from the estuarine smelt, *Atherina boyei* on the French Mediterranean coast. Comparing the present species with *G. atherinae*, the major characteristics, including spore morphology, structure and the size, structure and location of xenomas closely resemble those of *G. atherinae*. The only difference is the spore size, which is 4.5-6.3 x 2.6-3.3 (5.8 x 3) μm in Berrebi's report. Since spores may vary more or less in different geographic areas, I can find no designating characters to preclude the present species from *Glugea atherinae*.

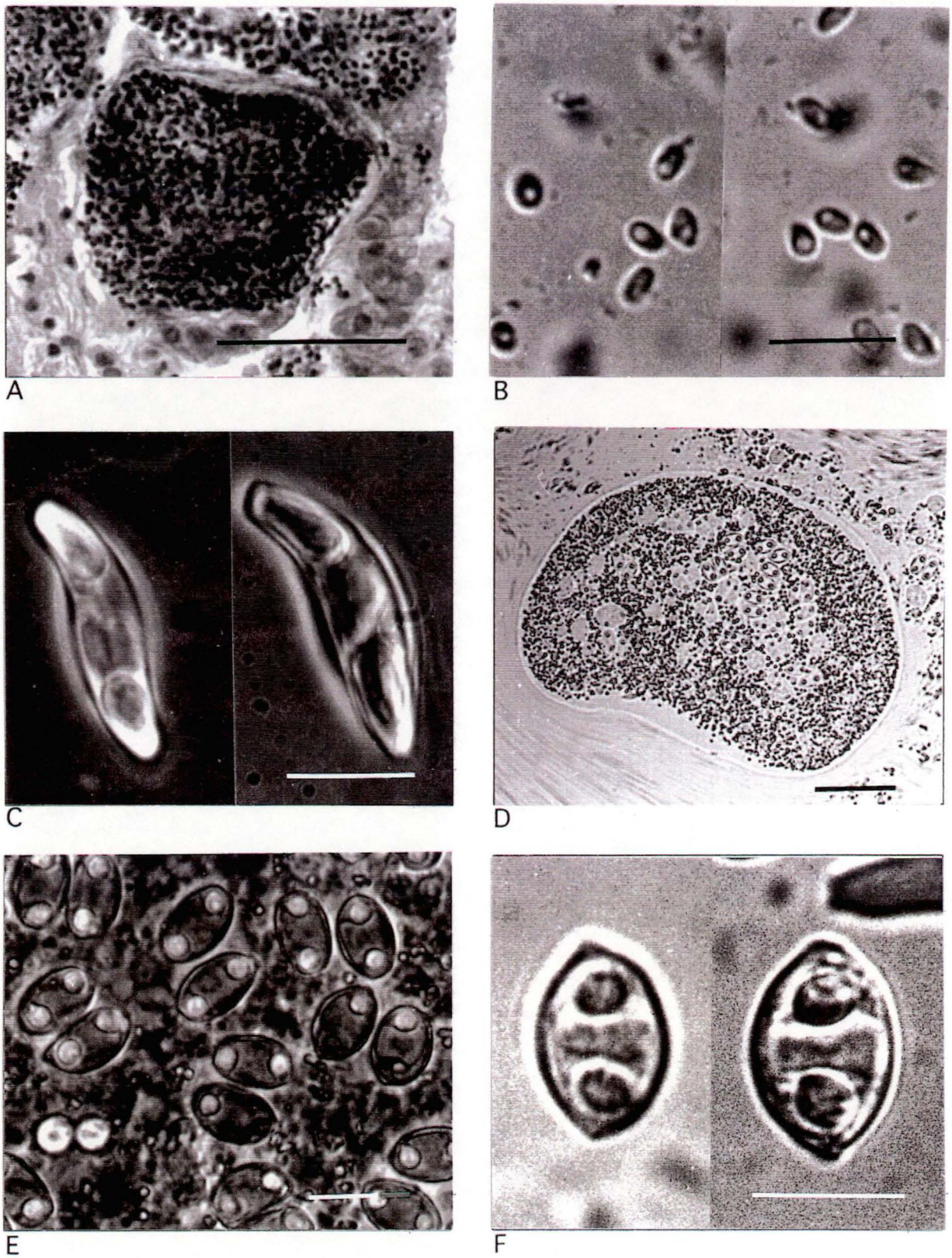



Plate 3.2. Microsporidian and myxosporeans. A. Xenoma of *Glugea atherinae*. B. Fresh spores of *G. atherinae*. C. Fresh spores of *Sphaeromyxa nesogobii*. D. Plasmodium of *Zschokkella leptatherinae*. E. Fresh spores of *Z. leptatherinae*. F. Enlargement of fresh spores of *Z. leptatherinae*. Bars = 50 μm in A, D and 10 μm in B, C, E, F.

### 3.3.2 Phylum Myxozoa

*Diagnosis*: Spores are of multicellular origin, with one or more polar capsules and sporoplasm; the spore wall is formed by two to seven valves. All species are parasitic, mostly in fish.

3.3.2.1 Genus *Sphaeromyxa* Thelohan, 1892

*Diagnosis*: Two polar capsules lie in the opposite, tapering and truncate ends of the elongated spores; they open in the level of the sutural ridge. Polar filament is not spirally wound but folded several times.

*Sphaeromyxa nesogobii* sp. nov. (Figs. 3.14, 3.15; Pl.3.2C)
Host: *Nesogobius* sp.1 (in Last *et al.*, 1983).
Location: Gall bladder.
Date: October 1990.
Type material: Holotype no. TMAG-K1301 is deposited in the TMAG.

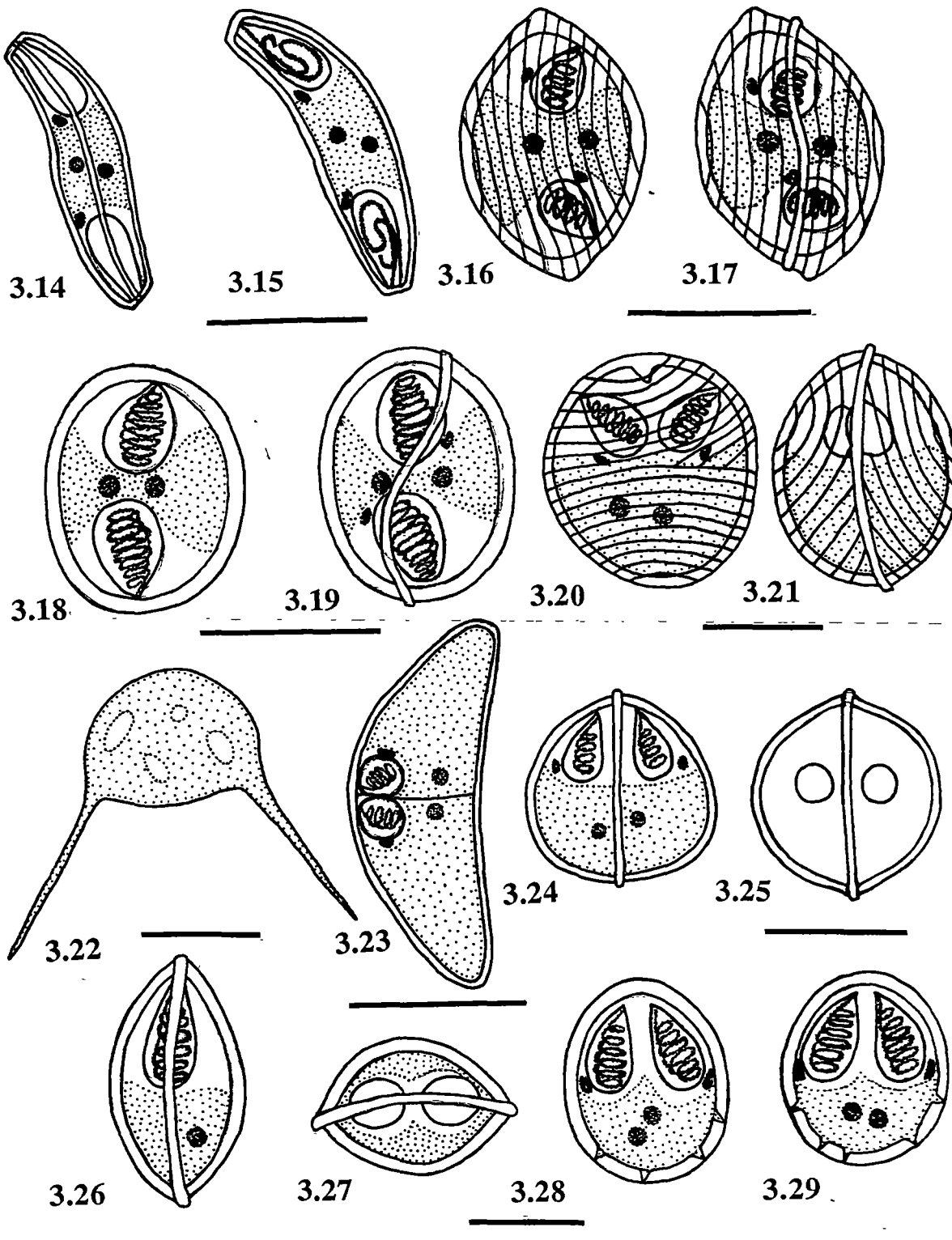
This myxosporean was found only in one of 23 *Nesogobius* sp.1 examined in the present study. The intensity of infection is relatively high, several hundreds of spores were observed in the gall bladder of host fish.

*Vegetative form*: Not found

*Spores*: The spores are elongate fusiform, arcuate in both valvular and sutural views. The ends of the spores are bluntly rounded to truncated, slightly curving in two directions. In valvular view, the spores are concave on one side and convex on the other. They are medium in size, measuring 20.5-23.7 (21.7) μm in length and 6.3-7.9 (6.6) μm in width. The spore valves are smooth, without striation. The sutural ridge is slightly curved. The two polar capsules are situated in the opposite poles of the spore. They are pyriform, 6.8-7.9 (7.6) μm in length and 3.1-3.3 (3.2) μm in width. The polar filaments fold 4-5 times parallel to the long axis of the spore within the polar capsules. The sporoplasm is situated in the space between the two polar capsules, two nuclei are visible amongst it. The capsular nuclei are often visible beneath the polar capsules.

*Remarks*

Laird (1953) divided the genus *Sphaeromyxa* into two groups. The*balbianii* group is characterized by straight or slightly curved fusiform or ovoid spores and ovoid polar




Figs. 3.14.-3.15. Spores of *Sphaeromyxa nesogobii.* 3.16-3.17. Spores of *Zschokkella leptatherinae.* 3.18-3.19. Spores of *Z. macrocapsula.* 3.20-3.21. Spores of *Ortholinea striateculus.* 3.22. Plasmodium of *Ceratomyxa arripica.* 3.23. Spores of *C. arripica.* 3.24-3.25. Spores of *Sphaerospora aldrichettae.* 3.26-29. Spores of *Myxobolus aldrichetti.* Bars = 10 µm in Figs. 3.14-3.19 and 3.22, 5 µm in Figs. 3.20-3.21 and 3.23-3.25.

capsules, while the *incurvata* group has the arcuate spores with pyriform polar capsules. It appears likely that the present species is a member of the *incurvata* group. Comparing the present myxosporean with other previously recorded species, *Sphaeromyxa nesogobii* resembles *S. parva* Dogiel, 1948 (Shulaman, 1966) from *Cololabis saira* and *Pholis pictus; S. hellandi* Auerbach, 1909 (Shulman, 1966) from *Phollis gunellus, Brosme brosme, Molva molva;* and *S. elegini* Dogiel, 1948 (Shulman, 1966) from *Eleginus gracilis* in having smooth spore valves and a slightly arcuate spore. However, *S. parva* and *S. elegini* are different markedly from those of the present species in having smaller spores (15.0-17.0 x 4.0 μm for *S. parva* and 17.0-20.0 x 6.0 μm for *S. elegini* ) and polar capsules (5.0 μm for *S. parva* and 5.0-6.0 μm for *S. elegini).* Although the dimensions of the spores and polar capsules of the present species are close to those of *S. hellandi,* the two species can be distinguished by two features: the spores of S. *hellandi* curve in one direction, while the spores of *S. nesgobii* curve in two different directions; the distance between the two polar capsules in *S. hellandi* is comparatively small and usually does not exceed the length of polar capsules, while in *S. nesogobii,* it is generally longer than the polar capsules and occasionally, it is slightly smaller than the polar capsules. In view of these morphological differences, geographical distribution as well as the host species; I designate the present species new and the name is given to refer to the host fishes.

3.3.2.2 Genus *Zschokkella* Auerbach, 1910

*Diagnosis:* Spores ellipsoidal in sutural view and slightly curved or semicircular in valvular view, with rounded or bluntly pointed ends; sutural ridge curved. Almost spherical capsules are situated at the opposite poles of the spore and open mostly slightly subterminally and both to one side.

*Zschokkella leptatherinae* sp. nov. (Figs. 3.16, 3.17; Pl. 3.2D-F).

Hosts: *Leptatherina presbyteroides, Atherinosoma microstoma, Kestratherina brevirostris, K. esox* and *K. hepsetoides.*

Location: Hepatic ducts and gall bladder.

Date: January 1990 to June 1992.

Type material: Holotype no. TMAG-K1310, from *L. presbyteroides* is deposited in the TMAG; paratypes no. 300367, from *A. microstoma;* no. 400038, from *K. esox* and no. 100061 from *K. brevirostris.*

Type host: *Leptatherina presbyteroides.*

This is a common parasite of atherinid fishes. It occurred very often during the study period. The prevalence of infection is up to 31% in *Leptatherina presbyteroides*. The intensity of infection is also high. Frequently, several thousands to several ten thousands of spores occurred in one individual fish.

*Vegetative form:* There are numerous plasmodia in the hepatic ducts of infected atherinids examined under the light microscope; they are rounded, ellipsoid or irregular (Pl. 3.2D). The plasmodia vary in size with the development of spores inside of them. Unsporulated plasmodia are slightly smaller (9-473 µm across) than sporulated plasmodia (38-520 µm across). Occasionally, some large plasmodia are visible with the naked eye. There are 7 to several thousands of spores in one plasmodium. The plasmodium has a thin surface membrane. The ectoplasm presents as a thin clear homogeneous layer. The endoplasm is coarse, basically dark grey with large clear areas and numerous yellow-brown vacuoles. Sporoblasts, in different developmental stages, are present in the endoplasm. Mature spores are found near the centre of plasmodium. Two spores are usually together suggesting a disporoblastic origin (Pl. 3.2E).

*Spores:* Mature spores are oval or ellipsoid in both valvular and sutural views, with slightly pointed ends when observed by oil immersion light microscope (Figs. 3.16, 3.17; Pl. 3.2F); they vary in length from 12.9 to 16.8 (15.3) µm and in width from 9.7 to 13.8 (11.8) µm. Both spore valves are vaulted and moderately thick. They meet meridionally to form a slightly curved sutural ridge which is approximately 1.5 µm wide. Ten to 12 fine longitudinal striations are visible on the spore valve. The polar capsules are almost oval with slightly attenuated distal ends. They are situated symmetrically at opposite poles of the spore, opening at some distance from the end of the spore and both to one side. They are equal in size, 3.3-5.0 (3.9) µm in length and 2.9-4.2 (3.4) µm in width. The polar filament is regularly coiled into a pyriform spiral, usually with four to five coils which lies perpendicular to the long axis of the capsules. When extruded, the length of polar filament measures 18.0-34.1 (22.5) µm in stained specimen. A large sporoplasm lies between the polar capsules, among it are two small spherical nuclei measuring 1.3-1.4 µm. Two capsulogenic nuclei lie on the periphery of the polar capsules.

*Remarks*

Moser *et al.* (1989) reported the first Australian species of *Zschokkella* from the Great Barrier Reef, Queensland. The present species differs markedly from that species, Z.

*heronensis*, in the position and opening of polar capsules and the dimension of the spore. Comparing the present species with other previously described species from the world, there are two which show close resemblance to the present species: *Z. orientalis* Konovalov and Shulman, 1966 in the gall bladder of *Oncorhynchus gorbuscha, O. kisutch, O. mykiss* (Walbaum), *O. tschawytscha, Salvelinus alpinus* (Shulman, 1966), and *S. leucomenes* from the former USSR (Shulman, 1966); and *Z. russelli* in the gall bladder and hepatic ducts of *Gaidropsarus mediterraneus* and *Ciliata mustela* from England and North America (Tripathi, 1948a; Davies, 1985). However, the spore of *Z. orientalis* is narrower and smaller than that of the present species. Although morphologically similar, *Z. leptatherinae* can be distinguished from *Z. russelli* in the following characteristics: the spores of *Z. leptatherinae* are oval or ellipsoid in valvular view and have narrow pointed ends whilst the spores of *Z. russelli* are gibbous in valvular view and have rounded ends; spore valves of *Z. leptatherinae* have 10-12 slightly curved longitudinal striations whilst those of *Z. russelli* have 9-18 straight striations; polar capsules of *Z. leptatherinae* are oval and open at some distance from the end of the spore whilst those of *Z. russelli* are spherical and open more or less terminally; extruded polar filaments of *Z. leptatherinae* are 18.0-34.1 µm in length whilst those of *Z. russelli* are 37-50 µm in length; moreover, the dimensions of plasmodia of *Z. leptatherinae* are 9-520 µm whilst those of *Z. russelli* are only 10-180 µm. Finally, *Zschokkella leptatherinae* seems to have a relatively high host specificity. At the study site it only infects atherinid fishes and six other sympatric fish species were not infected. For these reasons I consider *Z. leptatherinae* to be a new species. The name is given following the fish in which the highest prevalence of infection was detected.

*Zschokkella macrocapsula* sp. nov. (Figs. 3.18, 3.19; Pl. 3.3A)
Hosts: *Leptatherina presbyteroides* and *Atherinosoma microstoma.*
Location: Gall bladder.
Date: August, September, November and December 1990.
Type material: Holotype no. TMAG-K1302 is deposited in the TMAG.
Type host: *Leptatherina presbyteroides.*

*Zschokkella macrocapsula* was found in two atherinid species. Only three of 589 *L. presbyteroides* and two of 514 *A. microstoma* examined were found to be infected and the intensity of infection is also low.

*Vegetative form:* Not found.

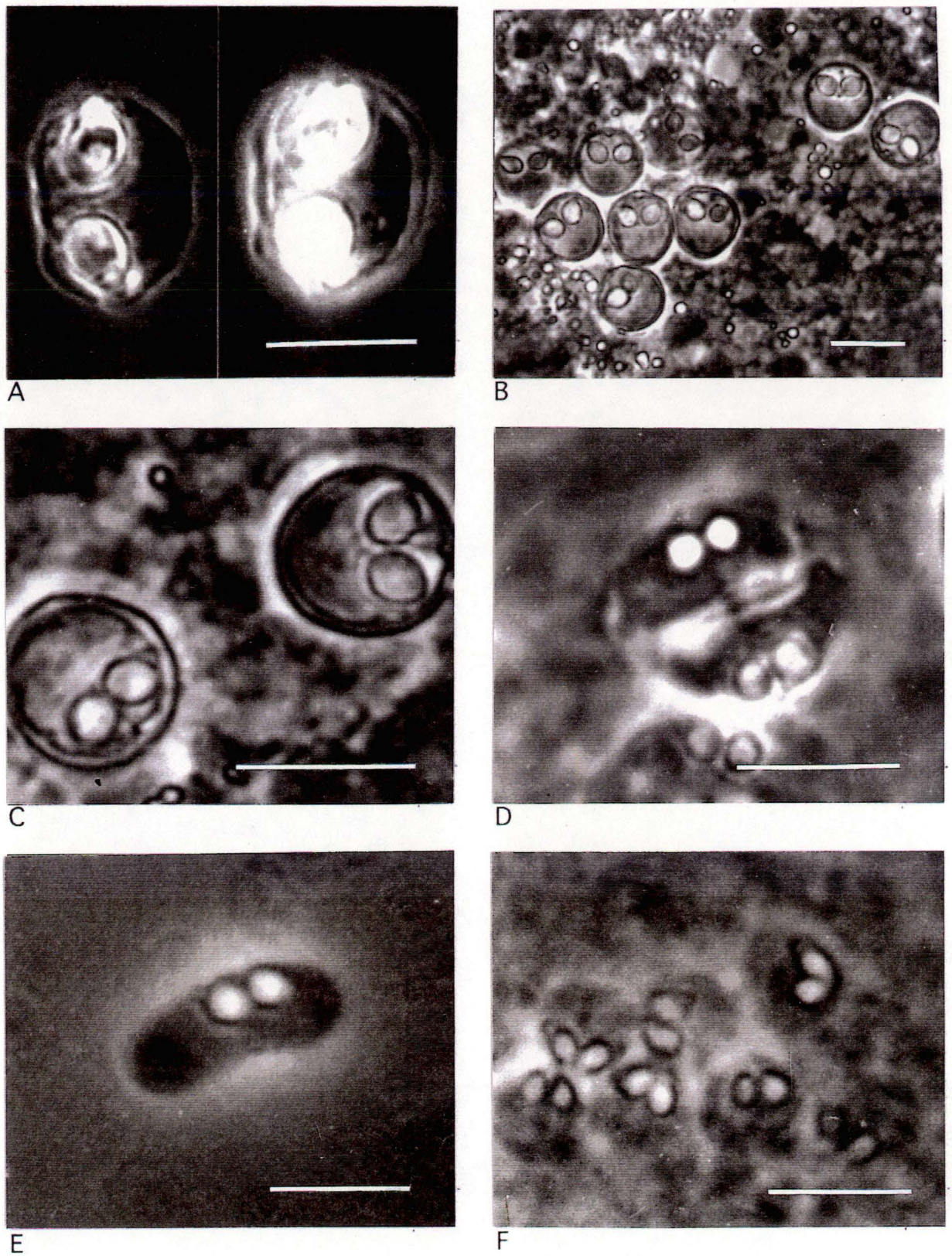



Plate 3.3. Fresh spores of myxosporeans. A. *Zschokkella macrocapsula*. B. *Ortholinea striateculus*. C. Enlargement of *O. striateculus*. D-E. *Ceratomyxa arripica*. F. *Sphaerospora aldrichettae*. All bars = 10 μm.

*Spores:* The spores are ellipsoidal or ovoid on both valvular and sutural views. The sutural ridge is indistinct, running from one pole of the spore to one side before reaching the other pole. The spore valves are smooth and thicken. Measurements were made on 20 fresh spores. The spores are 14.0-17.5 (16.4) µm long and 12.6-14.6 (12.9) µm wide. Two polar capsules are ovoid or pyriform in shape and open near the poles of spore. They are relatively large and both are equal in size, measuring 6.3-8.4 (6.9) µm in length and 4.3-6.2 (5.1) µm in width. The polar filaments coil 8-10 times within the polar capsules. The sporoplasm is situated between the two polar capsules. Two nuclei are present among homogeneous sporoplasm; the diameter of nuclei is c.1.4 µm.

*Remarks*

The overall morphological characters of spores of the present species resembles those of *Z. embiotocidis* Moser and Haldorson, 1976 from the gall bladder of *Damalichthys vacca* and *Embiotoca lateralis* in USA and *Z. rovigenesis* Nemeczek, 1922 from the urinary bladder of *Scorpaena scrofa* and *S. porcus* in Italy. However, *Z. macrocapsula* differs from those two species in the size of the polar capsule and the number of coils of the polar filament. Both *Z. embiotocidis* and *Z. rovigenesis* have smaller polar capsules and fewer coils in the polar filament than *Z. macrocapsula.* The measurements of spores of *Z. macrocapsula* most closely resemble those of *Z. meglitschi* Moser and Noble, 1976 in macrourid fish from California. However, the shape in sutural view of the two species is distinctly different and the dimensions of the polar capsule are greater in the present species. Moreover, the polar filament in *Z. meglitschi* has 6-8 coils. In view of these morphological differences and geographical distribution, I consider the species described here to be new. Its trivial name is proposed to refer to the size of the polar capsules.

3.3.2.3 Genus *Ortholinea* Shulman, 1962

*Diagnosis*: Spherical or subspherical spores are slightly flattened parallel to the sutural plane; the two polar capsules are set wide apart in a sutural plane, with capsular foramina directed away from each other at an angle.

*Ortholinea striateculus* sp. nov. (Figs. 3.20, 3.21; Pl. 3.3B, C)

Host: *Leptatherina presbyteroides.*

Location: Ureta.

Date: November 1990.

Type material: Holotype no. TMAG-K1303 is deposited in the TMAG.

*Ortholinea striateculus* was only found in one atherinid species. Both the prevalence and intensity of infection are low.

*Vegatative form:* Not found

*Spores:* The spores are subspherical in valvular view and oval in sutural view. Measurements were made on 20 fresh spores. The size of the spores range from 9.1 to 10.5 (10.1) μm in length and 8.9 to 10.4 (10.0) μm in width. There are 18-20 striations on the surface of the spore valves. A conspicuous triangular intercapsular process is visible at the anterior and of the spore. The sutural ridge is straight. The two polar capsules are equal in size and pyriform in shape, with proximal ends close to each other but the openings are widely separated from each other at the anterior and of the spores. The polar capsules are 3.4-3.6 (3.5) μm long and 2.8-3.1 (2.9) μm wide. The polar filaments coil 5-7 times. The sporoplasm occupies almost all the extra-capsular space, just below the polar capsules. Two sporoplasm nuclei are visible even in the fresh specimens. When exposed to freshly-prepared Lugol's solution, no iodinophilous vacuole was found in the sporoplasm.

*Remarks*

The genus *Ortholinea* was established by Shulman in 1962. To date, only eight species have been reported: *O. polymoroha* Davis, 1917 (Lom and Dykova 1992) from the urinary bladder of *Opsanus tau* and *O. beta* in USA; *O. divergens* Thelohan, 1895 (Shulman 1966) from the uriniferous tubules of kidney and urinary bladder of *Hippoglossoides platessoides limandoides, Blennius folis, Crenilabrus melops, C. pava* and *Pleuronichthus verticalis* in the former USSR, Norway and USA; *O. orientalis* Shulman and Shulman-Albova, 1953 (Shulman 1966) from the urinary bladder and gall bladder of *Clupea harengus pallasi, C. harengus pallasi n. maris-albi, Eleginus navaga* and *E. gracilis* in the former USSR; *O. irregularis* Kabata, 1962 from the urinary bladder of *Drepanopsetta platessoides* in Great Britain; *O. gobiusi* Naidenova, 1968 from the urinary bladder of *Gobius ophiocephalus* in the Black Sea (Lom and Dykova 1992); *O. undulans* Meglitsch, 1970 from the urinary bladder and ureters of *Caulopsetta scapha* and *Peltorhamplus novaezelandiae* in New Zealand; *O. alata* Kent and Moser, 1990 from the lumens of the renal tubules and collecting ducts of *Chaetodon rainfordi* in Australia; *O. australis* Lom *et al.,* 1992 from the hepatic ducts and gall bladder of *Acanthopagrus australis* and *Rhabdosargus* sarba in Australia.

The present species differs markedly from *O. alata* in the absence of wing-like projections at the posterior end of the spore, the latter may belong to a different order according to

Lom and Dykova (1992). It can also be distinguished from *O. australis* in having a triangular intercapsular process and the arrangement of polar capsules. The number of striation on the surface of spore valves, the coil of polar filaments as well as the location of *O. striateculus* and *O. australis* are also different. The spores of *O. orientalis* and *O. gobiusi* can be distinguished from the present species in the shape of spores, which taper to a sharp point at their posterior ends and *O. irregularis* differs from the present species in having irregular, pyriform spores. The absence of intercapsular process, different hosts and geographical distribution separates the present species also from *O. polymorpha* and *O. divergens*. The spores of *O. dundulans* differ in the same way and also the sutural ridge is undulant in this species. Based on these differences, I consider *Ortholinea striateculus* to be new. The name refers to the characteristics of spore valves.

3.3.2.4 Genus *Ceratomyxa* Thelohan, 1892

*Diagnosis*: Spores greatly drawn out in direction perpendicular to suture. Width exceeds more than twice their length. Subspherical polar capsules usually open on one pole of the spore.

*Ceratomyxa arripica* sp. nov. (Figs. 3.22, 3.23; Pl. 3.3D, E)

Host: *Arripis trutta.*

Location: Gall bladder.

Date: January 1991, March 1992.

Type material: Holotype no. TMAG-K1304 is deposited in the TMAG.

This Myxosporean was found in two of eight host fish, *Arripis trutta.* The intensity of infection is relevativley low, approximately 20 plasmodia were found in the smear preparations of the gall bladder.

*Vegetative form:* The plasmodia are typically rounded or ovoid with one or two elongated pseudopodia (Fig. 3.22). The plasmodia are 8.3-12.6 (10.3) μm long and 5.0-7.9 (6.3) μm wide. The length of pseudopodia is 8.2-25.3 (15.7) μm. Two spores are often seen lying side by side within one plasmodium (Pl. 3.3 D).

*Spores:* The spores are crescent-shaped, small and symmetrical. In sutural view, the anterior margin is arched and the posterior margin is usually nearly straight, with a very slight concavity which is sometimes more evident near the suture (Fig. 3.23, Pl. 3.3D, E). The spore valves are thin and delicate; the ends are rounded and direct laterally. A

straight suture line runs through the middle of the spore. Measurements were made on 20 fresh spores. The length of the spore is 4.8-5.1 (4.9) μm and the width is 9.3-11.3 (10.2) μm, more than twice the length. The two polar capsules are equal and spherical or subspherical. They are located close to each other in the anterior part of the spore next to the suture line and are 1.8-2.0 (1.9) μm in diameter. The polar filaments show 3-4 indistinct coils within the polar capsules. The length of extruded filaments is 31.1-50.6 (44.6) μm. Two sporoplasm nuclei and two capsular nuclei are visible.

*Remarks*

Two *Ceratomyxa* species have been previously reported from Australia: *Ceratomyxa sprenti* Moser *et al.*, 1989 and *C. rohdei* Moser *et al.*, 1989. Both species were found in the gall bladder of several fish species from Heron Island, Queensland. The species described here is readily distinguished from those two species in the size and shape of spores. About 140 *Ceratomyxa* species have been previously reported. Comparing the present species with other described species from elsewhere, there are four which show close resemblance to the present species in general features of the spores: *C. americana* Wierzbicka, 1987a from the gall bladder of *Scomber scomber; C. inconstans* Jameson, 1929 from the gall bladder of *Scomber japonica, Usacaranx lutescens, Trachurus novae-zelandiae* and *Helicolenus percoides* (Meglitsch, 1960); *C. faba* Meglitsch, 1960 from the gallbladder of *Caulopsetta scapha* and *C. gobioidesi* Chakravarty, 1939 from the gallbladder of *Odantoamplyopus rubicundus*. However, the spores of *C. americana* differ greatly from the present described species in having sharpened spores ends and they direct downward. *C. inconstans* can be distinguished from the present species in having subequal or unequal spore valves and the posterior margin varies from convex to very concave. Although the spores of *C. fabe* and *C. gobioidesi* resemble, to some extent, those of *C. arripica,* they are distinguished from the present species in having larger spores and polar capsules. Moreover, the spore valves of *C. fabe* generally terminate in extremely broad ends. Based on these morphological differences and the host records, the present species is considered distinct and designated as *Ceratomyxa arripica* following the fish it infected.

3.3.2.5 Genus *Sphaerospora* Thelohan, 1892

*Diagnosis:* Spores spherical or subspherical. Two polar capsules located along one pole of spore in a plane perpendicular to the plane of suture. Valves smooth or with ridges, often with lateral protuberances or bumps. Polar capsules subspherical or pyriform.

*Sphaerospora aldrichettae* sp. nov. (Figs. 3.24, 3.25; Pl. 3.3F)
Host: *Aldrichetta forsteri.*
Location: Gall bladder.
Date: February 1991, May 1992.
Type material: Holotype no. TMAG-K1305 is deposited in the TMAG.

This myxosporean was found in three of 11 *Aldrichetta forsteri.* The intensity of infection is lower. The maximum number of spores observed in one fish was 30.

*Vegetative form:* Not found

*Spores:* The spores are spherical or subspherical in both valvular and sutural views. The spore valves are thin, smooth and equal in size. Measurements were made on 20 fresh spores. The spores are 6.3-7.0 (6.7) µm long and 6.2-7.0 (6.6) µm wide. The sutural ridge is straight. The two polar capsules are situated at one pole of the spores. They are equal in size and pyriform in shape, and are 2.1-2.8 (2.5) µm long and 1.5-1.8 (1.6) µm wide. Usually 4 coils of the polar filaments can be seen inside the polar capsules. Two capsulogenous nuclei are situated below the polar capsules. The sporoplasm is underneath the two polar capsules; two small compact nuclei are visible within the sporoplasm.

*Remarks*
To date, 48 *Sphaerospora* species have been reported from fish (Lom and Dykova, 1992). Most of these are coelozoic in the urinary system. Some are histozoic and parasitize a variety of tissues; others are coelozoic in the gall bladder (Arthur and Lom, 1985; Supamattaya *et al.,* 1991). Nine *Sphaerospora* species were described from marine or estuarine fishes. The present described species is distinguished from *S. elegans* Thelohan, 1892 in *Gasterosteus aculeatus* (Shulman, 1966); *S. renalis* Bond, 1938 in *Fundulus heteroclitus; S. sphaerica* Dogiel, 1948 in *Sphaeroides pardale* (Shulman, 1966); *S. brevis* Polyansky, 1955 in *Myoxocephalus scorpius* (Shulman, 1966); *S. araii* Arthur and Lom, 1985 in *Raja rhina; S. epinepheli* Supamattaya *et al.,* 1991 in *Epinephelus malabaricus* and *S. testicularis* Sitja-Bobadilla and Alvarez-Pellitero, 1990 in *Dicentrarchus labrax* by the small size of spores, site of infection and geographical distribution. Two species have been reported previously from the gall bladder of marine fishes. But both are morphologically different from *S. aldrichettae.* The spores of *S. periophami* Fantham and Porter, 1943 from *Periophthalmus koelreuteri* and

*Boleophthalmus dentatus* are larger and those of *S. mayi* Moser *et al.*, 1989 from *Atherinomous capricornensis* are smaller than new species. The sutural ridge is central in *S. aldrichettae*, but not in *S. mayi*. Furthermore, most species of *Sphaerospora* appear to be highly host specific and are known only from a single host species (Arthur and Lom, 1985). Based on the above reasons, I consider *S. aldrichettae* to be new; the trivial name refers to the host fish.

3.3.2.6 Genus *Myxobolus* Butschli, 1882

*Diagnosis:* Spores ellipsoidal, ovoid or rounded in valvular view, and biconves in sutural view. Spore valves smooth. Two polar capsules mostly pyriform, lie solely in the sutural plane. Most species have an iodinophilous vacuole.

*Myxobolus aldrichetti* sp. nov. (Figs. 3.26-3.29; Pl.3.4A, B)
Host: *Aldrichetta forsteri.*
Location: Gills.
Date: February 1991.
Type material: Holotype no. TMAG-K 1306 is deposited in the TMAG.

One of 11 fish examined were found to be infected by *Myxobolus aldrichetti.*

*Cysts:* The cysts appear as ellipsoidal, opaque whitish pustules in the gill tissue. There are 8-21 cysts per infected gill arch and there are 55 cysts in the infected fish. The diameter of cysts ranging from 850 µm to 1 mm. Cysts often protrude from the surface of fish gills, and can be detected with the naked eye (Pl. 3.4A). Under the microscope, thousands of spores can be seen in each cyst.

*Spores:* The spores are very regular in shape; they are ellipsoidal or ovoid in valvular view and broad lenticular in sutural and end views (Figs. 3.26-3.29; Pl. 3.4B). The length of the spores is 8.4-10.5 (9.7) µm and the width is 7.0-8.4 (7.7) µm. The spore valves are symmentrical and uniform in thickness, with 4-5 triangular folds on the posterior margin. The sutural ridge is distinct, broad, uniform and straight. The intercapsular process is absent. The two polar capsules are elongated pyriform and occupy at least the anterior half of the length of the spores. The two polar capsules are of equal size, with dimensions of 4.2-5.2 (4.7) µm in length and 2.8-3.0 (2.8) µm in width. The polar filament usually coil 6-7 times and remains in a tight and regular

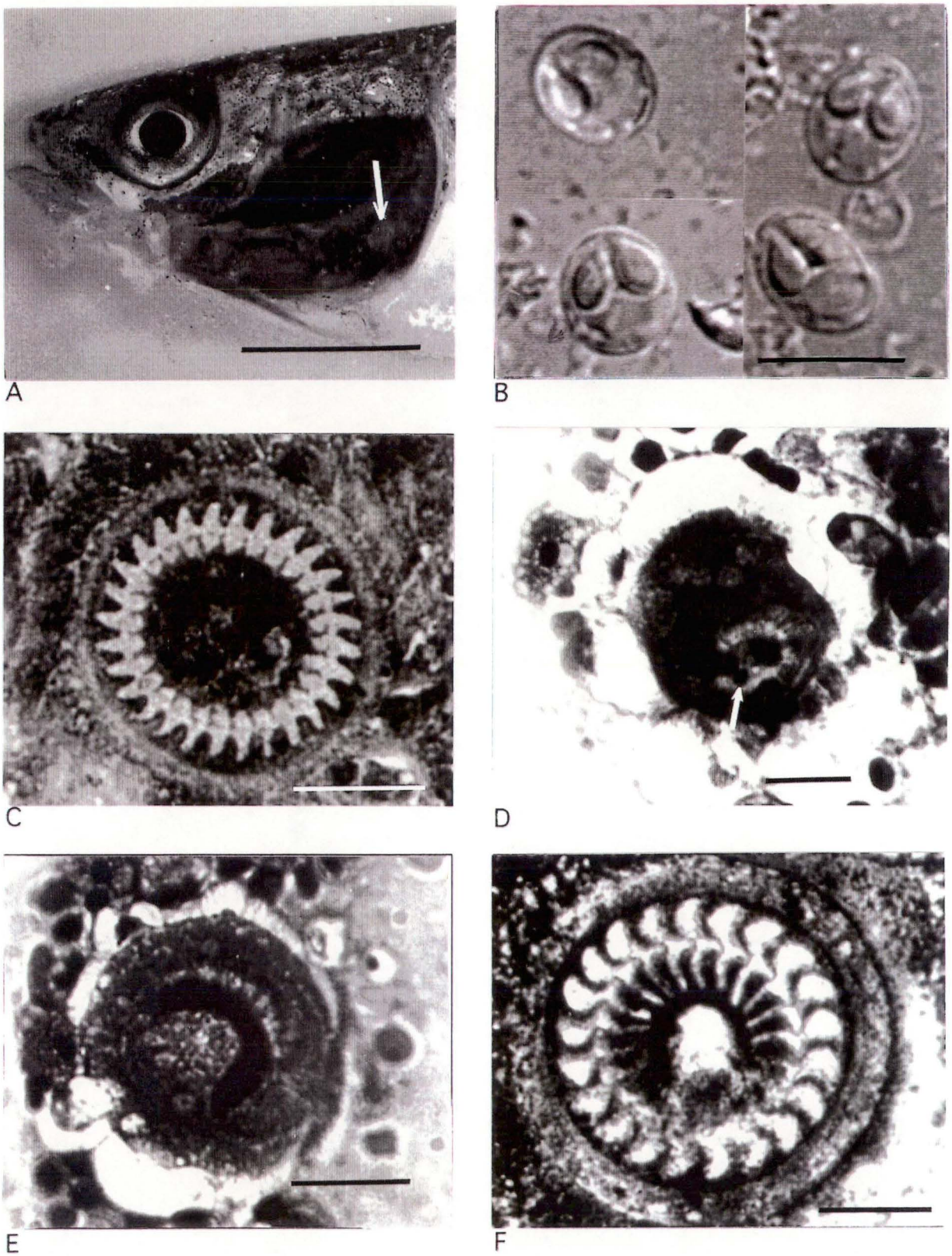



Plate 3.4. Myxosporeans and trichodinids. A. Cysts of *Myxobolus aldrichetti* on the gills of *Aldrichetta forsteri*. B. Fresh spores of *M. aldrichetti*. C. Silver impregnated *Paratrichodina tasmaniensis*. D-E. H & E stained *P. tasmaniensis* showing the macro- and micronucleus (arrow). F. Silver impregnated *Trichodina australis*. Bar = 1 cm in A and 10 μm in B to F.

fashion inside the polar capsules. Two rod-shaped compact capsulogenous nuclei are situated below the two polar capsules. The sporoplasm occupies the whole space behind the polar capsules. Two spherical nuclei are amongst the sporoplasm. An iodinophilous vacuole is not revealed by staining with Lugol's solution.

*Remarks*

To date, four species of *Myxobolus* have been described in freshwater fishes from Australia. These are: *Myxobolus (Myxosoma) ogilbyi* Johnston and Bancroft, 1918 on the gill arches of *Macquaria ambigua; M. plectroplites* Johnston and Bancroft, 1918 in the gall bladder and kidney of *Macquaria ambigua; M. gadopsii* Langdon, 1990 in the muscle and connective tissue of *Gadopsis bispinosus* and *G. marmoratus;* and *M. galaxii* Langdon, 1990 in the spinal cord of *Galaxias olidus*. Although the infection of marine fish by *Myxobolus* species have been previously reported (Moser *et al.*, 1989; Rothwell and Langdon, 1990), the present species is the first described species of *Myxobolus* from Australian marine fish. This species can be readily distinguished from *M. plectroplites* in having no iodinophilous vacuole and from *M. ogilbyi* and *M. gadopsii* in the shape and dimensions of spores. Although the general appearance of *Myxobolus aldrichetti* is similar to that of *M. galaxii* and the dimensional ranges fall within those of that species, the present species differs from *M. galaxii* in its location and having only 4-5 triangular folds on the posterior margin of spore valves; while *M. galaxii* with 8-9 folds around. In a comparison of new species with other previously reported marine and estuarine species from the world, the present species shows close resemblance to *Myxobolus (Myxosoma) squamalis* Iversen, 1954 from the scales of *Oncorhynchus mykiss*, *O. keta* and *O. tshawytscha* and *Myxobolus (Myxosoma) intestinalis* Narasimhamurti, 1969 from the intestine of *Mujil waigensis*. However, both these species are distinguished from the species described here in having no any fold of spore valves. Also, the polar filaments of *Myxobolus squamalis* only coil 4 times and the dimensions of the polar capsules of *Myxobolus intestinalis* are smaller than those of the present species. Furthermore, *Myxobolus intestinalis* has not been found on external surface of fish. In view of these differences, the present form is described as a new species and the name *Myxobolus aldrichetti* is proposed following the host fish.

### 3.3.3 Phylum Ciliphora

*Diagnosis:* Possess cilia at least one stage of life cycle, two types of nuclei; most parasitic forms are on gills and body surface, only a few species occur in the viscera and the blood.

3.3.3.1 Genus *Paratrichodina* Lom, 1963

*Diagnosis:* Adoral spiral makes an incomplete turn of 150°-280°. Denticles of the *Trichodina*-type; if there is an anterior projection at the base of the blade it does not interlock with the notch in the blade of the preceding denticle.

*Paratrichodina tasmaniensis* sp. nov. (Fig. 3.30-3.31, Pl. 3.4 C, D, E)

Hosts: *Atherinosoma microstoma, Leptatherina presbyteroides, Kestratherina brevirostris, K. esox* and *K. hepsetoides.*

Location: Gills.

Date: January 1990 to March 1993.

Type material: Holotype no. TMAG-K1290, from *Atherinosoma microstoma* is deposited in the TMAG; paratypes no. 901004, from *Leptatherina presbyteroides* and no. 901008, from *Kestratherina brevirostris* are in the collection of author.

Type host: *Atherinosoma microstoma.*

This is a common parasite of atherinids during the warm seasons. The prevalence and intensity of infestation are both high. Frequently, several thousands to several ten thousands of the ciliate occur in one individual fish.

The ciliate is small with disc-shaped body in both adoral and aboral views, 28.4-42.7 (32.7) µm in diameter (Pl. 3.4C). The adoral ciliary spiral turns about 150-200° around the oral zone (Fig. 3.30). The adhesive disc consists of denticulate ring, border membrane and radial pins. The adhesive disc is concave, 23.7-31.5 (27.0) µm in diameter; surrounded by a border membrane, which is 2.1-2.8 (2.5) µm in width. The centre of adhesive disc is dark in silver impregnated preparations. The diameter of the denticulate ring is 8.4-16.8 (13.1) µm. The number of denticles is 26-29 (27). Each denticle is 1.7 (1.5-1.8) µm long. The blades are well-developed, triangular in shape and slanted anteriorwards. The thorns are stunted and 1.1 (0.9-1.4) µm in length. The central parts are indistinct and closely spaced, 0.6 (0.4-0.7) µm wide (Fig. 3.31). There is a small dot in the blade, which corresponds to the ellipsoidal foramen in fresh specimens and scanning electron micrographs. There are 4-6 (5) radial pins per denticle. The macronucleus is crescent-shaped or horseshoe-shaped, measuring 15.8-25.3 (20.2) µm in length and 4.9-6.3 (5.7) µm in width. The distance between the two ends of the arm of the macronucleus is 14-20 (16) µm. The micronucleus is oval or rounded, usually situated towards one end of the macronucleus. The round micronucleus measures 1.8-

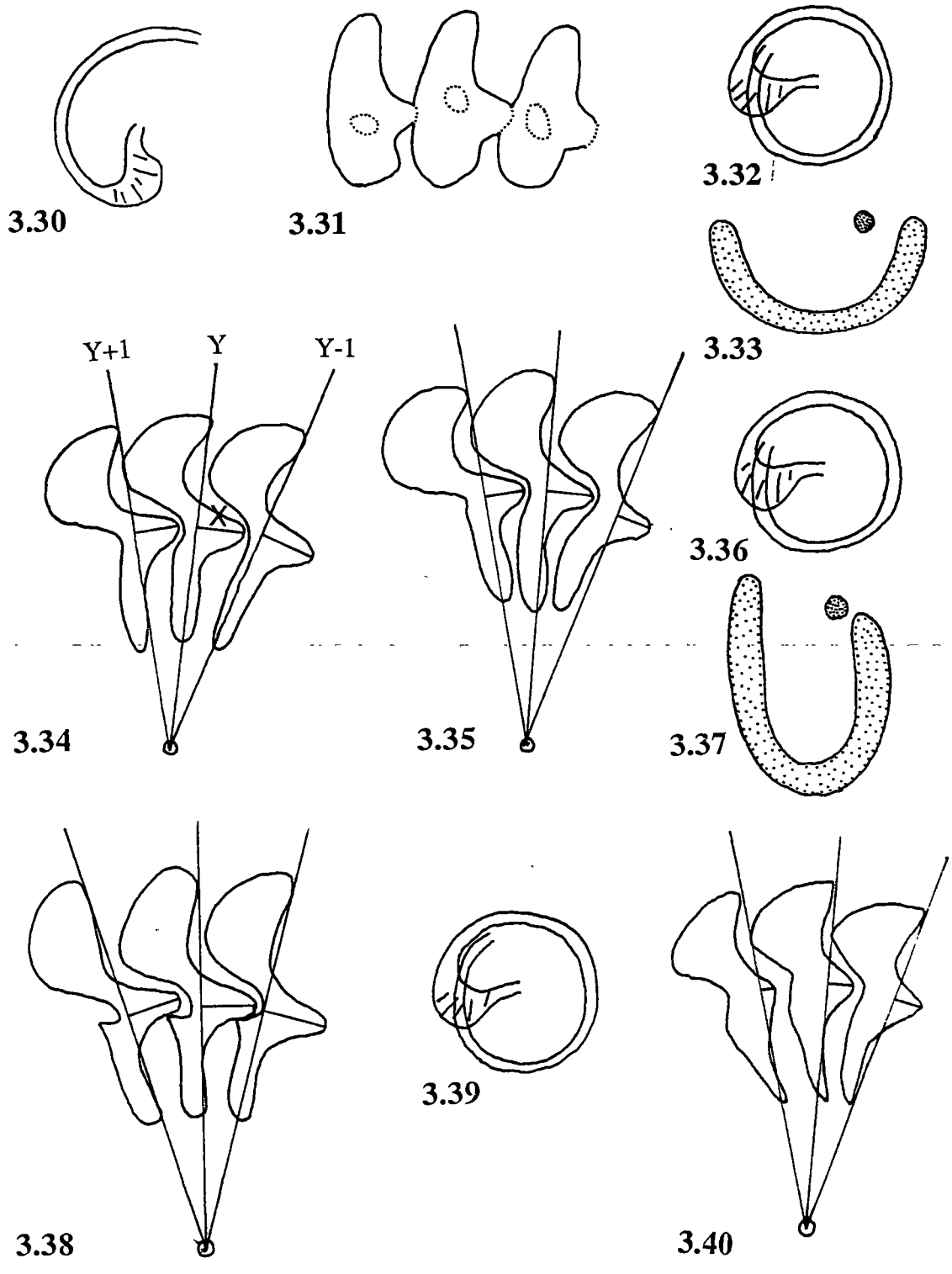



Fig. 3.30. Oral zone of *Paratrichodina tasmaniensis.* 3.31. Denticles of *P. tasmaniensis*. 3.32. Oral zone of *Trichodina australis.* 3.33. Nuclei of *T. australis.* 3.34-3.35. Denticles of *T. australis.* 3.36. Oral zone of *T. jadranica.* 3.37. Nuclei of *T. jadranica.* 3.38. Denticles of *T. jadranica.* 3.39. Oral zone of *T. nesogobii.* 3.40. Denticles of *T. nesogobii.*

3.2 (2.8) μm across and the oval micronucleus is 3-3.2 (3.1) μm long and 1.7-2.0 (1.9) μm wide (Pl. 3.4 D, E).

*Remarks*

The taxonomy of mobile peritriches is based upon two main criteria: development of the oral apparatus and structure of the adhesive disc (Lom, 1963; Raabe, 1963; Lom and Haldar, 1977). The adoral ciliary spiral makes an incomplete turn in four fish-invading genera of the family Trichodinidae (Basson and Van As, 1989). The ciliate described here is seems to be a member of the *Paratrichodina* since it has the *Trichodina* -type denticles.

To date, 10 *Paratrichodina* species have been reported from fish. Seven of these were found on the gills and three were described in the urinary tract. *Paratrichodina tasmaniensis* is markedly different from all described species of this genus in having a foramen in the denticle. Also, the stunted thorns and indistinct central parts can easily separate the present species from other described members. Based on these, I consider it to be new and the species name given alludes to the locality.

3.3.3.2 Genus *Trichodina* Ehrenberg, 1831

*Diagnosis:* Denticles consist of blades, central parts and thorns. Blades straight or curved, thorns rod-like, spine- or needle-shaped of various lengths. Central parts lack anteriorly directed projections. Adoral spiral of various lengths. 360-540°.

*Trichodina australis* sp. nov. (Figs. 3.32-3.35, Pls. 3.4F, 3.5A-B)

Hosts: *Atherinosoma microstoma, Leptatherina lebysteroides, Kestratherina brevirestris, K. esox* and *K. hepsetoides*.

Location: Gills.

Data: January 1990 to March 1993.

Type material: Holotype no. TMAG-K1299, from *A. microstoma;* paratype no. 903012, from *L. presbyteroides* is in the collection of author.

Type host: *Atherinosoma microstoma.*

This ciliate was recorded from the same hosts as *Paratrichodina tasmaniensis*. In some cases, these two species occur simultaneously on the same host. However, the prevalence and intensity of infestation of *Trichodina australis* is lower than those of *Paratrichodina tasmaniensis*. Between four and 2032 of this ciliate were recorded from

infested atherinids.

The ciliate is disc-shaped (Pls. 3.4F, 3.5A). The adoral ciliary spiral turns about 370-380° (Fig. 3.32). The body is medium-sized with a diameter of 41.5-53.7 (45.2) µm. The adhesive disc is concave, 31.6-44.2 (35.7) µm in diameter. The diameter of the denticulate ring is 17.4-22.0 (20) µm. The number of denticles is 20-24 (22). The length of denticles is 5.0-6.0 (5.3) µm. The blades are broad, sickle-like curved with rounded outward ends, filling the large portion between the y and y + 1 axes. The tangent points are rounded, situated at the same level as the flat distal surface or slightly below. The posterior margin indentation forms a crescent arch with the y axis. The deepest point is below the apex of the anterior margin. The anterior margin of the blade extends to the y + 1 line, sometimes extending beyond. The anterior and posterior apophyses of the blade are not visible. The sections connecting the blade and central part are thin. The tips of the central part are rounded and extend more than halfway towards the y -1 line. The width of the central part is 0.9-1.4 (1.2) µm. The length of the blade is 4.1-4.5 (4.4) µm. The thorns are slightly slanted, pointing in the posterior direction on the y axis and in most cases, extend beyond the y line. The thorn tapers slightly to a round tip. The sections connecting the central part and the thorn are slightly thicker than those connecting the blade and central part. There are no anterior and posterior apophyses on the thorn. The length of the thorn is 5.3-5.8 (5.5) µm. The ratio between the denticle above and denticle below the x axis is 0.89-1.00 (0.97) (Figs. 3.34-35, Pls. 3.4F, 3.5A). A well developed border membrane surrounds the denticulate ring; the width of the membrane is 4.7-5.8 (5.2) µm (Pl. 3.4F). The radial pins are not always defined in the silver impregnated preparation, but in the fresh specimen or under the SEM, 6-8 radial pins per denticle are clearly visible (Pl. 3.5B). There is a central circle in the centre of the adhesive disc; the diameter is 6.5-7.8 (6.8) µm (Pls. 3.4F, 3.5A). The macronucleus is horseshoe shaped, 28.4-44.2 (36.6) µm in diameter. The distance between the two ends of the arm of the macronucleus is 7.9-14.8 (11.9) µm. The micronucleus is ovoid and measures 3.0-3.2 (3.1) x 1.6-2.0 (1.8) µm across (Fig. 3.33).

*Remarks*

Although infestations by trichodinid protozoans have been reported from freshwater and marine fish in Australia (Langdon and Humphrey, 1985; Langdon, 1988; Rowland and Ingram, 1991), no species were named or comparative descriptions given. This is the first described *Trichodina* species from Australia. In comparison with other previously

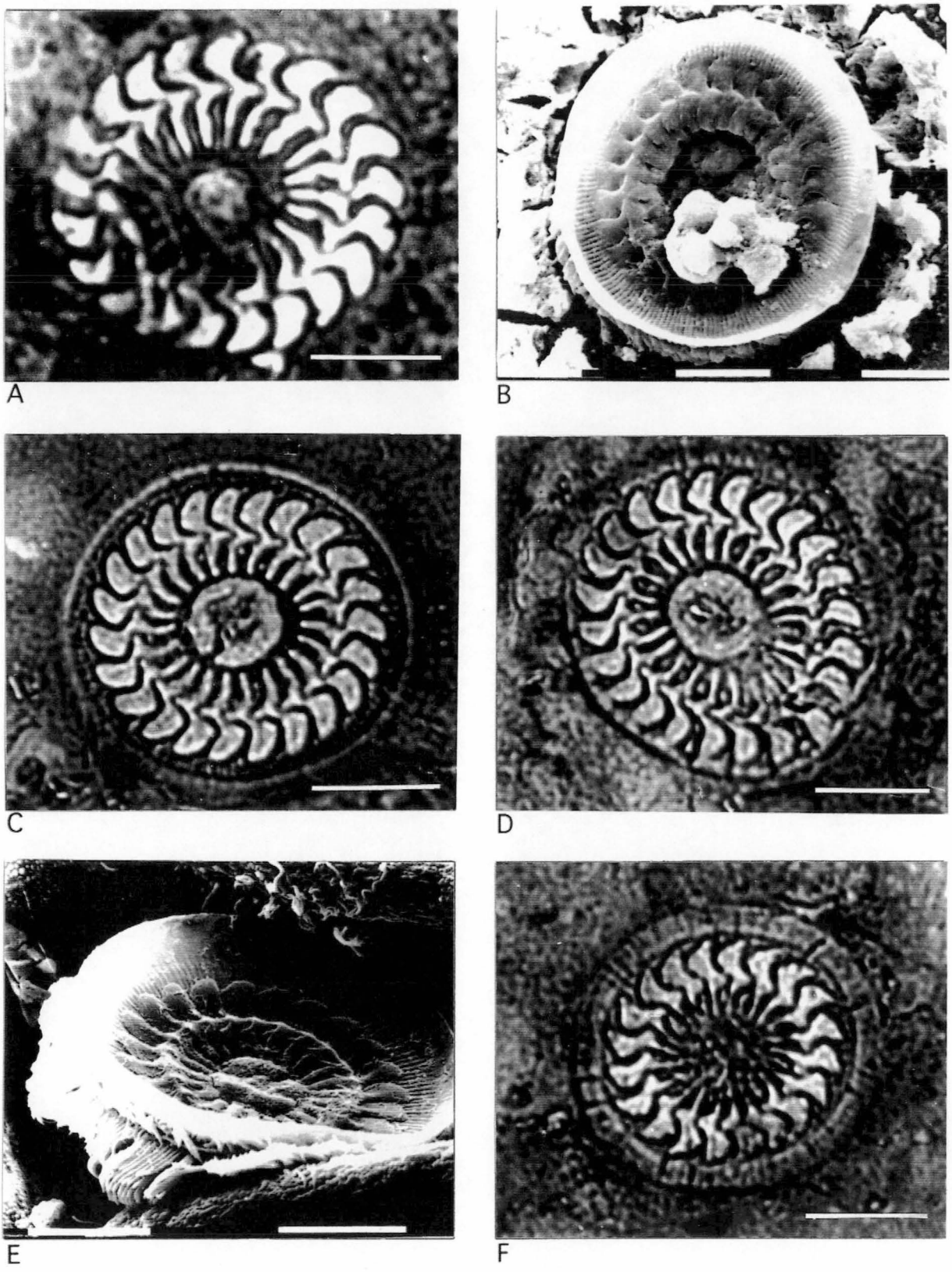



Plate 3.5. Trichodinids. A. Silver impregnated *Trichodina australis*. B. SEM micrograph of *T. australis*. C-D. Silver impregnated *T. jadranica*. E. SEM micrograph of *T. jadranica*. F. Silver impregnated *T. nesogobii*. All bars = 10 µm.

recorded species in the world, two species were found to have resemblance to *T. australis*. The dimaeters of the body, adhesive disc and denticulating ring of *Trichodina cottidarum* Dogiel, 1948 from the gills of *Myoxocephalus scorpius* and *M. octodecemspinosus* (Lom and Laird, 1969) are close to those of *T. australis*, but the shape and number of the denticles in the two species are quite different. Although the morphology of denticles of *T. ovonucleata* Raabe, 1958 from the gills of *Ophidion rochei, Gaidropsarus mediterranaeus, Crenilabrus quinquemacula, C. griseus, Radulinus asprellus* and *Blennius tentacularis* (Lom, 1970a) is similar to that of *T. australis*, *T. ovonucleata* is well distinguished from the new species in the oval shape of the macronucleus and lack of a central circle. In view of these difference, I consider *T. australis* to be new; the trivial name reflects the country in which it is found.

*Trichodina jadranica* Raabe, 1958. (Figs. 3.36-3.38; Pl. 3.5C, D, E)
Host: *Nesogobius* sp.1.
Location: Gills.
Date: October 1990, April 1992.
Specimens: Slide no. TMAG-K1301 is deposited in the Tasmanian Museum and Art Gallery, Argyle Street, Hobart, Tasmania, Australia 7000. Slides no. 800005 is in the collection of author.

This species is very common and occurs in great numbers on the gills of host fishes. Approximately 90% of *Nesogobius* sp.1 examined were infected with this ciliate. Several hundreds of parasites have been detected on a single gill arch.

A medium-sized parasite with a disc shaped body, 39.5-55.3 (45.1) µm in diameter. The adoral zone of the ciliate forms a spiral of about 370-380° (Fig. 3.36). The adhesive disc is concave, 23.7-36.7 (32.3) µm in diameter and is surrounded by a finely striated border membrane, which is 3.0-3.3 (3.1) µm in width. The diameter of the denticulate ring is 12.6-20.5 (16.6) µm and consists of 20-24 (23) denticles. The length of denticles is 4.2-4.9 (4.5) µm. The blades are broad, sickle-like, curved with rounded distal ends, filling the large portion between the y and y - 1 axes. The tangent points to the y axis are generally lower than the distal surface of the blade. The posterior margin curve forms a small narrow arch with the y axis. The apexes of the anterior margin extend to the y + 1 line and are situated above the deepest point of the posterior margin curves. The anterior and posterior apophyses of the blade are not visible. The sections connecting the blade and central part are thin. The central parts of denticles are triangular projections. The tips of the central part are rounded, extending more than half way past the y axis and fitting

tightly into the following denticles. The width of the central part is 0.8-1.5 (1.1) μm. The thorns are slender, straight, tapering slightly to a rounded point. The sections connecting the central parts and thorns are thicker than those connecting the blades and central parts. There is an anterior apophysis on the thorn. The length of the blades is 4.2-5.3 (4.7) μm and the length of the thorns is 3.2-4.4 (3.7) μm. The ratio between the denticle above and the denticle below the x axis is 1.13-1.27 (1.21) (Fig. 3.38). There are 6-9 (7) radial pins to each denticle on the periphery of the denticulate ring, which are clearly visible under the SEM (Pl. 3.5E). A central circle is present and the diameter is 7.0-8.4 (7.5) μm (Pl. 3.5C, D). The nuclear apparatus is comprised of a horseshoe-shaped macronucleus and an ovoid micronucleus. The micronucleus is usually located close to one end of the macronucleus. The macronucleus is 31.6-61.6 (48.3) μm long and 4.7-6.3 (5.2) μm wide. The distance between the two ends of the arm of the macronucleus is 16-27 (22) μm. The micronucleus is 4.7-11.1 (7.9) μm in length and 4.8 (3.2-6.3 ) μm in width (Fig. 3.37).

*Remarks*

*Trichodina jadranica* was originally described from the marine fish, *Mullus barbatus* (Raabe, 1958) and then from several other marine fishes (Raabe, 1959; Zaika, 1966). It is also a common parasite in freshwater eels and a pathogen (Lom, 1986). This is the first report of *T. jadranica* occurring on atherinid fish.

*Trichodina nesogobii* sp. nov. (Figs. 3.39, 3.40; Pls. 3.5F, 36A, B)
Host: *Nesogobius* sp.1.
Location: Gills.
Data: January 1991 to March 1992.
Type material: Holotype no. TMAG-K1300 is deposited in the TMAG; paratype no. 800001 is in the collection of author.

This ciliate is found on the same host as *Trichodina jadranica* Raabe, 1958. These two trichodinids usually occur in a mixed infestation on an individual fish. However, the prevalence and intensity of this species is lower than those of *Trichodina jadranica* and each gill arch harbours about one hundred parasites.

The ciliate is disc-shaped (Pls. 3.5F, 3.6A). The adoral zone forms a spiral of about 370-400° (Fig. 3.39). The diameter of body is 31.6-37.9 (34.7) μm. The adhesive disc is concave, 29.6-33.2 (29.8) μm in diameter and surrounded by a striated border membrane, which measures 2.9-3.2 (3.0) μm in width. The diameter of the denticulate ring is 11.5-15.8 (13.9) μm, which is composed of 19-23 (20) denticles. The length of denticles is

3.5-4.2 (3.7) μm. The blades are broad and fill a large portion of the sectors between the y and the y + 1 axes. The length of the blade is 3.2-4.3 (3.8) μm. The distal surface of the blade is flat and the distal points tangential to the y axis. The posterior margin indentation forms a small narrow space with the y axis. The deepest point of indentation is lower than the apex of the anterior margin. The anterior and posterior apophyses of the blade are not present. The anterior margin, in most cases, is extended to the y + 1 axis. The sections connecting the blade and central part are thick. The central parts are small. The tips of the central part are less than half way between the y and y - 1 axes. The points of the central part are roundly sharp and fit loosely into the following denticles. The width of the central part is 1.0-1.4 (1.2) μm. The thorns are relatively long and narrow, 3.5-4.7 (4.4) μm in length. The anterior and posterior apophyses of the thorns are not present. The posterior margin of the thorn extends to the y - 1 axis and in most cases extends beyond this line (Fig. 3.40, Pls. 3.5F, 3.6A). The points of the thorns are sharp. The sections connecting the central part and thorn are generally thinner than those connecting the blade and central part. The length of the denticle above the x axis is shorter than the length below, the ratio is 0.74-0.86 (0.80). The central circle is not present (Pls. 3.5F, 3.6A). The number of radial pins per denticle is 4-6 (5), which is well-defined under the SEM. The nuclear apparatus consists of a crescent macronucleus and a small oval micronucleus. The macronucleus measures 9.0-23.7 (21.4) μm in length and 9.5-11.1 (10.2) μm in width. The micronucleus lies above or on one side of the macronucleus and is 5.9-6.4 (6.3) μm long and 3.0-3.4 (3.2) μm long (Pl. 3.6B).

*Remarks*

*Trichodina nesogobii* is distinguished from *T. jadranica* in the diameters of the body, adhesive disc and denticulate ring. The structure of denticles is also different in the two species. The thorns are longer than the blades in *T. nesogobii*, while in *T. jadranica*, the blades are longer than the thorns. The ratio between the denticle above and the denticle below x axis is 0.74-0.86 in *T. nesogobii*, while it is 1.13-1.27 in *T. jadranica*; the section connecting the blade and the central part is wider in *T. nesogobii* and there is no anterior apophysis on the thorn, while in *T. jadranica*, this section is narrow and an anterior apophysis of the thorn is present. Furthermore, a central circle is present in *T. jadranica* but not in *T. nesogobii*. Of other described species of *Trichodina*, only *T. caspialosae* Dogiel, 1940 on the gills of *Alosa braschnikowimeotica* from the Rumanian Black Sea Coast (Lom, 1962) shows a close resemblance to *T. nesogobii* in the number and shape of denticles. However, the shape of macronucleus and the ratio between the blades and thorns are different in the two species. Therefore I designate it new; the trivial name refers to the fish it infects.

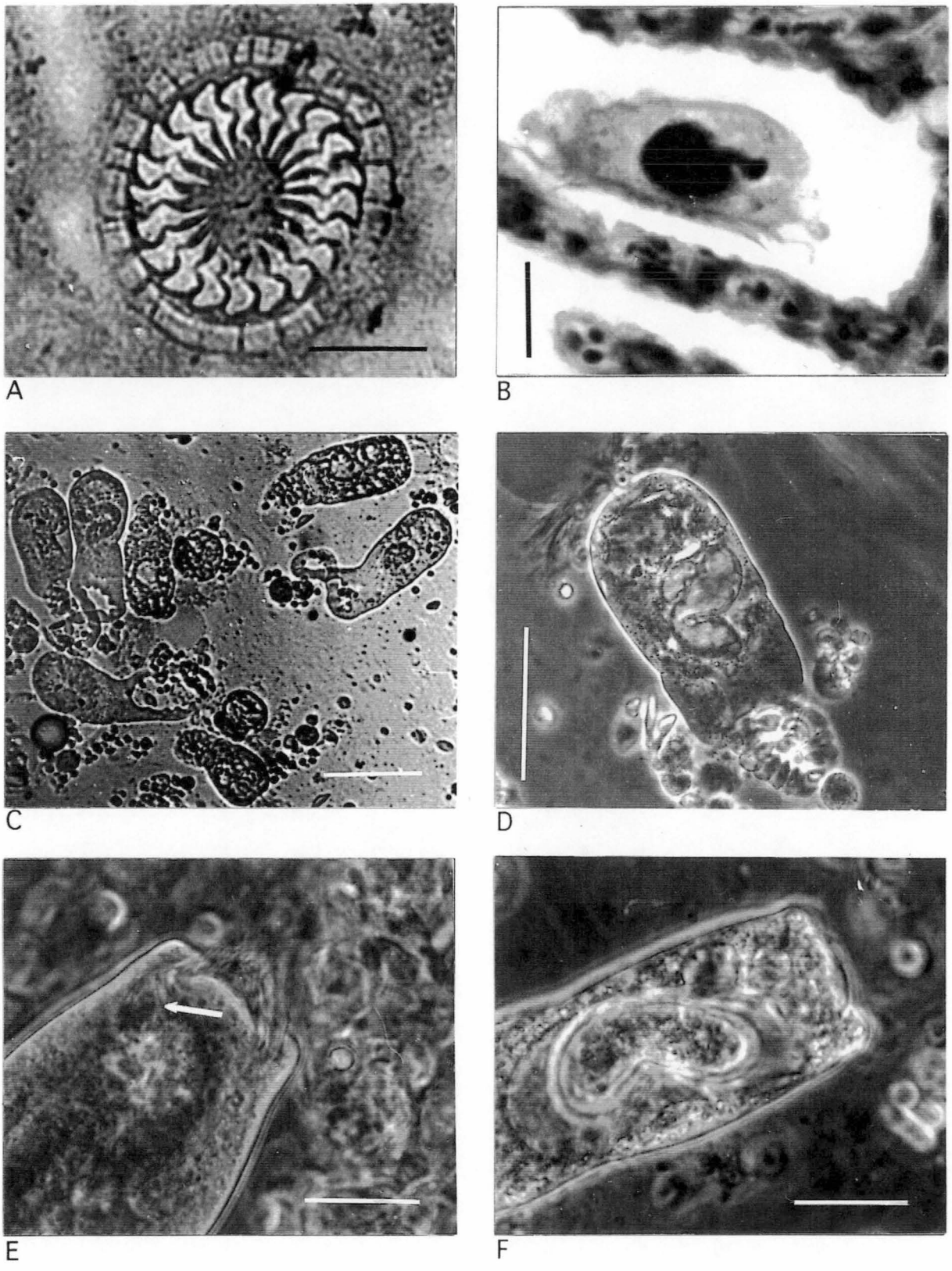



Plate 3.6. Trichodinid and ellobiophryid. A. Silver impregnated *Trichodina nesogobii*. B. H & E stained *T. nesogobii* showing the nuclei. C. *Clausophrya branchialis*. D. Enlargement of *C. branchialis* showing the macronucleus. E. Enlargement of the adoral end of *C. branchialis* showing the peristome and infundibulum (arrow). F. Part of *C. branchialis* showing the macronucleus. Bars = 10 μm in A, B, E, F and 50 μm in C, D.

3.3.3.3 Genus *Clausophrya* Naidenova and Zaika, 1969

*Diagnosis*: Body large cylindrical and possesses a large peristomal disc and a contractile cinctum with two joined limbs which have no an internal rod-like organelle (central axis).

*Clausophrya branchialis* sp. nov. (Figs. 3.41, 3.42; Pls. 3.6C, D, E, F; 3.7A, B)

Hosts: *Atherinosoma microstoma, Leptatherina lebyteriodies, Kestratherina brevirestris* and *K. esox.*

Location: Gills.

Date: January 1990 to April 1992.

Type material: Holotype no. TMAG-K1311 is deposited in the TMAG.

Type host: *Atherinosoma microstoma.*

In most instances during the study period, a light to medium infestation of this ciliate was observed; occasionally the heavy infestation occurred. The ciliate occurred either separately or in group. However, even in the latter situation, each ciliate is independent of its neighbours and is secured in position by means of the cinctia (Pl. 3.6C).

The body consists of two major regions: the body proper (soma) and the bipartite, ring-like holdfast (cinctum). The soma of the ciliate is cylindrical or barrel-shaped, often tapering slightly towards the aboral end where the two opposing limbs of the cinctum join it (Fig. 3.41). The surface of the soma is invested by a smooth pellicle, which appears to lack annuli. The soma is 56.9-120.1 (80.8) μm in length and 28.4-55.3 (36.9) μm in width. Numerous granules are distributed throughout the cytoplasm of the soma. The food particles are more or less greenish or yellowish, indicate algal origin (Pl. 3.6D). A large peristomal disc is visible in the adoral end of the soma. An infundibulum runs from the peristome to the cytopharynx which ends in the cytostome (Fig. 3.42; Pl. 3.6E). In living specimens, a contractile vacuole can be observed near the cytostome. The macronucleus is a long, thick sausage or horseshoe-shape. It is usually situated more or less centrally. The length of it is 39.4-75.8 (49.5) μm. The micronucleus is not visible (Pl. 3.6F).

The cinctum is made up of two slender, opposing extensions of the aboral portion of the body. The two limbs of the cinctum are of equal length. They have no terminal connecting organelle, but are simply rounded off distally (Pl. 3.7A). A central axis is not present; therefore, the cinctum is capable of contraction. The limb of the cinctum is

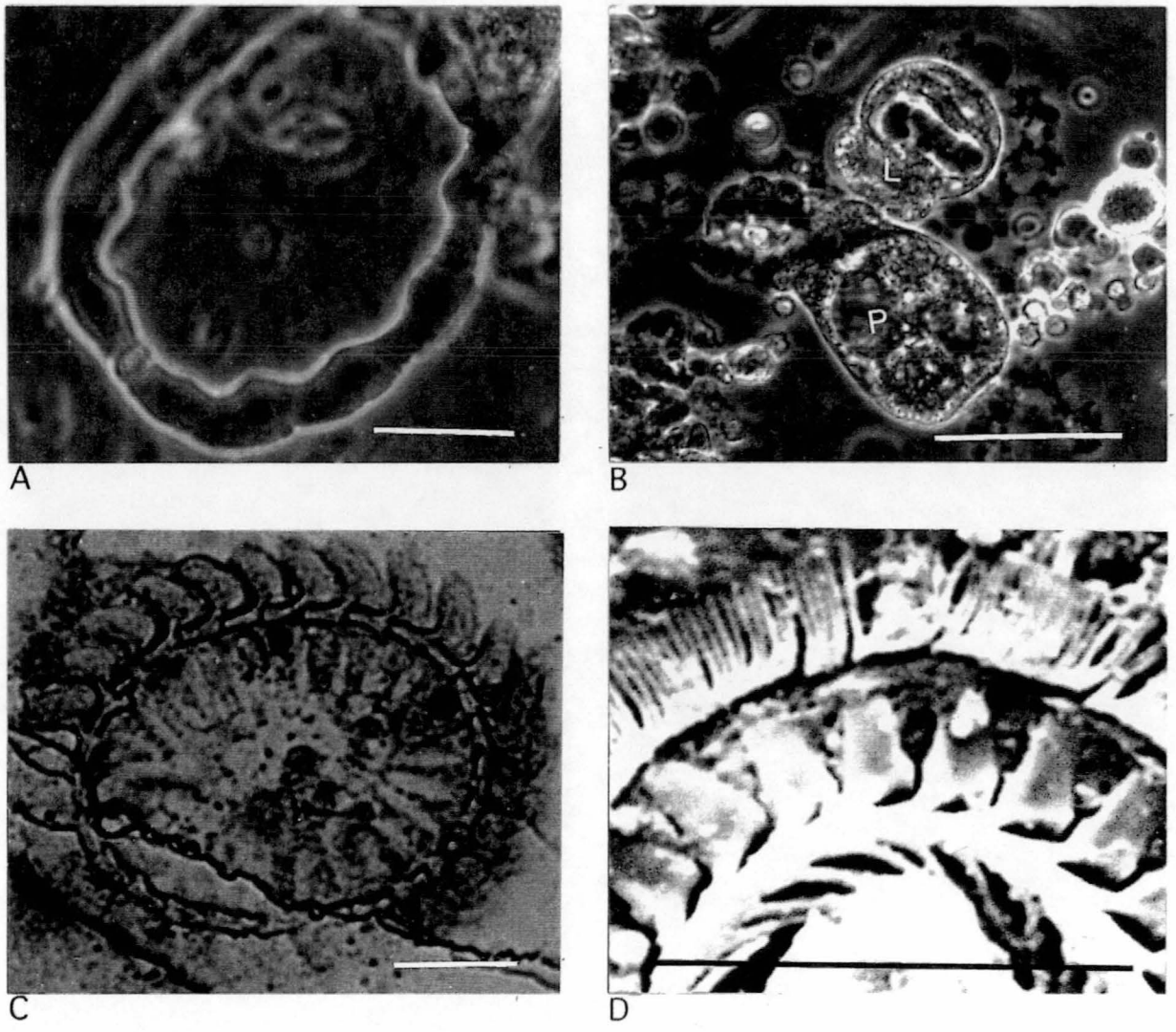
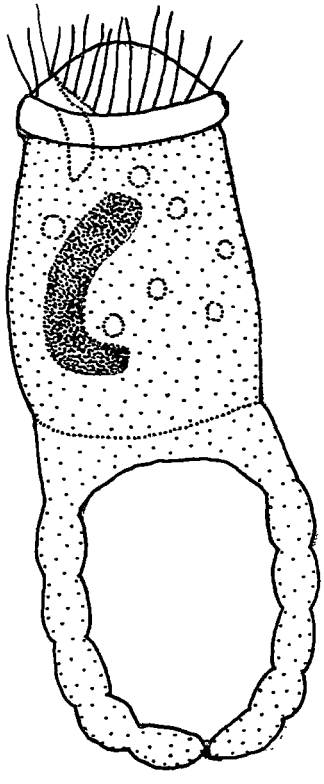
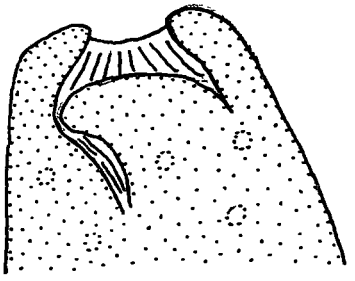



Plate 3.7. Ellobiophryid and trichodinid. A. Enlargement of cinctum of *C. branchialis*. B. *C. branchialis*. L, larva; P, parent. C. Silver impregnated *Trichodina* sp. D. SEM micrograph of *Trichodina* sp. Bars = 10 μm in A, C, D and 50 μm in B.



3.41



3.42

Fig. 3.41. *Clausophrya branchialis*. Fig. 3.42. Adoral end of *C. branchialis*. Bar = 20 µm.

usually 36.0-79.8 (59.5) μm long. When extended completely, the length is up to 150.1 μm. The cytoplasm of the cinctum is homogeneous and is clearly demarcated from that of the soma in living specimens.

The fixed and stained samples show the same general contours as do living ones. The soma ranges from 51.1 to 114.9 by 25.4 to 54.7 μm. The two limbs of the cincum are 31.4-76.2 μm long and they are separated in some instances during the processe of staining.

*Larvae:* Telotrochs of *Clausophrya branchialis* are spherical to subspherical. They can secrete a temporary stalk (or scopula). This stalk is used as an anchor to attach telotrochs on the aboral surface of the parent body (Pl. 3.7B). When mature, this stalk disappears and the offspring separates from the parent's body.

*Remarks*

Since the first species, *Ellobiophrya donacis,* was reported from the marine lamellibranch mollusc, *Donax vittatus* (Chatton and Lwoff, 1923, 1929), only five species in three genera have been described in the family Ellobiophryidae. They are: *Caliperi longipes* Laird, 1953 from the gills of the marine fish, *Oliverichthys melobesia* and *Ericentrus rubrus* in New Zealand; *C. brevipes* Laird, 1959 from the gills of the marine fish, *Raja erinacea* in Canada; *Clausophrya oblida* Naidenova and Zaika, 1969 from the mucus of body surface of the marine fish, *Proterorhinus marmoratus* in the former USSR; *Ellobiophrya conviva* from the marine ectoprocts, *Bugula neritina* and *B. turrita* (Clamp, 1982). Comparing the present described species with those previously reported species, only *Clausophrya oblida* Naidenova and Zaika, 1969 shows a resemblance to *C. branchialis* in having a large peristomal disc and a contractile cinctum without central axis. However, *C. oblida* can be distinguished from the present species in having a larger soma, which is 180 x 36.5 μm and two limbs of the cinctum being cemented to one another at their tips. In view of these differences, and also the host species and geographical distribution, I consider *Clausophrya branchialis* to be new and the name given refers to the location of the ciliate on the host fish.

### 3.3.4 Unidentified forms

**Phylum Sarcomastigophora**

*Dignosis:* One type of nucleus in most species; sexually, when present, essentially syngamy; flagella, pseudopodia or both types of locomotor organelles.

Genus *Cryptobia* Leidy, 1846

*Dignosis:* The recurrent flagellum is attached to the body surface at the side designated as ventral and sometimes raises the pellicle to form a short, narrow undulating membrane.

3.3.4.1 *Cryptobia* sp

Hosts: *Atherinosoma microstoma, Leptatherina lebysteroides, Kestratherina brevirestris* and *K. esox.*

Location: Skin and gills.

Date: February to September 1990.

Specimen: Slide no. 400080 is in the collection of author.

The shape of the living flagellate is pyriform or ellipsoid, 8.0-9.5 (9.1) μm in length and 3.5-4.9 (4.2) μm in width. The body possesses an anterior flagellum and a posteriorly deflected, winding recurrent flagellum, which is adherent to the flattened ventral part of the body. The flagella are unequal in length. The anterior flagellum is 12-17 (14) μm long; the posterior flagellum is almost twice as long as the anterior one and measures 27-34 (30) μm in length. The undulating membrane and contractile vacuole are absent.

*Remarks*

This is the only flagellate observed in the present study. It was only found occasionally and the intensity of infection was always low. The stained specimens were scarce and far from being perfect, therefore, the comparison between this species with other previously reported species was not possible.

**Phylum Ciliophora**

Genus *Trichodina* Ehrenberg, 1831

3.3.4.2 *Trichodina* sp. (Pl. 3.7C, D)

Hosts: *Atherinosoma microstoma, Leptatherina lebysteroides* and *Ketratherina esox.*

Location: Gills.

Date: January, 1990 to June 1992.

Specimen: Slide no. 904006 is in the collection of author.

This ciliate was found on the same hosts as *Paratrichodina tasmaniensis* and *Trichodina australis*. The prevalence and intensity of infestation are the lowest of the three species. Usually one to several hundreds of ciliate occurred in infested fish, occasionally up to

3400 were found.

This is the largest trichodinid observed in the present study. The diameter of the body is 50.6-63.2 (57.5) μm. The adoral zone of the ciliate turns about 380°. The adhesive disc is concave, 44.2-55.3 (49.1) μm in diameter, surrounded by a border membrane, which is 4.7-6.3 (5.8) μm in width. The diameter of the denticulate ring is 31.6-39.5 (33.5) μm, consisting of 24-30 (27) denticles. The centre of the denticulate ring is bright. The blades are broad with rounded distal ends, without anterior or posterior apophyses. The length of the blade is 6.3-7.9 (6.6) μm. The sections connecting the blades and the central parts are thin. The tips of the central parts are also rounded. The thorns are relatively long and narrow, 7.9-11.8 (9.3) μm in length, without anterior or posterior apophyses. The taper of the thorn is sharp. The number of the radial pins per denticle is 8-10 (9) (Pl. 3.7C, D). The macronucleus is horseshoe-shaped, 58.5-64.8 (61.7) μm in length. The micronucleus is not visible.

*Remarks*

This ciliate is readily distinguished from *Paratrichodina tasmaniensis* and *Trichodina australis* by the size of the body and the shape of the denticles. However, successfully impregnated specimens were scarce and far from being perfect, so that I cannot be sure about the correct systematic position at this stage.

### 3.4 Discussion

From the results of the present study, it appears likely that protozoans are common parasites of the fish in North-West Bay. Sixteen species of parasite have been found from 12 fish species during the 22 month study period. Each fish species had at least one parasitic protozoa, *Leptatherina presbyteroides* had up to nine species of parasitic protozoans. This may be due to the distribution of the host fish. It is interesting to note that all the fish species except for *Arripis trutta* are demersal which are in contact with the bottom of the water. This would bring the fish into frequent contact with the infective stages of protozoan parasites, or with invertebrates which can concentrate the spores or act as intermediate hosts for the transmission of some parasite species. Investigations of Markiw and Wolf (1983), Wolf and Markiw (1984), El-Matbouli and Hoffman (1989) and Hedricks (cited by Lom and Dykova, 1992) have implicated that the transmission of *Myxobolus cerebralis*, *M. cotti* and *M. pavlovskii* require an invertebrate as intermediate host. In the previous study, both littoral and benthic fish have been reported to harbour more species of metazoan parasites than their pelagic counterparts (cited by Khan *et al.*,

1986).

In his monograph on Myxosporea, Kudo (1920) noted that "by the study of the geographical distribution of Myxosporida, it is shown that few spices are common to both American and European waters or Asiatic and European waters, while the majority of the Myxosporidia are localised in definite and limited regions." The present result generally supports Kudo's view, it is shown that the majority of protozoan parasites in Australia are distinctive although two of them have been previously found in other parts of the world. These are *Glugea atherinae,* which was original reported from *Atherina boyei* in France (Berrebi, 1978), and *Trichodina jadranica,* which was found on *Calionymus lyra, Mullus barbatus, Gobius minutus* and *Liparis cyclopus*, variously from the Adriatic, Baltic, Black, Mediterranean and Carribean Seas and the Northern Pacific; it has also been found in freshwater eels (Lom, 1986). It is expected that more new data will be reported along with the development of taxonomic studies of protozoan parasites in Australia.

The Myxosporea is the most abundant group in the present study. Of 16 parasitic protozoan species, seven are myxosporeans. Nine of 12 fish species were infected by these parasites and the prevalence is 75%. Trichodinids is the second abundant group. Five species were observed from six fish species, the prevalence is 50%. These results are consistent with Lom's (1986) conclusion "the most frequently encountered marine fish protozoans are ciliates and myxosporeans."

The present results also show that atherinids harbour more parasites than other fish species. Four parasite species were recorded in *Kestratherina hepsetoides* and *K. brevirostris*, seven were found in *K. esox,* and eight and nine were reported in *Atherinosoma microstoma* and *Leptatherina presbyteroides* respectively. Other fish species harbour only one to three species of parasites. The explanation for this probably is due to the large number of atherinid fish sampled over a long period (22 months), while other fishes only have been collected for a few months. The role of the physiological condition and behaviour of the host fish played in the determination of parasite fauna requires further study.

Generally, the host specificity to microsporidians tend to be closely related hosts, although *Pleistophora hyphessobryconis* has a wide host range. Most microsporidians, e.g. *Glugea anomala* were only found in one or two closely related host species and

*Glugea stephani* were found in one or two related host genera (Canning, 1977). In comparison with microsporidians, myxosporeans have a relatively low host specificity. A number of myxosporeans have been found in various fish species belonging to different families (Shulman, 1966). About the host specificity of trichodinids, according to Stein (1985), most marine species have a wide host specificity, they usually infect fishes of different families (cited by Poynton and Lom, 1989). For freshwater trichodinids, Vas As and Basson (1992) found that the host specificity varies with their ecological niches on the fish. Skin-infecting species generally has a wide host specificity, one species can infect fishes of different families, up to eight families have been reported to be infected by *Trichodina heterodentata* in southern Africa. In contrast, gill-infecting trichodinids have a narrow host specificity, they are restricted to certain genera or even to a single host species.

In view of the present results, 16 protozoan parasites can be divided into three groups on the basis of the host specificity. The first group has a low host specificity, which parasitizes fishes of two families. This group includes only one species, *Microsporidium hepaticum*. The second group has a medium host specificity, which parasitizes fishes of two or more related genera. This group includes *Glugea atherinae, Zschokkella leptatherinae, Z. macroscapsule, Trichodina australis, Trichodina* sp., *Paratrichodina tasmaniensis, Clausophrya branchialis* and *Cryptobia* sp. The third group has a high host specificity, which infects only one host species. This group includes *Ortherilinea striateculus, Sphaerospora aldrichettae, Myxobolus aldrichetti, Ceratomyxa arripica, Sphaeromyxa nesogobii, Trichodina jadranica* and *Trichodina nesogobii.*

The results of the present study also extend the known host and geographic range of *Glugea atherinae* and *Trichodina jadranica.* Both species have not been previously reported from Australia.

The genera *Myxosoma* and *Myxobolus* are identical in the morphology and structure of spore except for the presence or absence of an iodinophilous vacuole in the sporoplasm. Therefore, the taxonomic separation of these two genera has been a controversial subject for many years. As early as 1960, Akhmerov questioned the validity of the genus *Myxosoma.* In the study of species of *Myxobolus* and *Henneyguya,* Walliker (1968b), Lom (1969b) and Podlipaev and Shulman (1978), found that a well defined "vacuole" was present only from 0% to 33% (Walliker, 1968b) or 80-90% spores (Podlipaev and Shulman, 1978) and a "vacuole" in moiety of spores varies greatly with the host species,

localities and organs. Therefore, Walliker (1968b) and Lom (1969b) suggested to synonymize *Myxosoma* with *Myxobolus*. However, Podlipaev (1974a, b), Shulman *et al.* (1978) and Haldar *et al.* (1981) recognized the reality of the iodinophilous vacuole in taxonomy of myxosporea. Haldar *et al.* (1981) indicated that since the morphological characters of the spore have immense value in myxosporidian taxonomy, the iodinophilous vacuole should be taken into consideration in the separation of the genera *Myxosoma* and *Myxobolus*. Spall (1974), Richard *et al.* ( 1981) and Inoue and Hoshina (1983) accepted this suggestion and continued to use *Myxosoma* in their papers. Recently, Lom and Noble (1984) reduced the genus *Myxosoma* to synonymy with *Myxobolus* and, accordingly, suppress the family Myxosomatidae Poche, 1913 as already proposed by Akhmerov (1960) and Walliker (1968b) because, as indicated by Lom and Noble (1984), "such an unstable feature is not sufficient to be used as the sole criterion for generic differentiation". In the present paper, I follow the classificational system of Lom and Noble (1984) and use *Myxobolus* as a compound genus.

Ellobiophryids were formerly included in the family Scyphidiidae (Chatton and Lwoff, 1929; Laird, 1953, 1959; Naidenova and Zaika, 1969). Corliss (1979) raised it as a family level. To date, the classification of ellobiophryids at the generic level is still uncertain. Three genera were reported previously, they are: *Ellobiophrya* (Chatton and Lwoff, 1929), *Caliperia* (Laird, 1953, 1959), *Clausophrya* (Naidenova and Zaika, 1969). In 1982, Clamp suggested reducing the number of genera to two based on the structure of cinctum. The genus *Ellobiophrya* Chatton and Lwoff, 1929 is characterized by the possession of a cinctum with joined limbs that is contractile to some degree and lacks an internal rod-like organelle. The second genus, *Caliperia* Laird, 1953 is characterized by the possession of a cinctum whose limbs are always free from one another and noncontractile and which contains an internal, elastic, rod-like organelle. The genus *Clausophrya* Naidenova and Zaika 1969, using this criterion, should be reassigned to *Ellobiophrya* according to Clamp (1982). Another opinion on the classification of ellobiophryids, suggested by Lom and Dykova (1992), is that the genus *Clausophrya* may be synonymous with *Caliperia*. Further study on the classification of the family Ellobiophryidae seems to be required. In this thesis, I still use *Clausophrya* as a separate genus. This status may need to be changed in the future.

# CHAPTER 4 - ULTRASTRUCTURE OF *ZSCHOKKELLA LEPTATHERINAE* AND *MICROSPORIDIUM HEPATICUM*, AND SURFACE TOPOGRAPHY OF *PARATRICHODINA TASMANIENSIS* AND *TRICHODINA NESOGOBII*

## 4.1 Introduction

The Protozoa is a complex group of organisms, placed at different levels of organisation. These organisms are very small. Some of them, especially the parasitic group, have complicated structures. The development of the electron microscope has allowed detailed study of these parasites and resulted in a better understanding of the phylogenetic origin, systematic position and the life cycles as well as the biology of members of this group .

Grasse (1960) was the first worker to use the electron microscope to study the vegetative stages of *Sphaeromyxa* and demonstrate the multicellular nature of myxosporeans. In the study of *Henneguya exilis*, Current and Janovy (1976, 1978) found that different species, and even different clinical types of a single species, have a different surface ultrastructure of the plasmodia and the pathogenicity of each form may be related to the differences between the interlamellar and intralamellar types of *H. exillis* (Current *et al.*, 1979)

The life cycle of myxosporeans has two phases. The parasitic phase represented by the trophozoite in the form of the multinucleate plasmodium which contains two types of nuclei, vegetative and generative nuclei. It reproduces asexually inside fish organs and the tissue. The other phase is represented by the multicellular spore which serves for distribution and the infection of a new host. The process of the formation of the spore from the generative cell is called sporogenesis and has been studied for relatively few species of different genera in Myxosporea. It is generally accepted that the binucleate pansporoblast is formed from the union or envelopment of two generative cells (Desser and Paterson, 1978b; Current, 1979; Current *et al.*, 1979; Pulsford and Matthews, 1982; Desser *et al.*, 1983), However, Hulbert *et al.* (1977) found that a single generative cell divided mitotically to form the binucleate pansporoblast in *Myxidium zealandicum* .

In this chapter, a study of the ultrastructure of *Zschokkella leptatherinae* sp. nov. from the hepatic ducts of *Leptatherina presbyteroides* is reported. This study aimed to eluciate how the binucleate pansporoblast is formed in this *Zschokkella* species. The

developmental stages from the generative cell to mature spore, the characteristics of sporogenesis, capsulogenesis, valvogenesis as well as the structure of plasmodia of this species are described from studies using transmission electron microscopy (TEM).

Microsporidians are small, spore-forming, intracellular parasites, which are difficult to identify and classify as a consequence of their minute dimensions. The majority of reported species were described from the characteristics of the spores observed through light microscopy. The development of the electron microscope allowed characters invisible with the light microscope to be visualised. Today, the electron microscope has become the most important tool in the study of microsporidians. It is now accepted that the genera and species of microsporidians can only be defined accurately using ultrastructural characters (Vavra, 1968; Loubes and Maurand 1975; Canning, 1977; Faye and Togyebate, 1990).

*Microsporidium hepaticum* sp. nov., a microsporidian parasite from the liver of *Rhomboselea tapirina*, was also examined using transmission electron microscopy. The ultrastructure of xenoma and mature spore are described in this chapter.

The detailed study on the surface topography and adhesive discs of ciliates of the family Trichodinidae have only been studied using scanning electron microscopy (SEM) for *Trichodina oviducti* (Khan *et al.*, 1974), *T. truttae* (Arthur and Margolis, 1984) and *T. japonica* (Imai *et al.*, 1991). The structure of the denticles was also examined using SEM for *Trichodina lucioperca* (cited by Lom and Dykova, 1992) and *Trichodina heterodentata* (Van As and Basson, 1987, 1989). In the *Trichodinella*-group, except for the brief description of the adhesive disc of *Trichodinella epizootica* (Raabe, 1950) Sramek-Husek, 1953 by Lom (1973), there is no detailed study on the surface topography and the structure of the adhesive disc of other species.

The ultrastructure of two trichodinid ciliates are reported in this chapter. These are *Paratrichodina tasmaniensis* sp. nov. from the gills of *Leptatherina presbyteroides* and *Atherinosoma microstoma* and *Trichodina nesogobii* sp. nov. from the gills of *Nesogobius* sp.1. The surface topography and the structure of the adhesive discs were examined using scanning electron microscopy.

## 4.2 Material and methods

The area and methods of collecting fish were the same as in the description in Chapter 3. The following methods were used in the preparation of samples for electron microscopy.

### 4.2.1 Transmission electron microscopy

Livers of fishes infected with parasites were fixed in 3% glutaraldehyde in 0.08 M cacodylate buffer (pH 7.2) for a minium of 60 min at 4° C, followed by several washes for a total of 10 min in the same buffer. The specimens were postfixed in 1% osmium tetroxide in 0.08 M cacodylate buffer for at least 60 min at room temperature. They were then washed several times in distilled water. The specimens were again post fixed in 4% uranyl acetate in water for 60 min and were then dehydrated in graded ethanol and embedded in resin. Semi-thin sections (0.5 µm) were cut with a glass knive and stained with toluidine blue. Ultrathin sections (0.05-0.1 µm) were cut with a diamond knife, mounted on famvar coated grids and stained with uranyl acetate and lead citrate. A Hitachi H300 electron microscope was used for observations and micrographs were taken with ILFORD EM technical film.

### 4.2.2 Scanning electron microscopy

Gills of fishes infected with ciliates were fixed in 3% glutaraldehyde buffered with 0.1M phosphate at pH 7.2 for 90 min; they were then rinsed in 0.1 M phosphate buffer. Postfixation was for 60 min in 1% osmium tetroxide solution in 0.1 M phosphate buffer (pH 7.2); the material was rinsed in the phosphate buffer. Dehydration was carried out in graded acetone; final drying was achieved in a Balzers Union CPD 020 critical point drier. The gills were then coated with ultra-pure gold in Balzer Union sputter coater to give a film thickness of 25 nm. Specimens were observed with Phillips 505 scanning electron microscope at accelerating voltage of 15 kV and a spot size of 20 nm. Images were recorded with Polaroid 667 and Ilford FP-4 120 film.

## 4.3 Results

### 4.3.1 Ultrastructure of *Zschokkella leptatherinae*

*4.3.1.1 Plasmodia:* The polysporous plasmodia occupy a large space in the lumen of hepatic ducts of *Leptatherina presbyteroides*. They do not contact with the epithelium directly and various distances between the parasites and epithelial cells can be seen (Pl. 4.1). The surface of the plasmodium is delimited by a single unit membrane. Numerous microvillous projections extend from the surface of plasmodium (Pl. 4.2). Pinocytotic

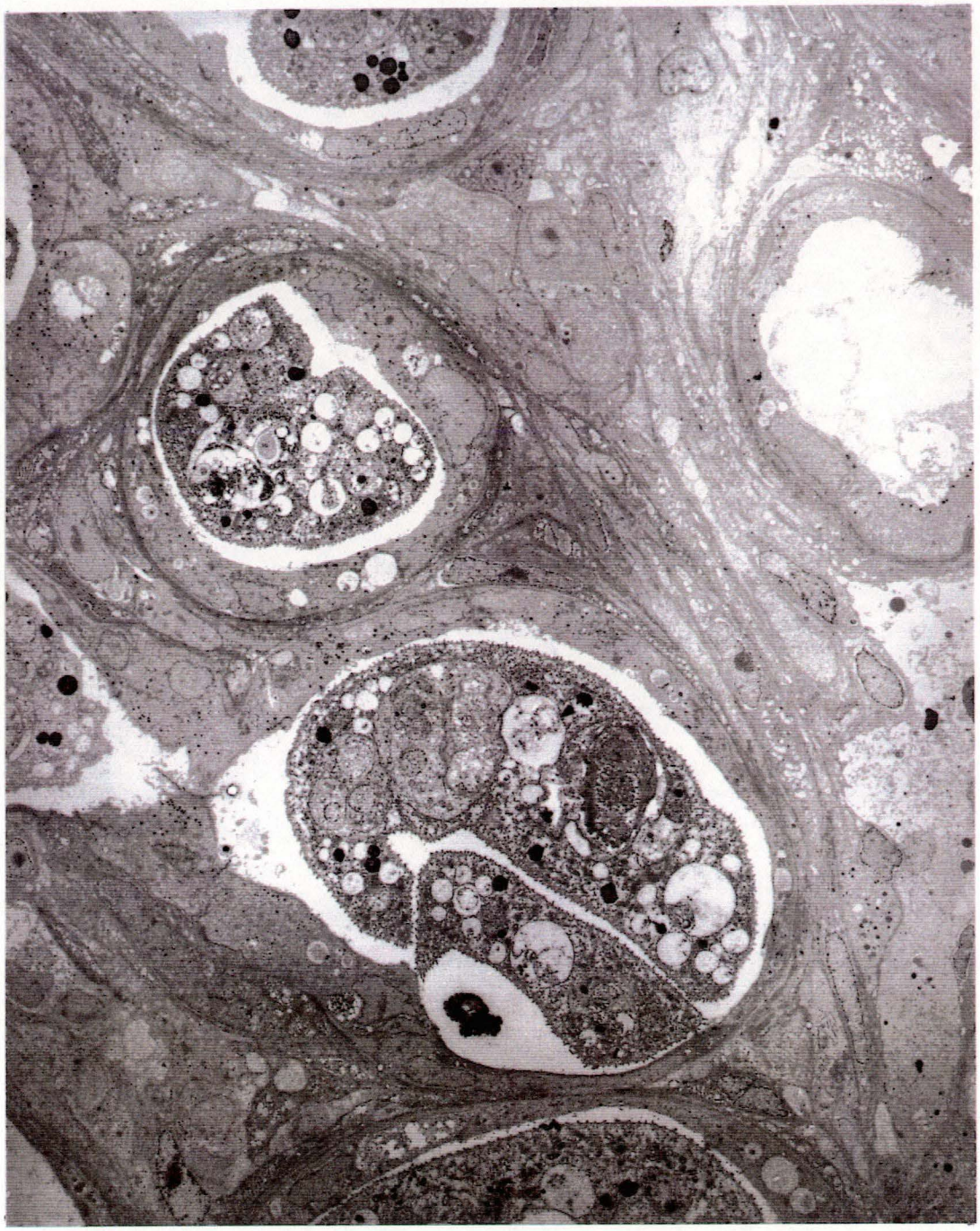


Plate 4.1. TEM micrograph of section of liver of *Leptatherina presbyteroides* showing plasmodia of *Zschokkella leptatherinae* within hepatic ducts, x 4600.

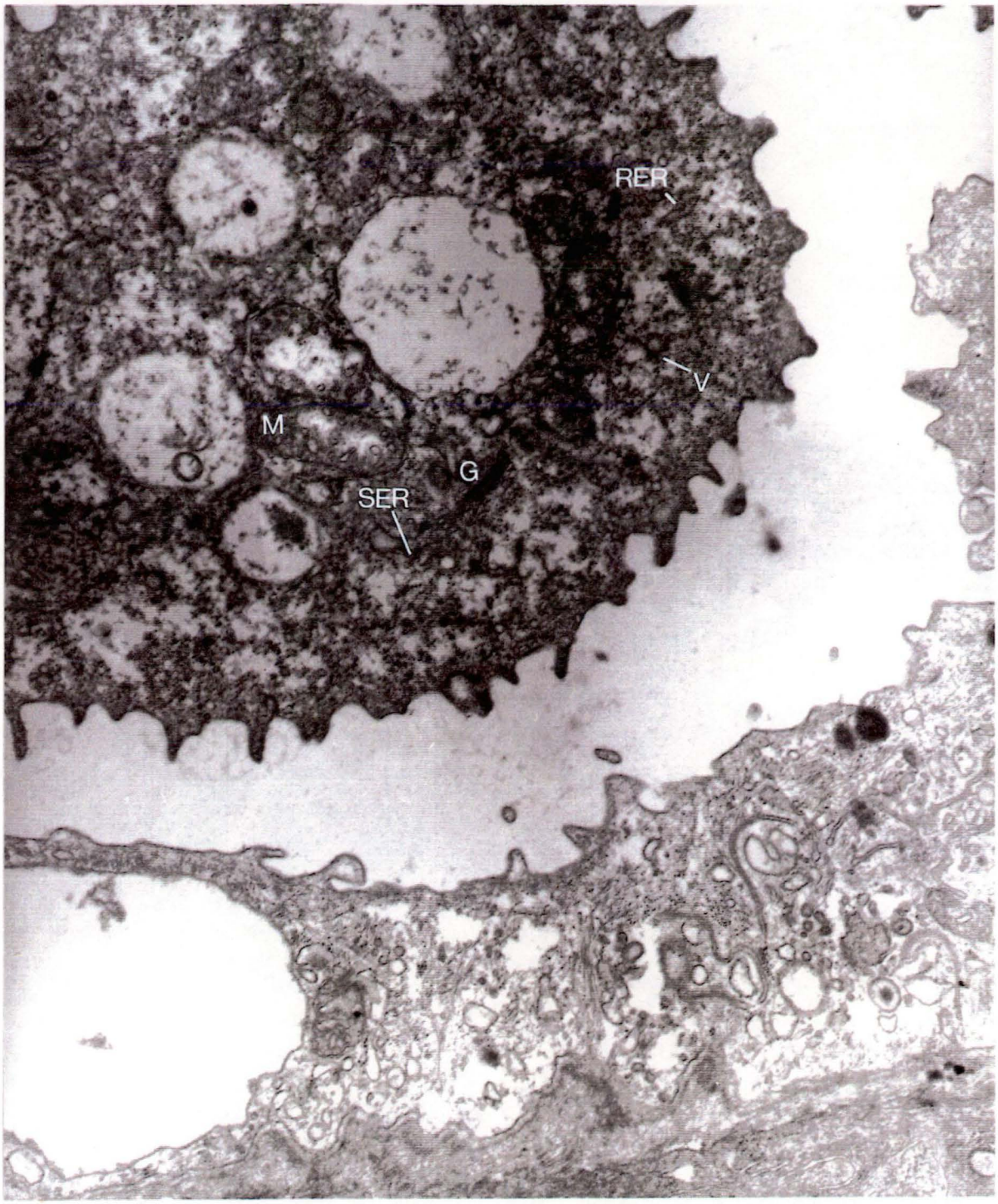



Plate 4.2. TEM micrograph of enlargement of part of plasmodium of *Zschokkella leptatherinae,* x 16100. M, mitochondria; G, Golgi body; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; V, vacuole.

canals, which have been reported from *Z. russelli* and many other myxosporeans (Desser and Paterson, 1978b; Current and Janovy, 1976; Current, 1979; Current *et al.*, 1979; Pulsford and Matthews, 1982; Davies and Sienkowski, 1988), are not found in this species. A number of vacuoles are present beneath the outer surface. Abundant mitochondria, Golgi bodies and rare fragments of cisternae of rough endoplasmic reticulum and smooth endoplasmic reticulum are also distributed in this area (Pl. 4.2). The inner cytoplasm contains the generative cells, vegetative nuclei, various stages of developing pansporoblasts and spores (Pls. 4.1, 4.3 ).

*4.3.1.2 Generative cell:* This cell occurs singly in plasmodium (Pl. 4.4). It is ellipsoidal with a small diameter. The cytoplasm is similar in electron density to the plasmodial cytoplasm. A large nucleus with an eccentric, electron-dense nucleolus is visible. Abundant mitochondria and rare cisternae of smooth endoplasmic reticulum are present within the cells (Pl. 4.4). Different morphological types of the generative cell are not found.

*4.3.1.3 Pansporoblast formation:* The earliest pansporoblast stage is recognised by two closely associated generative cells (Pl. 4.5). One of these cells is finally enveloped by another (Pl. 4.6). Later, the former becomes the sporont while the latter remains as an enveloping cell, eventually being reduced to the pansporoblastic membrane. The young sporont subsequently undergoes further karyokinesis and cytokinesis to form two to eight undifferentiated sporont progeny cells of various size which are compartmentalised within an enveloping cell. A 3-cell pansporoblast, apparently resulting from division of the sporont within an envelop cell is shown in Pl 4.7. A later pansporoblast stage, composed of 4 or 7 sporont progeny cells within an envelope cell are seen in Pls. 4.8, 4.9.

The pansporoblast is disporic. Undifferentiated cells then differentiate and divide into two spore-producing units; each has four cells including one capsulogenic cell, two valvogenic cells and one sporoplasm. In Pl. 4.10, one unit comprises only three cells, namely, one capsulogenic cell, one valvogenic cell and one sporoplasm. It is probable that another valvogenic cell is situated at a different level with other cells. In the next stage, the capsulogenic cell divides into two cells; therefore, each unit has five cells (Pls. 4.12, 4.13, 4.15). These two units are still compartmentalised within an enveloping cell (Pls. 4.14, 4.15).

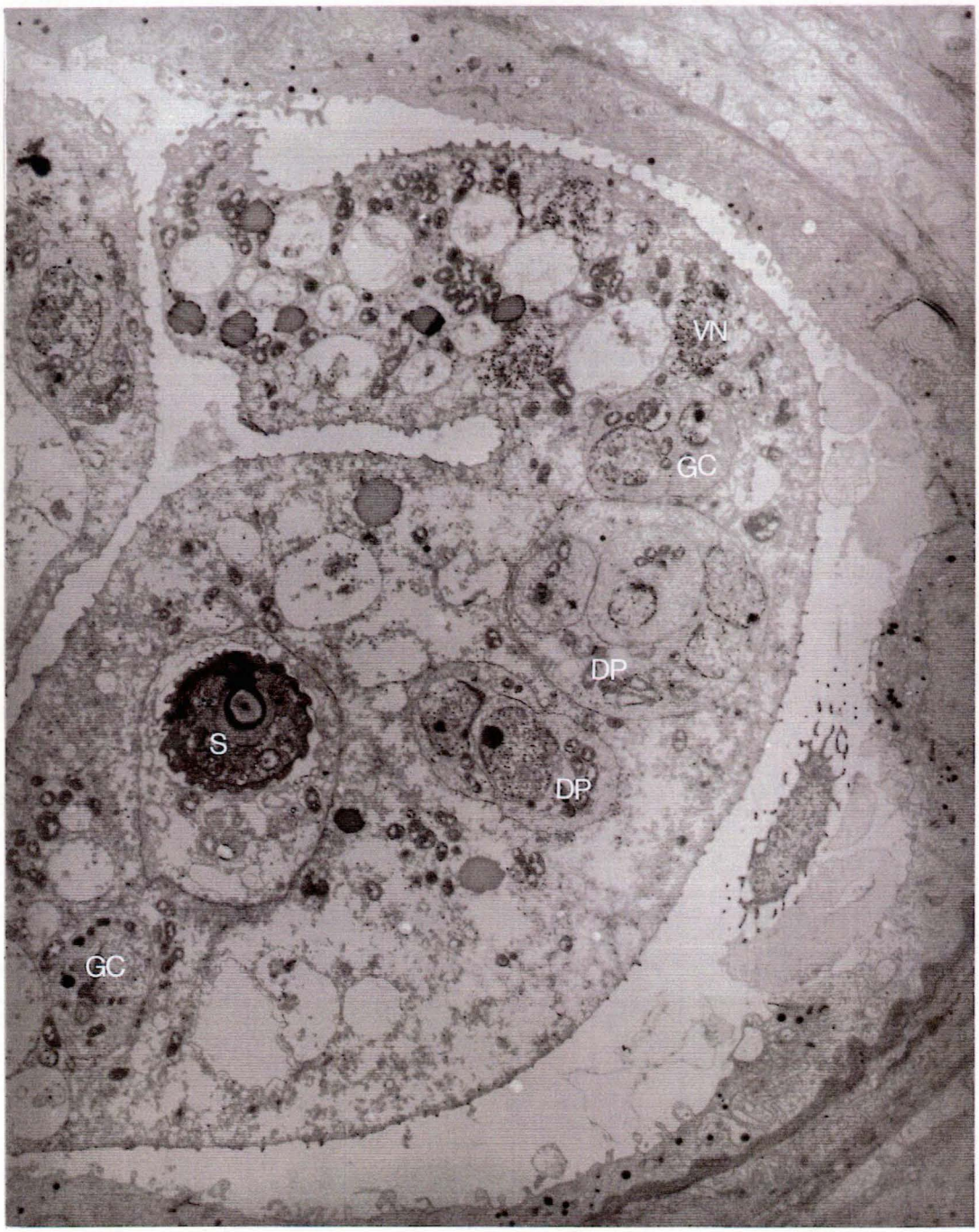



Plate 4.3. TEM micrograph of plasmodium of *Zschokkella leptatherinae,* x 6900. GC, generative cell; VN, vegetative nucleus; S, spore; DP, developmental pansporoblast.

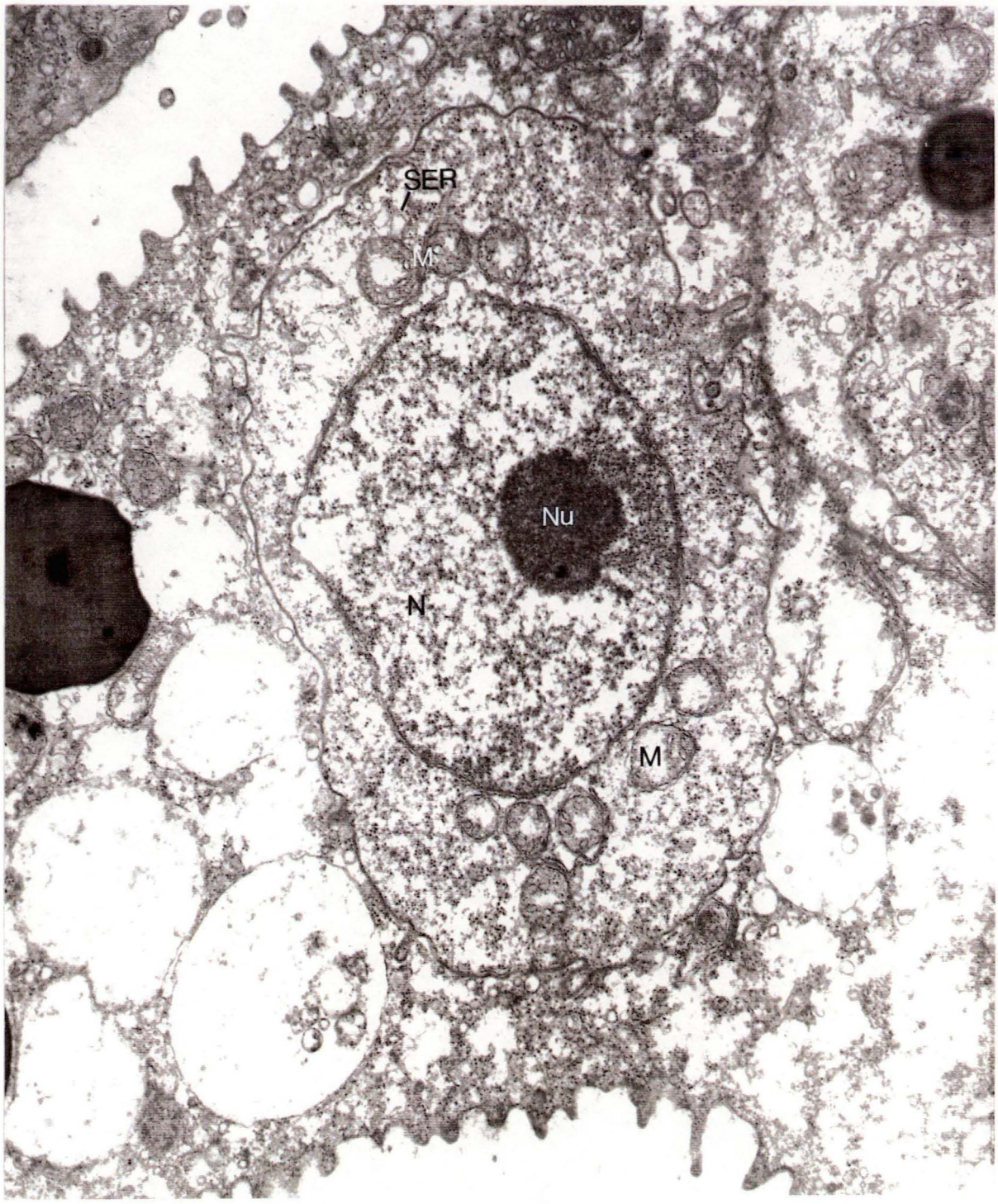



Plate 4.4. TEM micrograph of generative cell of *Zschokkella leptatherinae,* x 23000. N, nucleus; Nu, nucleolus; M, mitochondria; SER, smooth endoplasmic reticulum.

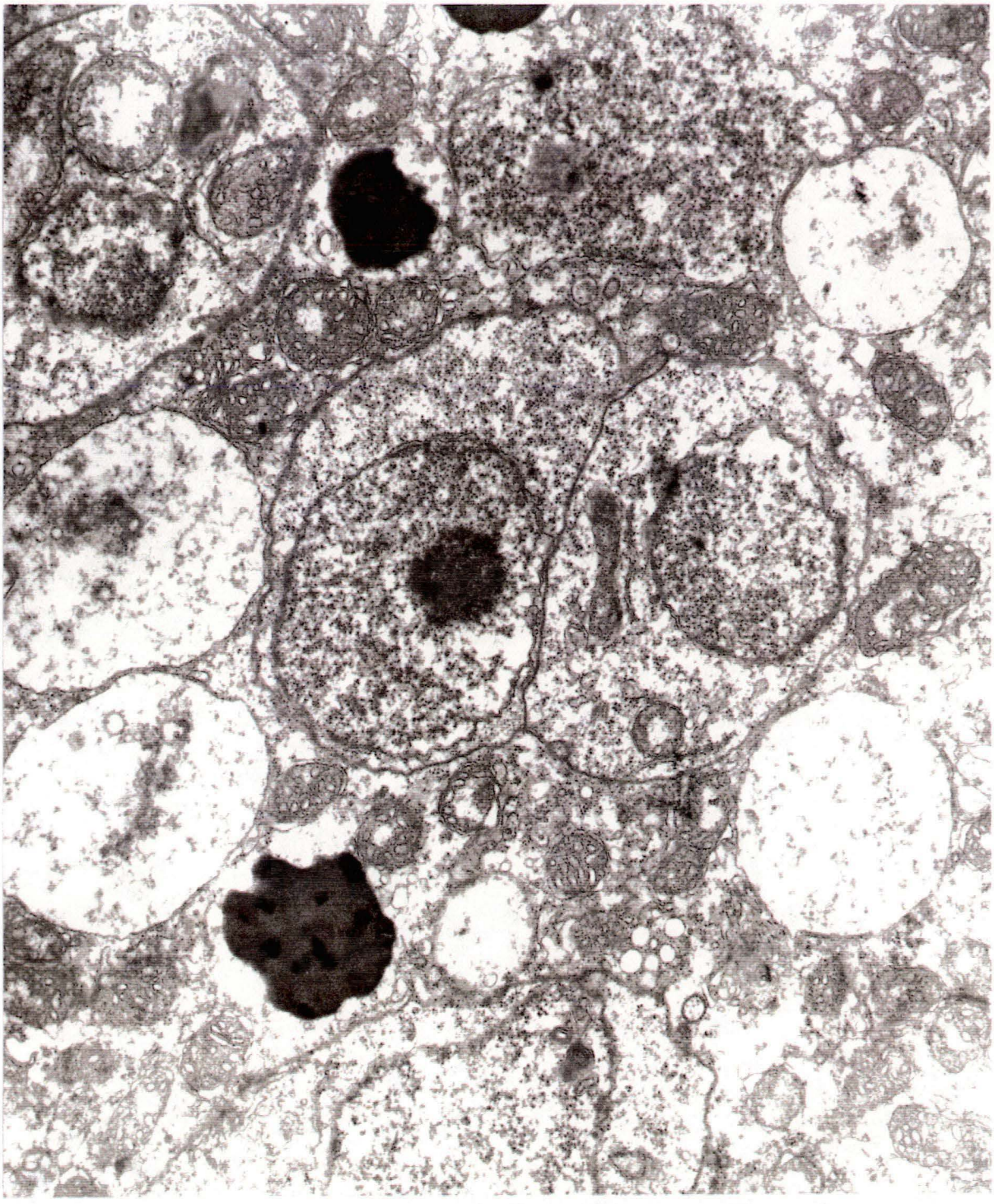


Plate 4.5. TEM micrograph of *Zschokkella leptatherinae* showing association of two generative cells, x 23000.

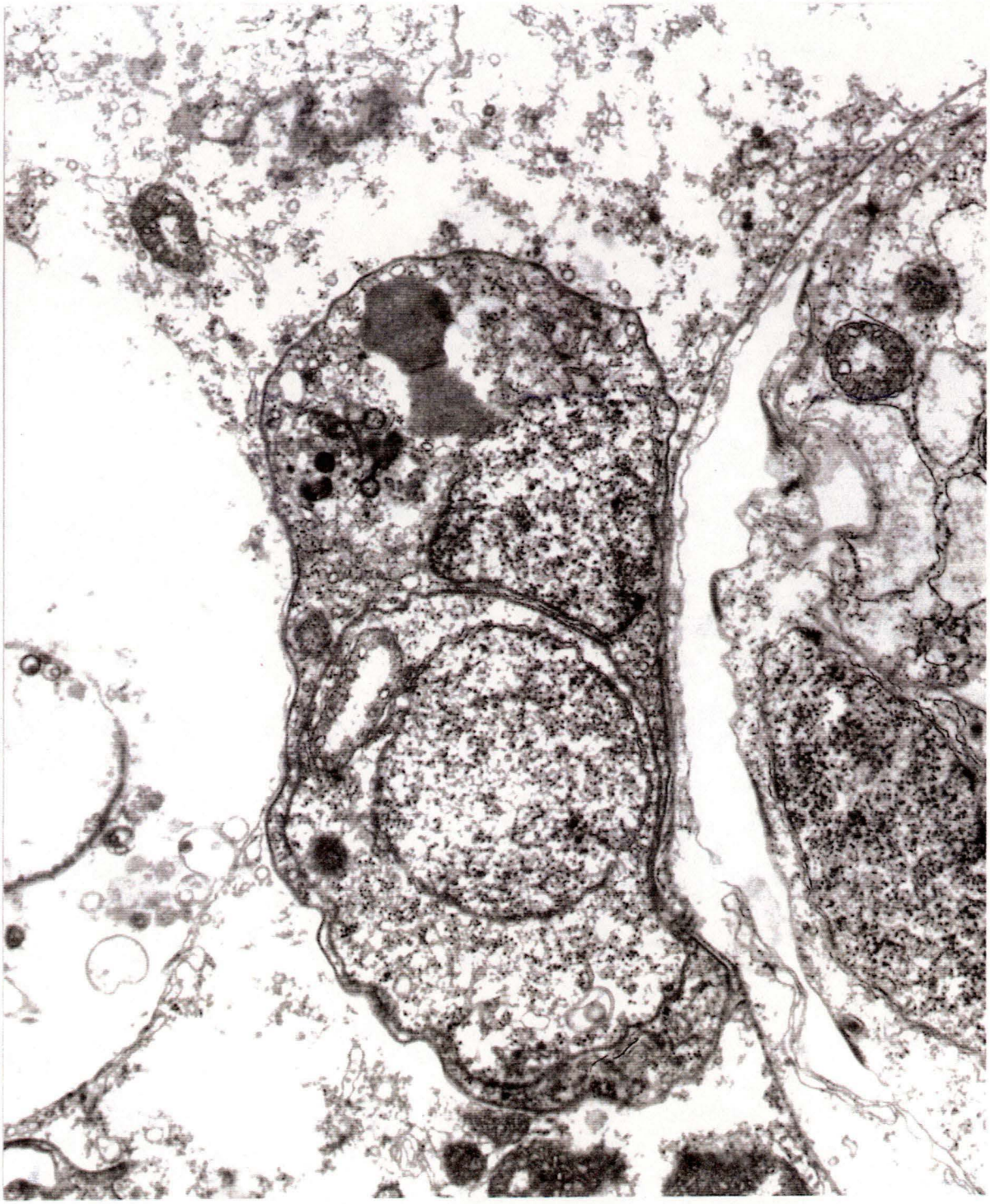


Plate 4.6. TEM micrograph of *Zschokkella leptatherinae* showing an early pansporoblast, x 25000.

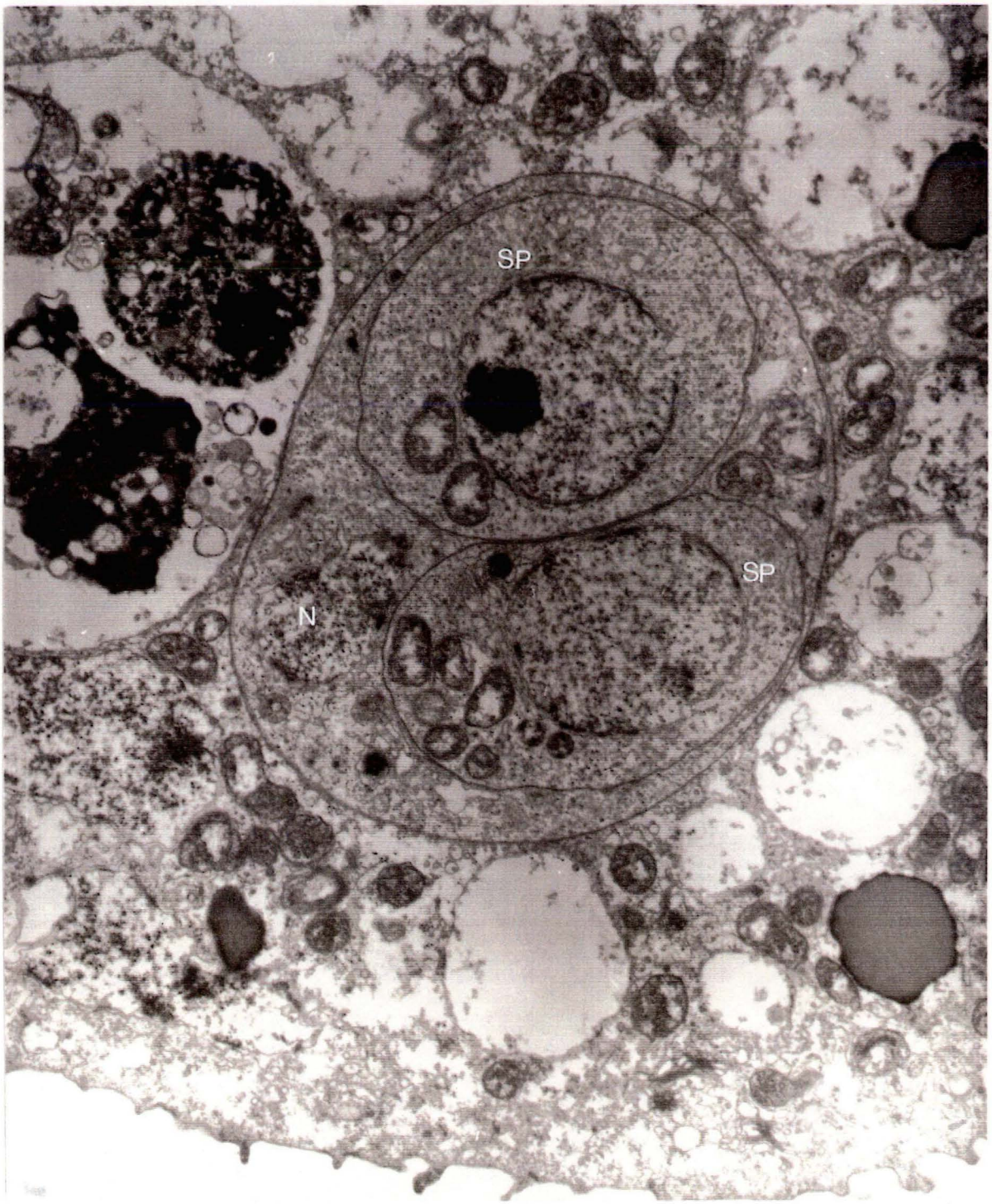



Plate 4.7. TEM micrograph of *Zschokkella leptatherinae* showing a 3-cell pansporoblast, x 23000. N, nucleus of envelope cell; SP, sporont progeny cell.

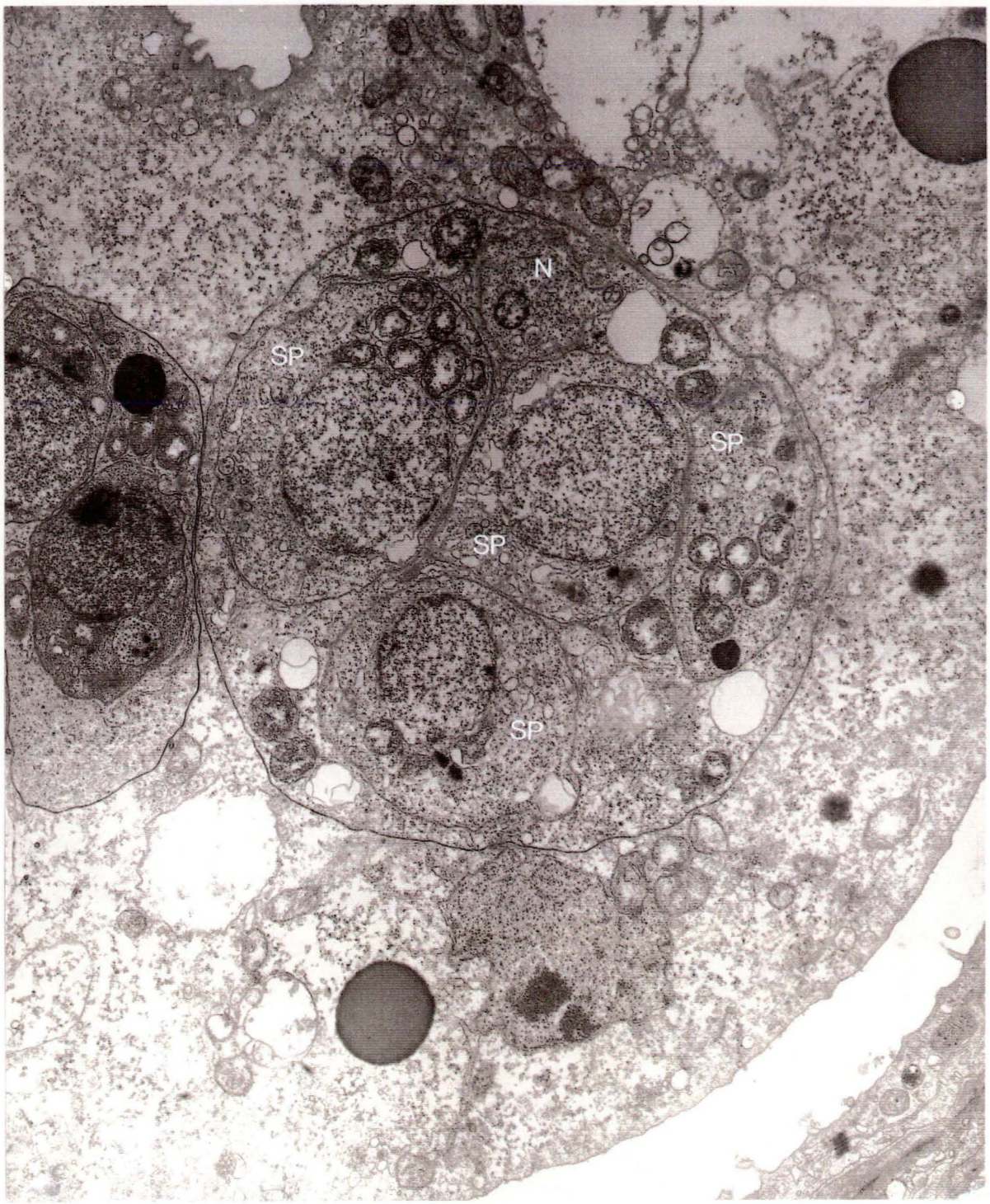



Plate 4.8. TEM micrograph of a 5-cell pansporoblast of *Zschokkella leptatherinae,* x 16100. N, nucleus of envelope cell; SP, sporont progeny cell.

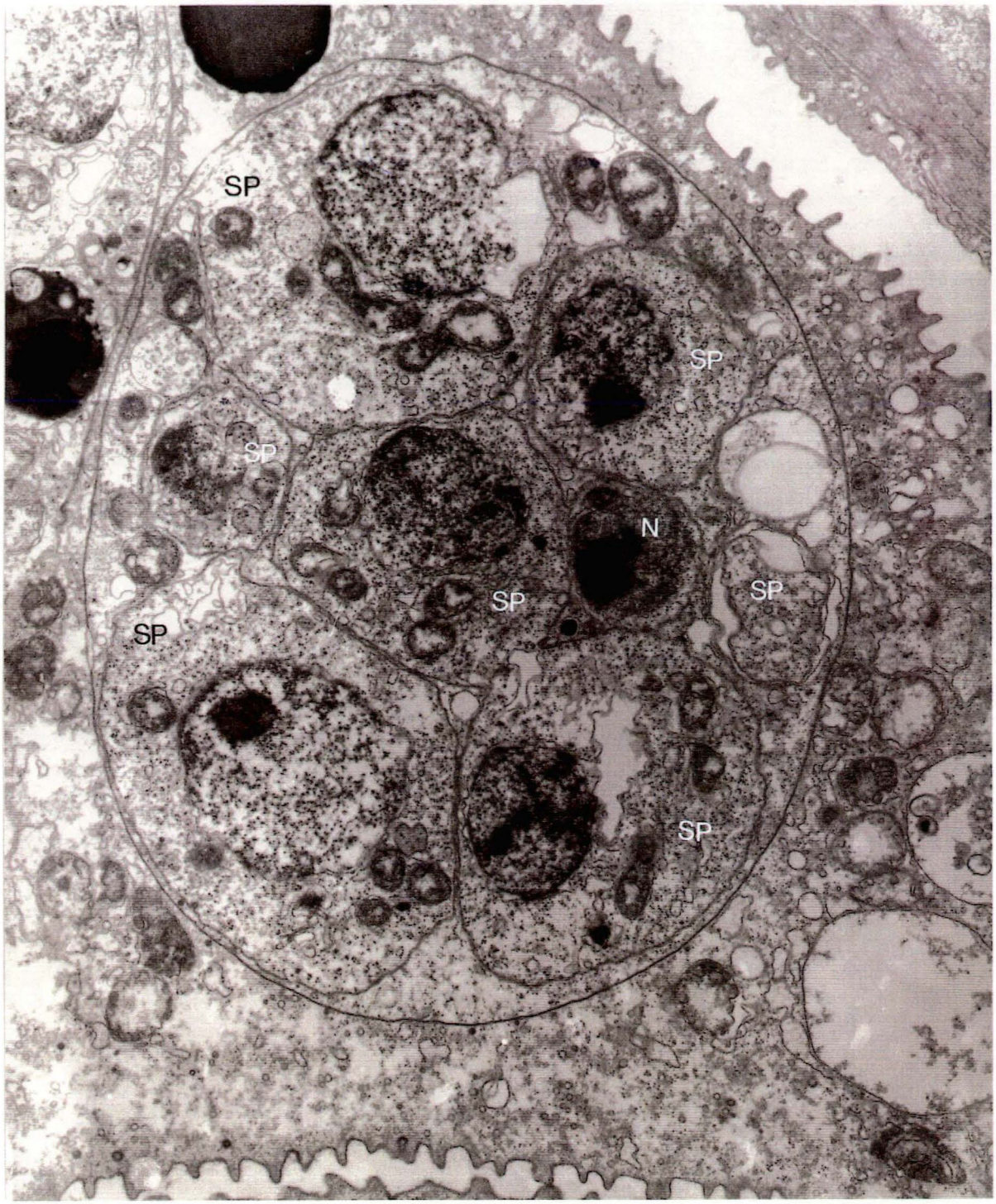



Plate 4.9. TEM micrograph of a 8-cell pansporoblast of *Zschokkella leptatherinae*, x 16100. N, nucleus of envelope cell; SP, sporont progeny cell.

*4.3.1.4 Capsulogenesis:* In the early stage of polar capsule formation, the cytoplasm of capsulogenic cells contains a large nucleus, some vacuoles, a few mitochondria and rough and smooth endoplasmic reticulum. One or two ovoid or pyriform capsular primordia can be recognised at this stage; they have an electron-dense granular core and an electron-lucent cortical region (Pl. 4.10). The presence of two capsular primordia within one capsulogenic cell indicates the one cell-origin of the two capsulogenic cells. With further development, an external tubule extends from the capsular primordium. The lumen of this tubule is filled with the electron-dense material like that seen within the capsular primordium; a girdle of microtubules is visible at the terminal region of the external tubule (Pl. 4.11). The area near the tubule's point of junction with the capsular primordium is reinforced by an electron-dense material, which lies beneath the limiting membrane (Pl. 4.11). The volume of the capsular primordia increases considerably and some large vacuoles occur at the further developmental stage. Two transverse sections of external tubules are visible close to the primordia (Pl. 4.12).

In the more advanced stage of capsulogenesis, several segments of the forming polar filaments are observed in the periphery of core material within the polar capsule (Pl. 4.13). The external tubule is absent after the rudimentary polar filaments appear.

In the subsequent stage of development of the polar capsule, the diameter of the polar filaments is enlarged and it coils 4-5 times within the polar capsules. Mitochondria are more numerous at this stage. The rudimentary polar capsule opening appears at a distance from the end of the spore and both openings occur on one side of the spore (Pl. 4.15); this is consistent with observations by light microscopy. The maturation of the two polar capsules is asynchronous. Two polar capsules at different size and different developmental stage can be seen in one spore (Pls. 4.14, 4.15).

Mature polar capsules are pyriform in shape and contain a dilated spirally-arranged filament which lies in a dense matrix (Pls. 4.16, 4.17). Beneath the limiting membrane of the polar capsule, a narrow, dense band surrounds the electron-lucent zone of the wall. At the apex of the capsule, the electron-lucent walls are reflexed inwards and connect with the wall of the polar filaments. A cork-like stopper plugs the opening of the polar capsule into the discharge channel. Underneath the discharge channel, numerous microfilaments are distributed on both sides of the polar capsule opening. Electron dense material fills the exit in spore valves (Pl. 4.16).

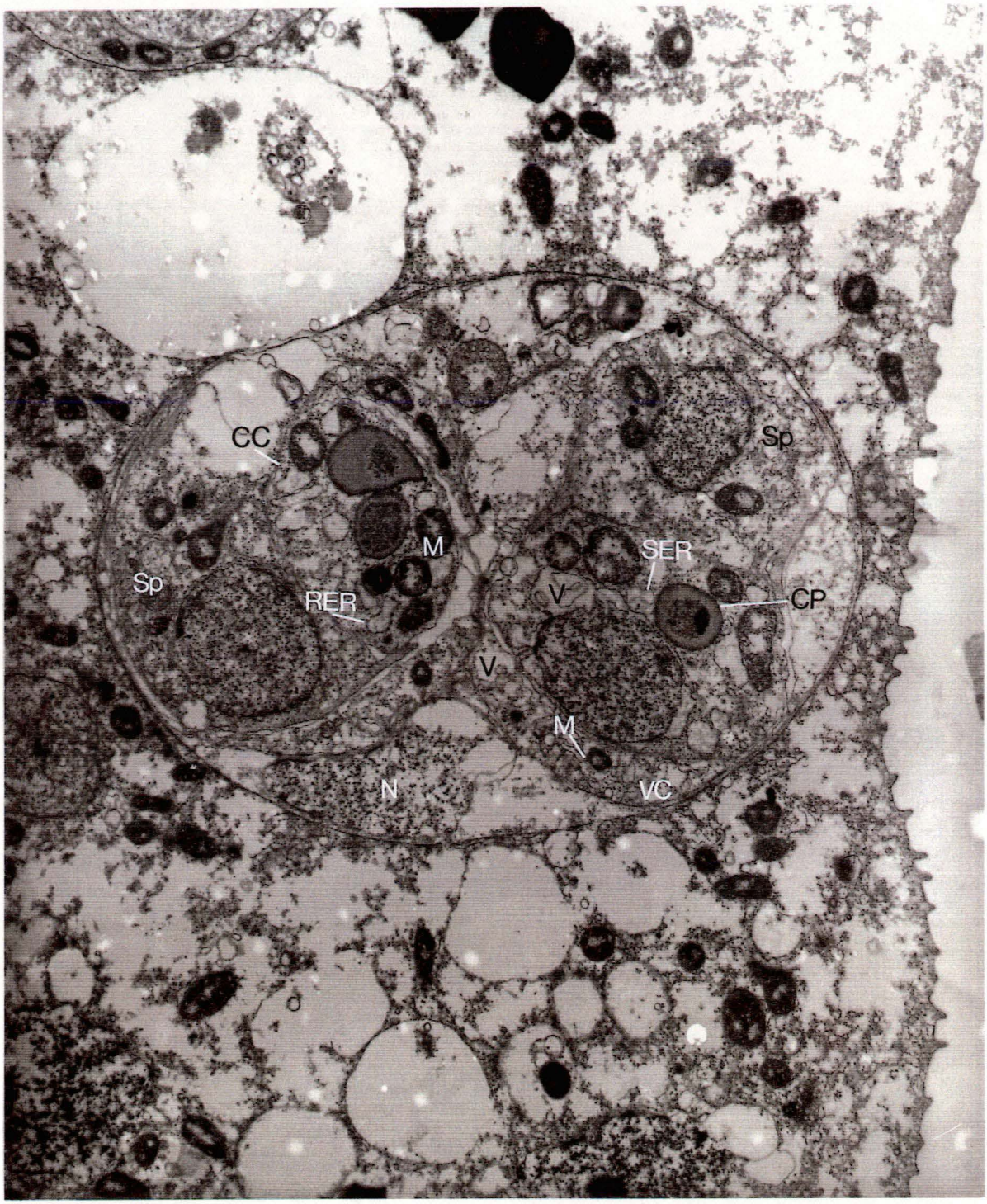



Plate 4.10. TEM micrograph of *Zschokkella leptatherinae* showing two spore-producing unites within one pansporoblast, x 11500. CC, capsulogenic cell; CP, capsular primordium; VC, valvogenic cell; Sp, sporoplasm; M, mitochondria; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; V, vacuole; N, nucleus of envelope cell.

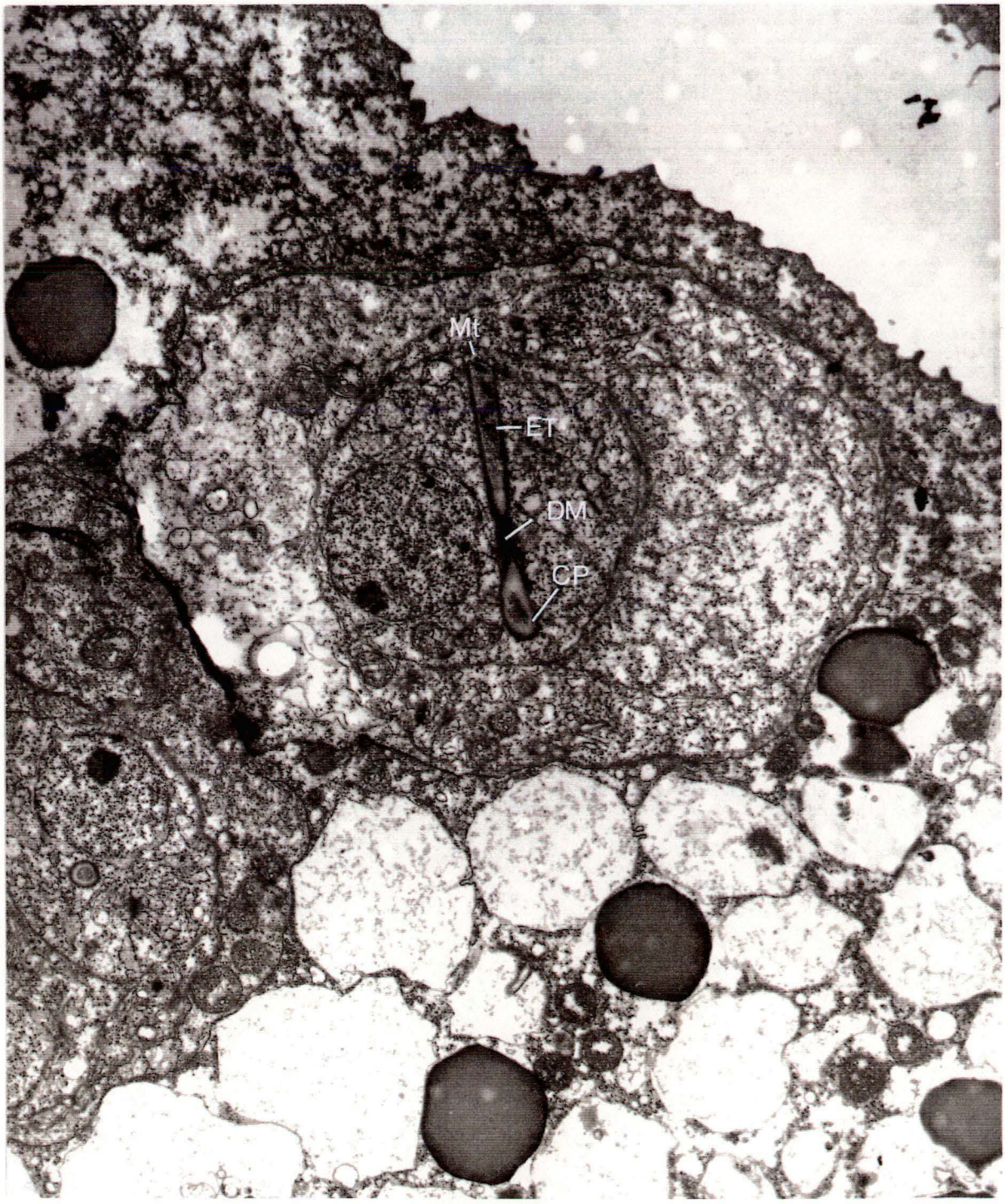



Plate 4.11. TEM micrograph of a developmental pansporoblast of *Zschokkella leptatherinae,* x 11500. ET, external tubule; CP, capsular primordium; Mt, microtubules; DM, electron-dense material.

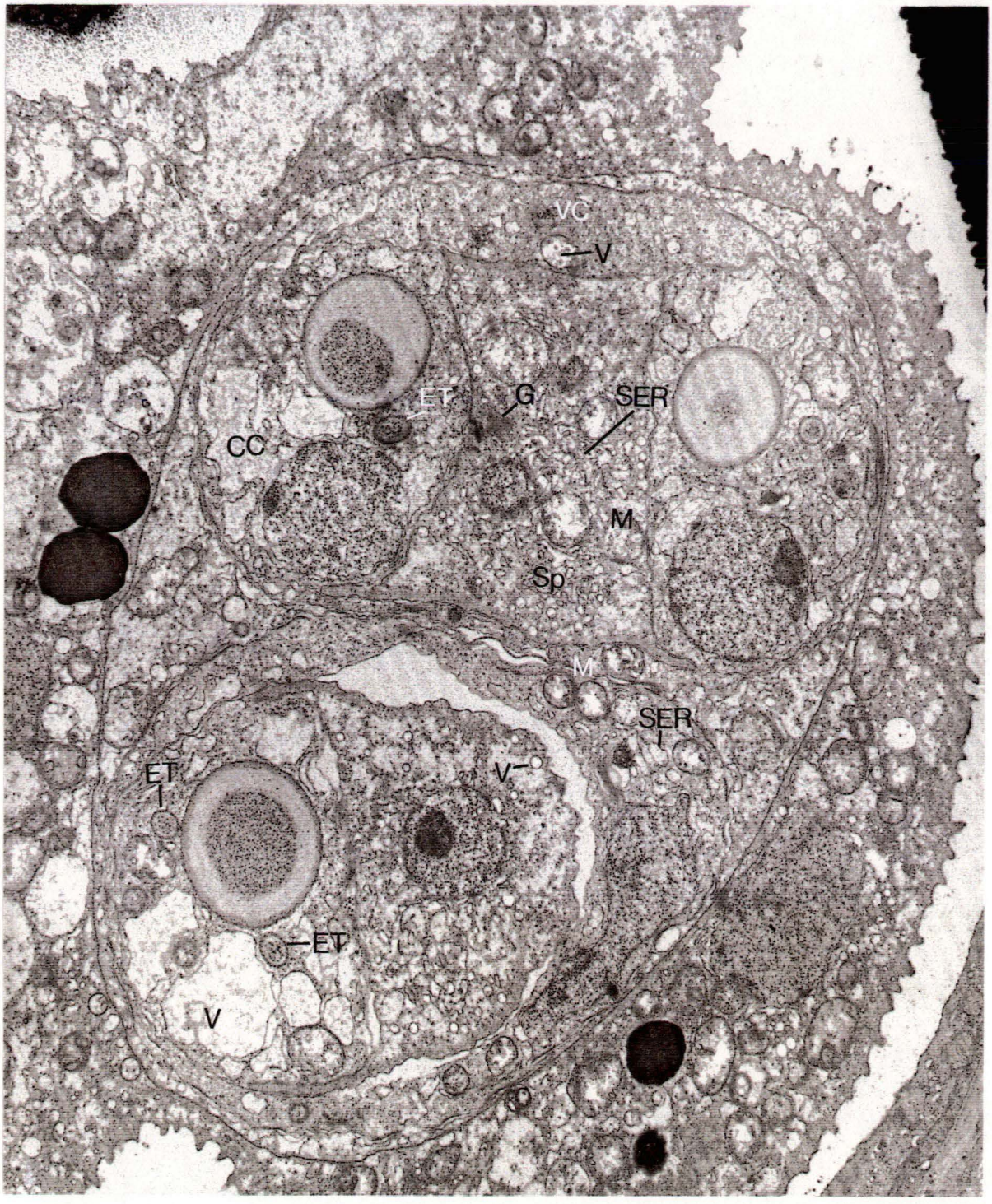



Plate 4.12. TEM micrograph of a developmental pansporoblast of *Zschokkella leptatherinae,* x 11500. ET, transverse section of external tubule; M, mitochondria; G, Golgi body; SER, smooth endoplasmic reticulum; CC, capsulogenic cell; VC, valvogenic cell; Sp, sporolasm; V, vacuole.

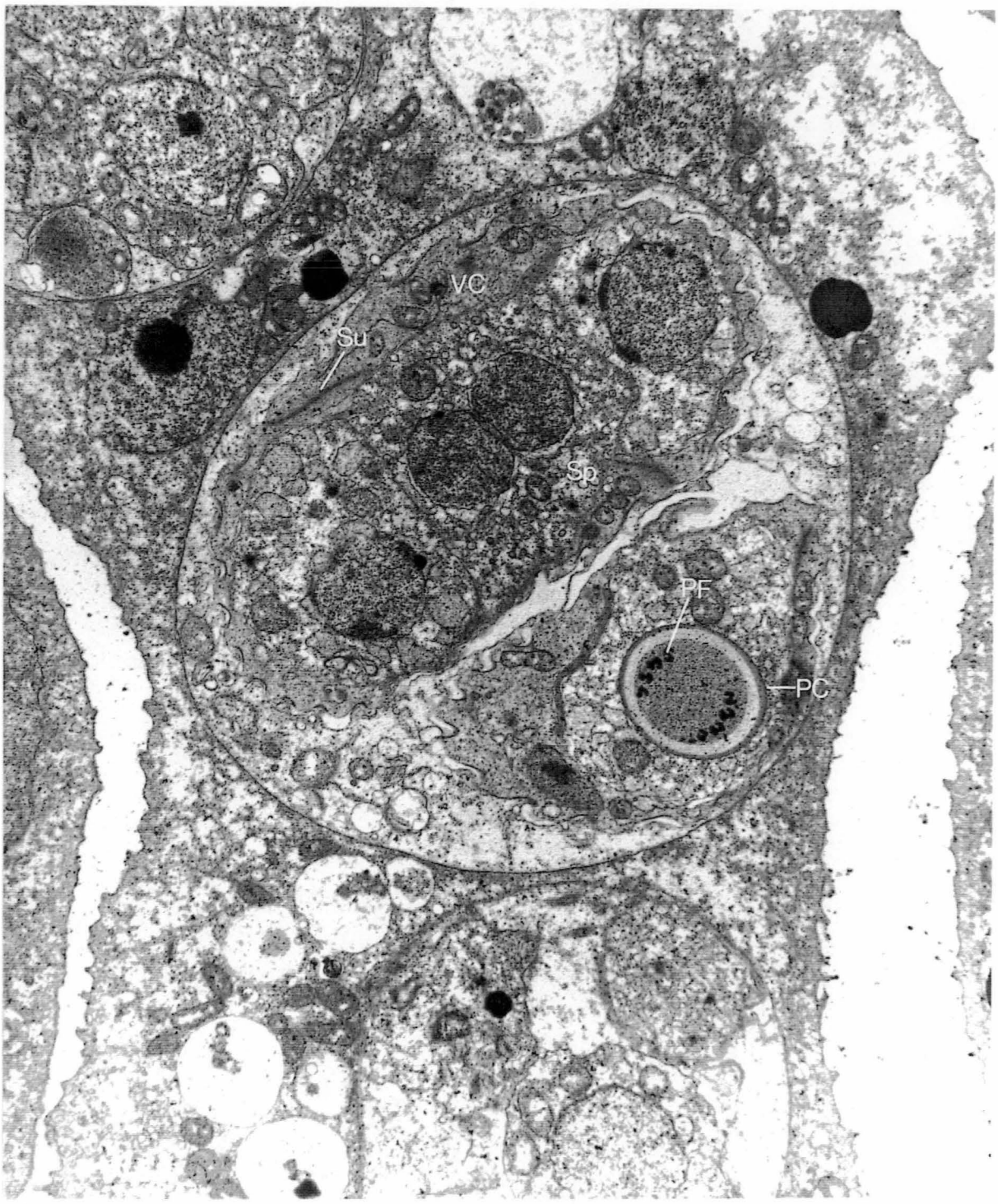



Plate 4.13. TEM micrograph of a developmental pansporoblast of *Zschokkella leptatherinae* x 9200. PF, segments of polar filament; PC, polar capsule; VC, valvogenic cell; Sp, sporoplasm; Su, suture of two valvogenic cells.

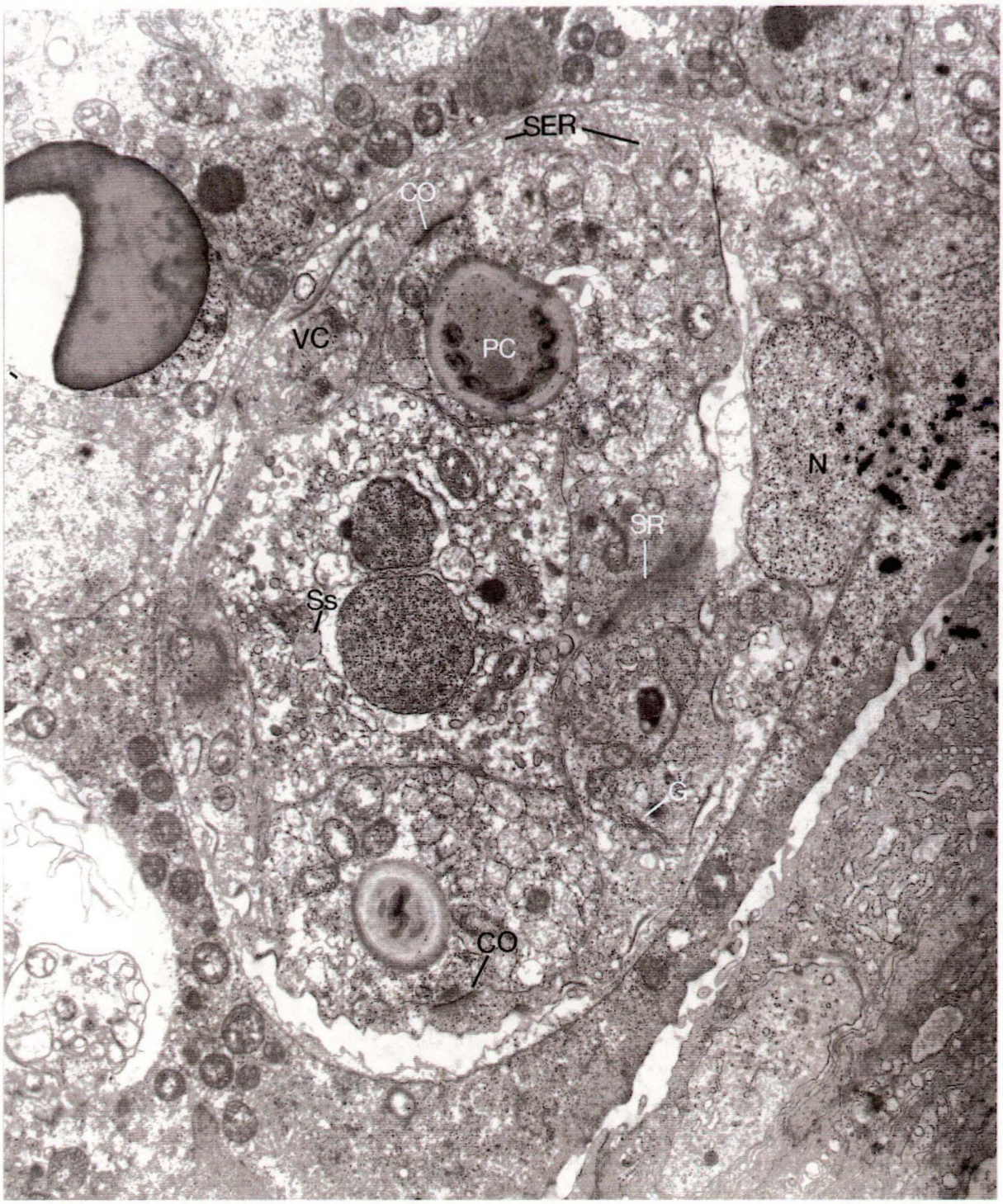



Plate 4.14. TEM micrograph of a developmental spore of *Zschokkella leptatherinae* within the pansporoblast, x 11500. CO, rudiment of polar capsule opening; PC, polar capsule; SR, sutural ridge; VC, valvogenic cell; G, Golgi body; SER, smooth endoplasmic reticulum; Ss, sporoplasmsome; N, nucleus of envelope cell.

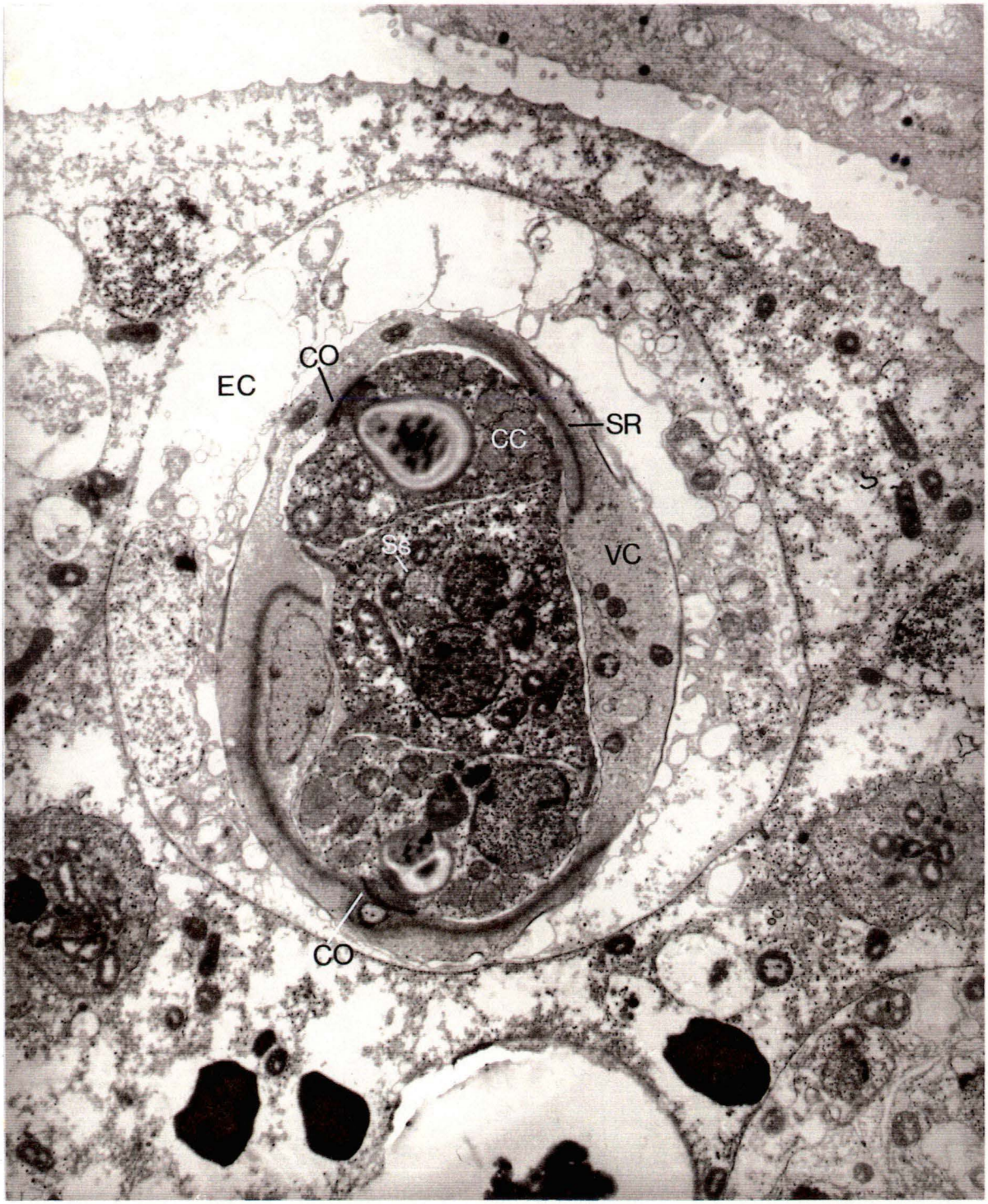



Plate 4.15. TEM micrograph of a developmental spore of *Zschokkella leptatherinae* within the pansporoblast, x 9200. CO, rudiment of polar capsule opening; SR, sutural ridge; VC, valvogenic cell; CC, capsulogenic cell; Ss, sporoplasmsome; EC, envelope cell.

*4.3.1.5 Valvogenesis:* The capsulogenic cells of early sporont are easily recognised by their capsular primordia; in contrast, no such cytoplasmic structures are observed in valvogenic cells. These cells can be identified, however, by their position relative to other cells within the sporont. The flattened valvogenic cells are situated on the side of the sporoplasm and capsulogenic cell in the early stage of development, each with an irregular nucleus, several small mitochondria, Golgi bodies, smooth endoplasmic reticulum and some vacuoles (Pls. 4.10, 4.12, 4.14). Later, the two valvogenic cells become flatter, and the ends of the cells join together to form the sutural ridge which is flanked by electron-dense material (Pls. 4.13, 4.14, 4.15).

In a more advanced stage, the valvogenic cells form the spore walls. These walls are composed of outer and inner layers (Pl. 4.16). The outer and inner valvular walls are continuous except in the sutural ridge. The spore walls in the sutural ridge are thickened and supported by the microtubules and microfilaments. The depressions between the irregular surface ridges are also supported by the microtubules (Pl. 4.16).

Eventually, the valvogenic cells become narrower and the cytoplasm appears electron-dense and the nuclei and other organelles degenerate. The microtubules and microfilaments can no longer be discerned. Ten to twelve surface ridges are distributed regularly on each spore valve at this stage (Pls. 4.16, 4.17, 4.19).

The wall of a mature spore consists of an outer coat and underneath bilayered electron-dense band, which is formed in the cytoplasm of the valvogenic cells. The plasmalemma of the underlying cells is closely applied to the dense band (Pl. 4.18).

*4.3.1.6 Sporoplasm maturation:* The earliest recognised uninucleate sporoplasm is located close to the capsulogenic cell in the young sporont (Pl. 4.10). It then divides into a binucleate sporoplasm between the two capsulogenic cells and is surrounded by two valvogenic cells (Pls. 4.13, 4.14, 4.15). The cytoplasm contains mitochondria, Golgi body, cisternae of the smooth endoplasmic reticulum and many small vacuoles (Pls. 4.12, 4.14). The dense inclusions in the sporoplasm called sporoplasmsomes (termed by Lom *et al.*, 1986) appear in the sporoplasm. They are spherical in shape and vary from 100 to 260 nm in diameter. The sporoplasmsomes are bound by a membrane and contain dense material in an electron-lucent matrix (Pls. 4.14, 4.15).

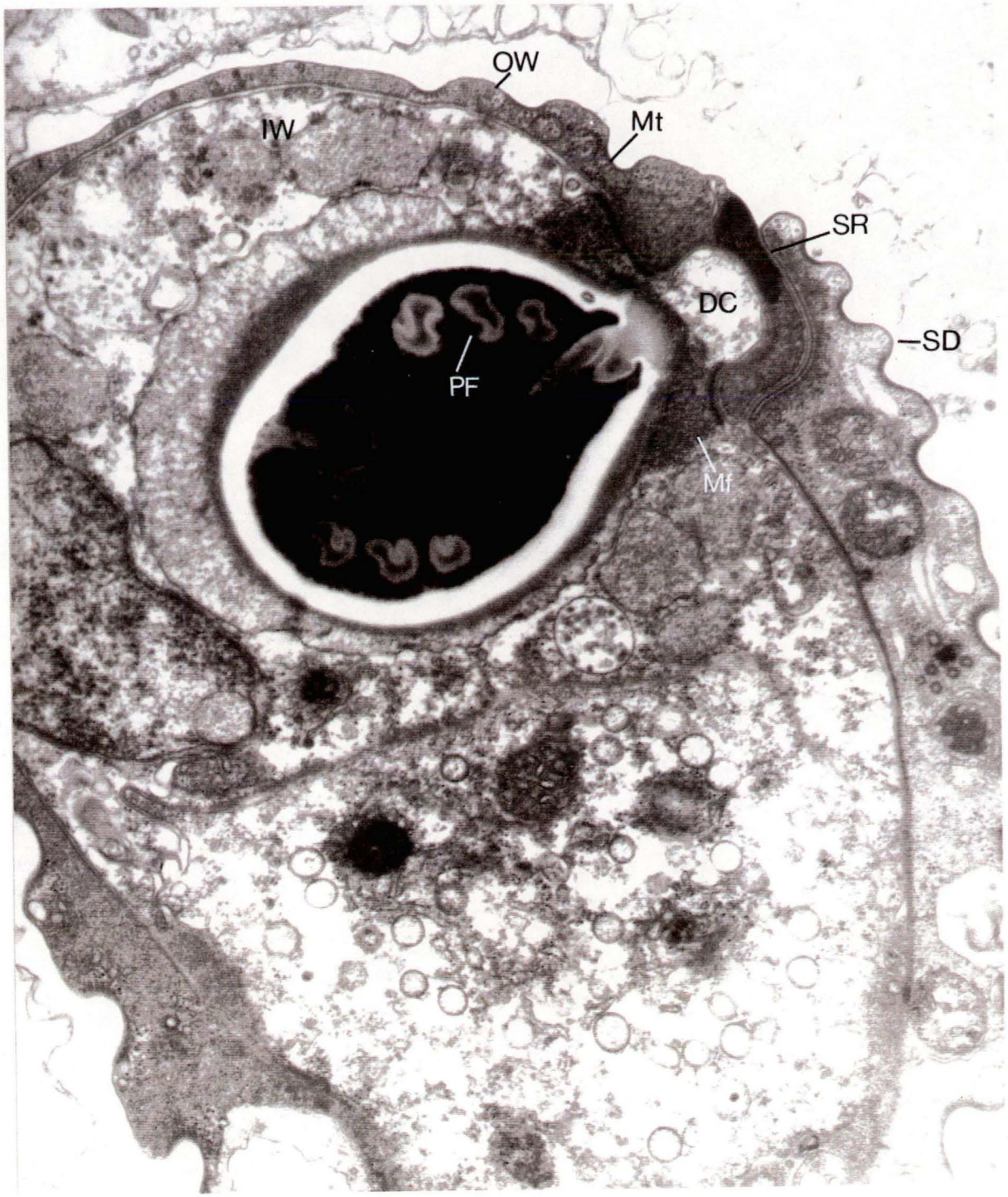



Plate 4.16. TEM micrograph of a nearly mature spore of *Zschokkella leptatherinae,* x 34500. PF, polar filament; DC, discharge channel; OW, outer valvular wall; IW, inner valvular wall; SR, sutural ridge; Mt microtubules; Mf, microfilaments; SD, surface ridge.

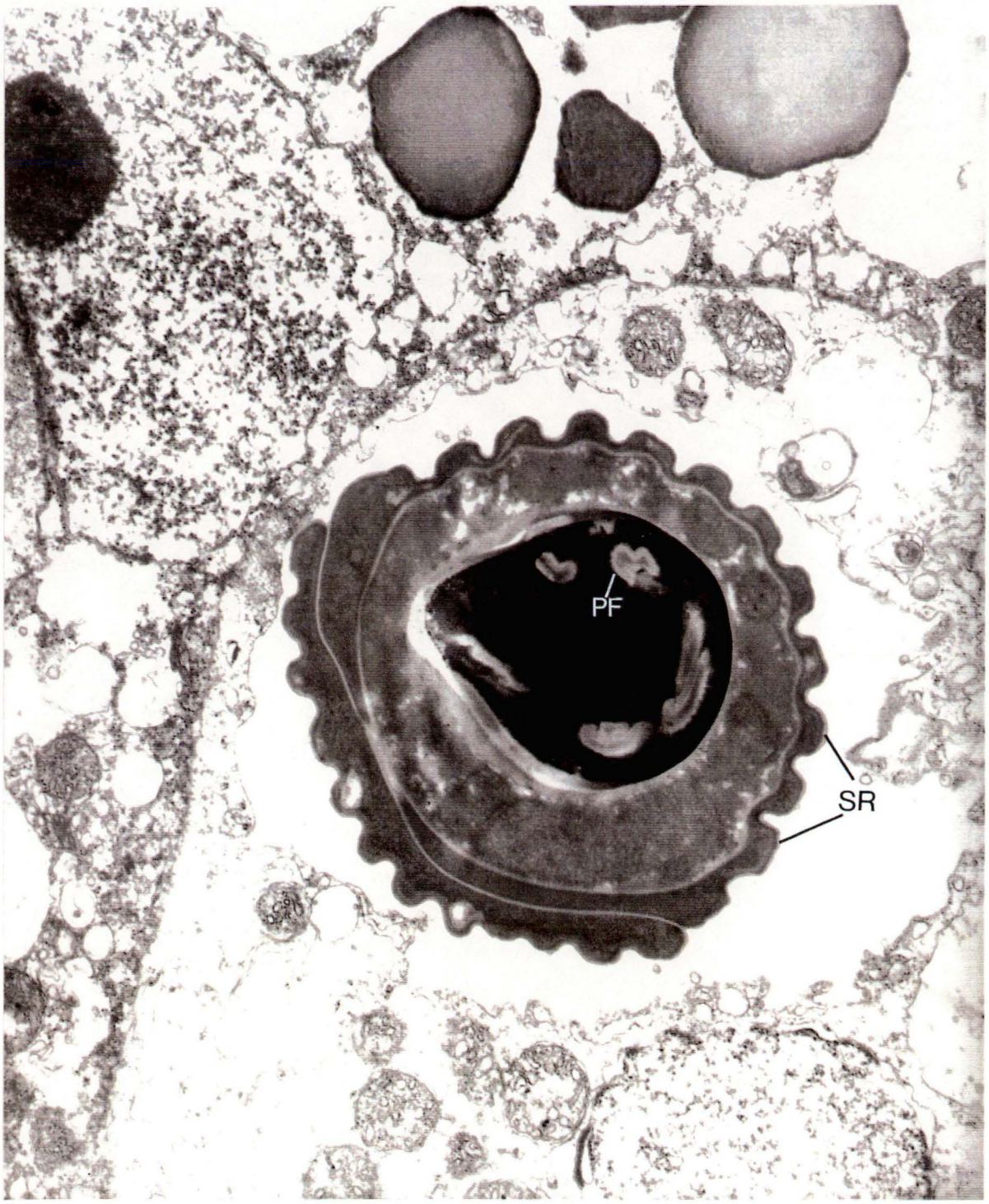



Plate 4.17. TEM micrograph of a mature spore of *Zschokkella leptatherinae,* x 23000. PF, polar filament; SR, sutural ridge.

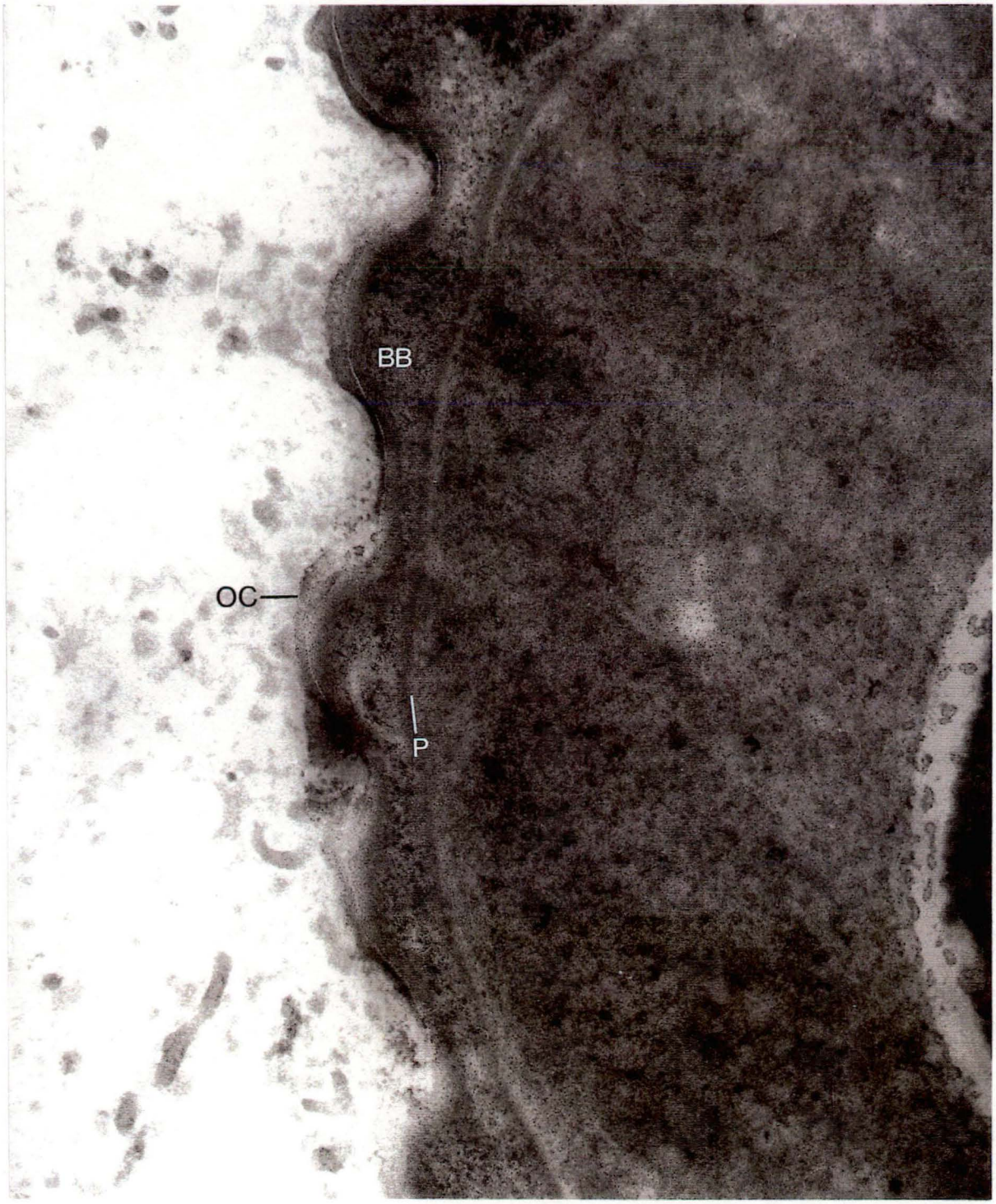



Plate 4.18. TEM micrograph of *Zschokkella leptatherinae* showing the wall of a mature spore, x 92000. OC, outer coat; BB, bilayered electron-dense band; P, plasmalemma.

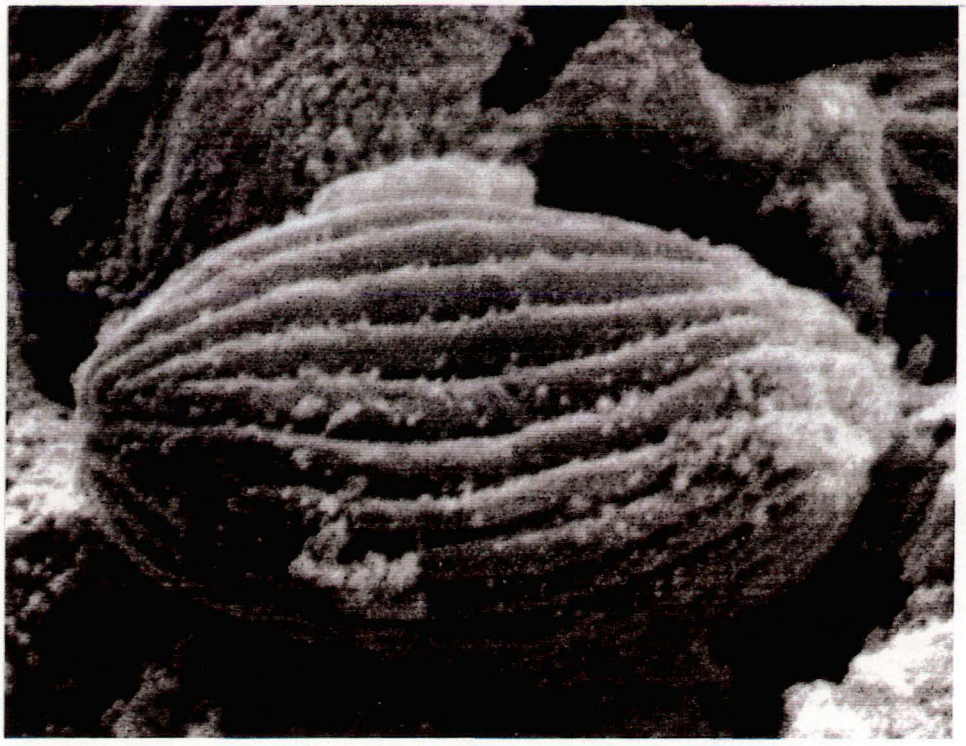


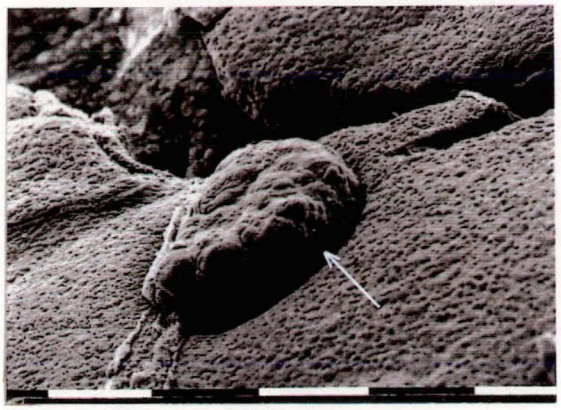
Plate 4.19. SEM micrograph of *Zschokkella leptatherinae* showing the surface ridges of spore, x 772.

4.3.2 Ultrastructure of *Microsporidium hepaticum*

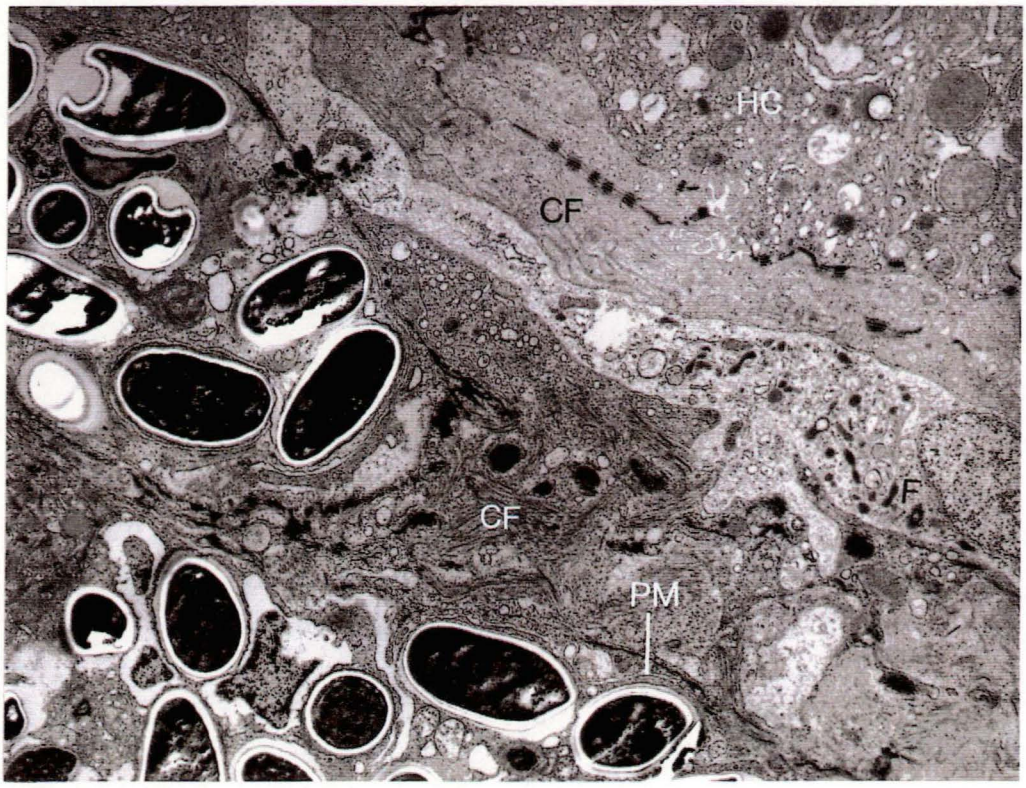
*Microsporidium hepaticum* is parasitic in the liver and gall bladder of flounder, *Rhombosolea tapirina,* and three leather jacket species: *Acanthaluteres spilemelanurus, Meuschenia freycineti* and *M. australis*. The ultrastructural study focuses on those from the liver of *Rhombosolea tapirina.*

*Xenoma:* The xenoma is distributed in the liver parenchyma of the host fish. Under the scanning electron microscope, these xenomas are spherical or ellipsoidal (Pl. 4.20A). Using the transmission electron microscope, the thickness of xenoma wall varies from 2.3-7.0 μm in different sections. The xenomas appear to be bound by layers of collagen fibres whose periodicity is clearly visible in places. The fibroblasts are situated between the layers of collagen fibres (Pl. 4.20B). Some xenomas are only bounded by layers of fibroblast and collagen fibres are not present (Pl. 4.21A). Passing inward towards the parasites, a single host-cell plasma membrane separates the wall from the contents of the xenoma (Pls. 4.20B, 4.21A).

No development stages were detected in this study. Examinations using transmission electron microscope reveals only giant aggregates of apparently free spores in the liver parenchyma. Large parts of liver tissue were replaced by a mass of spores (Pl. 4.21A). The mature spore is ovoid, pyriform or elongate in shape, narrowing slightly towards the anterior end (Pls. 4.21B, 4.22A, B). It measures 4.5 x 2.6 μm from electron micrographs; these measurements vary slightly from those obtained from fresh preparations using light microscopy. The spore wall consists of a thin electron-dense exospore and a thick electron lucent endospore (Pls. 4.21B, 4.22A, B; 4.23A, B). A small vacuole appears in the posterior end of the spore (Pls. 4.22A, 4.23A, B). The polar filaments are often discharged in the preparation for SEM (Pl. 4.24A). The undischarged filaments show obviously 6-10 coils under TEM aligned in two rows in the peripheral region of cytoplasm (Pls. 4.21B, 4.22B, 4.23A, B). The section through the mature polar filament reveals a hollow tubular structure (Pl. 4.24B). The polarplast consists of two different parts. Immediately behind the polar sac, the membranes are closely packed and form a lamellar area. The enlarged lamellae are distributed at the back of the lamellar polarplast, leaving a vesicular area in the polarplast (Pl. 4.22A). The anchoring disc or polar sac is present in the anterior end of the spore (Pls. 4.22A, 4.23A). The anterior end of the polar filament is just beneath the anchoring disc (Pls.

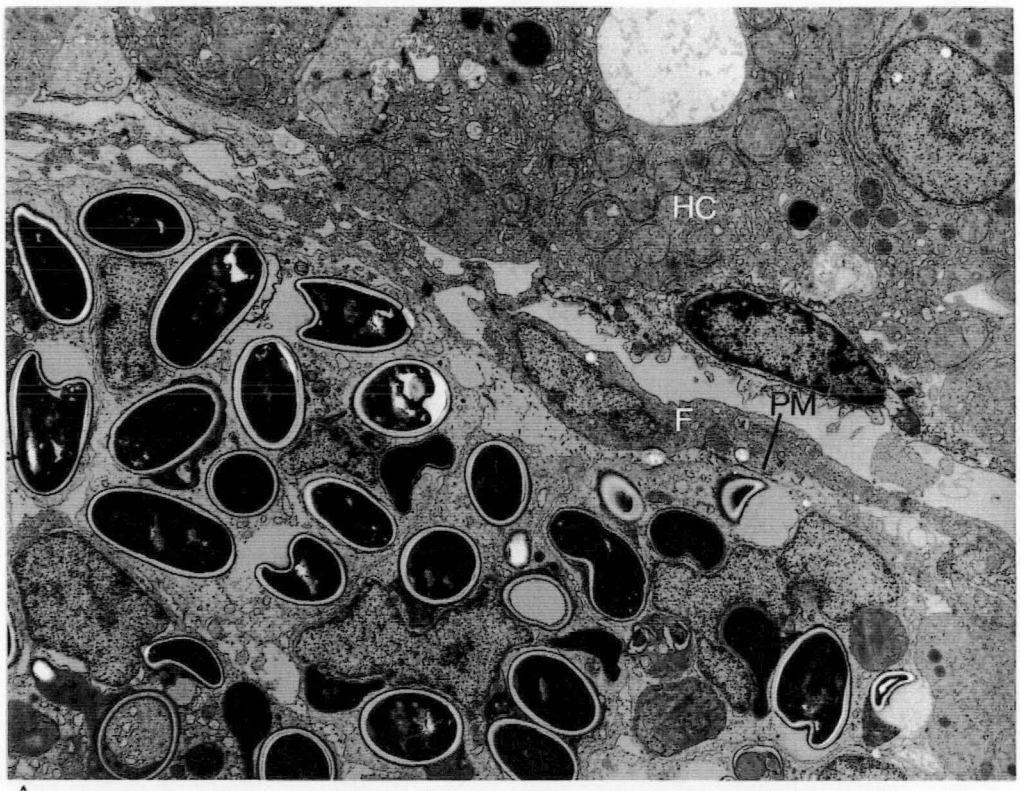


A

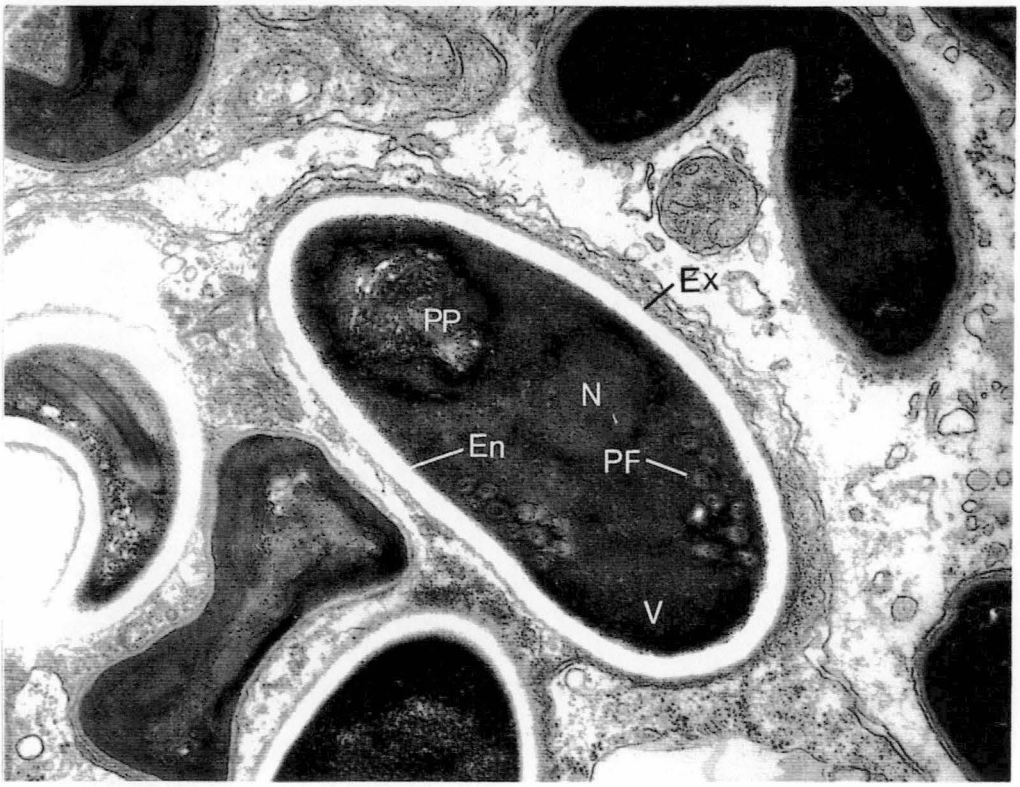



B

Plate 4.20. SEM and TEM micrographs of *Microsporidium hepaticum*. A. SEM micrograph of xenoma (arrow), bar = 10 μm. B. TEM micrograph showing xenoma wall, x 8000. CF, collagen fibres; F, fibroblast; HC, host cell; PM, plasma membrane of host cell.

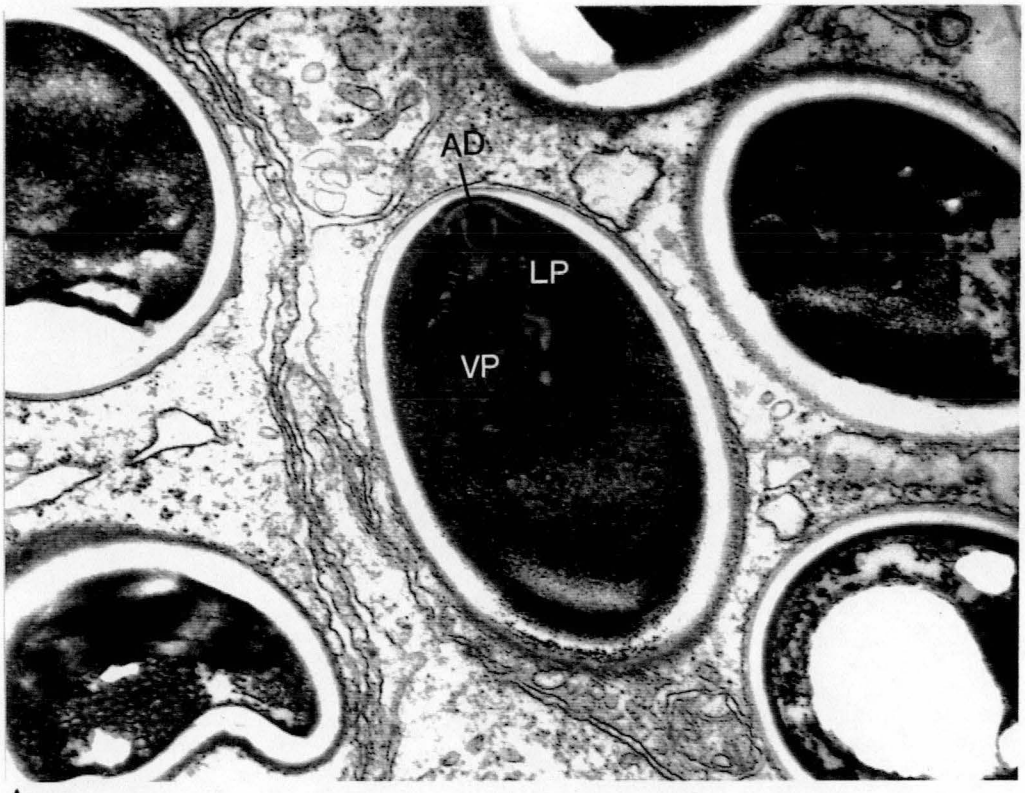



A

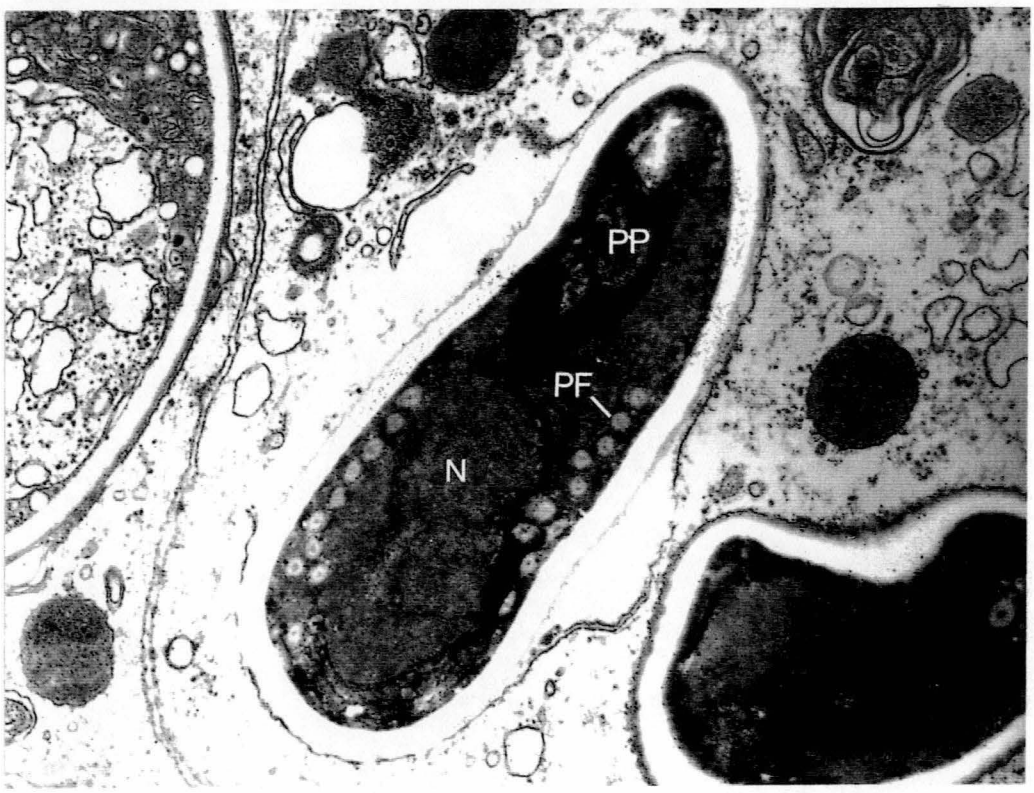



B

Plate. 4.21. TEM micrographs of *Microsporidium hepaticum.* A. Xenoma wall, x 8000. F, fibroblast; HC, host cell; PM, plasma membrane of host cell. B. Spore, x 32000. PP, polarplast; N, nucleus; PF, polar filament; V, vacuole; Ex, exospore; En, endospore.

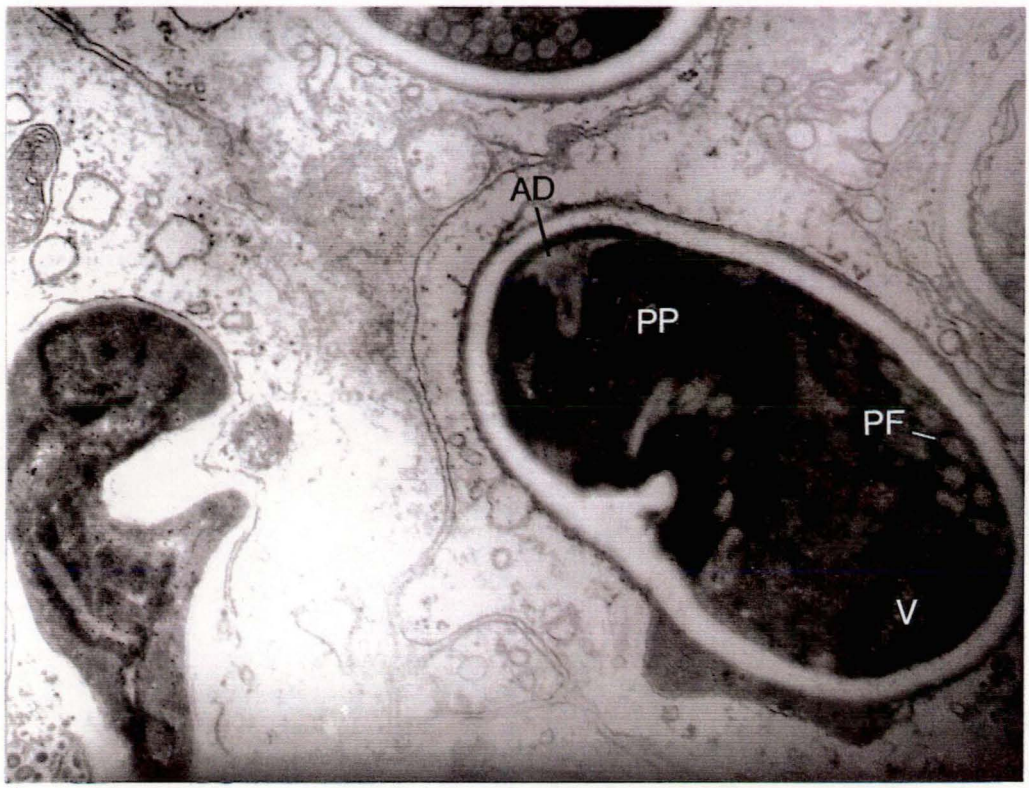



A

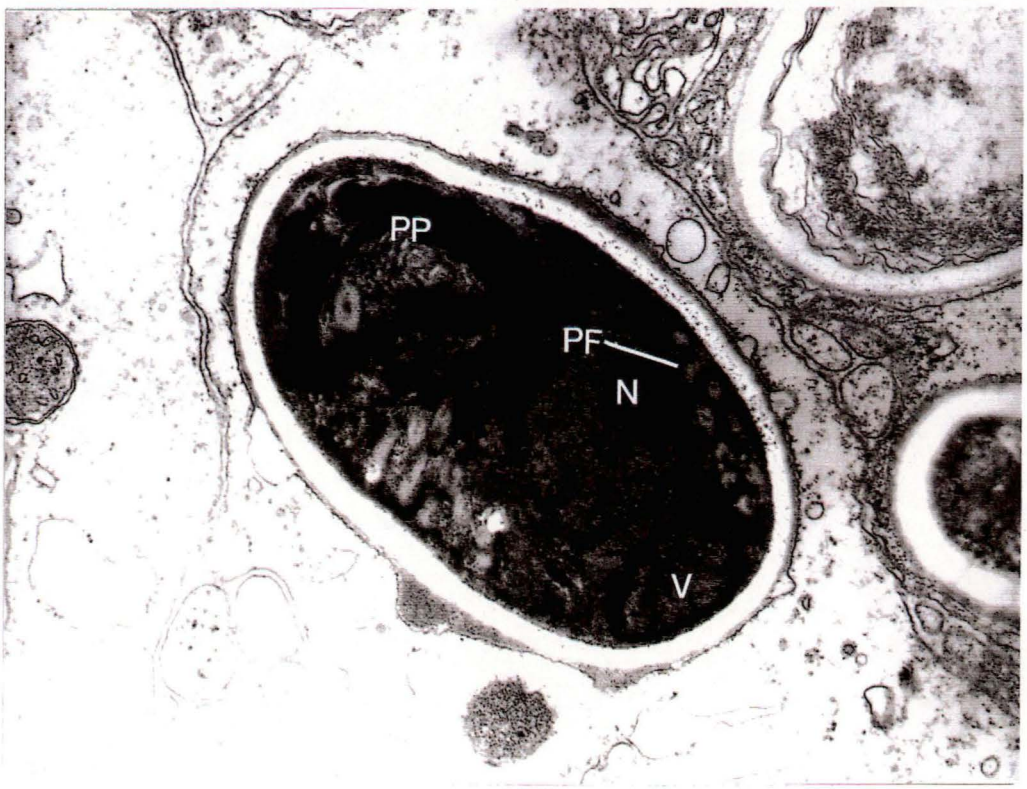



B

Plate 4.22. TEM micrographs of spores of *Microsporidium hepaticum*. A. Spores, x 32000. LP, lamellar polarplast; VP, vesicular polarplast; AD, anchoring disc. B. Spore, x 32000. N, nucleus; PF, polar filament; PP, polarplast.

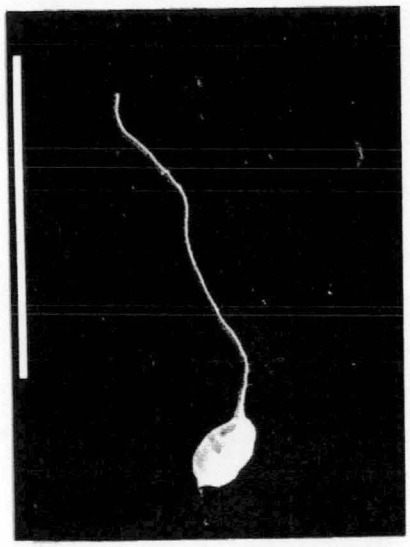



A

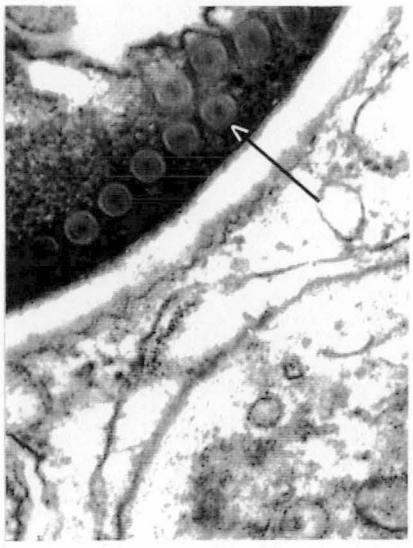



B

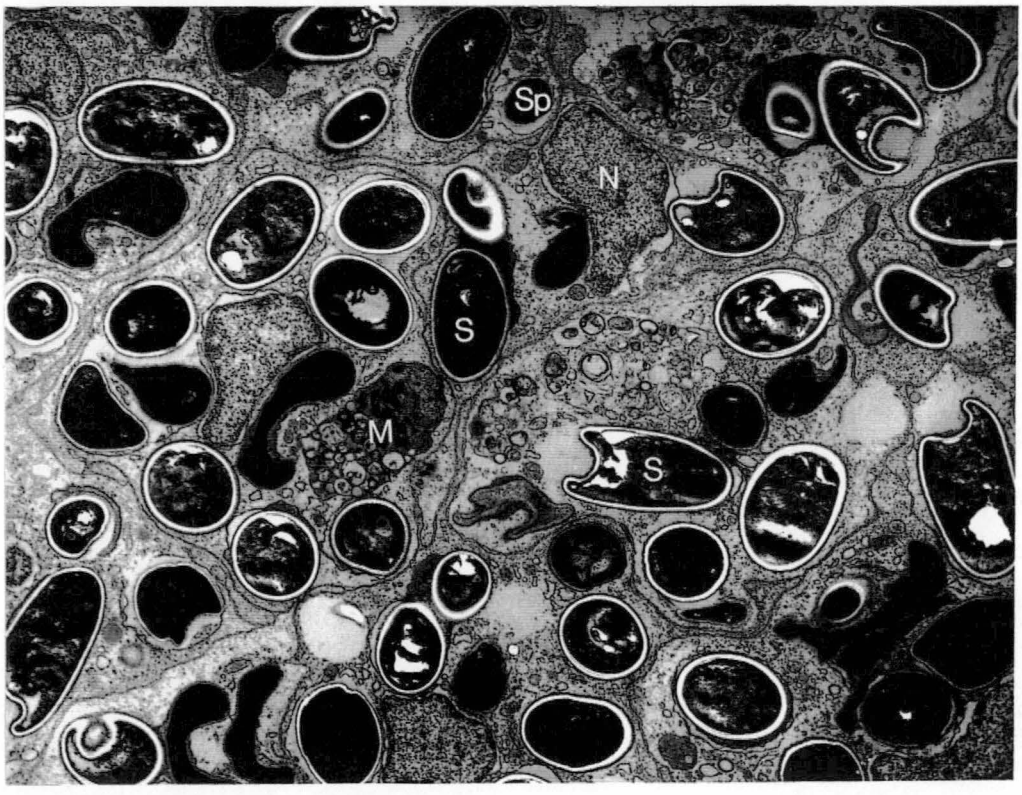
Plate 4.23. TEM micrographs of spores of *Microsporidium* ***hepaticum***. A. Spore, x 32000. PP, polarplast; PF, polar filament; V, vacuole; AD, anchoring disc. B. Spore, x 32000. PP, polarplast; N, nucleus; V, vacuole; PF, polar filament.



A



B




C

Plate. 4.24. SEM and TEM micrographs of *Microsporidium hepaticum*. A. SEM micrograph of spore showing the extruded polar filament. B. TEM micrograph showing the transverse section of polar filament (arrow), x 48000. C. The macrophages with infected spore mass in the liver of *Rhombosolea tapirina*, x 8000.

4.23A). The nucleus appears roughly spherical or ellipsoidal and is situated in a slightly posterior position or in the centre of the spore (Pls. 4.22A, B; 4.23A). The only host reaction observed is phagocytosis of spores. The appearance of macrophages is uniform with cytoplasm containing many spores (up to 16 spores are visible in section) (Pl. 4.24C).

4.3.3 The surface topography of *Paratrichodina tasmaniensis* and *Trichodina nesogobii*

*4.3.3.1 Paratrichodina tasmaniensis:* The shape of this ciliate observed by scanning electron microscopy corresponds with the observations gained by light microscopy. It is disc-like in the aboral view (Pl. 4.25A) and dome-shaped in the lateral view (Pl. 4.25B). The concave aboral (lower) face bears an adhesive disc for attachment to the host (Pl. 4.25A). The vaulted adoral (upper) surface is mainly smooth and naked but has an incomplete fringe of oral ciliary spiral (Pl. 4.25B).

The adhesive disc consists of denticulate ring, border membrane and radial pins. The denticulate ring is composed of 26–29 denticles (Pl. 4.25C). The blades are well-developed, triangular in shape and slanted anteriorwards. The thorns are stunted. The central parts are indistinct and closely spaced. An ellipsoidal foramen is situated in the posterior half of the blade. This foramen corresponds to the small dot in the silver impregnation preparations (Pl. 4.25C, D). There is a well developed border membrane around the circumference of the adhesive disc (Pl. 4.25A); this membrane is invested by closely spaced peripheral pins giving it a striated appearance (Pl. 4.25E). Four to six radial pins overlie each denticle blade and support the border membrane (Pl. 4.25C). The border membrane is comparatively sturdy; the attempt to remove this membrane using both the potassium hydroxide solution and the technique of etching was not successful.

There are two ciliary bands around the body surface. The adoral ciliary spiral turns $150^0$-$200^0$ around the oral zone in the base of the adoral dome (Pls. 4.25A, F; 4.26A). The aboral ciliary complex passes around the entire body immediately above the border membrane. The adoral ciliary spiral consists of four closed rows of long cilia (about 7.4 µm) (Pl. 4.25B). The aboral ciliary band has three distinct sets of cilia: basal ciliary ring, locomotory ciliary wreath and marginal ciliary ring. The basal ciliary ring is made up of a single row of relatively short cilia (about 3.1 µm), immediately adjacent to border membrane (Pls. 4.25A, 4.26B, C). The locomotory ciliary wreath is situated adorally of the basal ciliary ring and separated from it by a basal septum; the ciliary wreath is composed of several rows of medium length cilia (about 5.2 µm) (Pls. 4.25A, 4.26B,

C). The marginal ciliary ring is adoral to the locomotory ciliary wreath and separated from it by a well developed anterior septum; the ring comprises a single band of sparse cilia (Pl. 4.25B).

*4.3.3.2 Trichodina nesogobii:* This ciliate is disc-like in aboral view (Pl. 4.26D) and flattened hat-shaped in lateral view (Pl. 4.26E). The adhesive disc is bound by a border membrane (Pls. 4.26F, 4.27B). This membrane has a striated appearance, indicating invested by the peripheral pins (Pl. 4.27B). The denticulate ring consists of 20 (19-23) denticles, in which three parts - blade, thorn and central part are all well developed (Pl. 4.27A).

The most evident feature by which *T. nesogobii* differs from *P. tasmaniensis* is the adoral ciliary spiral which turns about 370-400° along the oral zone (Pl. 4.26E). The adoral ciliary spiral consists of relatively long cilia, which is about 5.4 μm and is located in at least two rows (Pls. 4.27C, D). Cilia in the outmost row are gathered into tufts with four to five individuals (Pl. 4.27D). The two ends of the ciliary spiral do not meet but diverge in the buccal region (Pl. 4.26E).

The aboral ciliary complex consists of three sets of cilia: basal ciliary ring, locomotory ciliary wreath and marginal ciliary ring (Pls. 4.26F, 4.27B, E). The basal ciliary ring is made up of a single row of relatively short cilia (about 1.3 - 1.4 μm), immediately adjacent to the border membrane (Pls. 4.26F, 4.27B). The locomotory ciliary wreath is situated adorally of basal ciliary and is separated from it by a well-developed two layered basal septum, composed of at least five rows of long cilia (about 4.3-6.7 μm ) (Pls. 4.26F, 4.27B). The marginal ciliary ring, adoral of the locomotory ciliary wreath and separated from it by a poorly developed anterior septum; comprises a single band of sparse cilia. The length of cilia is 2.8-3.1μm, between those of the basal ciliary ring and locomotory ciliary wreath (Pl. 4.27E).

## 4.4 Discussion

4.4.1 *Zschokkella leptatherinae*

*4.4.1.1 Structure of Plasmodium:* In the previous ultrastructural studies on myxosporeans, Current *et al.* (1979) indicated that the ultrastructure of plasmodium may depend upon the location of the parasite within the host. Coelozoic plasmodia have a

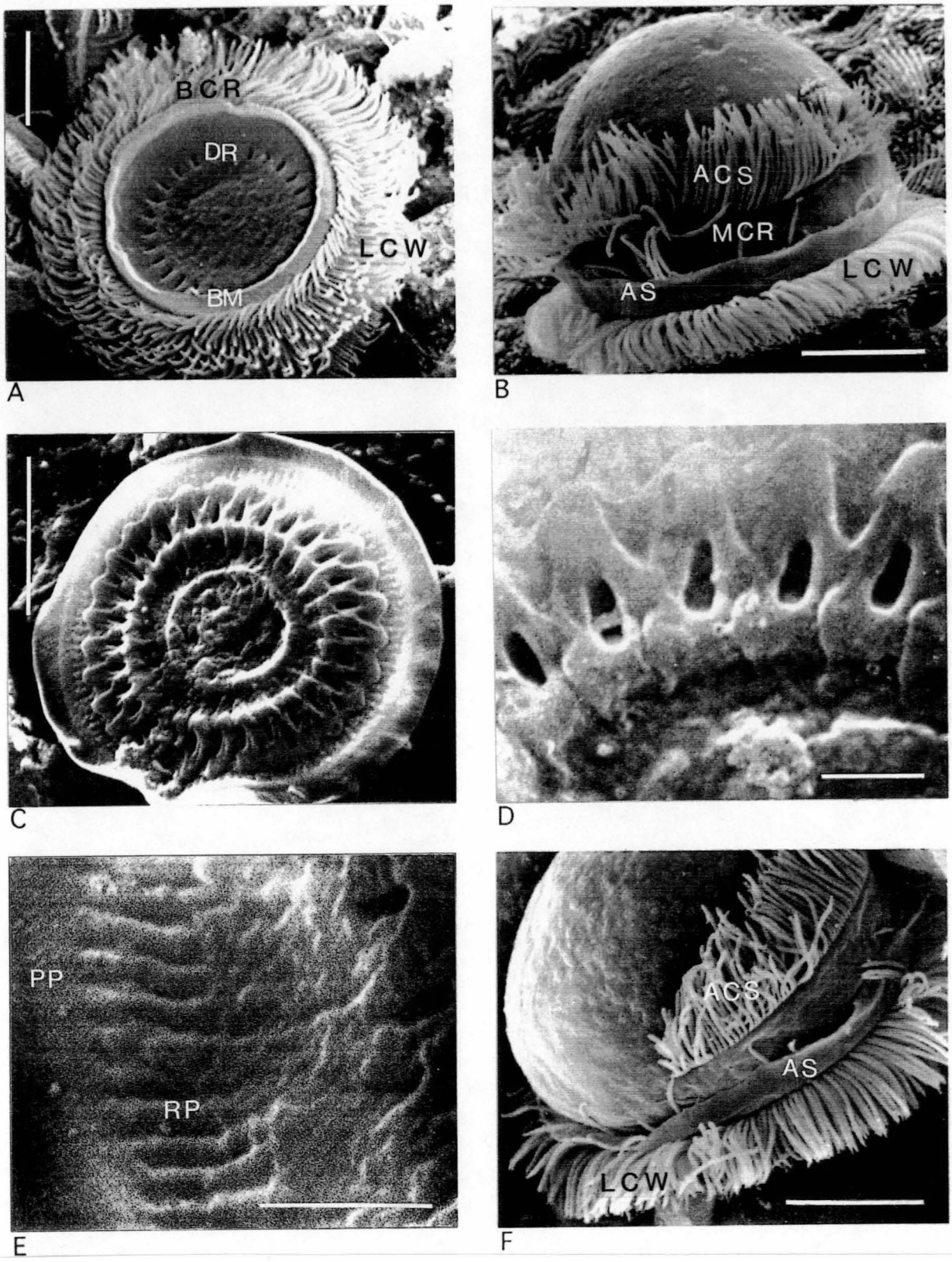



Plate 4.25. SEM micrographs of *Paratrichodina tasmaniensis*. DR, denticulate ring; LCW, locomotory ciliary wreath; BM, border membrane; BCR, basal ciliary ring. ACS, adoral ciliary spiral; AS, anterior septum; MCR, marginal ciliary ring. RP, radial pins; PP, peripheral pins. A. Aboral view of specimen. B. Lateral view of specimen. C. The adhesive disc showing an ellipsoid foramen in the denticle. D. Enlargement of the adhesive disc. E. Part of the adhesive disc showing peripheral pins. F. Lateral view of specimen. Bars = 10 μm in A, B, C, F, and 2 μm in D, E.

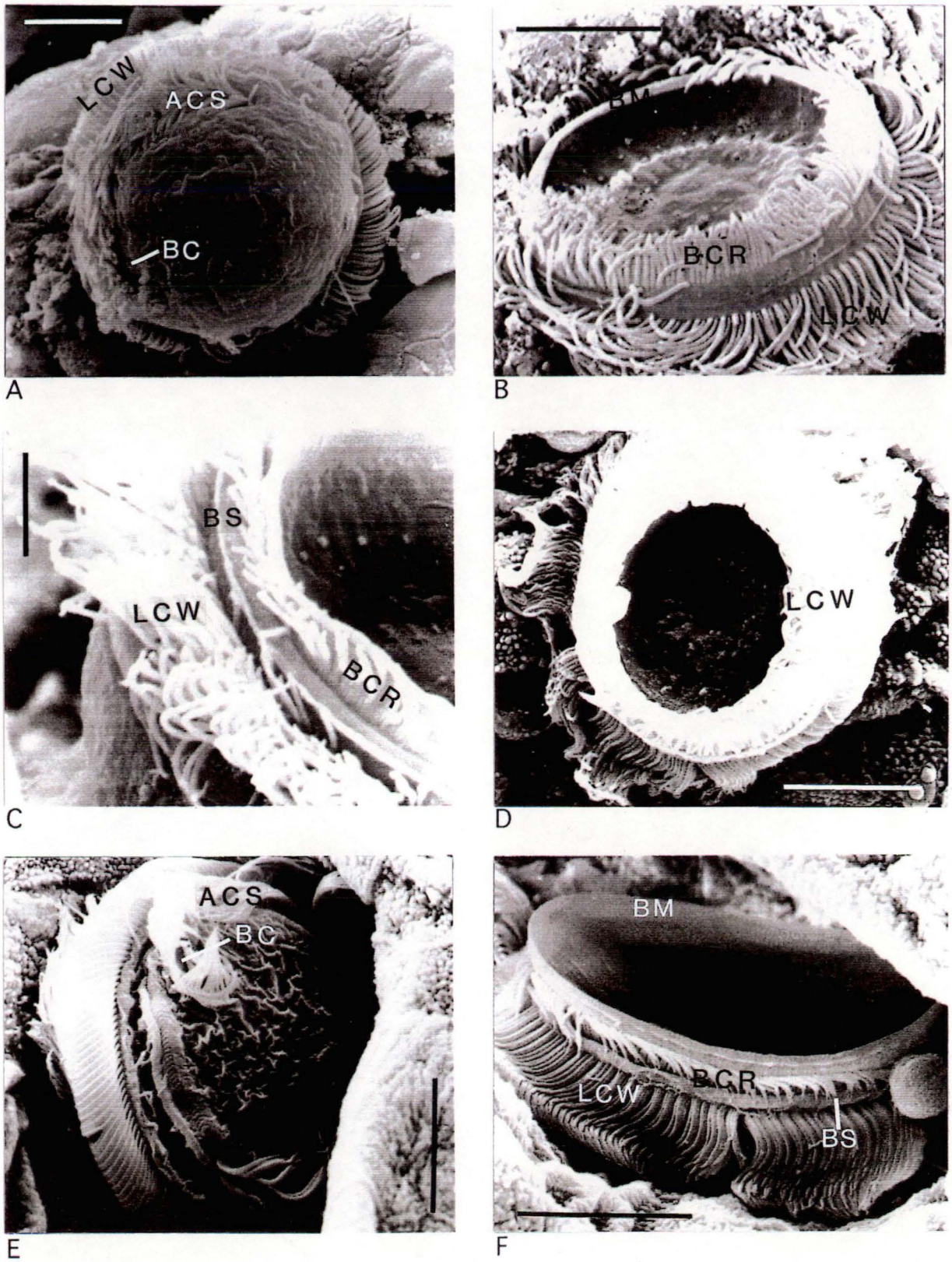



Plate 4.26. SEM micrographs of *Paratrichodina tasmaniensis* and *Trichodina nesogobii.* ACS, adoral ciliary spiral; LCW, locomotory ciliary wreath; BC, buccal cavity; BCR, basal ciliary ring; BM, border membrane; BS, basal septum; DR, denticulate ring; MCR, marginal ciliary ring; AS, anterior septum. A. Adoral surface of *P. tasmaniensis.* B. Lateral view of *P. tasmaniensis.* C. Enlargement of aboral ciliary complex of *P. tasmaniensis.* D. Aboral view of *T. nesogobii.* E -F. Lateral view of *T. nesogobii.* Bars = 10 μm in A, B, D, E, F and 5 μm in C.

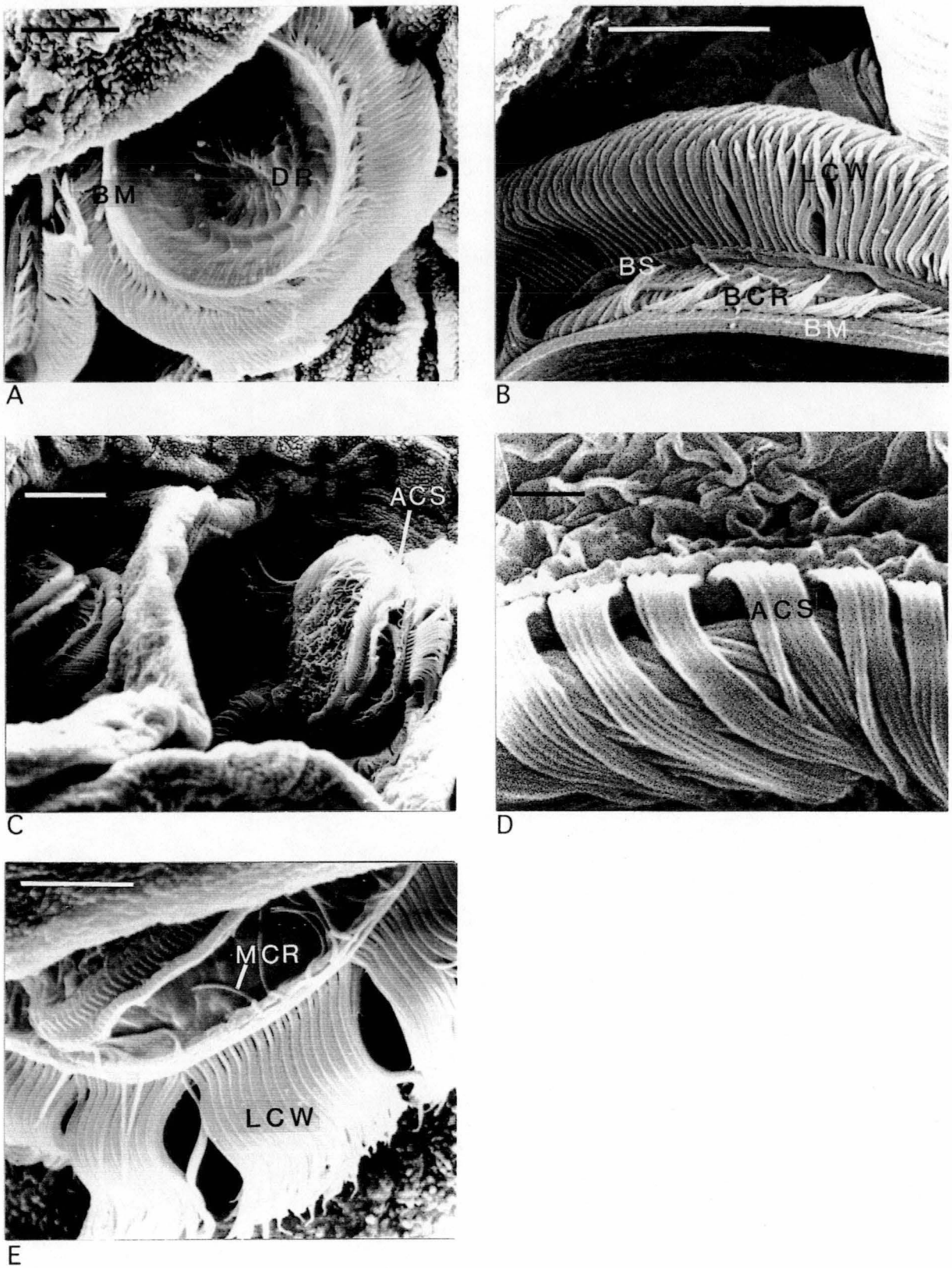



Plate 4.27. SEM micrographs of *Trichodina nesogobii*. BM, border membrane; DR, denticulate ring; BCR, basal ciliary ring; BS, basal septum; LCW, locomotory ciliary wreath; ACS, adoral ciliary spiral; MCR, marginal ciliary ring; AS, anterior septum. A. Aboral view of specimen. B. Enlargement of part of specimen. C. Lateral view of two specimens on the gills of host fish. D. Enlargement of part of adoral ciliary spiral. E. Enlargement of part of specimen. Bars = 10 μm in A, C, 5 μm in B, E and 1 μm in D.

single outer unit membrane which forms numerous microvilli-like projections which extend into the host fluid. Histozoic plasmodia have one or two unit membrane which forms pinocytotic canals extending into the parasite ectoplasm. However, Paperna *et al.* (1987) observed the pinocytotic system in the coelozoic plasmodia of *Myxidium giardi* and he indicated that the coelozoic and histozoic plasmodia may differ in their fine structures of the pellicle of plasmodium instead of the appearance of pinocytotic systems. Moreover, the pinocytotic systems have also been found in some other coelozoic plasmodia (Lom and de Puytorac, 1965b, Davis and Sienkowski, 1988). The ultrastructural study on *Z. leptatherinae* reveals that a single unit membrane with a number of microvilli projections cover the plasmodium externally and no pinocytotic canals were observed. This suggests that the occurrence of the pinocytotic system may vary with the species and is not related to the location of the parasite.

*Zschokkella russelli*, which is parasitic in the hepatic ducts and gall ducts of *Ciliata mustela*, is the member of the genus which has had it's ultrastructure examined in detail (Davies and Sienkowski, 1988). The pinocytotic system is present in this species. A fuzzy mucus layer covers the external surface of microvilli projections; these projections are bound by membranes. Another feature of the plasmodium of *Z. russelli* is that the fine tubular structures which lie externally to young sporonts and infrequently accompany some mature sporonts. These three features are not present in *Z. leptatherinae*.

There are abundant larger mitochondria beneath the outer surface of the plasmodia of *Z. leptatherinae*. The arrangement of the microvilli and adjacent mitochondria, according to Hulbert *et al.* (1977), is a characteristic of specialised transport cells such as those found in kidney tubules, crab gills and avian salt glands. The significance of this arrangement in myxosporean plasmodia is unclear. It may reflect a transport function in the plasmodia of *Z. leptatherinae* as in crabs and birds.

*4.4.1.2 Formation of pansporoblast and development of sporont:* The process of sporogony in *Z. leptatherinae* is essentially similar to those described by Desser and Paterson (1978a) for *Myxobolus* sp., Current (1979) for *Henneguya adiposa* ; Current *et al.* (1979) for *Myxobolus funduli* ; Pulsford and Matthews (1982) for *Myxobolus exiguus* and Desser *et al.* (1983) for *Thelohanellus mikolskii*. The young binucleate pansporoblast stage is formed from the envelopment of one generative cell by another.

The enclosed cell divides to form a sporont and the enveloping cell eventually forms the pansporoblastic membrane. The function of the enveloping cell has been compared with the nurse cell which accompanies oocyst development in annelids, sponges and insects (Desser and Paterson, 1978a). During the sporogenesis, the enveloping cell may provide structural and nutritive support and possibly supply molecular regulators which control sporogenesis (Pulsford and Matthews, 1982). In the ultrastructural studies of *Zschokkella,* the union of two generative cells has been observed in *Z. nova* Klokacheva, 1914 by Lom and de Puytorac (1965b). However, in an examination of *Z. russelli,* Davis and Sinekowski (1988) did not detect this process; the earliest stage that they observed was in young sporont undergoing internal organisation.

The enclosed cell begins to divide soon after the binucleate pansporoblast is formed. Generally, the stages of this process are the division of the single cell into 4, 6, and 10 undifferentiated cells. The 10 cells then differentiate into two 5-cell spore-producing units which develop into two spores (Desser and Paterson, 1978a; Current, 1979; Current *et al.*, 1979, Pulsford and Matthew, 1982; Desser *et al.*, 1983). In the present examination of *Z. leptatherinae*, two primordia of polar capsules were observed in one capsulogenic cell and they were separated into two capsulogenic cells at a later stage. This suggests that the 8-cell sporont may first differentiate and arrange into two spore-producing units. They could then divide into 10 cells, 5 in each unit.

*4.4.1.3 Development of polar capsules and polar filament:* The development of the polar capsule begins early in the sporogenesis. The origin of the capsular primordium was reported by Desser and Paterson (1978a) and Pulsford and Matthews (1982) as from the rough endoplasmic reticulum in immature capsulogenic cells. No trace of the origin of the capsular primordium has been found in the current study. In *Z. leptatherinae*, the capsular primordia appear earlier than the external tubule and the latter is formed by the extension of the capsular primordium. This result is consistent with the report of *Myxobolus exiguus* and *Myxobolus* sp. by Pulsford and Matthews (1982) and Desser and Paterson (1978a). But in *Thelohanellus nikolskii*, Desser *et al.* (1983) found that the capsular primordia and the external tubule occur simultaneously in the sporont. In the examination of some other myxosporeans, Lom and de Puytorac (1965a) found a cylindrical structure which contains microtubules which they considered to be an earlier capsular primordium. This structure is probably the external tubule recognised by other authors.

The pattern of formation of the polar filament is not clear in the present study. However, after the rudiment of the polar filament appears, the external tubule is absent. Also, as can be seen in Pl. 4.16, the invagination of the wall of the polar capsule results in the formation of the wall of the internalised filament. These two examples provide the evidence that the external tubule may be withdrawn into the capsular primordium and that its components form the filament. This may be a very rapid process so no details have been revealed. Desser and Paterson (1978a) have observed the membranous elements of the external tubule in the capsular primordium.

*4.4.1.4 Sporoplasmosomes and sporoplasm:* Sporoplasmosomes were first examined ulatrastructurally and named by Lom *et al.* (1986) for the dense inclusion in the sporoplasm of *Hofferellus gilsoni*. Subsequently, these small structures have been found in many other species (Yamamoto and Sanders, 1979, Mitchell *et al.*, 1985, Lom *et al.*, 1989). The structure of sporoplasmosomes in *Z. leptatherinae* is different from that reported from other myxosporeans species. However, as indicated by Lom *et al.* (1989), this difference is not useful in the systematics of the taxonomic position of the myxosporeas since the structure of sporoplasmosomes varies irrespective of the genus.

In the ultrastructural study of *Z. russelli,* Davies and Sienkowski (1988) observed the finger-like extensions of the sporoplasm. Similar extensions have been found in *Ceratomyxa shasta* (Yamamota and Sanders, 1979). These extensions were not observed in the present study of *Z. leptatherinae.*

In conclusion, the ultrastructure of *Z. leptatherinae* is essentially the same as that of *Z. russelli* (Davies and Sienkowski, 1988). But there are some features different from those of *Z. russelli.* These features are: (1) the plasmodium has no pinocytotic system and no fuzzy mucus layers cover the external surface of microvillus projections; (2) there is no fine tubular structure lying external to the young sporont; (3) the 8-cell sporot differentiates first and then divides into 10-cell sporont; (4) the polar capsules often develop asynchronously within each spore and also within each sporont. (5) finger-like extensions of the sporoplasm do not exist in developed or mature spore; and (6) 10-12 sutural ridges are found on the spore valves.

4.4.2 *Microsporidium hepaticum* sp. nov.

A perusal of existing descriptions indicates that this is a new species. To date, no microsporidia have been described from marine fish in Australia. Eight species have been previously reported from the liver of fish. They are *Pleistophora priacanthusis* Hua and Dong, 1983; *Glugea depressa* Thelohan, 1895; *G. machari* (Jirovec) Sprague, 1977; *G. pseudotumefaciens* Pflugfelder, 1952; *Ichthyosporidium giganteum* Thelohan, 1895; *Microsporidium ovoideum* Thelohan, 1895; *Microgemma hepaticus* Ralphs and Matthews, 1986; *Microsporidium zhanjiangensis* Hua and Zhang, 1988. All of those species have no comparable spore structure, host records and geographical distribution.

The generic assignment of the present parasite requires further study since the developmental stages is unknown. In accord with Sprague (1977b), I place it in a collective group, *Microsporidium*. This is an assemblage of identifiable species of which the generic positions are uncertain and treated as a generic group for taxonomic convenience. The name of *Microsporidium hepaticum* is proposed referring to the site of infection.

4.4.3 *Paratrichodina tasmaniensis* sp. nov. and *Trichodina nesogobii* sp. nov.

In 1958, Lom suggested using Klein's silver impregnation technique to demonstrate the components of the adhesive disc. Since then, this method has been universally employed in the study of trichodinids. In the present study, the denticle of *Paratrichodina tasmaniensis* shows an obvious ellipsoidal foramen under the scanning electron microscope. However, only a small dot is shown in the silver impregnated specimens. This suggests that, for some species, examination using scanning electron microscopy is important and it can provide additional data for the species identification.

Seven genera of the family Trichodinidae have been found from fish, these are *Dipartiella* Stein, 1961; *Paratrichodina* Lom, 1963; *Trichodinella* Sramek-Husek, 1953; *Tripartiella* Lom, 1959; *Trichodina* Ehrenberg, 1830; *Vauchomia* Mueller, 1938; *Hemitrichodina* Basson and Van As, 1989 (Basson and Van As, 1989). The *Trichodinella*-group comprises four genera: *Dipartiella, Paratrichodina, Tripartiella* and *Trichodinella* (Lom, 1963; Lom and Haldar, 1977; Basson and Van As, 1989). This group differs from other genera of trichodinids in three aspects: the reduced adoral ciliary spiral is less than $280^0$, the particular structure of the aboral adhesive disc, and the smaller size. The present examinations based on SEM reveal that the adoral ciliary spiral

turns 150-200$^0$ along the oral zone in *Paratrichodina tasmaniensis*, while in *Trichodina nesogobii*, it forms a 370-400$^0$ spiral along the oral zone. This result is consistent with the observations on genera *Trichodina* and the*Trichodinella*-group by light microscopy (Basson and Van As, 1989). Other characteristics including the structure of adoral ciliary spiral, the structure and the distribution of aboral ciliary complex seem to have no fundamental differences between *P. tasmaniensis* and *T. nesogobii*.

Although more than 150 species of the family Trichodinidae have been reported, the general surface topography has been previously investigated only for *Trichodina oviducti* (Khan *et al.*, 1974), *T. truttae* (Arthur and Margolis, 1984) and *T. japonica* (Imai *et al.*, 1991). The structure of the denticles was examined using SEM for *Trichodina lucioperca* (cited by Lom and Dykova, 1992) and *Trichodina heterodentata* (Van As and Basson, 1987, 1989). The present study of *Paratrichodina tasmaniensis* is the second using scanning electron microscopy to describe the species of the*Trichodinella*-group. The only previous use of SEM for a member of this group was by Lom (1973) who briefly described the structure of the adhesive disc of *Trichodinella epizootica*. The surface topography of *P. tasmaniensis* described here agrees enough with this species to be placed into the *Trichodinella*-group. However, the new species has an anterior septum and marginal ciliary ring which are lacking in *T. epizootica*. The general surface topography and the arrangement of the adoral and aboral ciliary rings of *P. tasmaniensis* closely resemble the observations on *T. japonica* from the gills of Japanese eel, *Anguilla japonica* by Imai *et al.* (1991). In both species and also in *T. nesogobii*, the basal ring cilia are clearly shorter than the locomotory wreath cilia. However, those in *T. truttae* (Arthur and Margolis, 1984) were proportionally longer than those in *P. tasmaniensis* and *T. nesogobii*. Cilia of the marginal ciliary ring in both present species are arranged sparsely, while they extended continuously in *T. pediculus* Muller, 1786 under the transmission electron microscope (Hausmann and Hausmann, 1981a). The anterior septum also shows a various degree of development in different species. Those in *T. urinicola* Dogiel, 1940 (Favard *et al.*, 963), *T. pediculus* (Hausmann and Hausmman, 1981a) and *T. nesogobii* are poorly developed, while in *T. truttae* (Arthur and Margolis, 1984), *T. japonica* (Imai *et al.*, 1991), as well as in *P. tasmaniensis*, they are well developed.

Van As and Basson (1989) developed a method to remove the soft material of trichodinids. In the present study, the potassium hydroxide solution at various

concentrations (5% - 20%), as well as an inert gas ion-etching technique of etching were attempted to remove the border membrane and isolate the denticles. However, neither of these was successful. This may indicate the sturdy nature of the border membrane of *Paratrichodina tasmaniensis*. Lom (1973) found that the injury caused by trichodinids to the host is completed by the border membrane cutting its sharp edge into the tissue and by pulling the cells into the vaulted disc to form a cup-like sucker. The present result suggests that *P. tasmaniensis* may have the potential to exert a relatively severe damage to its host as a result of a strong sucker-like action induced by a sturdy border membrane.

In the study of *Trichodina truttae,* Arthur and Margolis (1984) reported that the profile of this ciliate is relatively flattened whereas that of an endoparasitic species, *T. oviducti,* is highly vaulted. They suggested this variation may be an adaptation to life on the external surface which decreases the possibility of the parasite being dislodged by water currents. The present two species, *Paratrichodina tasmaniensis* and *Trichodina nesogobii* are both exposed to the water movements involved in ventilating the gills. However, *P. tasmaniensis* is clearly vaulted and *T. nesobogii* is more or less flattened. Therefore, some doubt must be cast on the explanation of profile differences of the two *Trichodina* species advanced by Arthur and Margolis (1984).

# CHAPTER 5 - BIOLOGY OF *ZSCHOKKELLA LEPTATHERINAE*, WITH PARTICULAR REFERENCE TO THE HOST FISHES, *ATHERINOSOMA MICROSTOMA* AND *LEPTATHERINA PRESBYTEROIDES*

## 5.1 Introduction

All the parasites have two living environments: microenvironment - the host's body, and macroenvironment - the environment surrounding the host. Therefore, the biological study of parasites include three main aspects: the parasite, the relationship between the parasite and the host, and the relationship between the parasite and the external environment. Although approximately 2420 species of fish-infectiong protozoa have been recorded (Lom and Dykova, 1992), relatively little is known concerning their ecology and pathogenicity. In the present study, the biology of two species; *Zschokkella leptatherinae* from the hepatic ducts and gall bladder of *Atherinosoma microstoma* and *Leptatherina presbyteroides* and *Paratrichodina tasmaniensis* from the gills of the same host species were studied.

In this chapter, the following topics will be covered: the frequency distributions of *Zschokkella leptatherinae* within the host populations; the relationships between *Z. leptatherinae* and host species including fish size and sex; the seasonal variations in prevalence and intensity of *Z. leptatherinae* infection; and the histopathology of *L. presbyteroides* with the infection of *Z. leptatherinae*. Similar aspects of the biology of *Paratrichodina tasmaniensis* will be studied in Chapter 6.

## 5.2 Material and methods

### 5.2.1 Collection of samples

A total of 514 *Atherinosoma microstoma* and 589 *Leptatherina presbyteroides* were collected in 19 and 22 samples between January 1990 and June 1992 respectively from Dru Point, south-east of Hobart, Tasmania. The area and methods of collecting fish were the same as given in Chapter 3. At each sampling date, salinity and temperature were measured using an optical refractometer and a mercury bulb thermometer respectively. Temperature varied seasonally between 10 and 21.5° C (Fig. 5.1). Salinity varied generally between 34 and 35 salinity units. It fell to 30 units during June and July of 1990 probably because of heavy rainfall (Fig. 5.2). In the laboratory, the fish were measured, weighted and sexed. For the investigation of frequency distribution, all the fish collected during the studied period were used, while in the study of seasonal variations and host-

parasite relationships, the fish caught at regular monthly intervals from January 1990 to April 1991 were used. The number of spores of *Zschokkella leptatherinae* was counted using a haemocytometer. The livers were first homogenised with Ringer's solution for 5-10 min.

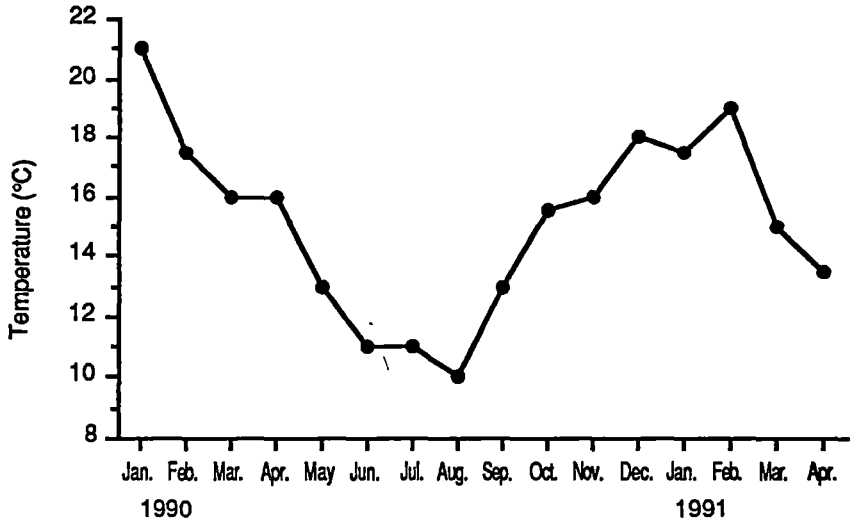



**Fig. 5.1** Monthly mean surface water temperatures at Dru Point.

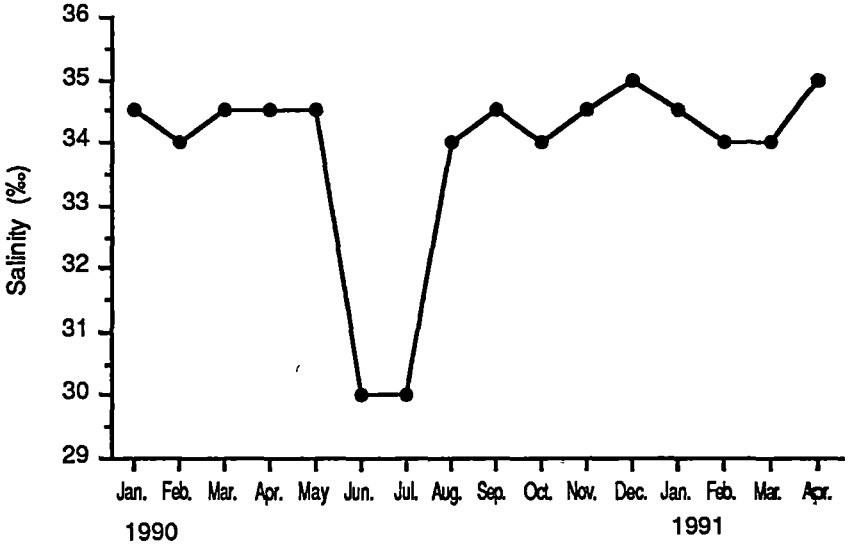



**Fig. 5.2** Monthly mean surface water salinities at Dru Point.

5.2.2 Statistical methods

All statistical analyses were conducted using *Statview* 512 software on a *Macintosh* computer. For the analysis of the frequency distribution of parasite, $\chi^2$ analysis was used to test if the expected distribution model fit the observed distribution. A fit was accepted when the $\chi^2$ value was not significant at the 95% level ($p > 0.05$). Variations in the prevalence of parasite infection in different fish species, and the fish of different lengths

and sexes, and in bimonthly samples were tested using a 2 x c Contingency Table. A secondary analysis, based on an examination of standardised residuals, was also established to judge the deviation of groups from the average. Magnitudes in excess of 2.0 are taken as indicators of significant deviation from the average. For comparing the prevalence between male and female samples of the host fish, 2 x 2 Contingency Table analysis was conducted. Variations in the intensity of parasite infection in fish of different lengths and in bimonthly samples using one-factor Analysis of Variance (ANOVA). A two-factor ANOVA was used to analyse the differences in mean fork length of host fish infected and uninfected with *Z. leptatherinae* in different months. A significant level of 95% ($p < 0.05$) or more was taken as a rejection of the null hypothesis which states that there was no dependence between the variables.

### 5.2.3 Histological techniques

The liver tissue and entire gall bladders from 10 fish which showed various infection degree were used. The procedures used were the same as given in Chapter 3 except that, as well as hematoxylin and eosin (H & E), the tissue section was also stained with Mallory's Triple to reveal the connective fibres.

All terminology in this chapter is consistent with the recommendations of Margolis *et al.* (1982). For convince, *mean intensity* was written as *intensity* in this chapter as well as in Chapter 6. The definition of prevalence, intensity and mean intensity of parasite infection is the same as described in Chapter 3.

## 5.3 Results

### 5.3.1 Frequency distributions of parasite within the host populations

A total of 84 infected *Atherinosoma microstoma* and 188 infected *Lepatherina presbyteroides* of mixed age and sex were investigated for the frequency distribution.

The number of parasites per individual *A. microstoma* was divided into 9 classes on a logarithmic scale at intervals of 8; the scale is given in Table 5.1. For *L. presbyteroides,* 10 classes on a logarithmic scale at intervals of 5 (Table 5.2) were used for the analysis. The observed numbers of fish infected by each group of *Z. leptatherinae* in both fish species are given in the tables.

**Table 5.1** The number of *Zschokkella leptatherinae* in *Atherinosoma microstoma* arranged on a log-normal scale at intervals of x 8.

| Class | Parasites. fish$^{-1}$ | No.fish (observed) S(R) | ln S(R) |
|---|---|---|---|
| 1 | 1-7 | 1 | 0.00 |
| 2 | 8-63 | 5 | 1.61 |
| 3 | 64-511 | 6 | 1.79 |
| 4 | 512-4095 | 15 | 2.71 |
| 5 | 4096-32767 | 28 | 3.40 |
| 6 | 32768-262143 | 20 | 3.00 |
| 7 | 262144-2097151 | 5 | 1.61 |
| 8 | 2097152-16777215 | 2 | 0.69 |
| 9 | 16777216-134217727 | 2 | 0.69 |
| Total | | 84 | 15.5 |

**Table 5.2** The number of *Zschokkella leptatherinae* in *Leptatherina presbyteroides* arranged on a log-normal scale at intervals of x 5

| Class | Parasites. fish$^{-1}$ | No.fish (observed) S(R) | ln S(R) |
|---|---|---|---|
| 1 | 3-12 | 2 | 0.69 |
| 2 | 13-62 | 5 | 1.61 |
| 3 | 63-312 | 14 | 2.64 |
| 4 | 313-1562 | 22 | 3.09 |
| 5 | 1563-7812 | 29 | 3.37 |
| 6 | 7813-39062 | 44 | 3.78 |
| 7 | 39063-195312 | 38 | 3.64 |
| 8 | 195313-976562 | 19 | 2.94 |
| 9 | 976563-4882812 | 10 | 2.30 |
| 10 | 4882813-24414062 | 5 | 1.61 |
| Total | | 188 | 25.68 |

In both speceis, a larger number of infected fish occurred in the middle classes of parasite intensity (class 5 in *A. microstoma* and class 6 in *L. presbyteroides* ).

Log-normal distributions are described by

$$S(R) = S_0 e^{(-a^2R^2)} \quad (5.1)$$

where S(R) is the number of fish in the Rth class from the mode, $S_0$ is an expected number of fish in the modal class (the class with the most fish), and parameter $\alpha$ is an inverse measure of the width of the distribution (i.e., $\alpha = {}^1/2\ \sigma$, where $\sigma$ is the standard deviation). R for *i* th class is given by

$$R = \log_2 (N_i / N_0) \quad (5.2)$$

Where $N_i$ is the species abundance in the *i* th class and $N_0$ is the species abundance in the modal class. From Eq. 5.2, it can be seen that R is always equal to 0 in the modal class since $N_i \doteq N_0$. Since each class represents a doubling of abundance, the ratio $N_i / N_0$ for the successive class to the right of the modal will be 2, 4, 8, 16, 32, etc., and R will be, respectively, 1, 2, 3, 4, 5, etc.; for the class to the left of the modal, the ratio $N_i / N_0$ for the successive class will be 0.5, 0.25, 0.125, 0.0063, 0.031, etc., and R will be, respectively, -1, -2, -3, -4, -5, etc.

Two parameters are required in order to present log-normal distribution model. An estimate of $\alpha$ is obtained from the equation

$$\alpha = \sqrt{\frac{\ln[(S(0) / S(R_{max})]}{R^2_{max}}} \quad (5.3)$$

Where S(0) is the observed number of fish in the modal class and $S(R_{max})$ is the observed number of fish in the class most distant from the modal class (indicated by $R_{max}$). An estimate of parameter $S_0$ is obtained by either fixing it at the observed value for the number of fish in the modal class, S(0), or by estimating it from the equation

$$S_0 = e^{(\overline{\ln S(R)} + a^2 \overline{R}^2)} \quad (5.4)$$

where $\overline{\ln S(R)}$ is the mean of the logarithms of the observed number of fish per class, $\alpha$ is estimated from Eq. 5.3, and $\overline{R}^2$ is the mean of the $R^2$.

The expected log-normal frequencies were computed using the two above parameters. R and $R^2$ value for each class are given in Table 5.3 for *A. microstoma*. $R_{max}$ = -4 and +4, $R^2_{max}$ =16. A value for $\alpha$ using both values of $S(R_{max})$ (1, and 2) in Eq.5.3 was computed and averaged.

$$\alpha_1 = \sqrt{\frac{\ln(28/1)}{16}} = 0.456$$

$$\alpha_2 = \sqrt{\frac{\ln(28/2)}{16}} = 0.406$$

$$\alpha = (\alpha_1 + \alpha_2) / 2 = 0.43$$

From Eq. 5.4

$$\overline{\ln S(R)} = \text{Total } \ln S(R) / 9 = 1.72$$

$$\overline{R}^2 = \text{Total } R^2 / 9 = 6.67$$

and, thus

$$S_0 = e^{[1.72 + (0.43)^2 \times 6.67]} = 19.17$$

The expected frequencies were computed using $\alpha = 0.43$ and $S_0 = 19.17$ and 28 respectively according to Eq. 5.1. The results are shown in Table 5.3. The values of observed and two sets of expected frequencies are plotted in Fig. 5.3.

The goodness of fit of the model to the observed frequencies is tested with a Chi-square statistic. The $\chi^2$ values for each group are computed from:

$$\chi^2 = \Sigma \frac{(O - E)^2}{E} \qquad (5.5)$$

where E = expected value, O = observed value.

When $S_0 = 19.17$ was used to calculate the expected frequencies, total $\chi^2 = 10.39$, $0.2 > p > 0.1$; $S_0 = 28.00$ was used in calculating the expected values, total $\chi^2 = 15.12$, $0.05 > p > 0.02$ (d.f = 7). Thus, the first parameter of estimate $S_0$ gives an acceptable fit to the distribution of *Z. leptatherinae* within the population of *A. microstoma.*

Table 5.2 shows the original data of *Zschokkella leptatherinae* in *Leptatherina presbyteroides.* The modal class is class 6 which has 44 infected fishes, that is S(0) = 44. From Table 5.4, the following values were obtained: $R_{max} = 5$, $S(R_{max}) = 2$, and $R^2_{max} = 25$.

**Table 5.3** Goodness-of-fit test for the log-normal model to observed data of *Zschokkella leptatherinae* in *Atherinosoma microstoma*. Expected values are given for two $S_0$ parameter estimates

| Class | R | $R^2$ | Observed S(R) | $S_0 = 19.17$ Expected S(R) | $\chi^2$ | $S_0 = 28$ Expected S(R) | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| 1 | -4 | 16 | 1 | 0.99 | 0.00 | 1.45 | 0.14 |
| 2 | -3 | 9 | 5 | 3.63 | 0.52 | 5.30 | 0.02 |
| 3 | -2 | 4 | 6 | 9.15 | 1.08 | 13.36 | 4.06 |
| 4 | -1 | 1 | 15 | 15.93 | 0.05 | 23.27 | 2.94 |
| 5 | 0 | 0 | 28 | 19.17 | 4.07 | 28.00 | 0.00 |
| 6 | 1 | 1 | 20 | 15.93 | 1.04 | 23.27 | 0.46 |
| 7 | 2 | 4 | 5 | 9.15 | 1.88 | 13.36 | 5.24 |
| 8 | 3 | 9 | 2 | 3.63 | 0.73 | 5.30 | 2.06 |
| 9 | 4 | 16 | 2 | 0.99 | 1.02 | 1.45 | 0.21 |
| Total | | 60 | 84 | 78.59 | 10.39 | 114.79 | 15.12 |

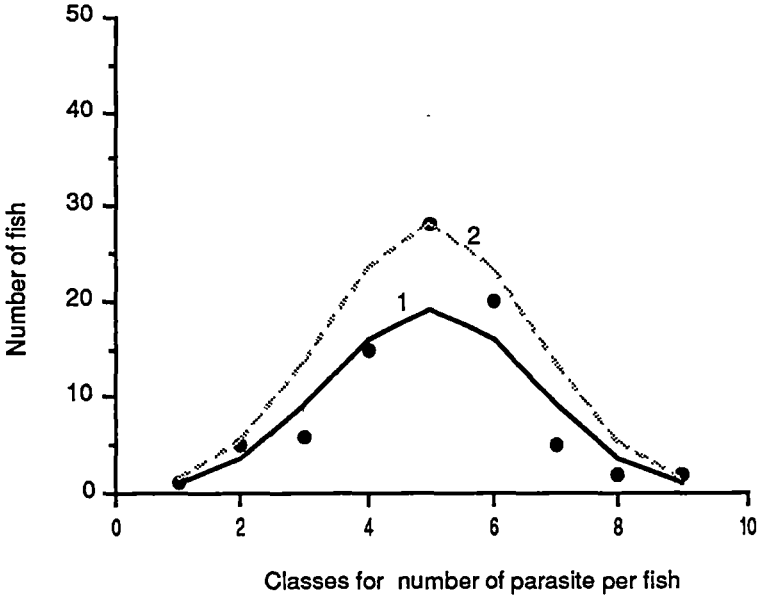



**Fig. 5.3** Frequency distribution of *Zschokkella leptatherinae* in *Atherinosoma microstoma*. Observed data shown as solid dots and fitted lognormal models as curves 1 (So=19.17), 2 (So=28.00).

Therefore

$$\alpha = \sqrt{\frac{\ln(44/2)}{25}} = 0.35$$

$$\overline{\ln S(R)} = \text{Total lnS(R)} / 10 = 2.57$$

$$\overline{R}^2 = \text{Total } R^2 / 10 = 8.5$$

From Eq. 5.4

$$S_0 = e^{[2.57+(0.35)^2 x 8.5]} = 37.01$$

The expected frequencies with $\alpha = 0.35$ and $S_0 = 37.01$ and 44 were computed respectively using Eq. 5.1. These results are summarised in Table 5.4 and plotted in Fig. 5.4. $\chi^2$ values for each group for goodness of fit were computed using Eq. 5.5. Total $\chi^2 = 3.93$ when $S_0 = 37.01$ and $\chi^2 = 7.76$ when $S_0 = 44$ (d.f = 8). Both log-normal curve 1 and curve 2 fit the present data. However, the curve 1 seems to be accepted as a better fit as $0.8 < p < 0.9$, but for curve 2, $0.3 < p < 0.5$.

**Table 5.4** Goodness-of-fit test for the log-normal model to observed data of *Zschokkella leptatherinae* in *Leptatherina presbyteroides,* Expected results are given fortwo $S_0$ parameter estimates.

| Class | R | $R^2$ | Observed S(R) | $S_0 = 37.01$ Expected S(R) | $\chi^2$ | $S_0 = 44$ Expected S(R) | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| 1 | -5 | 25 | 2 | 1.73 | 0.04 | 2.06 | 0.00 |
| 2 | -4 | 16 | 5 | 5.21 | 0.01 | 6.20 | 0.23 |
| 3 | -3 | 9 | 14 | 12.29 | 0.24 | 14.61 | 0.03 |
| 4 | -2 | 4 | 22 | 22.67 | 0.02 | 26.96 | 0.91 |
| 5 | -1 | 1 | 29 | 32.74 | 0.43 | 38.93 | 2.53 |
| 6 | 0 | 0 | 44 | 37.01 | 1.32 | 44.00 | 0.00 |
| 7 | 1 | 1 | 38 | 32.74 | 0.84 | 38.93 | 0.02 |
| 8 | 2 | 4 | 19 | 22.67 | 0.60 | 26.96 | 2.35 |
| 9 | 3 | 9 | 10 | 12.29 | 0.43 | 14.61 | 1.45 |
| 10 | 4 | 16 | 5 | 5.21 | 0.01 | 6.20 | 0.23 |
| Total | | 85 | 188 | 184.58 | 3.93 | 219.44 | 7.76 |

5.3.2 The relationships between the parasite and the host fish

*5.3.2.1 Host specificity to Zschokkella leptatherinae:* In the present study, *Zschokkella leptatherinae* was found in five atherinid fish species. Another six fish species occurring sympatrically with the atherinids were not infected. Table 5.5 shows the species of fish infected with *Z. leptatherinae* and the prevalence of infection in different locations of fish.

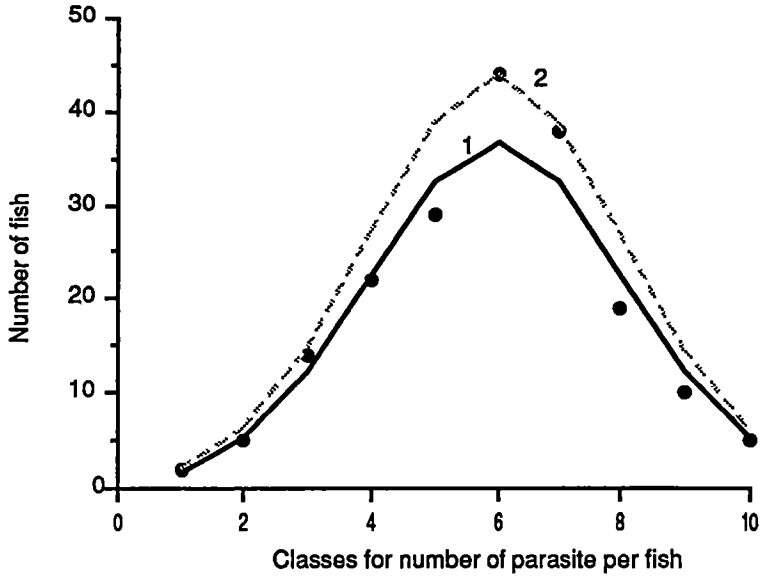



**Fig. 5.4** Frequency distribution of *Zschokkella leptatherinae* in *Leptatherina presbyteroides*. Observed data shown as solid dots and fitted lognormal models as curves 1 (So=37.01), 2 (So=44.00).

**Table 5.5** Species of fish infected with *Zschokkella leptatherinae* and the prevalence of parasite infection.

| Fish species | Prevalence of liver infection | Prevalence of gall bladder infection | Number of fish examined |
|---|---|---|---|
| *Atherinosoma microstoma* | 16.3% | 5.6% | 514 |
| *Lepatherina presbyteroides* | 31.9% | 18.3% | 589 |
| *Kestratherina esox* | 6.3% | 3.1% | 64 |
| *K. brevirostris* | 5.2% | 2.0% | 116 |
| *K. hepsetoides* | 40.0% | 20.0% | 5 |

There is a significant variation in the prevalence of infection among fish species (2 x c Contingency Table analysis, $\chi^2$ =72.353, $p < 0.001$). Except for *Kestratherina hepsetoides*, for which only five individuals were examined, *Atherinosoma microstoma* and especially *Leptatherina presbyteroides* are apparently the preferred host species for *Z. leptatherinae*.

In addition to the host specificity, *Z. leptatherinae* also shows an obligate organ specificity. It was only found in the hepatic ducts and gall bladder of atherinid fishes. No spores were observed in the intestine, kidney, blood, spleen, reproductive organs and

adipose tissue. Not every liver-infected fish has gall bladder infection but all fishes with gall bladder infection have liver infection. This suggests that the gall bladder might be the secondary site for *Z. leptatherinae.*

*5.3.2.2 Variations of parasite infection with the length of fish:* The total samples of 495 *Atherinosoma microstoma* and 445 *Leptatherina presbyteroides* were examined for the variations in prevalence and intensity of *Zschokkella leptatherinae* infection. The fish were divided into 13 groups at mostly 3 mm fork length intervals. There were very few fish smaller than 54 mm and larger than 88 mm; therefore, they were grouped into the two size classes. The size of samples examined in each length group and the data related to the prevalence of parasite infection are given in Appendix Table A-1, A-2.

Figure 5.5 shows the relationship between the prevalence and intensity of *Z. leptatherinae* infection and the length of *A. microstoma.* No infection was found in fish less than 60 mm. The prevalence then increased with the length of fish to an apparent peak in fish of 85-87 mm. Afterwards it declined in the fish of 88-93 mm. However, only a small number of (8) fish were examined in the last size group.

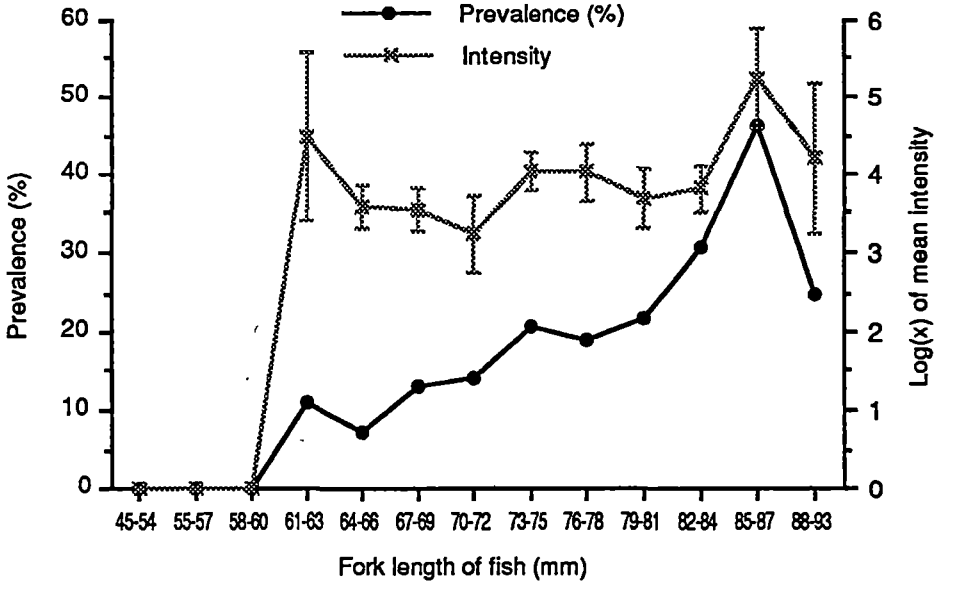



**Fig. 5.5** Prevalence and intensity of *Zschokkella leptatherinae* infection in *Atherinosoma microstoma* of different lengths (bars indicate standard error).

The Contingency Table analysis indicates that the variation in prevalence of infection in fish of different lengths is significant ($p < 0.01$; Table 5.6). A secondary analysis using the standardised residuals shows that 82-84 and 85-87 mm groups of fish have a significantly higher ($r_{sij} > 0.2$) prevalence of infection and 58-60 mm group of fish have

a significantly lower ($r_{sij} < -0.2$) prevalence than other size group of fish (Appendix Table A-1).

The initial examination indicated that the observed data of intensity of infection was not distributed normally. A plot of residuals against fitted values indicated that the logarithmic transformation of data was the appropriate transformation for the intensity of *Z. leptatherinae* infection. Therefore, all the data of intensity of infection used in this chapter are logarithmically transformed data.

The intensity of infection was high and even between fish of 61 and 93 mm. The result of ANOVA shows that there were no significant variations in intensity of infection with the length of fish ($p > 0.05$; Table 5.6).

**Table 5.6** Results of analysis of variations in prevalence and intensity of *Zschokkella leptatherinae* infection in *Atherinosoma microstoma* of different lengths.

| Prevalence of infection (2 x c Contingency Table ) | Intensity of infection (ANOVA) |
|---|---|
| DF = 12 | DF = 9 (between groups), 72 (within groups) |
| $\chi^2 = 28.161$ | F = 1.33 |
| p = 0.0052 | p = 0.2368 |

The relationship between the prevalence and intensity of *Z. leptatherinae* infection and the length of *L. presbyteroides* is displayed in Fig. 5.6. Fish under 54 mm were not infected. The prevalence rose steadily in fish of 55-57 to 79-81 mm and remained at a high level in fish between 79-81 to 85-87 mm. The level then dropped sharply in fish of 88-93 mm. Again, there only very few (10) fish were examined in the last size group.

The variation in prevalence of infection in *L. presbyteroides* is statistically significant ($p < 0.01$; Table 5.7). The results of standardised residuals (Appendix A-2) demonstrate that the deviations of the three groups with lengths from 79 to 87 mm range are significantly higher ($r_{sij} > 0.2$) than the average, while the deviation of 44-54 and 58-60 mm length group are significantly lower ($r_{sij} < -0.2$) than the average.

The intensity of *Z. leptatherinae* infection was low in *L. presbyteroides* under 55-57 mm in length. It rose rapidly in fish between 58 and 63 mm and then remained at a constant high level until in 88-93 mm group of fish. However, this variation is non-significant ($p > 0.05$) when the ANOVA is conducted (Table 5.7).

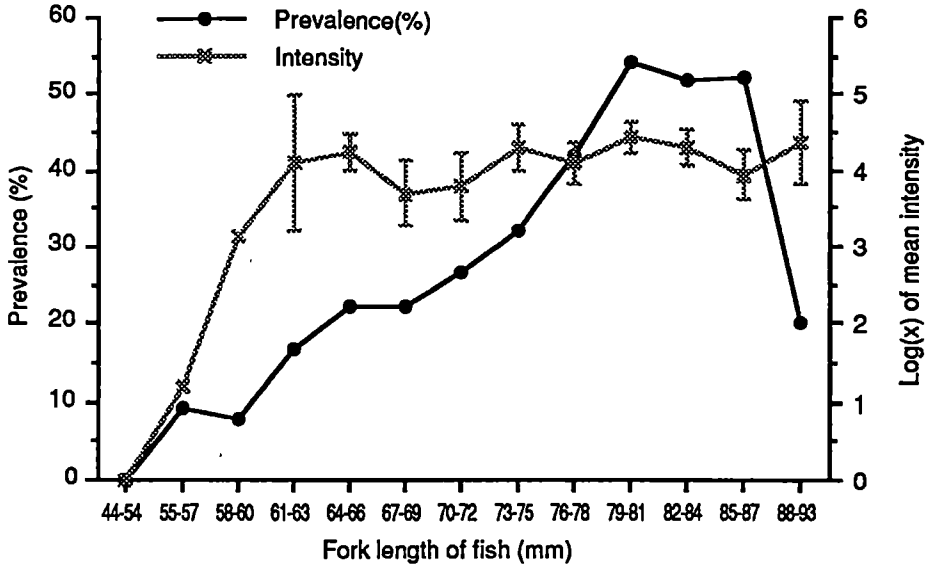



**Fig. 5.6** Prevalence and intensity of *Zschokkella leptatherinae* infection in *Leptatherina presbyteroides* of different lengths (bars indicate standard error).

**Table 5.7** Results of analysis of variations in prevalence and intensity of *Zschokkella leptatherinae* infection in *Leptatherina presbyteroides* of different lengths.

| Prevalence of infection (2 x c Contingency Table ) | Intensity of infection (ANOVA) |
|---|---|
| DF = 12 | DF =11 (between groups), 130 (within groups) |
| $\chi^2 = 46.314$ | F = 1.005 |
| p = 0.0001 | p = 0.4458 |

To investigate if any effect of infection on fish growth and/or survival, the length of fish in the bimonthly samples with parasites were compared to those without parasites (Figs. 5.7, 5.8). In both fish species, the fish infected with the parasite are larger than those uninfected fish except for *A. microstoma* obtained in January/February, 1991 and *L. presbyteroides* obtained in March/April 1990.

The results of a two-factor ANOVA (Tables 5.8, 5.9) show that, in both fish species, the lengths of fish vary with the month ($p < 0.01$); the lengths between infected fish and uninfected fish are significantly different ($p < 0.01$); and the differences in the length between infected and uninfected fish are independent of the month ($p > 0.05$). A one-factor ANOVA is then conducted to test the differences in the length between bimonthly infected and uninfected samples. Although a significant difference only existed between the fish obtained in March/April 1990 for *A. leptatherinae,* infected *L. presbyteroides*

were significantly longer than those lacking parasite from May/June to November/December 1990 and March/April 1991 (Appendix Table B-1, B-2).

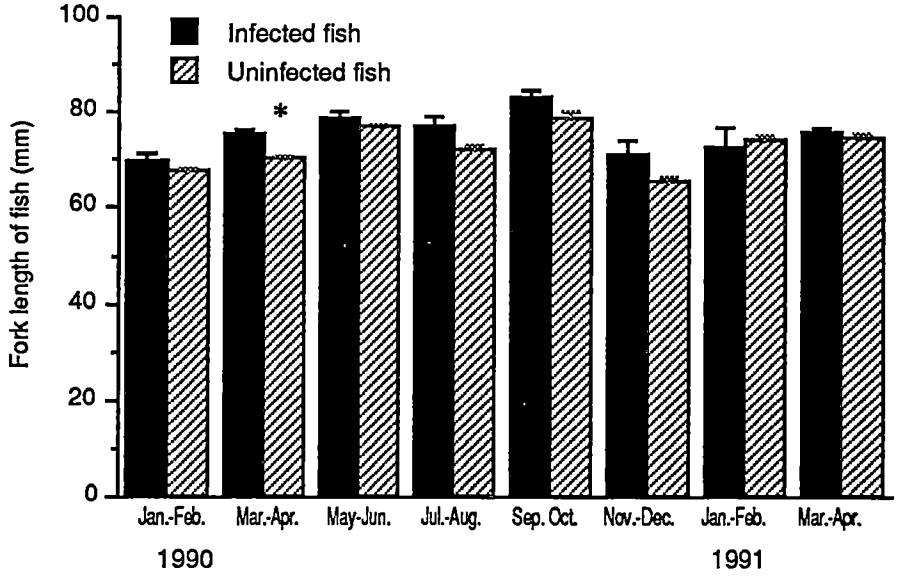



**Fig. 5.7** Comparisons of mean fork lengths of *Atherinosoma microstoma* infected and uninfected with *Zschokkella leptatherinae* (bars indicate standard error, * $p < 0.05$).

**Table 5.8** Analysis of variance of differences in mean fork length of *Atherinosoma microstoma* infected and uninfected with *Zschokkella leptatherinae* in different months.

| Source | DF | Sum of Squares | Mean Square | F-test | p value |
|---|---|---|---|---|---|
| Month (A) | 7 | 3061.592 | 437.37 | 9.101 | 0.0001 |
| Inf./Uninf. (B) | 1 | 439.785 | 439.785 | 9.152 | 0.0026 |
| AB | 7 | 249.048 | 35.578 | 0.740 | 0.6378 |
| Error | 479 | 23018.67 | 48.056 | | |

**Table 5.9** Analysis of variance of differences in mean fork length of *Leptatherina presbyteroides* infected and uninfected with *Zschokkella leptatherinae* in different months.

| Source | DF | Sum of Squares | Mean Square | F-test | p value |
|---|---|---|---|---|---|
| Month (A) | 7 | 3168.797 | 452.685 | 6.441 | 0.0001 |
| Inf./Uninf. (B) | 1 | 1445.144 | 1445.144 | 20.561 | 0.0001 |
| AB | 7 | 877.869 | 125.410 | 1.784 | 0.0886 |
| Error | 429 | 30152.654 | 70.286 | | |

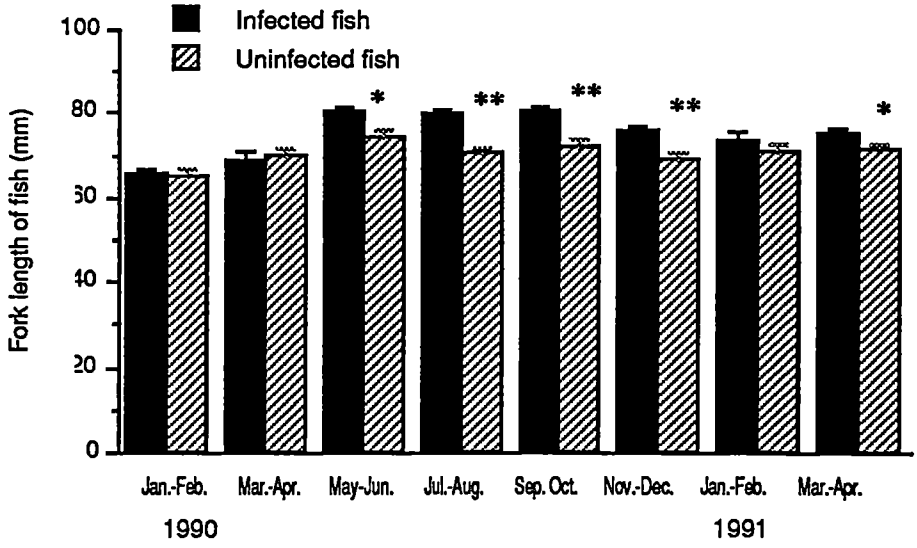



**Fig. 5.8** Comparisons of mean fork lengths of *Leptatherina presbyteroides* infected and uninfected with *Zschokkella leptatherinae* (bars indicate standard error, ** $p < 0.01$, *$p < 0.05$).

*5.3.2.3 Variations of parasite infection with the sex of fish:* The bimonthly sample size of male and female *Atherinosoma microstoma* and the date related to the prevalence of *Zschokkella leptatherinae* infection are given in Appendix Tables A-3 and A-4.

Figure 5.9 displays the differences in bimonthly prevalence of *Z. leptatherinae* infection between male and female samples of *A. microstoma*. Although male fishes showed a higher prevalence than female ones in September/October 1990, January/February and March/April 1991, in most months, female fish showed a higher prevalence than male ones. These variations are statistically non-significant ($p > 0.05$; Table 5.10).

The differences in intensity of *Z. atheirnosomus* infection between bimonthly samples of male and female *A. microstoma* are plotted in Fig. 5.10. The number of infected fish, particularly male ones, in each samples is very small; in most cases only less than five individuals were available. Therefore, data of intensity of infection are not robust enough to permit statistical analysis.

The analysis of variations in prevalence of *Z. leptatherinae* infection between bimonthly samples of the same sex is also conducted. The results are given in Table 5.11.

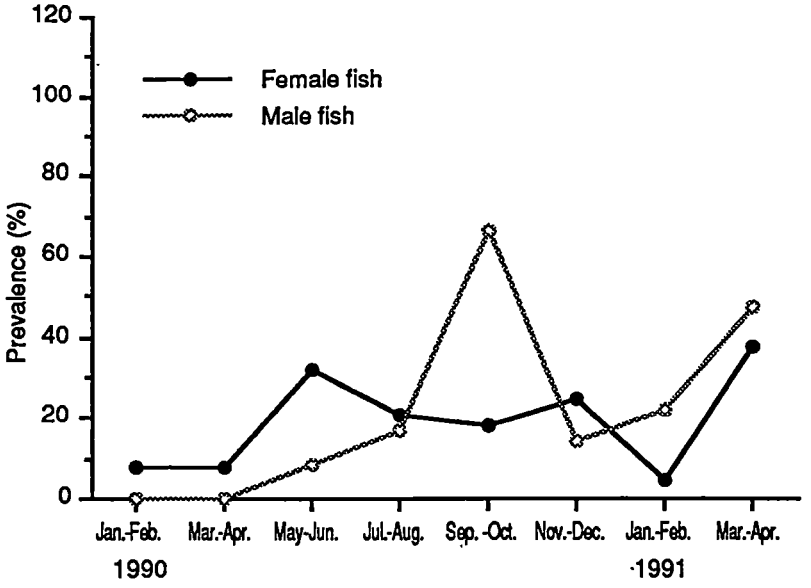



**Fig. 5.9** Variations of prevalence of *Zschokkella leptatherinae* infection in male and female *Atherinosoma microstoma.*

**Table 5.10** Results of analysis of variations in prevalence of *Zschokkella leptatherinae* infection between bimonthly male and female samples of *Atherinosoma microstoma.*

| Month | Prevalence (2 x 2 Contingency Table) | | |
|---|---|---|---|
| | DF | $\chi^2$ | p |
| Jan.-Feb. 1990 | 1 | 0.359 | 0.5489 |
| Mar.-Apr. | 1 | 0.037 | 0.8481 |
| May-Jun. | 1 | 1.589 | 0.2074 |
| Jul.-Aug. | 1 | 0.009 | 0.9237 |
| Sep.-Oct. | 1 | 1.263 | 0.2610 |
| Nov.-Dec. | 1 | 0.032 | 0.8577 |
| Jan.-Feb. 1991 | 1 | 0.635 | 0.4256 |
| Mar.-Apr. | 1 | 0.239 | 0.6251 |

For female fish, the prevalence of infection was low between January and April 1990. It rose in May/June and remained at a high level before dropping in January/February 1991. In March/April 1991, the prevalence rose again to a high level. The Contingency Table analysis indicates that this variation is significant ($p < 0.001$). The results of a secondary test show that the deviations of May/June 1990 and March/April 1991 are significantly higher than the average, while the deviations of January/February and March/April 1990 are significantly lower than the average (Appendix Table A-3).

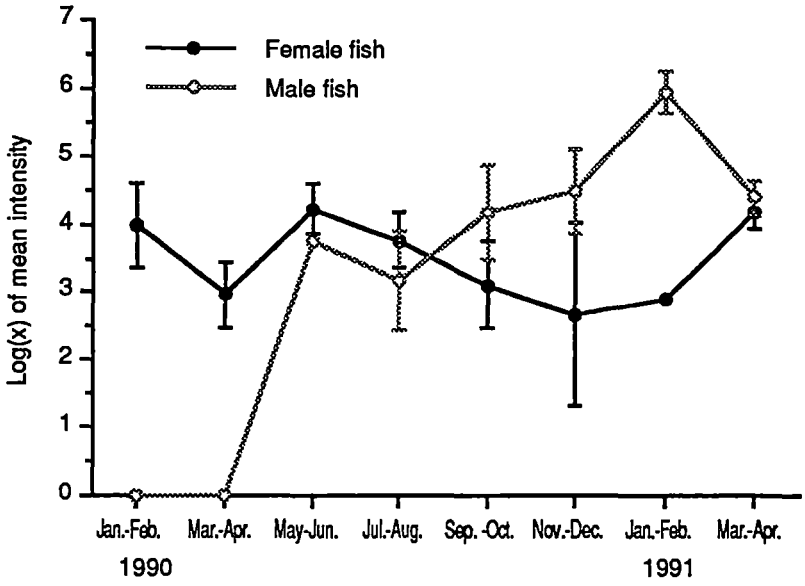



**Fig. 5.10** Variations of intensity of *Zschokkella leptatherinae* infection in male and female *Atherinosoma microstoma* (bars indicate standard error).

**Table 5.11** Results of analysis of seasonal variations in prevalence of *Zschokkella aerinosomus* infection in male and female *Atherinosoma microstoma.*

| Test | Female | Male |
|---|---|---|
| Prevalence | DF = 7 | DF =7 |
| (2 x c Contingency Table) | $\chi^2 = 32.997$ | $\chi^2 = 22.25$ |
| | p = 0.0001 | p = 0.0023 |

No male *A. microstoma* were infected in the first four months between January and April 1990. Low values of prevalence occurred from May to August. It rose in September/October and then declined in November/December 1990. In March and April 1991, it rose again to a high level. This variation is statistically significant ($p < 0.05$). The results of the secondary analysis show that the deviation of September/October 1990 and March/April 1991 are significantly higher ($r_{sij} > 0.2$) than the average, while the deviations of January/February 1990 is significantly lower ($r_{sij} < -0.2$) than the average (Appendix Table A-4).

The sample sizes of male and female *L. presbyteroides* examined bimonthly and the date related to the prevalence of *Z. leptatherinae* infection are given in Appendix Tables A-5 and A-6.

The differences in bimonthly prevalence of *Z. leptatherinae* infection between the male and female *L. presbyteroides* are plotted in Fig. 5.11. The levels of prevalence of infection of the two sexes are close except for March/April and November/December 1990 where the prevalence of infection is higher in male fishes than in female ones. Although the difference in March/April is significant ($p < 0.05$; Table 5.12), only three male fish were examined in those months.

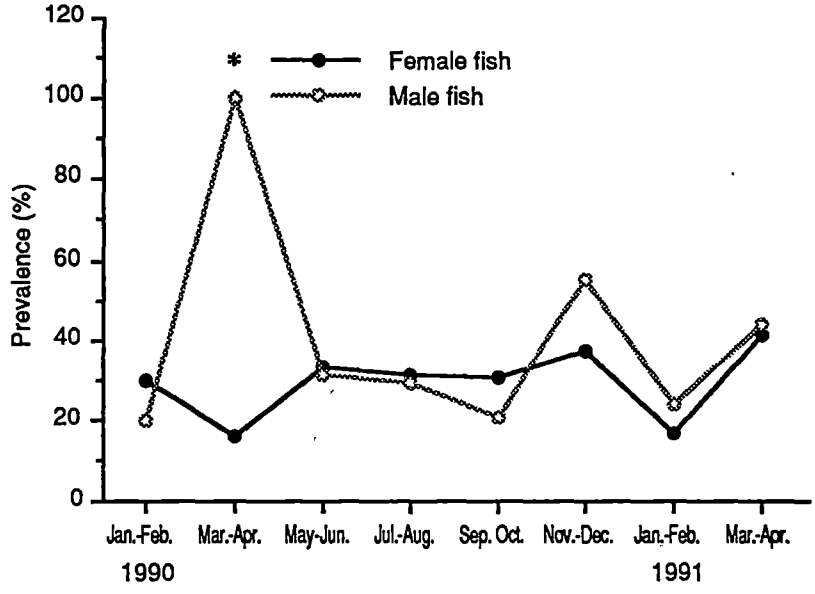



**Fig. 5.11** Variations of prevalence of *Zschokkella leptatherinae* infection in male and female *Leptatherina presbyteroides* (*$p < 0.05$).

**Table 5.12** Results of analysis of variations of prevalence of *Zschokkella leptatherinae* infection between bimonthly male and female samples of *Leptatherina presbyteroides*.

| Month | Prevalence (2 x 2 Contingency Table) | | |
|---|---|---|---|
| | DF | $\chi^2$ | p |
| Jan.-Feb. 1990 | 1 | 0.009 | 0.9238 |
| Mar.-Apr. | 1 | 6.098 | 0.0135* |
| May-Jun. | 1 | 0.030 | 0.8631 |
| Jul.-Aug. | 1 | 0.007 | 0.9340 |
| Sep.-Oct. | 1 | 0.514 | 0.4732 |
| Nov.-Dec. | 1 | 0.514 | 0.4732 |
| Jan.-Feb. 1991 | 1 | 0.050 | 0.8227 |
| Mar.-Apr. | 1 | 0.027 | 0.9919 |

Figure 5.12 shows the variations in intensity of *Z. leptatherinae* infection between bimonthly male and female *L. presbyteroides*. As in *A. microstoma*, no statistical analysis was attempted due to the small number of mature infected fish in each sample.

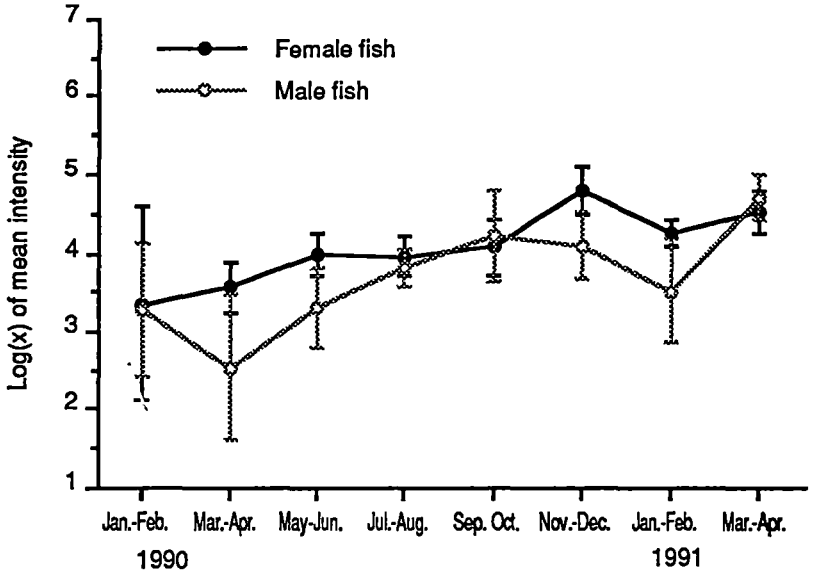



**Fig. 5.12** Variations of intensity of *Zschokkella leptatherinae* infection in male and female *Leptatherina presbyteroides* (bars indicate standard error).

The temporal variations in prevalence of *Z. leptatherinae* infection in the same sex of *L. presbyteroides* were also analysed. The results are given in Table 5.13. Only the variation in prevalence in males is significant ($p < 0.05$).

**Table 5.13** Results of analysis of seasonal variations of prevalence of *Zschokkella leptatherinae* infection in male and female *Leptatherina presbyteroides*.

| Test | Female | Male |
|---|---|---|
| Prevalence | DF = 7 | DF =7 |
| (2 x c Contingency Table) | $\chi^2 = 7.489$ | $\chi^2 = 17.741$ |
| | p = 0.3798 | p = 0.0132 |

5.3.3 Seasonal variations in the prevalence and intensity of infection

The seasonal variations in the prevalence and intensity of *Zschokkella leptatherinae* infection were investigated by pooling the data into two monthly group. The size of samples examined in each two month and the data on the prevalence of the parasite's infection are given in Appendix Table A-7 for *A. microstoma* and Table A-8 for *L. presbyteroides*.

The prevalence and intensity of *Z. leptatherinae* infection against months are shown in Fig. 5.13 for *A. microstoma*. The prevalence of infection was low between January and April 1990. It rose rapidly in May/June 1990, then remained at an even level until January/February 1990. In March/April 1991, the level rose markedly to a peak value.

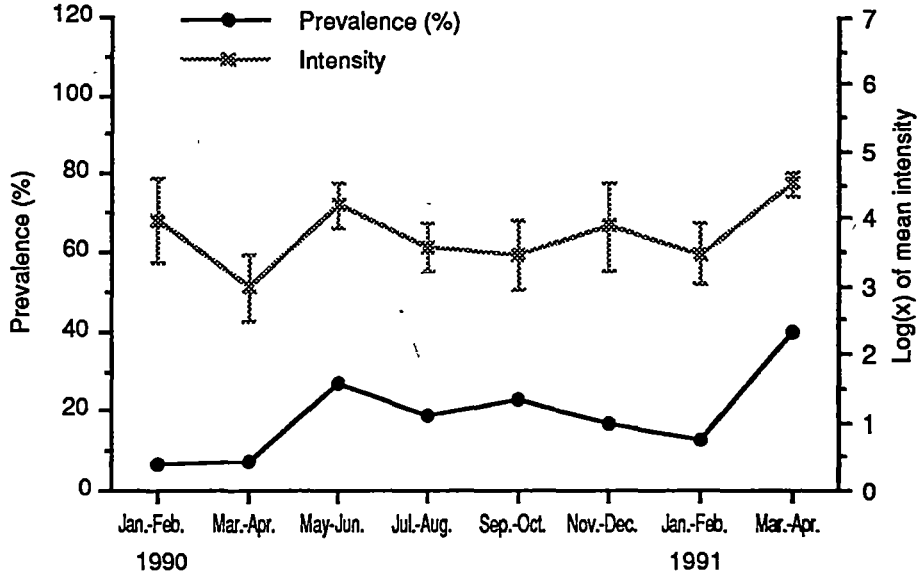



**Fig. 5.13** Seasonal variations of the prevalence and intenstiy of *Zschokkella leptatherinae* infection in *Atherinosoma microstoma* (bars indicate standard error).

The result of Contingency Table analysis indicates that the variation in prevalence of infection between bimonthly samples of *A. microstoma* is significant ($p < 0.01$; Table 5.14). A secondary analysis using the standardised residuals shows that the deviations of values in May/June 1990 and March/April 1991 are significantly higher than the average ($r_{sij} > 2$) and those in January/February and March/April 1990 are significantly lower than the average ($r_{sij} < -2$) .

In most months, the intensity of *Z. leptatherinae* infection was even except a slightly low level in March/April 1990 and a high level in March/April 1991 (Fig. 5.13). The ANOVA shows no significant ($p > 0.05$) difference between bimonthly levels of intensity of infection (Table 5.14).

The prevalence and intensity of *Z. leptatherinae* infection against months are shown in Fig. 5.14 for *L. presbyteroides*. The prevalence was higher and varied less between bimonthly samples than that in *A. microstoma*. There was no significant variation ($p > 0.05$) between the bimonthly levels of prevalence of infection. No significant ($p > 0.05$)

difference was shown between the bimonthly levels of intensity of infection either (Table. 5.15).

**Table 5.14** Results of analysis of seasonal variations in prevalence and intensity of *Zschokkella leptatherinae* infection in *Atherinosoma microstoma.*

| Prevalence of infection (2 x c Contingency Table ) | Intensity of infection (ANOVA) |
|---|---|
| DF = 7 | DF = 7 (between groups), 74 (within groups) |
| $\chi^2$ = 42.595 | F = 1.941 |
| p = 0.0001 | p = 0.0749 |

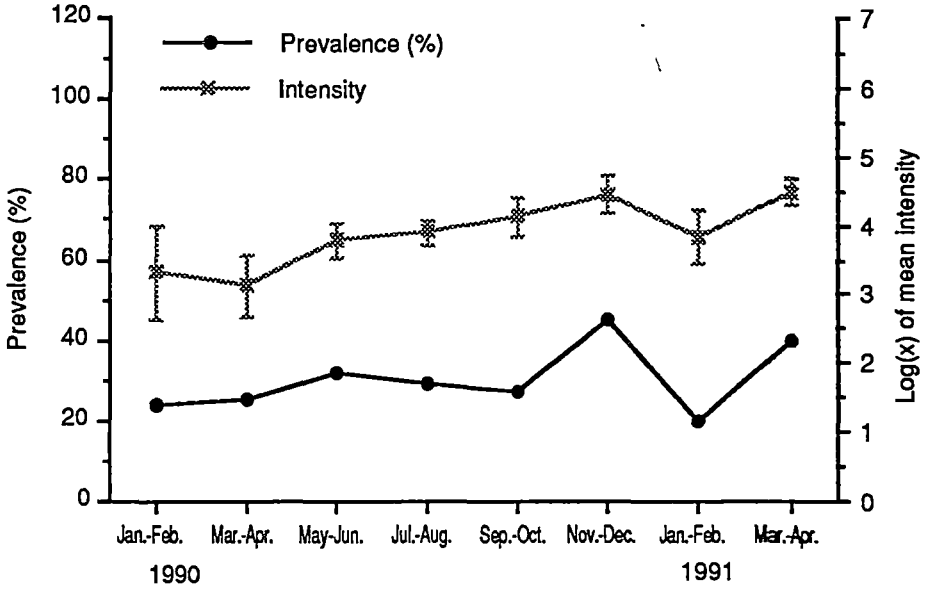



**Fig. 5.14** Seasonal variations of the prevalence and intenstiy of *Zschokkella leptatherinae* infection in *Leptatherina presbyteroides* (bars indicate standard error).

**Table 5.15** Results of analysis of seasonal variations in prevalence and intensity of *Zschokkella leptatherinae* infection in *Leptatherina presbyteroides.*

| Prevalence of infection (2 x c Contingency Table ) | Intensity of infection (ANOVA) |
|---|---|
| DF = 7 | DF = 7 (between groups), 134 (within groups) |
| $\chi^2$ = 12.585 | F = 2.053 |
| p = 0.0829 | p = 0.0 530 |

5.3.4 Histopathology of *Leptatherina presbyteroides* with the infection of *Zschokkella leptatherinae*

*5.3.4.1 Liver infection:* The colour of the liver of *Leptatherina presbyteroides* varied from pale yellow, light pink to reddish brown in both infected and uninfected fish. Therefore, liver colour was not a reliable indicator of infection. No obvious change in the external appearance of infected fish has been found.

Uninfected liver tissue consists of numerous multiangle hepatic cells which are distributed evenly. Small hepatic ducts lined by columnar epithelium can be observed among these cells (PL. 5.1A). Liver sections of infected fish usually contain plasmodia of *Zschokkella leptatherinae* in proliferous hepatic ducts (Pl. 5.1B). In comparison with uninfected liver, infected hepatic ducts are enlarged and the epithelial cells are lower than the normal cells (Pl. 5.1C). One to five layers of connective tissue fibres are found around the ducts; these tissue envelopes vary in thicknesses from 4.7 to 6.3 μm, occasionally 9.5 μm. These fibres are most obvious when stained with Mallory's Triple stain (Pl. 5.1D). Some hepatic ducts are so filled with parasites that they are blocked off (Pl. 5.1D). Liver parenchyma adjacent to these ducts show obvious atrophy (Pl. 5.1C). In more advanced infections, over 85% of the liver tissue is replaced by the spores (Pls. 5.1C). Concurrently, the sinusoidal spaces are abnormally wide (Pl. 5.2A). Finally necrosis of liver tissue appears (Pl. 5.2B).

*5.3.4.2 Gall bladder infection:* Gall bladder infection varies from a few spores and/or plasmodia to thousands of spores and plasmodia. Bile from infected gall bladder shows slightly more viscosity than that from uninfected gall bladder. Usually, spores are found free-floating in bile although a few scattered spores were found infrequently on the wall of the gall bladder. Histological observations show no obvious changes in tissue structures.

## 5.4 Discussion

The distribution of a number of metazoan parasites has been reported to be an overdispersion (variance greater than mean) and can be described adequately by the negative binomial distribution model (Crofton, 1971; Pennycuick, 1971a; Anderson, 1974). Only three fish-infecting protozoan species have been previously described by this model; they are *Myxosoma funduli* (Knight *et al.*, 1977, 1980), *Trichodina* sp. (Adams, 1980), and *Ichthyophthirius multifiliis* (McCallum, 1982).

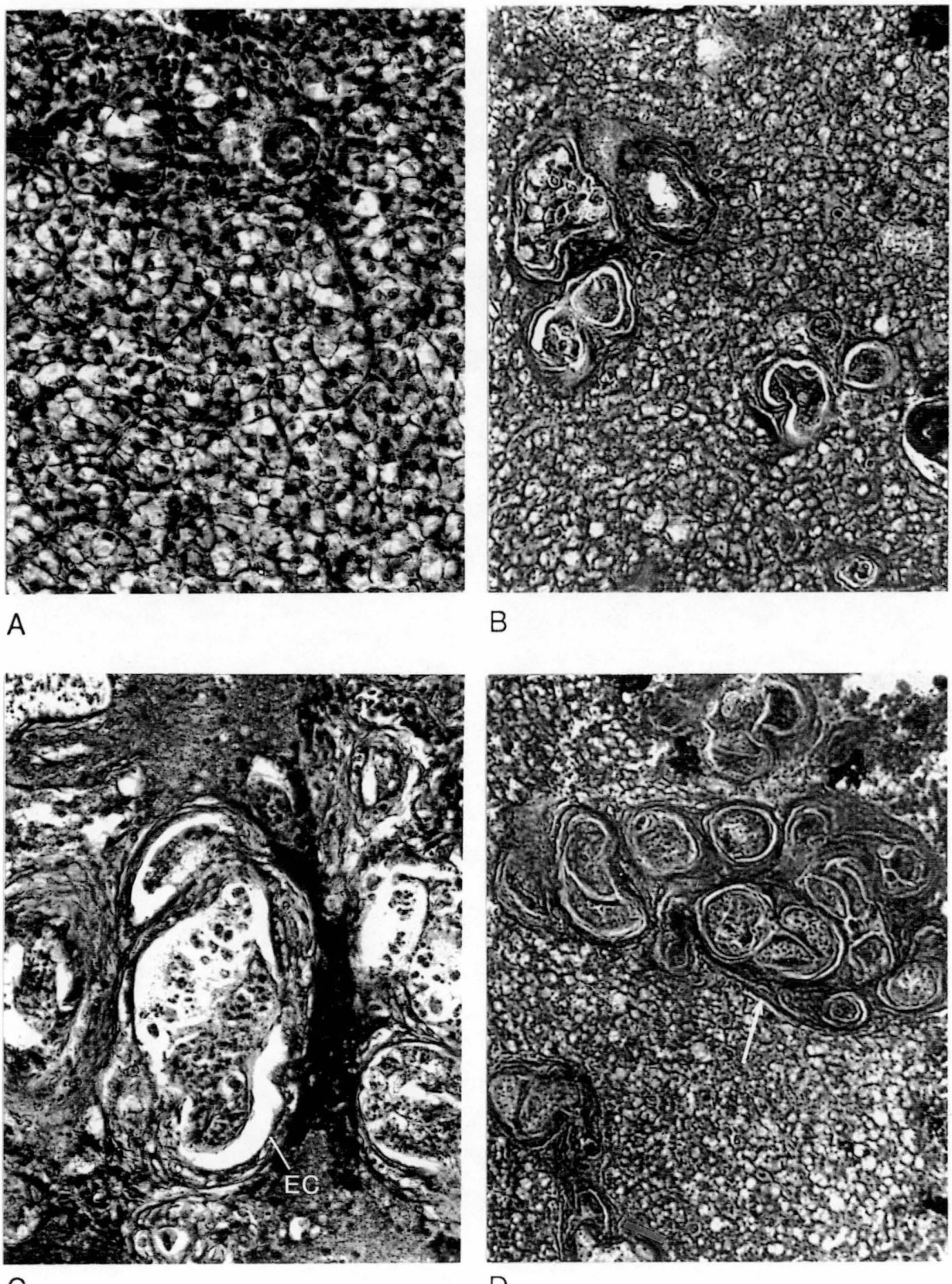
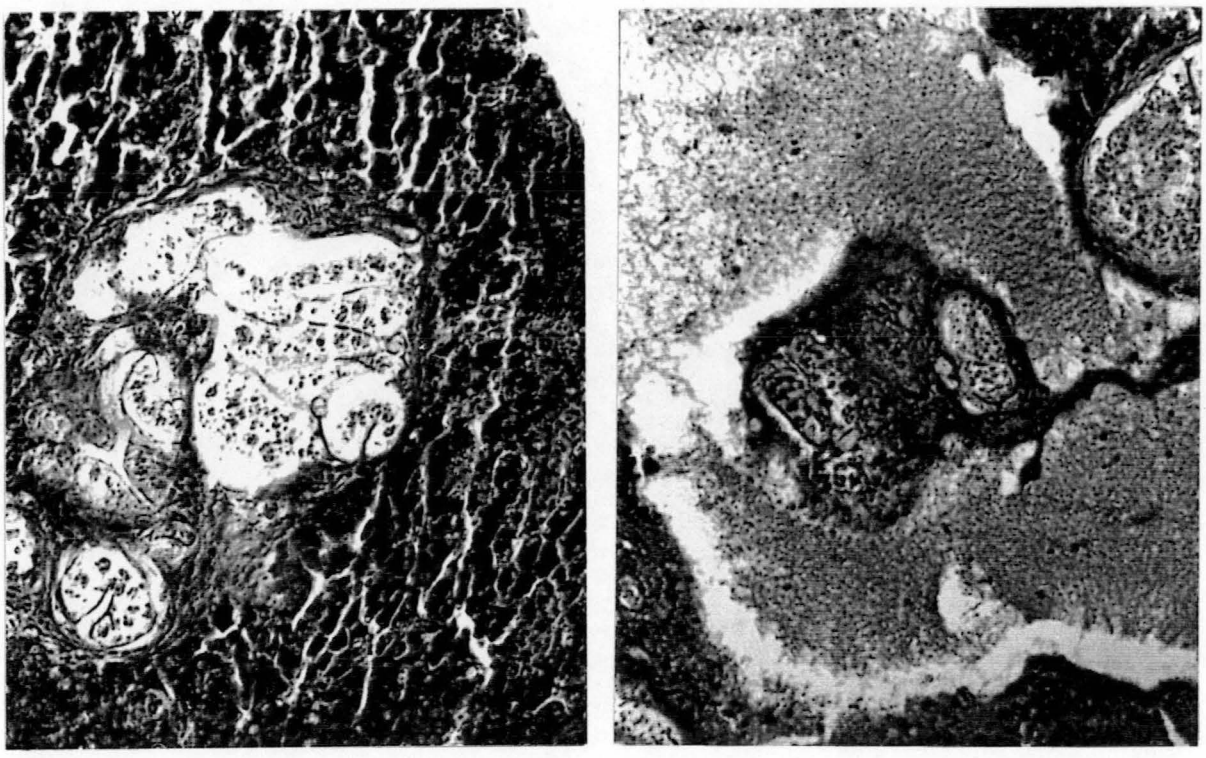



Plate 5.1. Sections of liver of *Leptatherina presbyteroides* infected or uninfected with *Zschokkella leptatherinae*. A. Uninfected liver, x 1388 (H & E). B. Infected liver showing proliferous hepatic ducts containing plasmodia of parasite, x 571 (Mallory's Triple). C. Infected liver showing enlarged hepatic ducts containing plasmodia, x 1388. EC, epithelial cells of hepatic ducts (H & E). D. Infected liver showing connective tissue fibres (arrow) around the plasmodia, x 571 (Mallory Triple).



A B

Plate 5.2. Sections of liver of *Leptatherina presbyteroides* infected with *Zschokkella leptatherinae*. A. Abnormally wide sinusoidal space in infected liver, x 571 (H & E). B. Necrosis of liver tissue around the plasmodia of parasite, x 571 (H & E).

*Zschokkella leptatherinae* displays a high overdispersion in the present study and the negative binomial distribution was first used to describe the distribution of this parasite. However, the model does not fit the data of the parasite population. This is probably due to the fact that the value of *k* is too small. Admas (1980) found that, when the numbers of the parasite were low, coupled with a small *k* value, the log-series would more likely provide a better fit to the observed data than the negative binomial. He also observed that the distribution of *Myxobolus (Myxosoma) fuluduli* Kudo, 1918 and *Trichodina* sp. can be described by the log-series distribution model although the negative binomial fitted the observed data better. Williams (1964) indicated that when the variation of the number of animals is geometric and not arithmetic, only the log-normal distribution model can provide a good fit.

The distribution of *Z. leptatherinae* in the present study can only be described by the log-normal. This is the first fish-infecting protozoan population described by this model. In the previous studies, malarial parasites in human blood and some other human diseases were described adequately by this distribution model (Williams, 1964). Since the reproduction of protozoan parasites usually occurs within the host and is rapid and the variation in the number of parasites is usually high, the log-normal model may describe adequately the distribution of many protozoan parasite populations.

The parasites are highly dependent on a number of factors in the external environment and on the mode of life of their host. The parasites regulate their interrelationship with the external environment through the medium of the host fish. From this arises their exceptional dependence on various aspects of the ecology and biology of the host.

The results of the present study show that the size of the host fish has a great effect on the infection of *Zschokkella leptatherinae;* the level of prevalence of infection generally increases with the size of host fish. Such relationship has been previously reported in other myxosporean species by Cone and Anderson (1977), Lucky (1978), Okada *et al.*, (1981), Mitchell (1989) and Brummer-Korvenkontio *et al.* (1991). A few microsporidians (Arthur *et al.,* 1982) and a haemoflagellate, *Cryptobia bullodki* (Burn, 1980) also followed this pattern.

Usually, the size reflects the age of fish. In analysis of the relationship between the parasite infection and the age of fish, several factors have been considered to relate to the variation in parasite infection. These include the change in composition and quantity of host diet with the age; the development of age-acquired immunity to certain species; the

accumulation with age of long lived species of parasites and the effect of host migration (Arthur *et al.*, 1982).

For *Atherinosoma microstoma* and *Leptatherina presbyteroides*, the fish less than 61 mm, are usually less than one year old. The fact that no infection was found in these fishes is most likely because the parasite being in a latent condition. In the study of *Myxobolus (Myxosoma) funduli* in *Fundulus kansae,* Knight *et al.* (1977) found that the young one year old fish were infected, however, the smallest young of the year old fish were not infected. The authors explained this by stating that the smallest fish may carry prepatent infections which become the source of infective spores for the young one year old fish.

The prevalence of *Z. leptatherinae* infection increased with the size of fish up to the 85-87 mm group in both fish species. This may be due to, firstly, the death of some small infected fish. In view of the variation in intensity of infection in both fish species, (except for fish of 55 to 60 mm in *L. presbyteroides*), the level of intensity of infection was comparatively constant in 10 size groups between 61 to 93 mm. This indicates that once the infection is established, the parasites reproduce rapidly inside the host's body. The large number of parasites occurred irrespective of the size of fish. Some small fish with heavy infection might not survive as a result of low immunity resistance. The histopathological study also demonstrates that heavy infections of *Z. leptatherinae* destroy a large portion of liver tissue. Secondly, the diet habitat of the host fish may contribute to the high prevalence of infection in large fish. It is known that the infective stages of endoparasitic myxosporeans are ingested with the food (Shulman, 1966). Therefore, the larger and older fishes ingest large quantity and variety of diet comparing with small ones. This will bring more parasites into fish since the invertebrates which compose the fish diet can play a role in concentrating spores (Li and Desser, 1985; Khan *et al*, 1986) or act as a intermediate host of parasite (Wolf and Markiw, 1984; El-Matbouli and Hoffmann, 1989).

Janovy and Hardin (1987) concluded that there are three types of relationship between the sex of host fish and parasite population from their observations on protozoan and metazoan parasites in *Fundulus zebrinus:* one in which infection is independent of sex, one in which sex-infection differences are probably of physiological origin, e.g., based on mucus, colour, or hormonal factors, and one in which the differences are likely ecological. To date, the sexual differences in fish-infecting protozoa were only reported in few ectoparasitic species, such as *Myxbolus funduli* from the gills of *Fundulus zebrinus* (Janovy and Hardin, 1987), *Trichodina* sp. from the gills of *Fundulus kansae* (Adams, 1980), *Scyphidia* sp. from the skin of *Salmo trutta* (Pickering and Christe, 1980). The

investigations by Price (1982) on *Pleistophora cepedianae* Putz *et al.*, 1965; Arthur *et al.* (1982) on *Pleistophora* sp., Alvarez-Pellitero *et al.* (1983) on *Myxidium carinae* and *M. macrocapsulae* as well as Alvarez-Pellitero and Sitja-Bobadilia (1993) on *Ceratomyxa* spp. showed that there were no significant differences between the sexes of host fish. The present results on *Zschokkella leptatherinae* are consistent with those of the previous studies on endoparasitic protozoans. In both *Atherinosoma microstoma* and *Leptatherina presbyteroides,* the prevalence and intensity of *Z. leptatherinae* infection showed non-significant difference between the sexes of fish.

Both the prevalence and intensity of *Zschokkella leptatherinae* infection varied independently of seasons in the present study. This may be related highly to the location of the parasite. *Zschokkella leptatherinae* inhabits the hepatic ducts and gall bladder of atherinid fishes; it has a relatively constant microenvironment. Bond (1939) obtained an even seasonal infection for *Myxobolus (Myxosoma) subtecalis* in the kidney and brain of *Fundulus heteroclitus* in spite of variation in the occurrence of this species on the fins. The similar situation was also reported in the infection of *Myxidium rhodei* and *Zschokkella nova* from *Rutilus rutilus* (Brummer-Korvenkontio *et al.,* 1991). Bond (1939) explained this as being due to the inability of the spore to release itself from the organs of the fish during the lifetime of the host, which possibly occurs only in the case of host death and decay.

In *A. microstoma,* the prevalence of infection varied significantly with the months. This may be related to the condition of the host fish. It was found that, from the original data, many small and young fish were examined from January to April 1990, while many large and old fish were examined in May/June 1990 and March/April 1991. Considering the relationship between the prevalence of infection and the length of fish, it is not difficult to understand the low level of prevalence occurring between January to April 1990 and the high level in May/June 1990 and March/April 1991.

Only three species of *Zschokkella* have been previously reported to cause medium to remarkable pathology in their fish hosts. *Z. resselli* from the hepatic ducts and gall bladder of *Ciliata mustela* can cause thickening of hepatic ducts, attenuation of the duct lining epithelium and pericholangitis (Davies, 1985); *Z. nova* inhabiting the bile and pancreatic ducts of *Cottus gobio* causes a proliferation and a considerable distension of the ducts as well as metaplastic flattening of duct epithelium. The mature spores of *Z. nova* also invade the liver parenchyma and cause necrosis (Bucher *et al.*, 1992). *Z. icterica* reported from the hepatic ducts and gall bladder of *Siganus iuridus* (Diamant and Paperna, 1992) even induces more severe liver disease. In addition to blocking the hepatic

ducts, producing cholestasis and breakdown of those ducts, it also infects liver parenchyma and causes hepatic necrosis and jaundice, occasionally ascites. Members of other myxosporean genera have also been reported as invading the hepatic ducts. *Myxidium folium* Bond, 1938 in *Fundulus heteroclitus* produces distension and blockage of hepatic ducts, atrophy of tubular epithelium as well as connective tissue reaction around infected tubules (Bond, 1938). *Myxidium oviforme* Parisi, 1912 in the gall bladder and hepatic ducts of Atlantic salmon, *Salmo salar*, causes intense inflammation of the liver (Walliker, 1968a).

The results of the present study show that *Zschokkella leptatherinae* can cause severe liver disease in *Leptatherina presbyteroides*. It not only induces the histopathological changes in the hepatic ducts, but also invades the liver parenchyma. Although no deaths of fish induced by *Z. leptatherinae* were observed directly during the study, this myxosporean can replace up to 85% of the host's liver tissue. It also blocks the hepatic ducts and causes necrosis of liver tissue. The function of the liver is almost certainly disturbed as a result of such a large portion of tissue being damaged. Death may happen in some situations.

The enveloping of plasmodia by connective tissue fibre is a common host response to myxosporean infections. This has previously been observed in the muscle (Stehr and Whitaker, 1986), liver (Bond, 1938; Davies 1985) and kidney (Dykova, Lom and Grupcheva, 1987). It has also been detected in the fish muscle infected by *Pleistophora*-type microsporidian (Dykova and Lom, 1980) and heart tissue infected by microsporidian *Loma morhua* (Morrison, 1983). Morrison (1983) suggested that the significance of this response is to "reduce the passage of oxygen to the spore, resulting in coagulative necrosis of the parasite". In the present study, mass plasmodia were observed inside the hepatic ducts and these ducts were surrounded by the connective tissue fibres in the early stage of infection, but only few plasmodia were seen after the hepatic necrosis had occurred. This indicates that the suggestion of Morrison may also apply to *Z. leptatherinae*.

# CHAPTER 6 - BIOLOGY OF *PARATRICHODINA TASMANIENSIS*, WITH PARTICULAR REFERENCE TO THE HOST FISHES, *ATHERINOSOMA MICROSTOMA* AND *LEPTATHERINA PRESBYTEROIDES*

## 6.1 Introduction

*Paratrichodina tasmaniensis* sp. nov. was found on the gills of five atherinid fish species. However, it was only encountered frequently on *Atherinosoma microstoma* and *Leptatherina presbyteroides*. In this chapter, I report on the frequency distribution of *Paratrichodina tasmaniensis* within the host populations, the relationships between *P. tasmaniensis* and the host species including fish size and sex, the seasonal variations in prevalence and intensity of *P. tasmaniensis* infestation, a preliminary study of the spatial distribution of *P. tasmaniensis* on the gills of host fish, the interspecific association and correlation between *P. tasmaniensis* and five other parasite species, and the pathological effects on *Atherinosoma microstoma* of the infestation of *P. tasmaniensis*. All the studies were conducted on *Atherinosoma microstoma* and *Leptatherina presbyteroides* except for the pathology, where only the former species was used.

## 6.2 Material and methods

A total of 514 *Atherinosoma microstoma* and 589 *Leptatherina presbyteroides* collected in 19 and 22 samples between January 1990 and June 1992 respectively were investigated for the frequency distribution of *Paratrichodina tasmaniensis* and the interspecific relationships. For the study of the host-parasite relationships and seasonal variations of infestation, 495 *A. microstoma* and 445 *L. presbyteroides* caught at regular monthly intervals were examined. A preliminary study of the spatial distribution of *P. tasmaniensis* on the gills of host fish was undertaken with 20 *A. microstoma* and 16 *L. presbyterodies*. The area and methods of collecting fish were the same as given in Chapter 3. The number of parasites was counted from the slides prepared from each arch.

The methods of statistical analysis for the frequency distribution, the relationships between the parasite and the host fish and the seasonal variations of parasite infestation were the same as in Chapter 5. Regression analysis was conducted in the investigation of correlation between the prevalence of infestation of *P. tasmaniensis* and water temperature. $\chi^2$ analysis was used in the study of interspecific association. For the study of the spatial distribution of parasite on the gills of fish, 2 x 2 Contingency Table analysis was conducted to analyse the variation of prevalence; analysis of variance

(ANOVA) was used to analyse the variation of intensity.

Twelve *Atherinosoma microstoma* which displayed a different degree of infestation were used for the light microscope examination of pathological effect of the parasites; five fish were used for the transmission electron microscope observations. The histological techniques were the same as given in Chapter 3 and the methods of preparing samples for transmission electron microscopy were the same as given in Chapter 4.

## 6.3 Results

6.3.1 Frequency distributions of *Paratrichodina tasmaniensis* within the host populations

A total of 191 infested *Atherinosoma microstoma* of mixed age and sex were first used to deduce the frequency distribution. However, the results show that the observed distribution of *Paratrichodina tasmaniensis* could not be described adequately by the log-normal distribution model ($p < 0.01$). Examination of the original data reveals that some fish with considerably larger number of parasites occurred in January 1990. The frequency distribution was then attempted without the January 1990 samples.

The number of parasites on individual *A. microstoma* were divided into 7 classes on a logarithmic scale at intervals of 4; the scale is given in Table 6.1. The observed numbers of fish infested by each group of *P. tasmaniensis* is given in the same table.

**Table 6.1** The number of *Paratrichodina tasmaniensis* in *Atherinosoma microstoma* arranged on a log-normal scale at intervals of x 4.

| Class | Parasites. $fish^{-1}$ | No.fish (observed) S(R) | ln S(R) |
|---|---|---|---|
| 1 | 2-9 | 30 | 3.40 |
| 2 | 10-45 | 36 | 3.58 |
| 3 | 46-181 | 25 | 3.22 |
| 4 | 182-725 | 20 | 3.00 |
| 5 | 726-2901 | 10 | 2.30 |
| 6 | 2902-11605 | 7 | 1.95 |
| 7 | 11606-46421 | 2 | 0.69 |
| Total | | 130 | 18.14 |

The equations used for calculating the log-normal distribution and the definition for the parameters is the same as given in Chapter 5.

The modal class has 36 infested fishes, that is S(0) = 36. The value of R and $R^2$ for each class are given in Table 6.2. The following parameters are obtained from Table 6.2: $R_{max} = 5$, $S(R_{max}) = 2$, and $R^2_{max} = 25$.

The expected log-normal frequencies were computed from Eq. (5.3).

$$\alpha = \sqrt{\frac{\ln(36/2)}{25}} = 0.34$$

and from Table 6.1

$$\overline{\ln S(R)} = \text{Total ln S(R)} / 7 = 2.59$$

From Table 6.2

$$\overline{R}^2 = \text{Total } R^2 / 7 = 8$$

Thus, from Eq. (5.4)

$$S_0 = e^{[2.59+(0.34)^2 x8]} = 33.61$$

The expected frequencies with $\alpha = 0.34$ and $S_0 = 36$ and 33.61 were computed respectively using Eq. 5.1. These results are summarised in Table 6.2 and plotted in Fig. 6.1. $\chi^2$ values for each class were computed using Eq. 5.5. When $S_0 = 36.00$ was used, total $\chi^2 = 2.90$ and $0.8 > p > 0.7$; $S_0 = 33.61$ was used in calculating the expected values, total $\chi^2 = 1.91$ and $0.9 > p > 0.8$ (d.f. = 5). Both log-normal curve 1 and curve a2 fit the present data. However, the curve 1 seems to be accepted as a better fit.

**Table 6.2** Goodness-of-fit test for the log-normal model to observed data of *Paratrichodina tasmaniensis* in *Atherinosoma microstoma*. Expected results are given for two $S_0$ parameter estimates.

| Class | R | $R^2$ | Observed S(R) | $S_0 = 36.00$ Expected S(R) | $\chi^2$ | $S_0 = 33.61$ Expected S(R) | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| 1 | -1 | 1 | 30 | 32.07 | 0.13 | 29.94 | 0.00 |
| 2 | 0 | 0 | 36 | 36.00 | 0.00 | 33.61 | 0.17 |
| 3 | 1 | 1 | 25 | 32.07 | 1.56 | 29.94 | 0.82 |
| 4 | 2 | 4 | 20 | 22.67 | 0.31 | 21.17 | 0.06 |
| 5 | 3 | 9 | 10 | 12.72 | 0.58 | 11.87 | 0.30 |
| 6 | 4 | 16 | 7 | 5.66 | 0.32 | 5.29 | 0.56 |
| 7 | 5 | 25 | 2 | 2.00 | 0.00 | 1.87 | 0.01 |
| Total | | 56 | 130 | 143.19 | 2.90 | 133.69 | 1.91 |

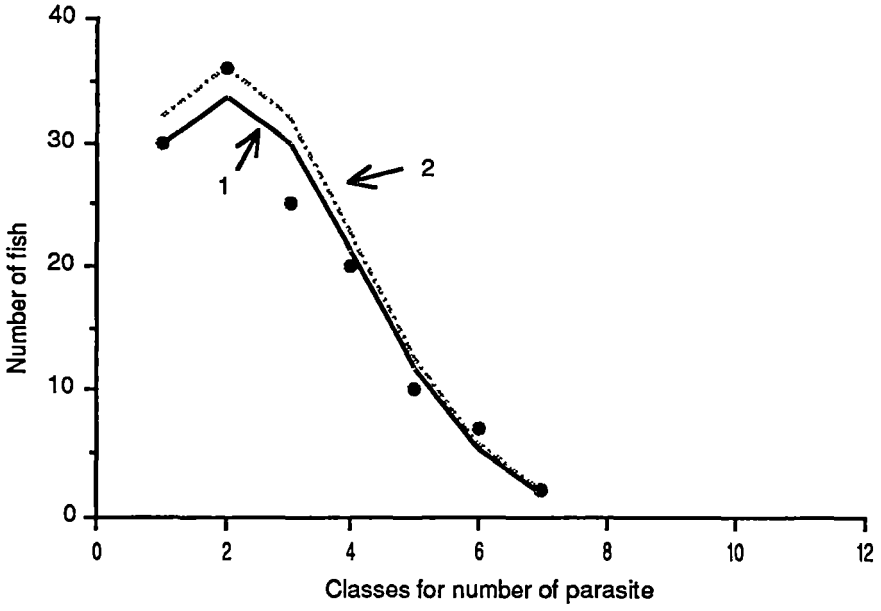



**Fig. 6.1** Frequency distribution of *Paratrichodina tasmaniensis* in *Atherinosoma microstoma*. Observed data shown as solid dots and fitted lognormal models as curves 1 (So=33.61), 2 (So=36).

A total of 136 infested *Leptatherina presbyteroides* was used to investigate the frequency distribution of *Paratrichodina tasmaniensis*. The number of parasites per fish was divided into 11 classes in *L. presbyteroides* on a log-normal scale at intervals of 2; the scale is given in Table 6.3. The observed number of fish infested by each group of *Paratrichodina tasmaniensis* is also given in that table.

**Table 6.3** The intensity of *Paratrichodina tasmaniensis* in *Leptatherina presbyteroides* arranged on a log-normal scale at intervals of x 2.

| Class | Parasites. fish$^{-1}$ | No.fish (observed) S(R) | ln S(R) |
|---|---|---|---|
| 1 | 1-2 | 1 | 0.00 |
| 2 | 3-4 | 5 | 1.61 |
| 3 | 5-8 | 14 | 2.64 |
| 4 | 9-16 | 15 | 2.71 |
| 5 | 17-32 | 22 | 3.09 |
| 6 | 33-64 | 30 | 3.40 |
| 7 | 65--128 | 22 | 3.09 |
| 8 | 129-256 | 12 | 2.48 |
| 9 | 257-512 | 7 | 1.95 |
| 10 | 513-1024 | 6 | 1.79 |
| 11 | 1025-2048 | 2 | 0.69 |
| Total | | 136 | 23.46 |

The modal class is the class 6 which has 30 infested fishes, that is, S(0) = 30. The following parameters can be obtained from Table 6.4: $R_{max}$ = -5 and +5, $R^2_{max}$ = 25. A value for a using both values of $S(R_{max})$ (1, and 2) in Eq (6.3) was computed and averaged.

$$\alpha_1 = \sqrt{\frac{\ln(30/1)}{36}} = 0.37$$

$$\alpha_2 = \sqrt{\frac{\ln(30/2)}{36}} = 0.33$$

$$\alpha = (\alpha_1 + \alpha_2)/2 = 0.35$$

From Table 6.3

$$\overline{\ln S(R)} = \text{Total ln S(R)} / 11 = 2.13$$

and Table 6.4

$$\overline{R}^2 = \text{Total } R^2 / 11 = 10$$

thus, from Eq. 5.4

$$S_0 = e^{[2.13+(0.35)^2 x10]} = 28.65$$

The expected frequencies are computed using $\alpha$ = 0.35 and $S_0$ = 28.65 and 30 respectively according to Eq. 5.1. The results are given in Table 6.4. The observed values and two sets of expected frequencies are plotted in Fig. 6.2.

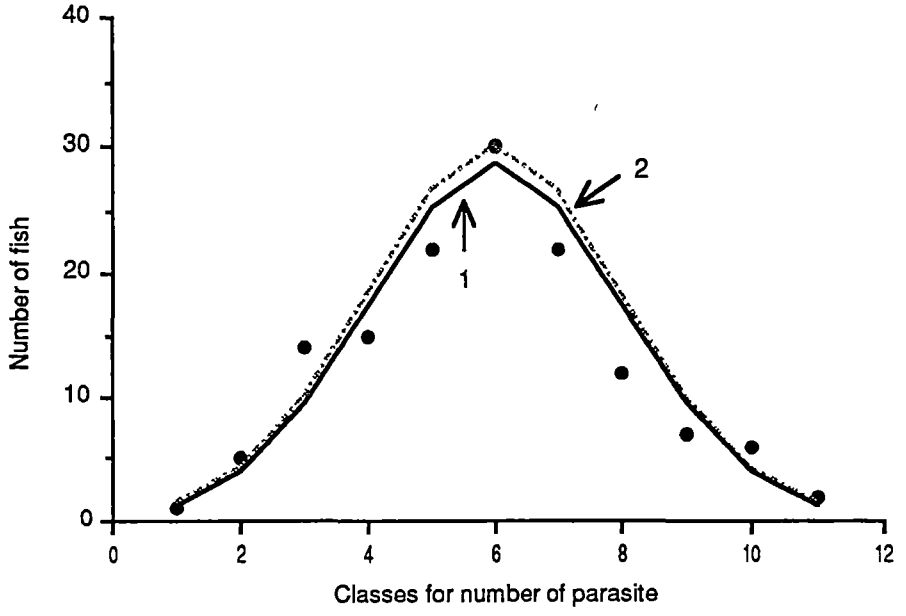



**Fig. 6.2** Frequency distribution of *Paratrichodina tasmaniensis* in *Leptatherina presbyteroides*. Observed data shown as solid dots and fitted lognormal models as curves 1 (So=28.65), 2 (So=30).

The goodness of fit of the expected model to the observed frequencies was tested using $\chi^2$ statistic analysis. When $S_0 = 30$ was used to calculate the expected frequency, total $\chi^2 = 8.16$; $S_0 = 28.65$ was used in calculating the expected values, total $\chi^2 = 7.45$. In both estimations, $0.7 > p > 0.5$ (d.f. = 9). This indicates that both curves 1 and 2 (Fig. 6.2) fit the present data of *P. tasmaniensis* within the population of *L. presbyteroides*.

**Table 6.4** Goodness-of-fit test for the log-normal model to observed data of *Paratrichodina tasmaniensis* in *Leptatherina presbyteroides*. Expected results are given for two $S_0$ parameter estimates.

| Class | R | $R^2$ | Observed S(R) | $S_0 = 30.00$ Expected S(R) | $\chi^2$ | $S_0 = 28.65$ Expected S(R) | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| 1 | -5 | 25 | 1 | 1.40 | 0.12 | 1.34 | 0.09 |
| 2 | -4 | 16 | 5 | 4.23 | 0.14 | 4.04 | 0.23 |
| 3 | -3 | 9 | 14 | 9.96 | 1.64 | 9.51 | 2.12 |
| 4 | -2 | 4 | 15 | 18.36 | 0.62 | 17.55 | 0.37 |
| 5 | -1 | 1 | 22 | 26.54 | 0.78 | 25.35 | 0.44 |
| 6 | 0 | 0 | 30 | 30.00 | 0.00 | 28.65 | 0.06 |
| 7 | 1 | 1 | 22 | 26.54 | 0.78 | 25.35 | 0.44 |
| 8 | 2 | 4 | 12 | 18.38 | 2.21 | 17.55 | 1.76 |
| 9 | 3 | 9 | 7 | 9.96 | 0.88 | 9.51 | 0.66 |
| 10 | 4 | 16 | 6 | 4.23 | 0.74 | 4.04 | 0.95 |
| 11 | 5 | 25 | 2 | 1.40 | 0.25 | 1.34 | 0.33 |
| Total | | 110 | 136 | 151.02 | 8.16 | 144.22 | 7.45 |

6.3.2 The relationships between the parasite and the host fish

*6.3.2.1 Host specificity to Paratrichodina tasmaniensis: Paratrichodina tasmaniensis* was found on five atherinid fish species in the present study. Another six fish species occurring sympatrically with the atherinids were not infested. Table 6.5 shows the species of fish infested with *P. tasmaniensis* and the prevalence of infestation in different hosts.

There is a significant variation in the prevalence of infestation among fish species (2 x c Contingency Table analysis, $\chi^2 = 69.439$, $p < 0.001$). Ignoring *Kestratherina hepsetoides*, for which only five individuals were examined, *Leptatherina presbyteroides, Kestratherina esox* and especially *Atherinosoma microstoma* are apparently the three preferred host species for *P. tasmaniensis*.

**Table 6.5** Species of fish infested with *Paratrichodina tasmaniensis* and the prevalence of parasite in different species

| Fish species | Prevalence of gill infestation | Number of fish examined |
|---|---|---|
| *Atherinosoma microstoma* | 37.16% | 514 |
| *Lepatherina presbyteroides* | 23.09% | 589 |
| *Kestratherina esox* | 20.31% | 64 |
| *K. brevirostris* | 2.59% | 116 |
| *K. hepsetoides* | 60.00% | 5 |

In addition to host specificity, *P. tasmaniensis* also shows an obligate organ specificity. It was only found on the gills of atherinid fishes. No ciliate occurred on the fins, skin and visceral organs.

*6.3.2.2 Variations of parasite infestation with the length of fish:* A total of 495 *Atherinosoma microstoma* and 445 *Leptatherina presbyteroides* were divided into 13 groups at mostly 3 mm fork lengths intervals. The size of samples examined in each length group and the data on the prevalence of parasite infestation are given in Appendix Table A-9 for *A. microstoma* and Table A-10 for *L. presbyteroides*.

Figure 6.3 shows the relationship between the prevalence and intensity of *P. tasamaniensis* infestation and the length of *A. microstoma*. The prevalence of infestation was low in fish of 45-54 mm. It increased rapidly in 55-60 and 58-60 mm length groups of fish and remained at a high level until 70-72 mm length group. A low level of prevalence of infestation occurred in fish of 73-75 and 82-84 mm before it rose to the second peak in fish of 85-87 mm. The prevalence of infestation dropped slightly in 88-93 mm group.

The variation in prevalence of infestation within fish of different lengths is significant ($p < 0.01$) when the 2 x c Contingency Table analysis was conducted (Table 6.6). A secondary analysis using the standardised residuals indicates that the deviations of 58-60 and 64-66 mm length groups are significantly higher ($r_{sij} > 0.2$) than the average, while the deviations of three groups with lengths from 76 to 84 mm range are significantly lower ($r_{sij} < -0.2$) than the average (Appendix Table A-9).

The original data of the intensity of *Paratrichodina tasmaniensis* infestation were not distributed normally. A plot of residuals against fitted values indicated that the logarithmic transformation of data was the appropriate transformation for the intensity of *Paratrichodina tasmaniensis* infestation. Therefore, all the data of intensity of infestation used in this chapter are transformed logarithmically.

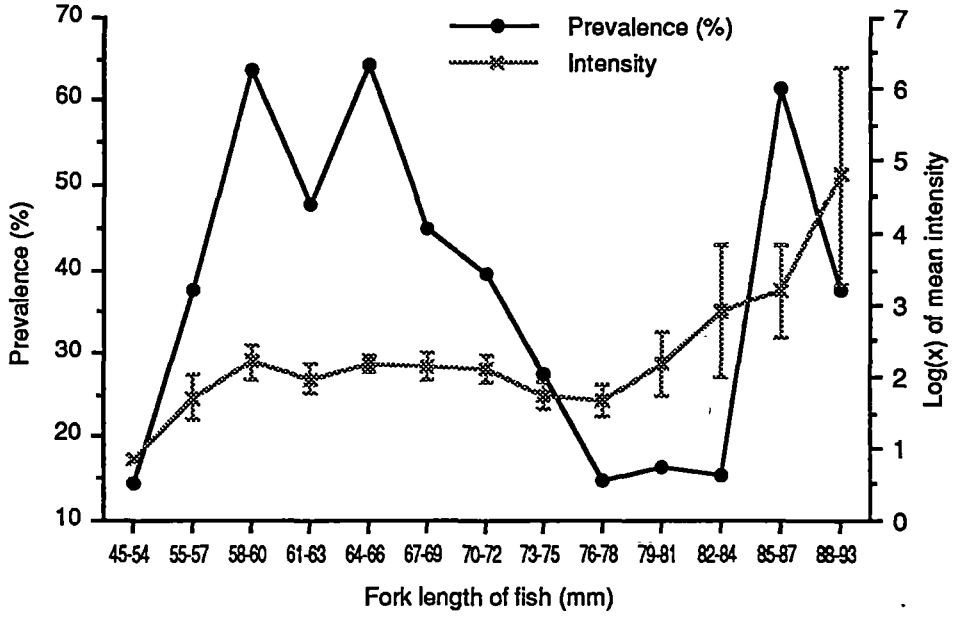



**Fig. 6.3** Infestation of *Paratrichodina tasmaniensis* in *Atherinosoma microstoma* of different length (bars indicate standard error).

The intensity of *P. tasmaniensis* infestation was low in *A. microstoma* less than 55 mm. A slight increase occurred in 55-57 and 58-60 mm length groups. The level of the intensity of infestation then remained at a relatively constant level until fish reached 78 mm. A steady rising of the intensity of infestation then occurred in fish with lengths from 79 to 93 mm range and a peak value appeared in fish of 88-93 mm. The ANOVA shows that the variation in intensity of infestation between different length groups of *A. microstoma* is significant ($p < 0.01$; Table 6.6). The results of Fisher PLSD test indicate that the intensity of infestation in fish of 88-93 mm is significantly ($p < 0.05$) different from those in other groups of fish; intensity of infestation in 85-87 mm group of fish is significantly different from those in fish between 45 and 78 mm; and the intensity of infestation in fish with length of 73 to 78 mm is significantly different from that in 82-84 mm group of fish (Appendix Table C-1).

The variations in prevalence and intensity of *P. tasmaniensis* infestation with the length of *L. presbyteroides* is displayed in Fig. 6.4. The prevalence of infestation was low in fish under 57 mm. It rose in 58-60 mm length group and remained at a high level until

fish of 70-72 mm. The level of prevalence of infestation then dropped in six groupsbetween 73 and 93 mm range.

**Table 6.6** Results of analysis of variations in prevalence and intensity of *Paratrichodina asmaniensis* infestation in *Atherinosoma microstoma* of different lengths.

| Prevalence of infestation (2 x c Contingency Table ) | Intensity of infestation (ANOVA) |
|---|---|
| DF = 12 | DF = 12 (Between groups), 163 (Within groups) |
| $\chi^2 = 62.06$ | F = 2.926 |
| p = 0.0001 | p = 0.0011 |

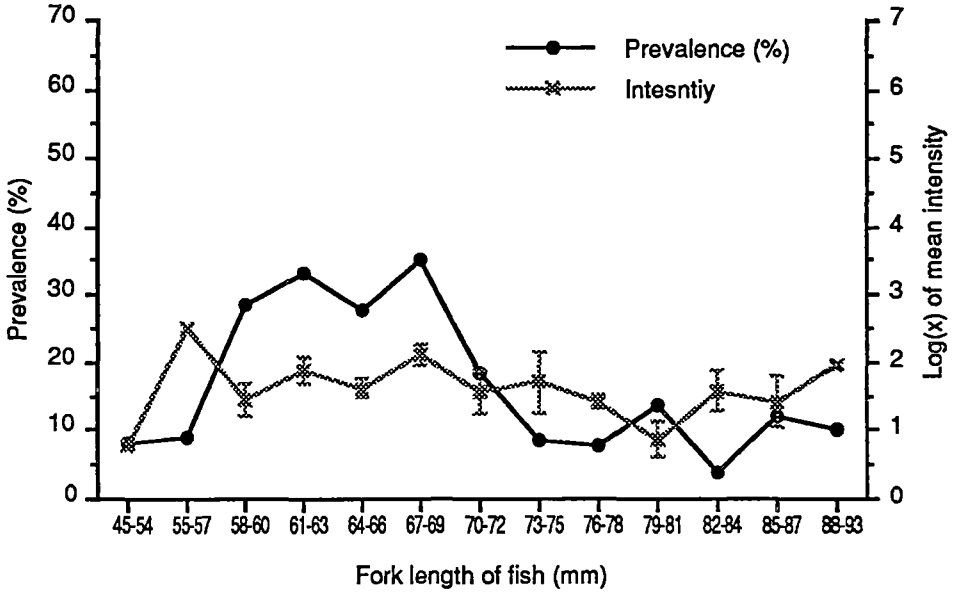



**Fig. 6.4** Infestation of *Paratrichodina tasmaniensis* in *Leptatherina presbyteroides* of different length (bars indicate standard error).

The variation in prevalence of *P. tasmaniensis* infestation is significant ($p < 0.01$) in *L. presbyteroides* of different lengths (Table 6.7). A secondary analysis using the standardised residuals indicates that the deviations of values in three length groups from 61 to 69 mm are significantly higher ($r_{sij} > 0.2$) than the average and the deviations of the value in fish of 82-84 mm is significant lower ($r_{sij} < -0.2$) than the average (Appendix Table A-10).

Although the result of ANOVA is significant ($p < 0.05$; Table 6.7), there was no clear trend between the intensity of parasite and the length of *L. presbyteroides*. The results of Fisher PLSD test of variations in intensity of *P. tasmaniensis* infestation between

different length groups of *L. presbyteroides* are given in Appendix Table C-2. The level of intensity of infestation in fish of 67-69 mm is significantly ($p < 0.05$) different from that in 45-54, 64-69 mm groups of fish; intensity of infestation in fish of 55-57 and between 61-69 are significantly different from that in fish of 79-81 mm .

**Table 6.7** Results of analysis of variations in prevalence and intensity of *Paratrichodina tasmaniensis* infestation in *Leptatherina presbyteroides* of different lengths.

| Prevalence of infestation (2 x c Contingency Table ) | Intensity of infestation (ANOVA) |
|---|---|
| DF = 12 | DF = 12 (between groups), 65 (within groups) |
| $\chi^2 = 37.054$ | F = 1.988 |
| p = 0.0002 | p = 0.0396 |

To investigate if any effect of infestation on fish growth and/or survival, the lengths of fish in the bimonthly samples with parasites were compared to those without parasites (Figs. 6.5, 6.6). The results of a two-factor ANOVA (Tables 6.8, 6.9) show that, in both fish species, the lengths of fish vary significantly with the month ($p < 0.01$). There were, however, no significant differences in the length between infested and uninfested fishes ($p > 0.5$) although the infested *L. presbyteroides* are significantly smaller than uninfested ones between January and April 1991.

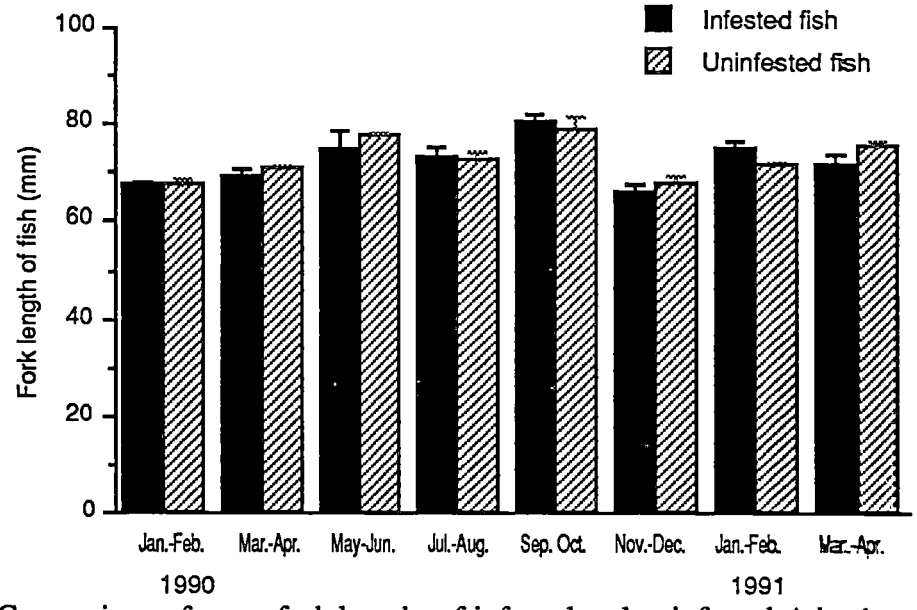



**Fig. 6.5** Comparison of mean fork lengths of infested and uninfested *Atherinosoma microstoma* with *Puntidina tasmaniensis*.

**Table 6.8** Analysis of variance of differences in mean fork length of *Atherinosoma microstoma* infested and uninfested with *Paratrichodina tasmaniensis* in different months.

| Source | DF | Sum of Squares | Mean Square | F-test | p value |
|---|---|---|---|---|---|
| Month (A) | 7 | 4055.898 | 579.414 | 11.769 | 0.0001 |
| Inf./Uninf. (B) | 1 | 113.383 | 13.383 | 0.272 | 0.6023 |
| AB | 7 | 199.397 | 28.485 | 0.579 | 0.7735 |
| Error | 479 | 23582.203 | 49.232 | | |

**Table 6.9** Analysis of variance of differences in mean fork length of *Leptatherina presbyteroides* infested and uninfested with *Paratrichodina tasmaniensis* in different months.

| Source | DF | Sum of Squares | Mean Square | F-test | p value |
|---|---|---|---|---|---|
| Month (A) | 7 | 1567.911 | 223.987 | 2.912 | 0.0055 |
| Inf./Uninf. (B) | 1 | 109.017 | 109.017 | 1.417 | 0.2345 |
| AB | 7 | 551.609 | 78.801 | 1.024 | 0.4131 |
| Error | 429 | 32999.54 | 76.922 | | |

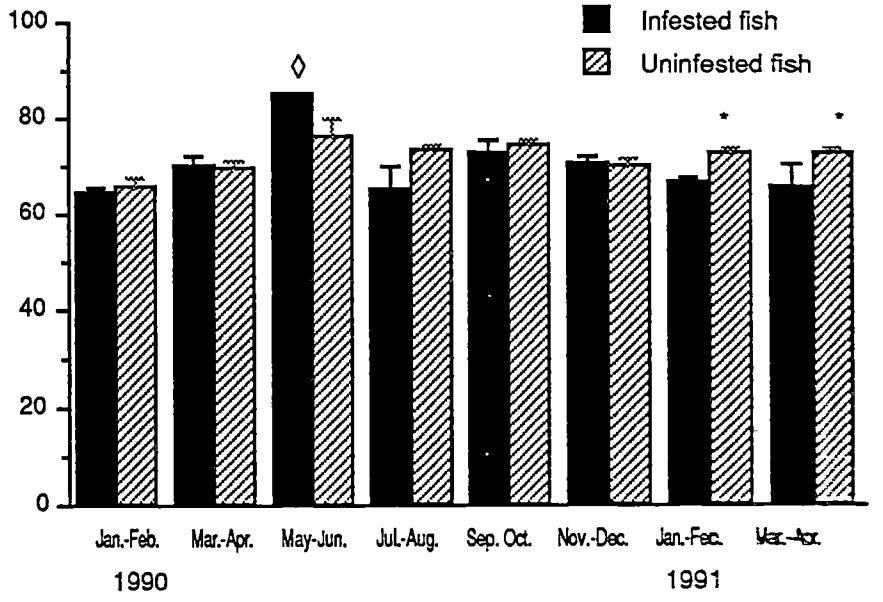



**Fig. 6.6** Comparison of mean fork lengths of infested and uninfested *Leptatherina presbyteroides* with *Puntidina tasmaniensis* (*p < 0.05, ◊ only one fish was examined).

6.3.3 Seasonal variations in prevalence and intensity of *Paratrichodina tasmaniensis* infestation

The sizes of samples examined bimonthly and the data on the prevalence of the parasite infestation are given in Appendix Table A-11 for *A. microstoma* and Table A-12 for *L. presbyterodies*.

The variations in prevalence and intensity of *P. tasmaniensis* infestation between bimonthly samples of *A. microstoma* are shows in Fig. 6.7. Both prevalence and intensity of infestation showed clear seasonal variations. The level of prevalence was high in January/February (summer) 1990. It was low between March/April (autumn) and July/August (winter) 1990. Prevalence increased in September/October (early spring) 1990 and it reached high levels in November/December (late spring/early summer) 1990 and January/February (summer) 1991. This was followed by a sharp drop in March/April (autumn) 1991.

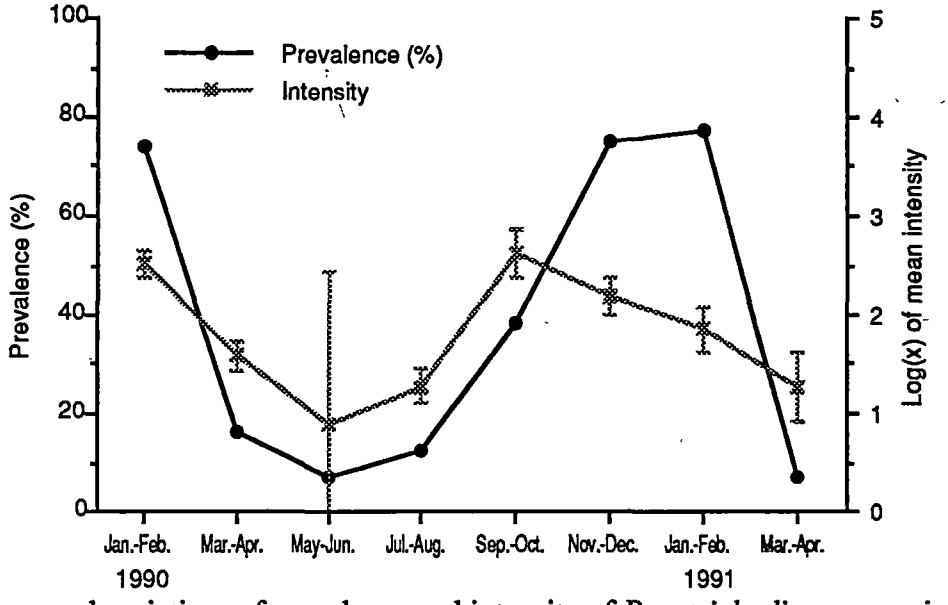



**Fig. 6.7** Seasonal variations of prevalence and intensity of *Paratrichodina tasmaniensis* infestation in *Atherinosoma microstoma* (bars indicate standard error).

The variation of prevalence of *P. tasmaniensis* infestation between the bimonthly samples of *A. microstoma.* is significant ($p < 0.001$; Table 6.10) A secondary analysis indicates that the deviations of values in January/February, November/December 1990 and January/February 1991 are significantly higher ($r_{sij} > 0.2$) than the average, while those in March/April, May/June 1990 and March/April 1991 are significantly lower ($r_{sij} < -0.2$) than the average (Appendix Table A-11).

The intensity of infestation varied in a similar pattern as the prevalence except that the second peak occurred between September/October (spring) 1990 and January/February (summer) 1991. The result of ANOVA indicates that the variation in intensity of *P. tasmaniensis* infestation is significant ($p < 0.001$; Table 6. 10). The results of Fisher PLSD test indicate that the intensity of infestation in January/February 1990 is significantly ($p < 0.05$) different from that between March/April and July/August 1990

and between January/February and March/April 1991; intensity of infestation in September/October 1990 is significantly different from those between March/April 1990 and January/February 1991; the levels of intensity in November/December 1990 is significantly different from those in May/June and July/August; and that in September/October 1990 is significant from that in March/April 1991 (Appendix Table C-3).

**Table 6.10** Results of analysis of seasonal variations in prevalence and intensity of *Paratrichodina tasmaniensis* infestation in *Atherinosoma microstoma.*

| Prevalence of infestation (2 x c Contingency Table ) | Intensity of infestation (ANOVA) |
|---|---|
| D F = 7 | DF = 7 (between groups), 168 (within groups) |
| $\chi^2$ = 192.118 | F = 4.392 |
| p = 0.0001 | p = 0.0002 |

Figure 6.8 shows the variations in prevalence and intensity of *P. tasmaniensis* infestation between bimonthly samples of *L. presbyteroides*. As in *Atherinosoma microstoma*, the prevalence and intensity of *P. tasmaniensis* infestation also displayed pronounced seasonal variations with higher values in summer and spring, and lower values in autumn and winter. The peaks of prevalence of infestation are, however, almost half that seen in *A. microstoma;* the levels of intensity of infestation are also lower.

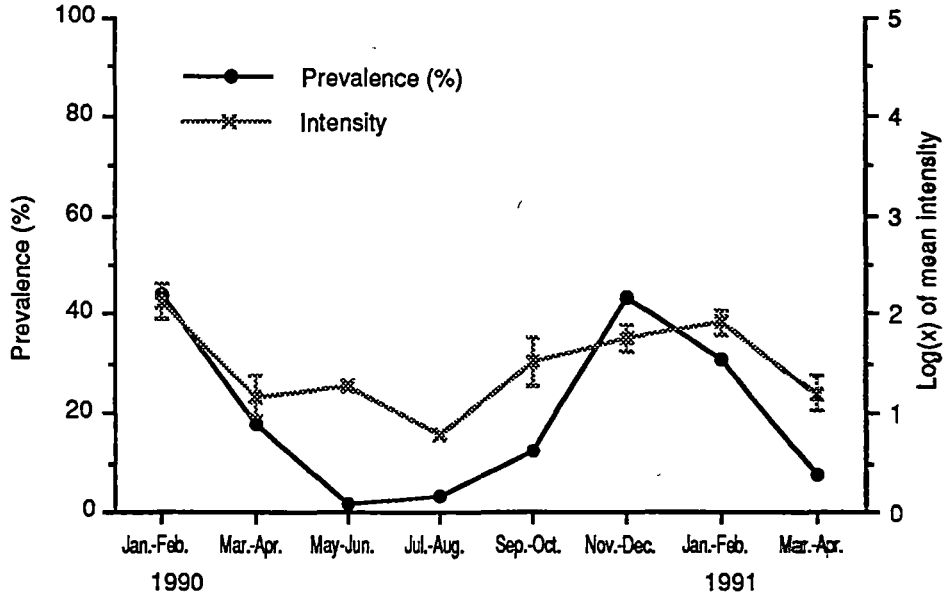



**Fig. 6.8** Seasonal variations of prevalence and intensity of *Paratrichodina tasmaniensis* infestation in *Leptatherina presbyteroides* (bars indicate standard error).

The prevalence of *P. tasmaniensis* infestation was significantly ($p < 0.001$; Table 6.11) different between the bimonthly level of infestation. A secondary analysis indicates that

the deviations of values in January/February 1990, November/December 1990 and January/February 1991 are significantly higher ($r_{sij} > 0.2$), and those in May/June, July/August 1990 and March/April 1991 are significantly lower ($r_{sij} < -0.2$) than the average (Appendix Table A-12).

The variations in intensity of *P. tasmaniensis* infestation between the bimonthly samples of *L. presbyteroides* are also significant ($p < 0.05$; Table 6.11). Fisher PLSD test shows that the intensity of infestation in January/February 1990 is significantly ($p < 0.05$) different from those in March/April, July/August and September/October 1990 and March/April 1991; the level in November/December 1990 is significantly different from that in July/August 1990; that in January/February 1991 is significantly different from those in March/April and July/August 1990; and the intensity in March/April 1991 is significantly different from those between November/December 1990 and January/February 1991.

**Table 6.11** Results of analysis of seasonal variations in prevalence and intensity of *Paratrichodina tasmaniensis* infestation in *Leptatherina presbyteroides*.

| Prevalence of infestation (2 x c Contingency Table ) | Intensity of infestation (ANOVA) |
|---|---|
| D F = 7 | DF = 7 (between groups), 70 (within groups) |
| $\chi^2 = 74.214$ | F = 2.797 |
| p = 0.0001 | p = 0.0126 |

To determine the relationships between the prevalence of parasite infestation and the water temperature, the prevalence of *P. tasmaniensis* infestation in both *A. microstoma* and *L. presbyteroides* is plotted against the water temperature in Figs. 6.9 and 6.10. The results of regression analyses show that there was a significantly ($p < 0.05$) positive correlation between the water temperature and the seasonal variation of prevalence of *P. tasmaniensis* infestation in both fish species (Tables 6.12, 6.13).

**Table 6.12** Result of regression analysis of correlation between prevalence of *Paratrichodina tasmaniensis* infestation in *Atherinosoma microstoma* and water temperature.

| Source | DF | Sum Squares | Mean Square | F-test |
|---|---|---|---|---|
| Regression | 1 | 4962.591 | 4962.591 | 12.986 |
| Residual | 6 | 2292.808 | 382.135 | p = .0113 |
| Total | 7 | 7255.399 | | |

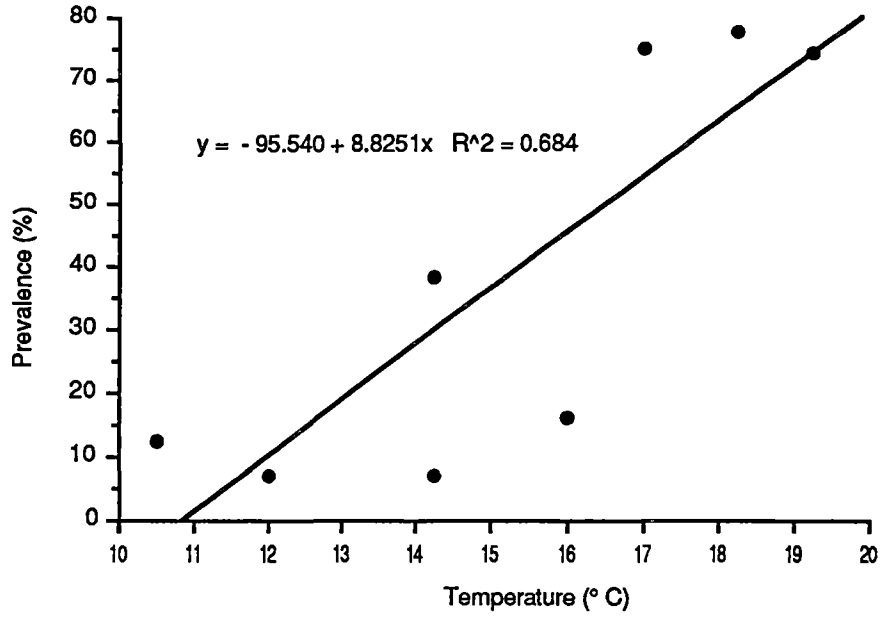



**Fig. 6.9** Correlation between prevalence of *Paratrichodina tasmaniensis* infestation in *Atherinosoma microstoma* and water temperature.

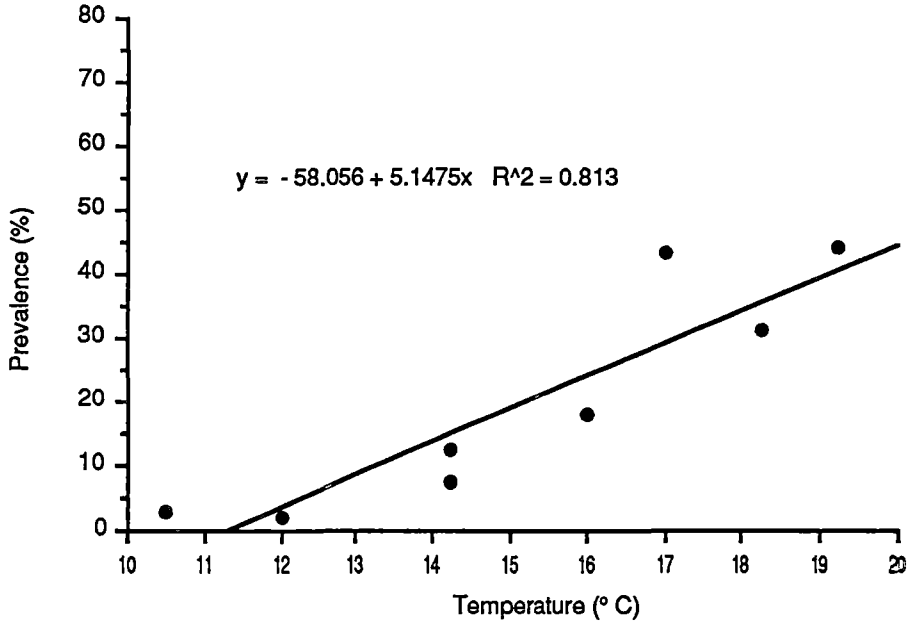



**Fig. 6.10** Correlation between prevalence of *Paratrichodina tasmaniensis* infestation in *Leptatherina presbyteroides* and water temperature.

**Table 6.13** Result of regression analysis of correlation between prevalence of *Paratrichodina tasmaniensis* infestation in *Leptatherina presbyteroides* and water temperature

| Source | DF | Sum Squares | Mean Square | F-test |
|---|---|---|---|---|
| Regression | 1 | 1688.355 | 1688.355 | 26.056 |
| Residual | 6 | 388.784 | 64.797 | p = .0022 |
| Total | 7 | 2077.139 | | |

6.3.4 Variations of parasite infestation with the sex of fish

The sample size of male and female *Atherinosoma microstoma* examined bimonthly and the data on the prevalence of *Paratrichodina tasmaniensis* infestation are given in Appendix Tables A-13 and A-14. Immature fish were excluded from the analyses in this section.

Figure 6.11 displays the variations of bimonthly prevalence of *P. tasmaniensis* between male and female samples of *A. microstoma*. The results of 2 x 2 Contingency Table analysis indicate that there were no significant differences ($p > 0.05$) in the prevalence of infestation between bimonthly male and female samples of *A. microstoma* (Table 6.14).

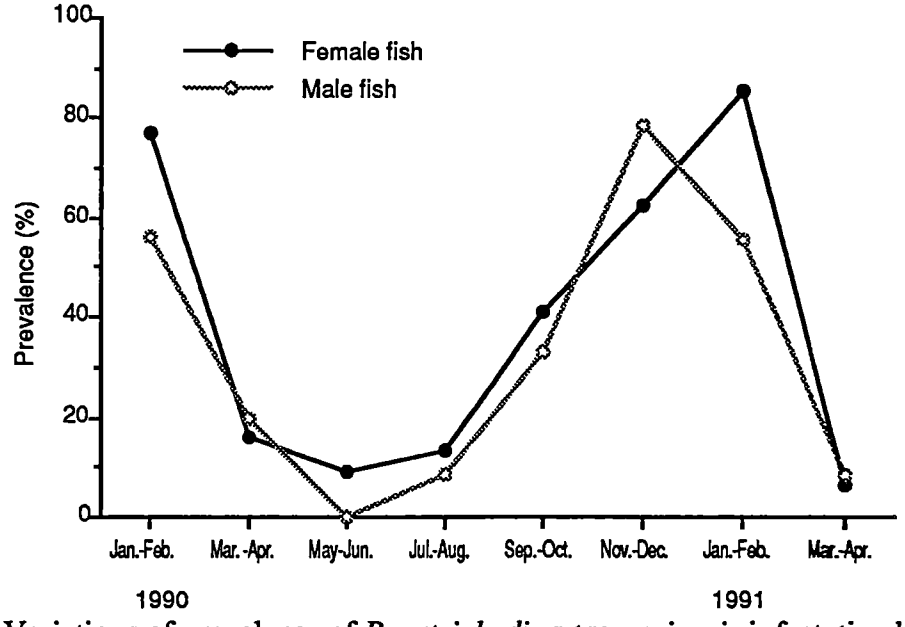



**Fig. 6.11** Variations of prevalence of *Paratrichodina tasmaniensis* infestation between male and female *Atherinosoma microstoma*.

**Table 6.14** Results of analysis of variations in prevalence of *Paratrichodina tasmaniensis* infestation between bimonthly male and female samples of *Atherinosoma microstoma*.

| Month | Prevalence (2 x 2 Contingency Table) | | |
|---|---|---|---|
| | DF | $\chi^2$ | p |
| Jan.-Feb. 1990 | 1 | 2.035 | 0.1537 |
| Mar.-Apr. | 1 | 0.155 | 0.6936 |
| May-Jun. | 1 | 0.209 | 0.6515 |
| Jul.-Aug. | 1 | 0.030 | 0.8629 |
| Sep.-Oct. | 1 | 0.142 | 0.7063 |
| Nov.-Dec. | 1 | 0.214 | 0.6434 |
| Jan.-Feb. 1991 | 1 | 1.739 | 0.1872 |
| Mar.-Apr. | 1 | 0.033 | 0.8557 |

The variations in intensity of *P. tasmaniensis* infestation between bimonthly male and female samples of *A. microstoma* are displayed in Fig. 6.12. The number of fish, especially male ones, in each sample is very small; in most cases only less than five infested individuals were available. Therefore, data of intensity of infestation are not robust enough to permit statistical analysis.

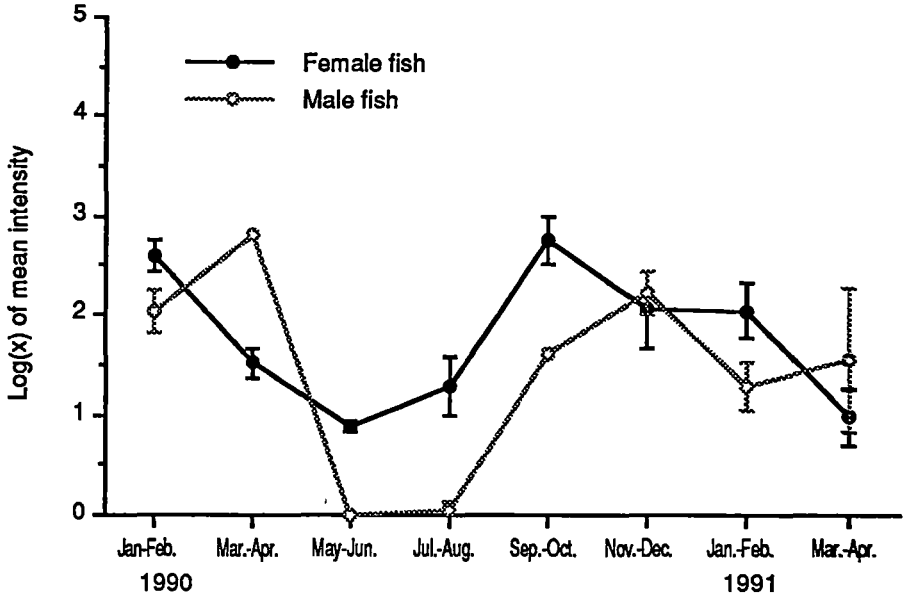



**Fig. 6.12** Variations of intensity of *Paratrichodina tasmaniensis* infestation between male and female *Atherinosoma microstoma* (bars indicate standard error).

The sample size of male and female *Leptatherina presbyteroides* examined bimonthly and the data on the prevalence of *P. tasmaniensis* infestation are displayed in Appendix Tables A-15, A-16.

The variations in bimonthly prevalence of *P. tasmaniensis* infestation between male and female populations of *L. presbyteroides* are plotted in Fig. 6.13. Male fish showed a higher level of prevalence of infestation than female ones throughout the study period except in May/June 1990. However, these differences are non-significant ($p > 0.05$) (Table 6.15).

Figure 6.14 shows the variations in intensity of *P. tasmaniensis* infestation between bimonthly populations of male and female *L. presbyteroides*. Again, due to the small number of mature fish in each sample, statistical analysis was not attempted.

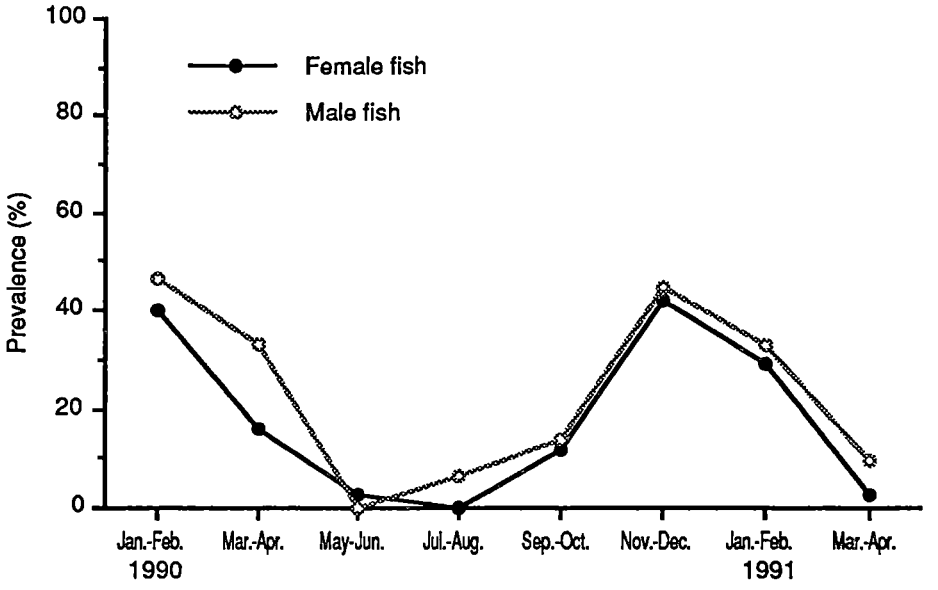



**Fig. 6.13** Variations of prevalence of *Paratrichodina tasmaniensis* infestation between male and female *Leptatherina presbyteroides*.

**Table 6.15** Results of analysis of variations in prevalence of *Paratrichodina tasmaniensis* infestation between bimonthly male and female samples of *Leptatherina presbyteroides*.

| Month | Prevalence (2 x 2 Contingency Table) | | |
|---|---|---|---|
| | DF | $\chi^2$ | p |
| Jan.-Feb. 1990 | 1 | 0.007 | 0.9345 |
| Mar.-Apr. | 1 | 0.003 | 0.9546 |
| May-Jun. | 1 | 0.177 | 0.6740 |
| Jul.-Aug. | 1 | 0.550 | 0.4584 |
| Sep.-Oct. | 1 | 0.003 | 0.9549 |
| Nov.-Dec. | 1 | 0.001 | 0.9793 |
| Jan.-Feb. 1991 | 1 | 0.0004 | 0.9828 |
| Mar.-Apr. | 1 | 0.520 | 0.4708 |

The variations in prevalence of *P. tasmaniensis* infestation between the bimonthly samples of the same sex of both fish species were also analysed by 2 x c Contingency Table analysis. In both *Atherinosoma microstoma* and *Leptatherina presbyteroides*, male and female fishes showed a significant ($p < 0.001$) variation in prevalence of infestation between bimonthly populations (Tables 6.16, 6.17).

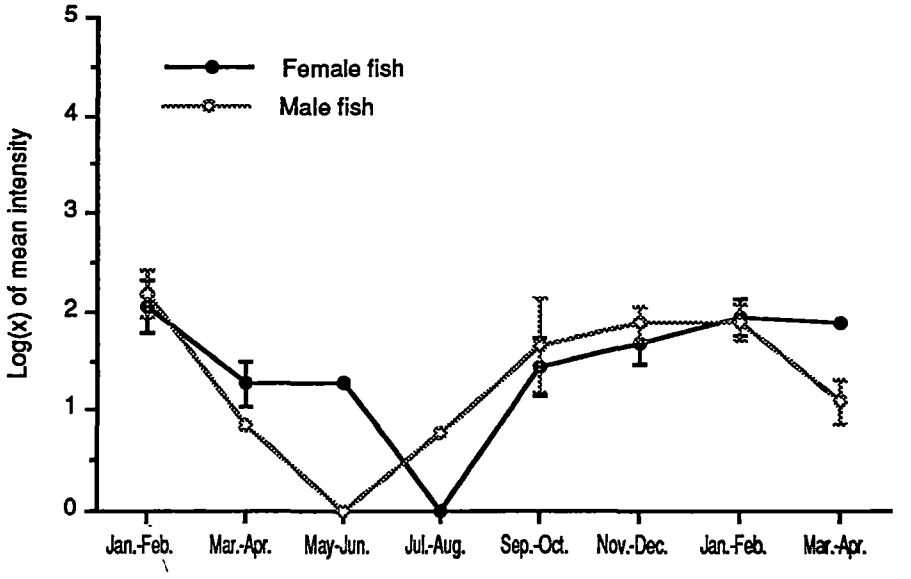



**Fig. 6.14** Variations of intensity of *Paratrichodina tasmaniensis* infestation between male and female *Leptatherina presbyteroides* (bars indicate standard error).

**Table 6.16** Results of analysis of seasonal variations in prevalence of *Paratrichodina tasmaniensis* infestation in male and female *Atherinosoma microstoma.*

| Test | Female | Male |
|---|---|---|
| Prevalence | DF = 7 | DF =7 |
| (2 x c Contingency Table) | $\chi^2$= 145.453 | $\chi^2$ = 49.67 |
| | p = 0.0001 | p = 0.0001 |

For both male and female *A. microstoma,* the prevalence of infestations showed a similar trend as that for all fish (i.e. including immature fish) as shown in Fig. 6.7. A secondary analysis using the standardised residuals shows that, for female fish, the deviations of values in January/February 1990 and January/February 1991 are significantly higher ($r_{sij}$ > 0.2) than the average, while the deviations of the values in March/April, May/June, July/August 1990 and March/April 1991 are significantly lower ($r_{sij}$ < -0.2) than the average; for male fish, the deviation of value in November/December 1990 is significantly higher ($r_{sij}$ > 0.2) than the average and the deviations of values in May/June, July/August 1990 and March/April 1991 are significantly lower than the average ($r_{sij}$ < -0.2) (Appendix Tables A-13, A-14).

**Table 6.17** Results of analysis of seasonal variations in prevalence of *Paratrichodina tasmaniensis* infestation in male and female *Leptatherina presbyteroides*.

| Test | Female | Male |
|---|---|---|
| Prevalence | DF = 7 | DF =7 |
| (2 x c Contingency Table) | $\chi^2$ = 45.091 | $\chi^2$ = 29.781 |
| | p = 0.0001 | p = 0.0001 |

Again, the trends of prevalence of infestation in both female and male *L. presbyteroides* were similar to that for all fish. The secondary analysis of variation in prevalence of infestation between female bimonthly samples indicate that the deviations of the values in January/February, November/December 1990 and January/February 1991 are significantly higher ($r_{sij} > 0.2$) than the average, while the deviations of the values in May/June, July/August 1990, and March/April 1991 are significantly lower ($r_{sij} < -0.2$) than the average (Appendix Table A-15). For male fish, the results of the secondary analysis indicate that the deviations of the values in January/February and November/December 1990 are significantly higher ($r_{sij} > 0.2$) than the average, while the deviations of the values in May/June and July/August are significantly lower than the average ($r_{sij} > 0.2$) (Appendix Table A-16).

As indicated before, the number of mature fish in each sample are not robust enough to permit statistical analysis for the data of intensity of infestation. However, the trends shown by Figs. 6.12 and 6.14 mirror that deduced from the analysis of all fish, i.e.immature and mature fishes.

6.3.5 Spatial distributions of *Paratrichodina tasmaniensis* on the gills of host fish

A preliminary investigation on the distribution of *P. tasmaniensis* on the gills of fish was conducted to reveal the site preference of *P. tasmaniensis*. A total of 20 *A. microstoma* and 16 of *L. presbyteroides* were examined for this study. The data of infestation of *Paratrichodina tasmaniensis* on the left and right gills of fish are given in Table 6.18 for *A. microstoma* and Table 6.19 for *L. presbyteroides*.

In both fish species, the left gills show a higher level of prevalence and intensity of *P. tasmaninesis* infestation than the right gills. However, only the difference in prevalence of infestation are significant ($p < 0.05$). The results of ANOVA indicate that there were no significant differences ($p > 0.05$) in the intensity of infestation between the left and

right gills in both fish species (Tables 6.20, 6.21).

**Table 6.18** Infestation of *Paratrichodina tasmaniensis* on the left and right gills of *Atherinosoma microstoma.*

| Gills | Number of gill arches infested | Number of gill arches uninfested | Prevalence (%) | Log(x) of mean intensity |
|---|---|---|---|---|
| Left | 68 | 12 | 85.0% | 1.191 |
| Right | 54 | 26 | 67.5% | 1.142 |
| Total | 122 | 38 | | |

**Table 6.19** Infestation of *Paratrichodina tasmaniensis* on the left and right gills of *Leptatherina presbyteroides*

| Gills | Number of gill arches infested | Number of gill arches uninfested | Prevalence (%) | Log(x) of mean intensity |
|---|---|---|---|---|
| Left | 57 | 7 | 89.06% | 1.122 |
| Right | 42 | 22 | 34.38% | 0.992 |
| Total | 99 | 29 | | |

**Table 6.20** Results of analysis of variations in prevalence and intensity of *Paratrichodina tasmaniensis* infestation on the left and right gills of *Atherinosoma microstoma.*

| Prevalence of infestation (2 x c Contingency Table ) | Intensity of infestation (ANOVA) |
|---|---|
| DF = 1 | DF = 1 (between groups), 120 (within groups) |
| $\chi^2 = 5.833$ | F = 0.152 |
| p = 0.0157 | p = 0.6972 |

**Table 6.21** Results of analysis of variations in prevalence and intensity of *Paratrichodina tasmaniensis* infestation on the left and right gills of *Leptatherina presbyteroides.*

| Prevalence of infestation (2 x c Contingency Table ) | Intensity of infestation (ANOVA) |
|---|---|
| DF = 1 | DF = 1 (between groups), 97 (within groups) |
| $\chi^2 = 10.031$ | F = 1.185 |
| p = 0.0015 | p = 0.279 |

6.3.6 Interspecific association between parasite species

A total of 9 species of protozoan parasites were found to infest atherinid fishes in the present study. Except *Zschokkella macrocapsula, Ortholinea striateculus* and *Cryptobia* sp., six other species were more or less commonly encountered; these are *Glugea atherinae, Zschokkella leptatherinae, Paratrichodina tasmaniensis, Trichodina australis, Trichodina* sp. and *Clausophrya branchialis*. Of these species, *G. atherinae* and *Z. leptatherinae* infest the internal organs of fish, while the four ciliates were found on the gills of fish. The interspecific association between these six parasite species in 514 of *Atherinosoma microstoma* and 589 of *Leptatherina presbyteroides* were investigated.

The data of the observed and expected coexistence among six parasite species are given in Tables 6.22 for *A. microstoma* and Table 6.23 for *L. presbyteroides*. Expected coexistences were calculated by the hypothesis that each parasite's probability of infestation was essentially random, being not affected by the presence of the other parasite species. The number of expected cases of coexistence of parasite A with B ($EC_{A,B}$) is given by the formula:

$$(EC_{A,B}) = (ON_A / N) \times ON_B \qquad (6.1)$$

where $ON_A$ = total number of observed fish infested with parasite A, $ON_B$ = total number of observed fish infested with parasite B, N = total number of observed fish infested with parasite ($ON_A$, $ON_B$, $ON_C$, $ON_D$, $ON_E$, $ON_F$), which is 845 in *A. microstoma* and 551 in *L. presbyteroides*.

The significance of $\chi^2$ statistic test was determined by comparing the observed and expected distribution (Eq. 5.5). The values of $\chi^2$ are also given in Tables 6.22 and 6.23.

In *A. microstoma*, a positive association exists between *P. tasmaniensis* and *T. australis*, while a negative association exists between *T. australis* and *Z. leptatherinae*, *Trichodina* sp. and *Z. leptatherinae* (Table 6.22). In *L. presbyteroides*, a negative association exists between *P. tasmaniensis* and *Z. leptatherinae*, *Clausophrya branchialis* and *Z. leptatherinae* (Table 6.23). The data of coexistence for other pairs of species are not significant ($p > 0.05$).

**Table 6. 22** The observed and expected coexistence among six parasite species in *Atherinosoma microstoma.*

| | Number o f infected fish | | | | | | | | | | | | | | | | | | Total no. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Paratrichodina tasmaniensis* | | | *Trichodina australis* | | | *Trichodina* sp. | | | *Zschokkella leptatherinae* | | | *Glugea atherinae* | | | Clausophrya *branchialis* | | | of infected |
| | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | fish |
| *Paratrichodina tasmaniensis* | 51 | 78.164 | ** 9.440 | 105 | 63.566 | ** 27.008 | 33 | 30.718 | 0.169 | 22 | 30.718 | 2.474 | 10 | 11.253 | 0.140 | 36 | 42.580 | 1.017 | 257 |
| *Trichodina australis* | 105 | 63.566 | ** 27.008 | 21 | 51.693 | ** 18.225 | 32 | 24.981 | 1.972 | 10 | 24.981 | ** 8.984 | 10 | 9.151 | 0.079 | 31 | 34.627 | 0.380 | 209 |
| *Trichodina* sp. | 33 | 30.718 | 0.169 | 32 | 24.981 | 1.972 | 5 | 12.072 | * 4.143 | 3 | 12.072 | ** 6.818 | 7 | 4.422 | 1.502 | 21 | 16.734 | 1.088 | 101 |
| *Zschokkella leptatherinae* | 22 | 30.718 | 2.474 | 10 | 24.981 | ** 8.984 | 3 | 12.072 | ** 6.818 | 54 | 12.072 | ** 145.619 | 2 | 4.422 | 1.327 | 10 | 16.734 | 2.710 | 101 |
| *Glugea atherinae* | 10 | 11.253 | 0.140 | 10 | 9.151 | 0.079 | 7 | 4.422 | 1.502 | 2 | 4.422 | 1.327 | 1 | 1.620 | 0.237 | 7 | 6.130 | 0.123 | 37 |
| *Clausohrya branchialis* | 36 | 42.580 | 1.017 | 31 | 34.627 | 0.380 | 21 | 16.734 | 1.088 | 10 | 16.734 | 2.710 | 7 | 6.130 | 0.123 | 35 | 23.195 | * 6.008 | 140 |

O, observed number of fish; E, expected number of fish.

*p < 0.05, **p < 0.01.

**Table 6. 23** The observed and expected coexistence among six parasite species in *Leptatherina presbyteroides*.

| | Number o f infected fish | | | | | | | | | | | | | | | | | | Total no. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Paratrichodina tasmaniensis* | | | *Trichodina australis* | | | *Trichodina* sp. | | | *Zschokkella leptatherinae* | | | *Glugea atherinae* | | | *Clausophrya branchialis* | | | of infected |
| | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | O | E | $\chi^2$ | fish |
| *Paratrichodina tasmaniensis* | 67 | 45.307 | 10.387** | 33 | 27.241 | 1.217 | 5 | 5.162 | 0.005 | 39 | 60.218 | 7.476** | 3 | 5.162 | 0.905 | 11 | 14.911 | 1.026 | 158 |
| *Trichodina australis* | 33 | 27.241 | 1.217 | 18 | 16.379 | 0.160 | 5 | 3.103 | 1.159 | 26 | 36.207 | 2.877 | 3 | 3.103 | 0.003 | 10 | 8.966 | 0.119 | 95 |
| *Trichodina* sp. | 5 | 5.162 | 0.005 | 5 | 3.103 | 1.159 | 1 | 0.588 | 0.289 | 2 | 6.861 | 3.443 | 1 | 3.855 | 2.114 | 4 | 1.699 | 3.118 | 18 |
| *Zschokkella leptatherinae* | 39 | 60.218 | 7.476** | 26 | 36.207 | 2.887 | 2 | 6.861 | 3.443 | 127 | 80.036 | 27.557** | 7 | 6.860 | 0.003 | 9 | 19.819 | 5.906** | 210 |
| *Glugea atherinae* | 3 | 5.162 | 0.905 | 3 | 3.103 | 0.003 | 1 | 3.855 | 2.114 | 7 | 6.860 | 0.003 | 1 | 0.588 | 0.289 | 3 | 1.699 | 0.997 | 18 |
| *Clausophrya branchialis* | 11 | 14.911 | 1.026 | 10 | 8.966 | 0.119 | 4 | 1.699 | 3.117 | 9 | 19.819 | 5.906** | 3 | 1.699 | 0.997 | 15 | 4.907 | 20.756** | 52 |

O, observed number of fish; E, expected number of fish.

*$p < 0.05$, **$p < 0.01$.

### 6.3.7 Pathology of *Atherinosoma microstoma* with the infestation of *Paratrichodina tasmaniensis*

The fish that harboured *Paratrichodina tasmaniensis* did not have the external indications of infestation in the present study. The infested *A. microstoma* which displayed a different degree of infestation under the light microscope were used for the pathological study. Mixed infestation with other parasites was not found in these fishes.

#### *6.3.7.1 Light microscopy*

The structure of uninfested gills of *Atherinosoma microstoma* is shown in Pl. 6.1A, B. Each gill filament or primary lamella bears a row of secondary (respiratory) lamellae on the left and right surfaces. The primary lamellae consists of several layers of epithelial cells overlying blood vessels. The secondary lamellae are lined externally by the epithelium and a row of red blood cells are situated between the layers of epithelium (Pl. 6.1B).

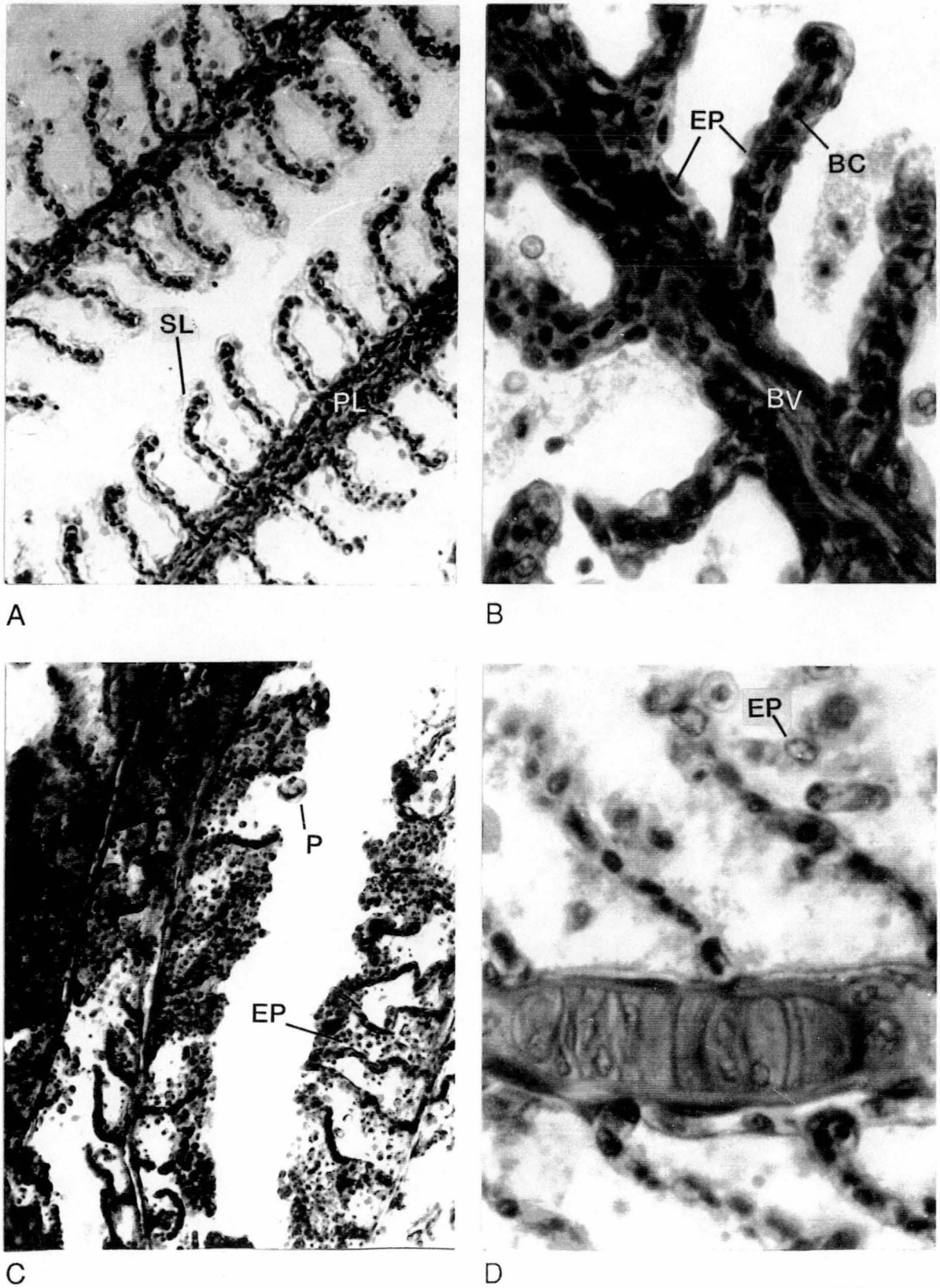
Individuals of *Paratrichodina tasmaniensis* are found attached to the epithelium of the secondary lamellae, they are not encapsulated by the gill tissue (Pl. 6.1C). The histopathology associated with this species shows that the structure of both the primary and secondary lamellae are altered by the presence of the parasite. The first sign, under the light microscope, is the sloughing of the epithelial layer (Pl. 6.1C, D).

Hyperplasia of the primary and secondary lamellar epithelium is often observed. This change, like others, appears to occur along the filaments and is not confined to the areas directly adjacent to the parasites. The hyperplasia results in the fusion of adjacent secondary lamellae (Pl. 6.2A).

#### *6.3.7.2 Transmission electron microscopy*

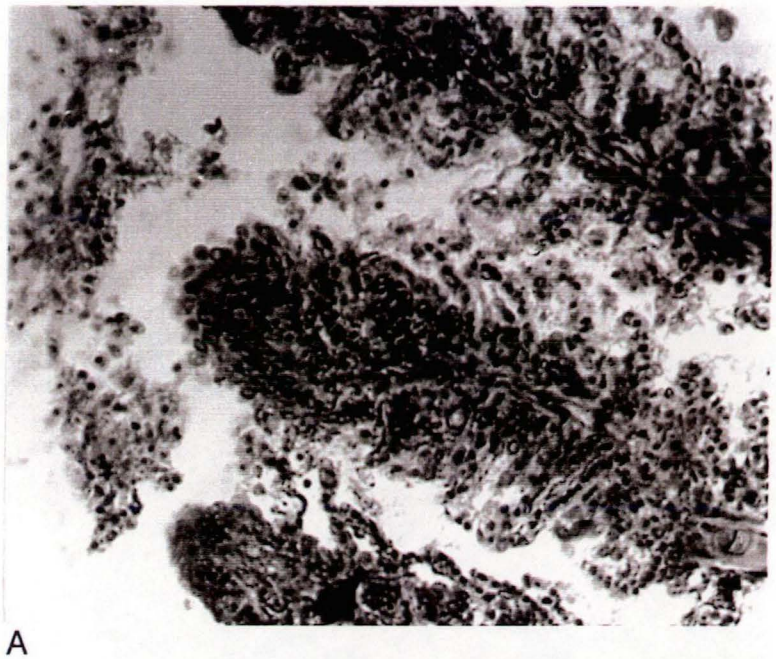
Plate 6.2B shows the ultrastructure of an uninfested gill filament of *Atherinosoma microstoma*. Two layers of epithelium are separated by a large number of pillar cells; their flanges meet and connect, delimiting the lamellar blood spaces. At the margin of the secondary lamellae the flanges of the pillar cells meet to form a continuous channel (Pl. 6.3A). Erythrocytes are distributed in the lamellar blood spaces. The outmost surface of the epithelial cells has a modified microvillous plasma membrane. A basement membrane lies immediately below each epithelium (Pl. 6.2B).

Hypertrophic epithelial cells of the secondary lamellae are often seen in the infested fish gills under the transmission electron microscope (Pl. 6.3B). These cells have an enlarged

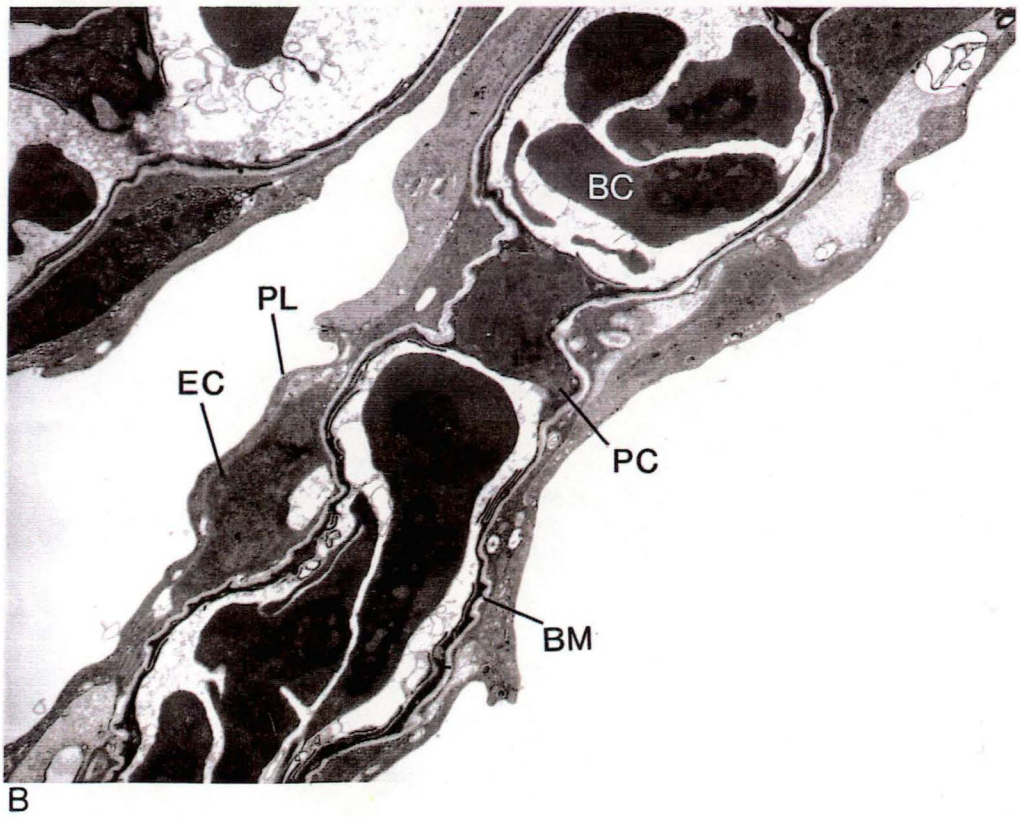



A B C D

Plate 6.1. Sections of gill filaments of *Atherinosoma microstoma* infested or uninfested with *Paratrichodina tasmaniensis*. A. Uninfested gill filaments, x 1208. SL, secondary lamella; PL, primary lamella. B. Enlargement of part of an uninfested gill filament, x 1760. EP, epithelial cells; BV, blood vessel; BC, red blood cell. C. Infested gill filaments, x 448. P, parasite; EP, sloughed epithelial cells. D. Enlargement of part of an infested gill filament, x 1671. EP, sloughed epithelial cell.

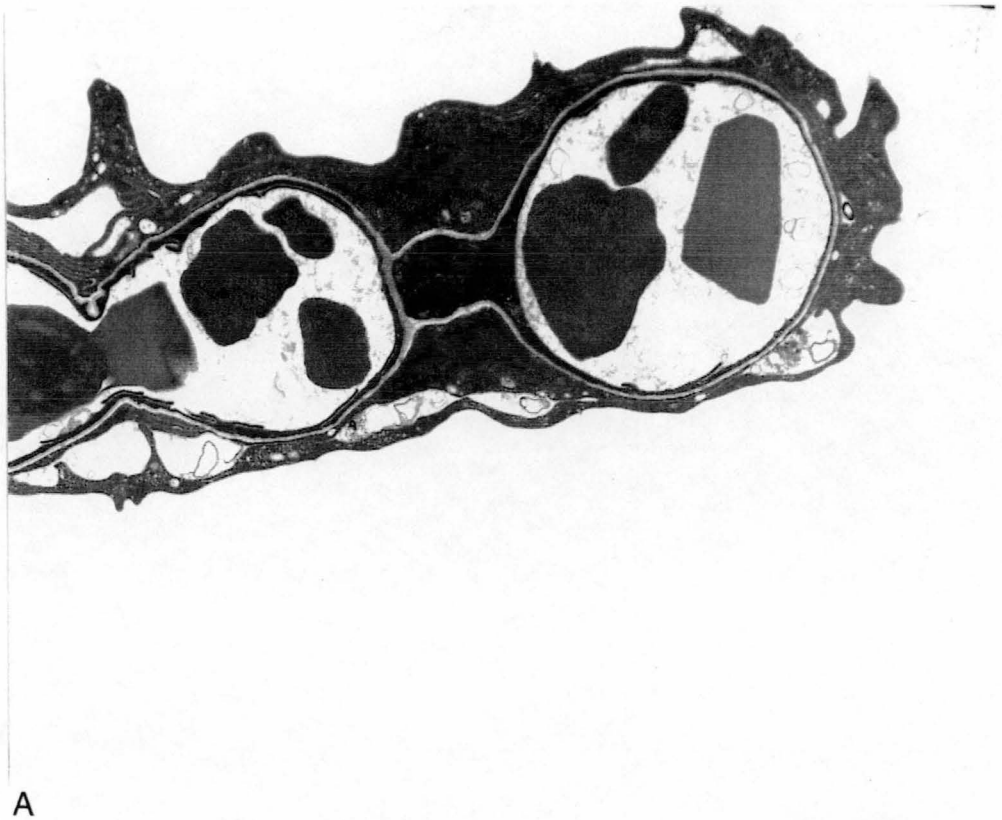


A

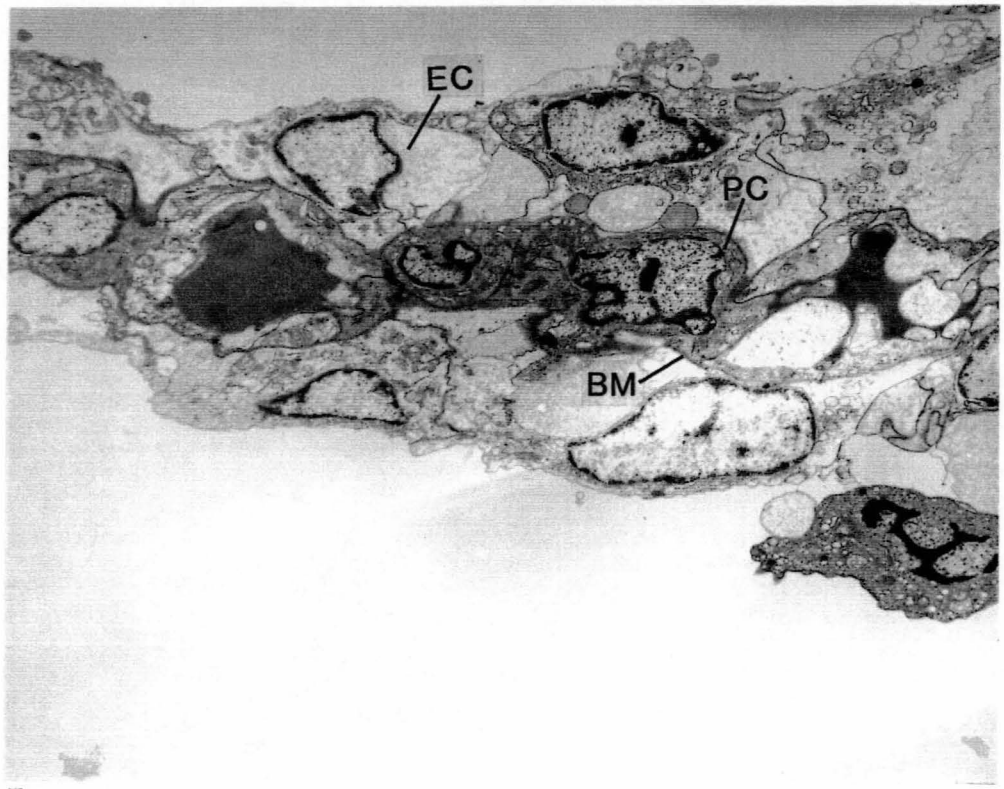



B

Plate 6.2. Sections of gill filaments of *Atherinosoma microstoma* infested or uninfested with *Paratrichodina tasmaniensis*. A. Infested gill filaments showing hyperplasia of epithelial cells and fusion of secondary lamellae, x 952. B. TEM micrograph of uninfested secondary lamellae, x 6000. EC, epithelial cell; PC, pillar cell; BM, basement membrane; BC, red blood cell; PL, plasma



A




B

Plate 6.3. TEM micrographs of secondary lamellae of *Atherinosoma microstoma* infested or uninfested with *Paratrichodina tasmaniensis*. A. Marginal end of an uninfested secondary lamella, x 6000. B. Part of an infested secondary lamella, x 4500. EC, epithelial cell; PC, pillar cell; BM, basement membrane.

cytoplasm with the majority of organelles restricted to the perinuclear region. The pillar cells are irregular in shape and the basement membrane is thinner than in uninfested fishes (Pls. 6.3B, 6.4A). The pattern of the surface ridges is disrupted in most infested secondary lamellae (Pl. 6.3B).

Lifting of the epithelial cells was found extensively in infested fish (Pl. 6.4B); this can subsequently lead to completely sloughing (Pl. 6.5A). In two infested fish, the hyperplasia of the pillar system and the epithelial cells occurred at the marginal end of the secondary lamellae. As a result, two layers of pillar system and three layers of epithelial cells were visible at the margin of the lamella (Pl. 6.5B).

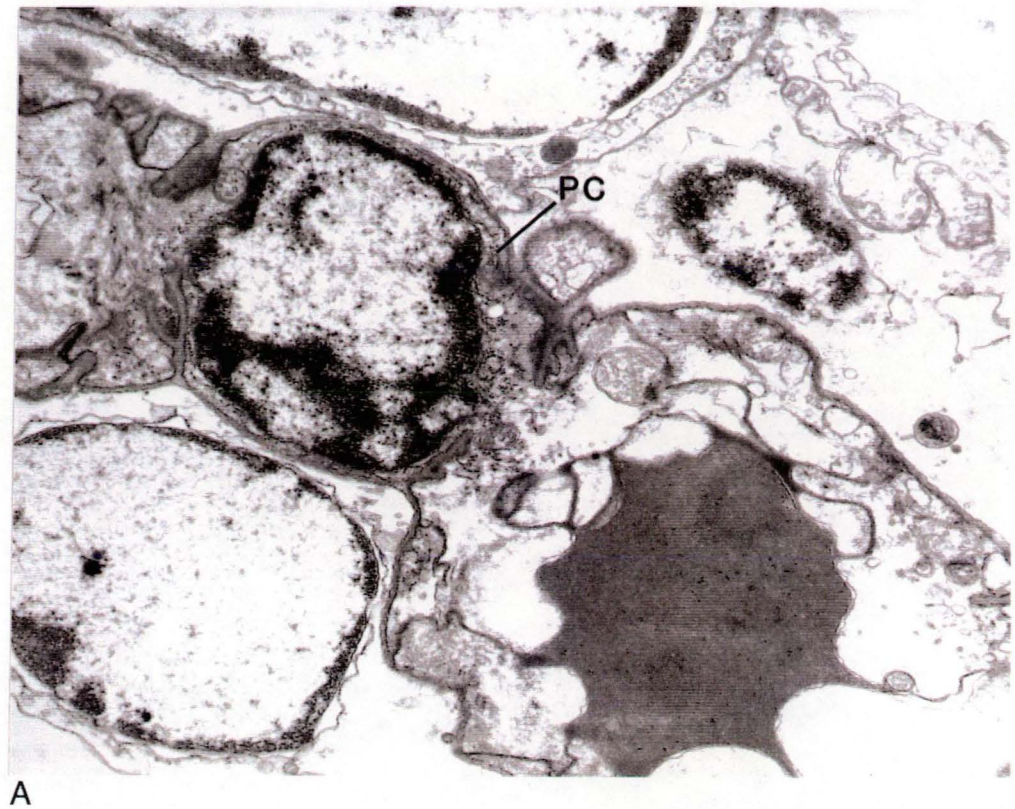
## 6.4 Discussion

### 6.4.1 Seasonal variations of *P. tasmaniensis* infestation

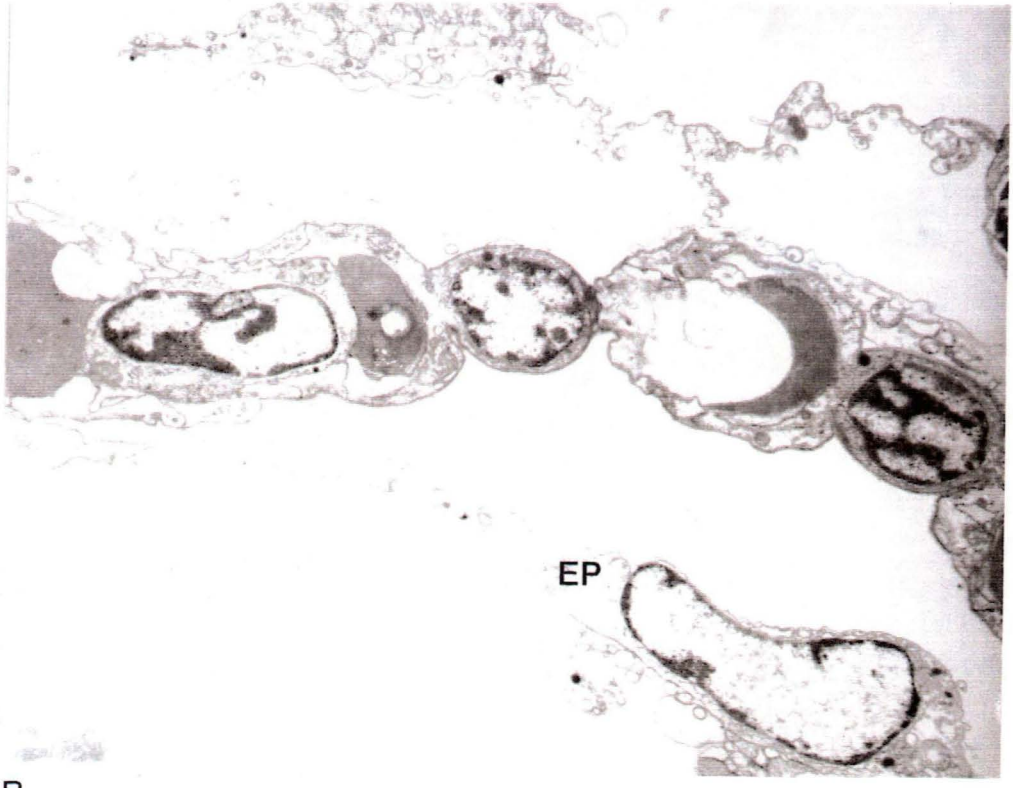
The prevalence and intensity of *Paratrichodina tasmaniensis* infestation showed a pronounced seasonal variation in both *Atherinosoma microstoma* and *Leptatherina presbyteroides*. The high level of the prevalence of infestation occurred in late spring and summer in both fish species and the low level occurred in autumn and winter in *A. microstoma*, while in *L. presbyteroides*, it appeared from autumn until spring. In both fish species, the high level of the intensity of infestation appeared between early spring and summer and the low level appeared from autumn to winter.

The parasite infestations are highly dependent on a number of factors in the external environment. Temperature is a major environmental factor and can have a pronounced influence on the ectoparasites of poikilotherm host (Kennedy and Hine, 1969; Lom and Laird, 1969; Rawson, 1976). Walkey (1967) indicated that "since fish are poikilothermic animals, temperature changes in the external environment are transmitted to the parasite fauna". The experiments of Schaperclaus (cited by Shulman, 1966) and Udey *et al.* (1975) demonstrated that a rise in temperature results in an increase of developmental rates of *Ceratomyxa shasta* Noble, 1950 and *Myxobolus cerebralis* Hofer, 1903. The infestation of *Glugea stephani* Hagenmuller, 1899 in winter flounder *(Pseudopleuronectes americanus)* rose correspondingly with increased water temperature (McVicar, 1975; Olson, 1976; Takvorian and Cali, 1984). Both McVicar (1975) and Olson (1976) were able to achieve laboratory induced infestations at water temperature over 15° C, while control fish maintained at 11° C did not develop infestations despite being fed *G. stephani*.

High temperatures in spring and summer are favourable for the growth of many protozoan parasites (Haley, 1954; Delisle, 1969; Olson, 1976, 1978; Takvorian and

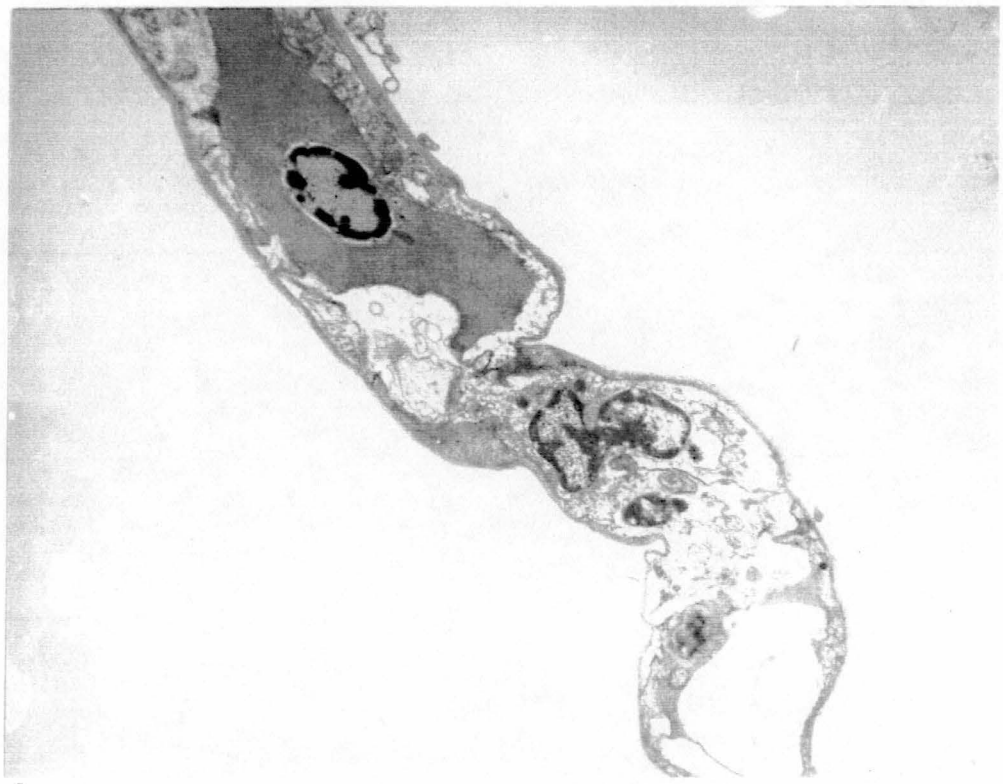



A

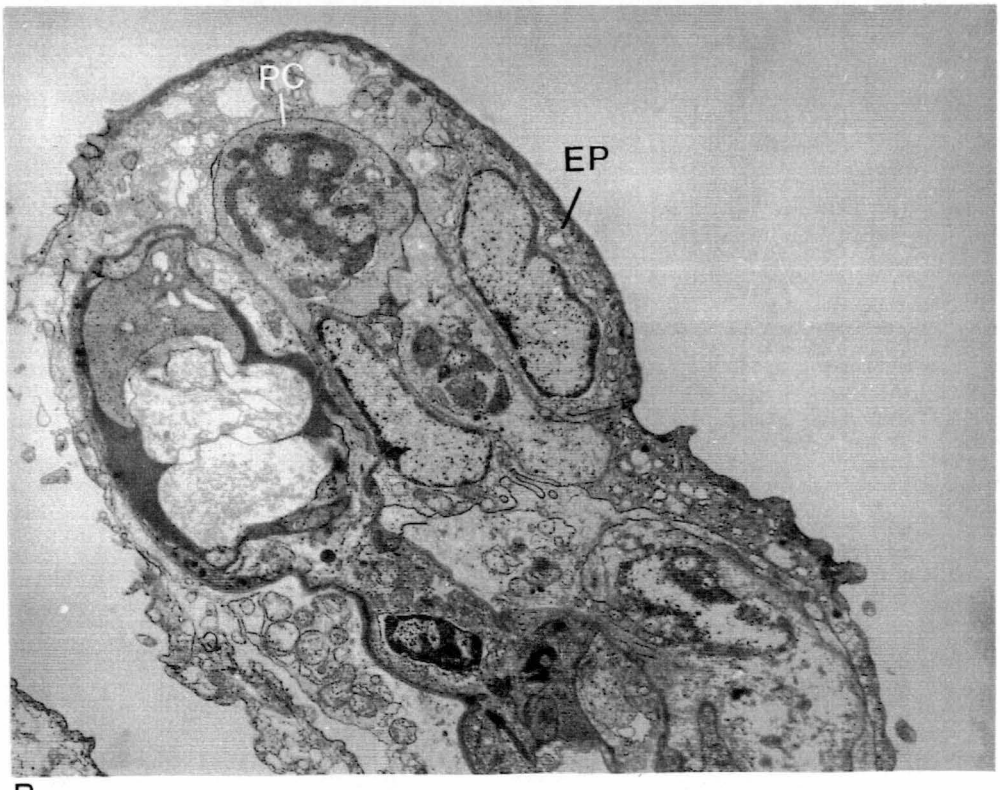



B

Plate 6.4. TEM micrographs of secondary lamellae of *Atherinosoma microstoma* infested with *Paratrichodina tasmaniensis*. A. Enlargement of part of a secondary lamella showing an irregular pillar cell (PC), x 15000. B. Part of a secondary lamella showing lifting epithelial cells (EP), x 6000.



A




B

Plate 6.5. TEM micrographs of secondary lamellae of *Atherinosoma microstoma* infested with *Paratrichodina tasmaniensis*. A. Part of a secondary lamella showing sloughing of epithelial cells, x 6000. B. Marginal end of a secondary lamella showing hyperplasia of pillar system and epithelial cells, x 6000. PC, pillar cell; EP, epithelial cell.

Cali, 1984). In the study of trichodinid infestations in marine and euryhaline fishes, Lom and Laird (1969) found that only five (16%) of 31 fish examined from the cold waters (temperature at the surface ranged from -1 to 5° C) were infested by trichodinids but that 9 of 16 (35%) fish were infested at times when surface temperatures averaged 15-20° C. The authors suggested that the low water temperature is a limiting factor for trichodinids. Willomitzer (1980) also reported that the highest prevalence and intensity of infestation of *Trichodina* sp. occur in the period from August to October (late summer/autumn) in grasscarp fry and fingerlings in Czechoslovakia.

A positive relationship between the water temperature and the prevalence of *P. tasmaniensis* infestation in both fish species was revealed in the present study. This indicates that the seasonal variations of *P. tasmaniensis* infestation are probably closely related to the water temperature. The present results support the suggestion of Lom and Laird (1969) given above.

6.4.2 Frequency distribution of *Paratrichodina tasmaniensis*

It appears that the seasonal variation of *Paratrichodina tasmaniensis* infestation may affect other aspects of parasite-host relationships. During the investigations on the frequency distribution, the log-normal distribution model was found not to fit the observed data of *P. tasmaniensis* within the population of *Atherinosoma microstoma* which were obtained throughout a 19 month period. However, when the data of January 1990, including some fish with very high intensity of infestation were removed from the analysis, the log-normal distribution model fitted well to the observed distribution. This may indicate that the environmental factors in January (summer) 1990 are so profitable for *P. tasmaniensis* that the extremely high intensity was achieved in few host fishes.

*Paratrichodina tasmaniensis* is the first fish-infesting ciliate described by the log-normal distribution model. Previously, the negative binomial distribution model was found to fit well the distribution of *Myxobolus funduli, Trichodina* sp. and *Ichthyophthirius multifiliis* (Knight *et al.*, 1977, 1980; Adams, 1980; McCallum, 1982). The distribution of the former two species could also be described adequately by the log-series distribution model (Adams, 1980). In the study of fish-infesting monogene*Schistocephalus solidus* Müller, 1776, the log-normal was found to be a better fit for the distribution by Pennycuick (1971a). The author indicated that the very high percentage infestation combined with relatively low mean and overdispersion mean that the log-normal is a better fit.

6.4.3 Infestation of *P. tasmaniensis* and the size and sex of host fish

*Paratrichodina tasmaniensis* exhibits a different pattern of infestation in *Atherinosoma microstoma* and *Leptatherina presbyterodies* with different lengths. In *A. microstoma,* the high level of prevalence of infestation was found in two size groups; the small fish with the length from 55 to 72 mm and the largest fish with the length between 85 and 93 mm. The variation in intensity of infestation was different from that of the prevalence of infestation; generally the level increased with the length of fish. In *L. presbyteroides,* only the small fish with the length from 58 to 72 mm showed a high level of prevalence and the intensity of infestation remained steadly in different size groups.

As stated in Chapter 5, there are a few factors which have been considered to be involved in the relationship of parasites and host fish. They are the amount and type of food, the development of age-acquired immunity to certain species, the accumulation of parasite with time and the effects of host migrations (Dogiel, 1958; Arthur *et al.*, 1982).

For *P. tasmaniensis*, the infestation of this ectoparasite is quite possible being completed by a direct contact. The relationships between *P. tasmaniensis* infestation and the size of *A. microstoma* seem to be highly related to the seasonal variations of infestation. The previous study by Kitchener (1985) on the life history of atherinid fish at the same site of this study revealed that the peak intensity of spawning for *A. microstoma* occurred from November to December. Accordingly, the fish caught in summer are usually small young fish and the largest old fish; while those with size 76 to 84 mm are mostly overwinter fish. Bearing this in mind, it is quite possible that the high level of prevalence of infestation in fish of 55 to 72 mm and both high prevalence and intensity in those of 85-93 mm reflect actually the summer infestation. The low level of prevalence of infestation in fish with size of 76 to 84 mm may represent a winter infestation.

There were two possibilities which may cause the variation of prevalence of *P. tasmaniensis* infestation in *L. presbyteroides* with different size. Similar as in *A. microstoma,* the peak intensity of spawning for *L. presbyteroides* was from October to November (Kitchener, 1985). Therefore, firstly, the relatively high prevalence of infestation in fish with size from 58 to 72 mm may actually be a summer peak of infestation. Secondly, the low level of age-acquired immunity in the small fish may also contribute to the high level of prevalence of infestation. The preference of trichodinids for the small host fish was previously reported by Aleshkina (1982) in the investigation of *Trichodina* sp. on the gills of *Merluccius capensis*. A lower prevalence in older fish has also been reported in a few myxosporean infestations (George *et al.*, 1977; Copland,

1981; Gonzalez-Lanza and Alvarez-Pellitero, 1984; Alvarez-Pellitero and Sitja-Bobadilia, 1993).

In both fish species, the smallest fish, less than 55 mm in *A. microstoma* and less than 58 mm in *L. presbyteroides* showed a lower level of infestation. The previous study demonstrated that these fish are younger than one year old (Kitchener, 1985). In view of the data of *P. tasmaniensis* infestation in other fish species (Table 6.5), a low prevalence of infestation was obtained in *Kestratherina brevirostris* compared with other fish species. It is interesting to note that this fish species has only a life cycle of one year and most fish examined in the present study were less than one year old. The low prevalence of infestation (2.59%) could suggest that the fish have lived in water for too short a time to be infested. This explanation may also apply to the infestation in *A. microstoma* and *L. presbyteroides*.

It is generally accepted that female vertebrates are less heavily infested with parasites than males (Thomas, 1964b). Kearn (1967) reported that some ectoparasites may locate their hosts by means of chemoreception. There was also some experimental evidence that corticosteroids facilitate skin infestations in salmonid fish (Robertson *et al.*, 1963; Roth, 1972). If this is correct, female fish would be more prone to the infestation as they may have a higher level of circulating corticosteroids (Pickering and Christie, 1980).

No significant differences in the prevalence of *P. tasmaniensis* infestation were found between sexes of both *A. microstoma* and *L. presbyteroides* in the present study. Available information indicated that protozoan parasites generally have no sexual difference in the infestation (Adams, 1980; Alvarez-Pellitero and Sitja-Bobadilia, 1993). In the study of *Trichodina* sp. from the gills of *Gasterosteus aculeatus*, Chappell (1969b) found no sexual difference in the infestation.

However, there are also examples showing different situations. High infestations of some ectoparasitic protozoans have been reported in female fish than in males (Adams, 1980; Pickering and Christie, 1980; Janovy and Hardin, 1987). *Syphidia* sp., from the skin of *Salmo trutta* occurs more frequently in male fishes than in females during the spawning seasons (Pickering and Christie, 1980).

6.4.4 Spatial distribution of the parasite on the gills of fish

Only little work has been done on the distribution of ectoparasitic protozoans on the gills of fish. The study of *Myxobolus funduli* on the gills of *Fundulus kansae* by Knight *et al.*

(1977, 1980) demonstrated that both the prevalence and intensity of infestation is independent of gill arches and gills. The authors indicated that the possibility of the fish being infested on the left side was virtually equal to that of the fish being infested on the right side. Adams (1980) also found no significant differences in infestation by six protozoan parasites between the right and left gills. Some metazoans also show the same distribution (Dickinson and Threlfall, 1975; Ramasamy *et al.*, 1985).

In contrast, Wootten (1974) found a significant bias for the infestation of the monogenean, *Dactylogyrus amphibothrium* Wagener, 1857 on the right gill of *Gymnocephalus cernua*. Wiles (1968) reported a similar result involving the monogenean *Diplozoon paradoxum* Nordmann, 1832 infesting the gills of *Abramis brama*. The present study shows that *P. tasmaniensis* has a higher affinity for the left gills in both *A. microstoma* and *L. presbyteroides*. No satisfactory explanation is available for this result. It is possible that there are some morphological or physiological differences between the two sides of the fish, or some type of behaviour of fish may be advantageous for parasites reach to the left gills.

6.4.5 Interspecific association between parasite species

The present results show that a positive association exists between *Paratrichodina tasmaniensis* and *Trichodina australis*, a negative association exists between *T. australis* and *Zschokkella leptatherinae*, *Trichodina* sp. and *Z. leptatherinae* in *A. microstoma*. In *L. presbyteroides*, a negative association was found between *P. tasmaniensis* and *Z. leptatherinae*, *Clausophrya branchialis* and *Z. leptatherinae*.

Interspecific association may exist for a number of reasons. Both species may select or avoid the same habitat or habitat factors; they have the same general abiotic and biotic environmental requirements; or one or both of the species has an affinity for the other, either attraction or repulsion (Alvarez-Pellitero *et al.*, 1983). In the present study, a positive association exists between two ectoparasites in *A. microstoma*. A similar situation was reported by Alvarez-Pellitero *et al.* (1983) in the coexistence of *Myxidium carinae* Alvarez-Pellitero *et al.*, 1983 and *Chloromyxum complicatum* Alvarez-Pellitero *et al.*, 1983 in *Barbus barbus bocagei*. Thomas (1964a) and Cloutman (1975) both recognised two possibilities for this relationship; either the presence of one parasite is advantageous to the other or the parasites have mutual requirements from the host or from ecological factors outside of the host. *P. tasmaniensis* and *T. australis* have a close relationship in their systematic status. They both inhabit the gills of fish, and they may have the similar food resources and oxygen demands. These factors determine the

positive association between them. In other pairs of ectoparasites, such as *P. tasmaniensis* vs *Trichodina* sp. and *C. branchialis, T. australis* vs *Trichodina* sp. and *C. branchialis, Trichodina* sp. vs *C. branchialis,* although their requirements for the habitat, food and oxygen may be similar, one parasite probably has no affinity for the other.

Moser (1976) observed the apparent positive or negative associations between different myxosporeans from macrourid fish. The author considered that these associations were probably due to the seasonal, geographic, depth, and habitat preferences of parasites. Considering the negative association between the ectoparasites and endoparasite in the present study, it is quite possible that the seasonal preference of parasites may contribute to the interspecific association. Although the patterns of seasonal variation of all ectoparasites are unknown, at least the occurrence of *P. tasmaniensis* in different seasons was known to be different from that of *Z. leptatherinae* in the same fish species.

### 6.4.6 Pathology of *Atherinosoma microstoma*

The present study demonstrates the pathogenic nature of *Paratrichodina tasmaniensis.* The pathological changes induced by *P. tasmaniensis* infestation result from two major processes. These are lifting and sloughing of epithelial cells of secondary lamellae and the hyperplasia of interlamellar epithelial cells, and the hypertrophy of epithelial cells of secondary lamellae.

The sloughing and hyperplasia of epithelial cells was observed previously by Paperna and Van As (1983) in the gills of cichlid fish infested by *Chilodonella hexasticha.* However, in that case, sloughing was caused by the degeneration and necrosis of the epithelium. In the infestation of *P. tasmaniensis,* it appears likely that the sloughing of epithelial cells of secondary lamellae in *A. microstoma* results from the lifting of the cells. This is similar to the process seen in fish affected by the heavy metals (Skidmore and Tovell, 1972).

Hyperplasia is a generalised response of the epithelial tissue to chemical irritation and physical trauma (Roberts and Bullock, 1976), and has also been reported in many ectoparasite infestations (Hines and Spira, 1974; Roger and Gains, 1975; Paperna and Van As, 1983). The hypertrophy of epithelial cells has also been observed as a response to the monogenean and copepod (Roubal, 1983).

The lifting and hypertrophy of the epithelial cells of secondary lamellae increase the diffusion distance from water to the non-marginal blood spaces. Under this situation, the

only practical route for the gas exchange is across the epithelium covering the distal edge of the marginal channel (Skidmore and Tovell, 1972). However, in fish which have a hyperplasic marginal pillar system and epithelial cells, it is almost certain that the gas exchange has been disturbed as a result of increased diffusion distance also existed in that area.

Skidmore and Tovell (1972) showed that the blood flow through the pillar cell system almost ceases when the lamellar epithelium becomes completely sloughed off. However, in the present study, normal-looking red blood cells are distributed in the lamellar blood spaces where the epithelium has sloughed off completely; this suggests that blood circulation is not completely disturbed.

Infested *A. microstoma* also show more or less fusion of the secondary lamellae because of hyperplasia of the interlamellar and secondary lamellar epithelial cells. This is a frequent response to non-specific injury of the fish gill (Eeller, 1975). Because of the fusion, the exposed surface of the secondary lamellae is reduced and this may affect the gas exchange.

Previous studies have revealed that parasites affect their hosts in two ways. Mechanical impact causes only focal damage and is limited to the parasitised cells; examples from ultrastructure studies including *Ichthyobodo necator* (Schubert, 1966) and *Amylooginium ocellatum* infestations (Lom and Lawer, 1973). Chemical impact, including changes induced by toxins, irritants or digestive enzymes secreted by the parasite, causes an extensive damage of fish gills (Paperna, 1980; Robertson *et al.*, 1981). The pathological changes of the gill of *A. microstoma* with the infestation of *P. tasmaniensis* is most likely induced by a chemical process since these changes are not limited to the parasitised cells.

# CHAPTER 7 - CONCLUSIONS AND RECOMMENDATIONS

An important contribution of this study is the demonstration that protozoans are common parasites in Tasmanian marine fish. A total of 16 parasitic species were obtained from 12 fish species in the present study. Of these, 12 are new to science; two of the other species have been previously reported from other parts of the world. This result, combined with the previous studies, brings the total number of fish-infecting protozoa known in Australia to 54 genera (in addition to the collective group, *Microsporidium*) and 77 species. It is expected that many more records and new forms of protozoan parasites will be found in the future when a greater number of different types of water body and variety of fish are investigated.

Examinations on the sporogenesis of *Zschokkella leptatherinae* sp. nov. revealed that the early pansporoblast of this myxosporean is formed by the union of two generative cells. This result supports the studies of other members of Myxosporea by several workers (Lom and de Puytorac, 1965b; Desser and Paterson, 1978a; Current, 1979; Current, *et al.*, 1979; Pulsford and Matthews, 1982; Desser, Molnar and Weller, 1983; Lom, 1987). However, it differs from the report of Hulbert *et al.*, 1977 on *Myxidium zealandicum* in which the pansporoblast was found to be formed by mitotic division of an uninucleate generative cell. The multicellular nature of both the plasmodium and spore of *Z. leptatherinae* has also been demonstrated in this study. This is a unique characteristic of the Myxosporea, which separates these organisms from the remaining groups of Protozoa except the Actinosporea.

To date, because the life cycle of Myxosporea are not fully understood, the identification of myxosporean species is mainly based on spore features, the host and/or tissue they infect, and their geographical distribution. Therefore, a lack of clarity and precision is sometimes characteristic of the process of identification of myxosporean species. More accurate methods, such as the cross-infection experiments and the molecular techniques, e.g. DNA sequencing, following from polymerase chain reaction amplication, are recommended for the future study. These techniques should provide a precise knowledge of taxonomy of Myxosporea.

There was some evidence from the previous study on macronuclear DNA of free-living ciliates suggesting that the regulation of transcription and the usage of the genetic code

are different in these ciliates from those in other organisms (Steinbrudk, 1990). Using the advantages of DNA technique may also help us in the understanding of the origin of Myxosporea.

*Paratrichodina tasmaniensis* and *Trichodina nesogobii* are placed in *Trichodinella*-group and genus *Trichodina* respectively. The present study demonstrates that there are no fundamental differences in the surface topography between these two species except the adoral spiral and the adhesive discs. This study, combined with the previous observations on other trichodinids, suggests that the length of the cilia comprising the basal ciliary ring, locomotory ciliary and marginal ciliary ring, as well as the development of the septum which separates each set of cilia may vary between the species. An obvious ellipsoidal foramen was revealed in the denticle of *P. tasmaniensis* using the scanning electron microscope. This indicates that, for some species, scanning electron microscope examinations are important since they can provide more information.

This study is the first to find that the log-normal distribution model can describe the frequency distributions of fish-infecting protozoans. Previously, this model was only used in malarial parasites in human blood, some other human diseases and fish-infecting cestode, *Schistocephalus solidus*. Since the reproduction of protozoan parasites usually occurs within the host and is rapid and the variation in the number of parasite is often high, the log-normal model may fit adequately the distribution of many protozoan parasite populations.

Ecological studies on *Zschokkella leptatherinae* and *Paratrichodina tasmaniensis* indicate that the seasonal variations of parasite infection are different between these two species. The infection of the liver parasite, *Z. leptatherinae* did not vary significantly with season, while the infestation of gill parasite, *P. tasmaniensis* displayed a pronounced seasonal variation. These results agree with those of Bond (1939) who obtained a clearly seasonal variation of *Myxobolus subtecalis* in the fins of fish, while the infection of the same species showed an even seasonal infection in the kidney and brain. Mitchell (1977) also indicated that the incidence (prevalence) of infestations of the body surface and gills and the occurrence of serious epizootics are often seasonal. The present study also reveals a significant positive correlation between the prevalence of *P. tasmaniensis*

infestation and the water temperature. This suggests that the water temperature is the main factor affecting the seasonal variation *P. tasmaniensis* infestation.

The infestation of *P. tasmaniensis* has a significant affinity for the left gill arches in both *Atherinosoma microstoma* and *Leptatherina presbyteroides*. This result is different from the previous reports on the ectoparasitic protozoans in which no differences were found between the left and right gills (Knight *et al.*, 1977; 1980; Adams, 1980). The preference for the right gill arches was reported in the infestation of two monogenean species by Wiles (1968) and Wootten (1974). No satisfactory explanation is available from the present study. Further comparing study on the morphology and physiology of left and right gills may provide evidence for this distribution.

Two species of protozoan parasites have been demonstrated to be pathogenic agents of the atherinid fishes. The histopathological and cytopathological studies show that the liver structure of infected *Leptatherina presbyteroides* and the gill structure of *Atherinosoma microstoma* infested by *Paratrichodina tasmaniensis* were damaged by the parasites. No death of fish induced by these two species of parasites were observed directly during the study period; however, from the pathological damage they caused, it is almost certain that the function of the liver and gills has been disturbed. The result of ecological study also supports this hypothesis. The prevalence of *Z. leptatherinae* infection in both *A. microstoma* and *L. presbyteroides* was high in larger fish compared with that in small ones. This may be, at least partly, due to death of small fish with heavy infection. It should be possible to investigate the disturbance of the liver function of *Leptatherina presbyteroides* infected by *Z. leptatherinae* and the respiratory function of *A. microstoma* infested by *P. tasmaniensis* in the future.

Previous reports suggested that some of protozoan diseases could present a risk to the development of mariculture because high stocking densities are maintained in cages in coastal waters. The best example in Tasmania is the paramoebic gill disease which is a severe disease in salmonid mariculture. When infestation occurs, all or most of the fish in a cage become infected over a period of several weeks. Infested fish suffer from anorexia and respiratory distress; the mortality can be up to 50% (Munday *et al.*, 1990).

Currently in Tasmania, commercial mariculture involves only introduced salmonids. However, research is now being conducted aimed at introducing native marine species,

e.g. the striped trumpeter, *Latris lineata*, into intensive culture. This gives further impetuous to gain an understanding of all potential pathogens, including parasitic protozoans, which may affect these fish and potentially reduce their production.

## BIBLIOGRAPHY


Adams, M. A. 1980. Parasites on the gills of the plains killifish, *Fundulus kansae*, in the South Platte River, Nebraska. *Transactions of the American Microscopical Society* 104, 278-284.

Adams, A. M. 1986. The parasite community on the gills of *Fundulus kansae* (Garman) from the South Platte River, Nebraska (USA). *Acta Parasitologica Polonica* 31, 47-54.

Ahmed, A. T. A. 1974. Kidney enlargement disease of gold fish in Japan. *Japanese Journal of Zoology* 17, 37-65.

Aleshkina, L. D. 1982. Parasite fauna of Cape Hake and dependence of its species composition on age of host. *Gidrobiologicheskii Zhurnal* 18, 65-568.

Alvarez-Pellitero, M. P., Gonzalez-Lanza, M. C. and Pereira-Bueno, J. M. 1983. Celozoic myxosporidians (*Myxidium* spp. and *Chloromyxum* spp.) of cyprinids from the River Esla (Leon, NW Spain). II. Population dynamics. *Angewandte Parasitologie* 24, 65-71.

Alvarez-Pellitero, P. and Sitja-Bobadilla, A. 1993. *Cetatomyxa* spp. (Protozoa: Myxosporea) infections in wild and cultured sea bass, *Dicentrarchus labrax*, from the Spanish Mediterranean area. *Journal of Fish Biology* 42, 889-901.

Anderson. R. M. 1974. Population dynamics of the cestode, *Caryophyllaeus laticeps* (Pallas, 1781) in the bream (Abramis brama L.). *Journal of Animal Ecology* 43, 305-321.

Arthur, J. R. and Lom, J. 1985. *Sphaerospora araii* n. sp. (Myxosporea: Sphaerosporidae) from the kidney of a longnose skate (*Raja rhin* Jordan and Gilbert) from the Pacific Ocean off Csanada. *Canadian Journal of Zoology* 63, 2902-2906.

Arthur, J. R. and Margolis, L. 1984. *Trichodina truttae* Mueller, 1937 (Ciliophora: Peritrichida), a common pathogenic ectoparasite of cultured juvenile salmonid fishes in British Columbia: redescription and examinatio by scanning electron microscopy. *Canadian Journal of Zoology* 62, 1842-1848.

Arthur, J. R. Margolis, L., Whitaker, D. J. and McDonald, T. E. 1982. A quantitative study of economically important parasites of walleye pollock *(Theragra chalcogramma)* from British Columbian waters and effects of postmortem handling on their abundance in the musculature. *Canadian Journal of Fisheries and Aquatic Science* 39, 710-726.

Ashburner, L. D. 1975. Coccidiosis in an Australian fish, the macquarie perch, *Macquaria australasica* Cuvier, 1830. *Bulletin of the Australian Society of Limnology* 6, 33-34.

Ashburner, L. D. and Ehl. A. S. 1973. *Chilodonella cyprini* (Moroff), a parasite of freshwater and its treament. *Bulletin of the Australian Society of Limnology* 5, 3-4.

Auerbach, M. 1910a. Die Cnidosporidien (Myxosporidien, Actynomyxidien, Microsporidien). *Eine Monographische Studie, Leipzig* 8, 261.

Auerbach, M. 1910b.Cnidosporidien studien. *Zoologischer Anzeiger* 35, 767-777.

Auerbach, M. 1910c. Biologische und morphologische Bemerkungen uber Myxosporidien. *Zoologischer Anzeiger* 35, 57-63.

Avtalion, R. R. 1981. Environmental control of the immune response in fish. *Critical Reviews in Environmental Control* 11, 163-188.

Azevedo, C., Lom, J. and Corral, L. 1989. Ultrastructural aspects of *Myxidium giardi* (Myxozoa, Myxosporea), parasite of the European eel *Anguilla anguilla*. *Diseases of Aquatic Organisms* 6, 55-61.

Balbiani, G. 1882. Sur les microsporidies ou psorospermies des articules. *Compte rendu de l'Academie des sciences* 129, 836-839.

Baska, F. 1990. *Chloromyxum inexpectatum* n. sp. and *Sphaerospora colomani* n. sp. (Myxozoa: Myxosporea) parasites of the urinary system of the sterlet, *Acipenser ruthenus* L. *Systematic Parasitology* 16, 185-193.

Basson, L. and Van As, J. 1987. Trichodinid (Ciliophora: Peritricha) gill parasites of freshwater fish in South Africa. *Systematic Parasitology* 9, 143-151.

Basson, L. and Van As, J. 1989. Differential diagnosis of the genera in the family Trichodinidae (Ciliophora: Peritrichida) with the description of a new genus ectoparasitic on freshwater fish from southern Africa. *Systematic Parasitology* 13, 153-160.

Basson, L. and Van As, J. 1991. Trichodinids (Ciliophora: Peritrichida) from a calanoid copepod and catfish from South Africa with nots on host specificity. *Systematic Parasitology* 18, 147-158.

Basson, L., Van As, J. G. and Paperna, I. 1983. Trichodinid ectoparasites of cichlid and cyprinid fishes in South Africa and Israel. *Systematic Parasitology* 5, 245-257.

Bauer, O. N., Musselius, V. A. and Strelkov, Y. A. 1973. *Diseases of Pond Fishes*. 2 nd ed. Legkaya i Pishchevaya Promyshlenost, Moscow.

Becnel, J. J. 1992. Horizontal transmission and subsequent development of *Amblyospora californica* (Microsporida: Amblyosporidae) in the intermediate and definitive hosts. *Diseases of Aquatic Organisms* 13, 17-28.

Bekhti, M. and Bouix, G. 1985. *Loma salmonae* (Putz, Hoffman and Dunbar, 1965) et *Loma diplodae* n. sp., microsporidies parasites des branchies des poissons telesteens; implanatation et donnees ultrastructurales. *Protistologica* 21, 47-59.

Berrebi, P. 1978. Contribution a l'etude biologique des zones saumatres du littoral mediterraneen francais. Biologie d'une Microsporidie: *Glugea atherinae* n. sp. parasite de l'Atherine: *Atherina boyeri* Risso, 1810 (Poisson-Teleosteen) des etangs cotiers. Thesis of University of Science and Technology of Languedoc, Monpellier.

Berrebi, P. 1979. Etude ultrastructurale de *Glugea atherinae* n. sp., microsporidie parasite de l'atherine *Atherina boyei* Risso, 1810 (Poisson Teleosteen) dans les Lagunes du Languedoc et de provence. *Zeitschift für Parasitenkunde* 60, 105-122.

Berrebi, P. and Bouix., G. 1978. Premieres observations sur une microsporidiose de l'Atherine des etangs languedociens, *Atherina boyeri* Risso. 1810 (Poissons Teleosteens). *Annales de Parasitologie Humaine et Comparee* 53. 1-20.

Beumer, J. P., Ashburner, L. D., Burbury, M. E., Jette, E. and Latham, D. J. 1982. *A Checklist of the Parasites of Fishes from Australia and Adjacent Terriitories*. Commonwealth Agricultural Bureaux. Eyre and Spottiswoode Ltd, Thanet Press, Margate.

Bond, F. F. 1938. Cnidosporidia from *Fundulus heteroclitus* Lin. *Transactions of the*

*American Microscopical Society* 57, 107-122.

Bond, F. F. 1939. The seasonal incidence of myxosporidian parasites infection *Fundulus heteroclitus* Lin. *Transactions of the American Microscopical Society* 58, 156-163.

Bower, S. M. and Margolis, L. 1984. Distribution of *Cryptobia salmositica,* a haemoglagellate of fishes, in British Columbia and the seasonal pattern of infection in a coastal river. *Canadian Journal of Zoology* 62, 2512-2518.

Broek, W. L. F. Van Den. 1979. Infection of estuarine fish populations by *Cryptocotyle lingua* (Creplin). *Journal of Fish Biology* 14, 395-402.

Brummer-Korvenkontio, H., Valtonen, E. T. and Pugachev, O. N. 1991. Myxosporea parasites in roach, *Rutilus rutilus* (Linnaeus), from four lakes in central Finland. *Journal of Fish Biology* 38, 573-586.

Bucher, F., Hofer, R. and El-Matbouli, M. 1992. Prevalence and pathology of *Zschokkella nova* (Myxosporea) in the liver of bullhead *Cottus gobio* from a polluted river. *Diseases of Aquatic Organisms* 14, 137-143.

Burn, P. R. 1980. The parasites of smooth flounder, *Liopsetta putnami* (Gill), from the Great Bay Estuary, New Hampshire. *Journal of Parasitology* 66, 532-541.

Burreson, E. M. 1989. Haematozoa of fishes from Heron I., Australia, with the description of two new species of *Trypanosoma. Australian Journal of Zoology* 37, 15-23.

Burreson, E. M. and Zwerner, D. 1982. The role of host biology, vector biology, and temperature in the distribution of *Trypanplasma bullocki* infections in the lower Chesapeake Bay. *Journal of Parasitology* 68, 306-313.

Butcher, A. D. 1941. Outbreaks of white-spot or ichthyophthirius (*Ichthyophthirius multifiliis* Fouquet, 1876) at the hatcheries of the Ballarat Fish Acclimatization Society with notes on laboratory experiments. *Proceedings of the Royal Society of Victoria* 53, 126-144.

Butcher, A. D. 1943. Observations of some phase of the life cycle of *Ichthyophthirius multifiliis* Fouquet, 1876, a ciliate protozoan parasite of freshwater fish. *Australian Journal of Zoology* 10, 125-137.

Butcher, A. D. 1947. Ichthyophthiriasis in an Australian trout hatchery. *Proceedings of Fish Culture* 9, 21-26.

Butschli, O. 1881a. Myxosporidien. *Zoologische Jahrbucher (Systematik.)* 1880, 1, 162-164.

Butschli, O. 1881b. Beitrage zur Kenntnis der Fischpsorospermien. *Biologisches Zentralblatt* 1, 294-297.

Butschli, O. 1881c. Beitrage zur Kenntnis der Fischpsorospermien. *Zeitschrift für Wissenschaftliche Zoologie* 35, 629-651.

Cali, A. 1971. Morphogenesis in the genus *Nosema.* In *Proceedings of the IVth International Colloquium of Insect Pathology.* pp. 431-438. College Park, Maryland.

Callinan, R. B. 1988. Deseases of Australian native fishes. In *Fish Diseases.* Proceedings of the 106th Refresher Course for Veterinarians, Sydney NSW, May 1988. Ed. by D. I. Bryden, pp. 459-472. University of Sydney. Sydney.

Canning, E. U. 1977. Microsporidia. In *Parasitic Protozoa.* Ed. by J. P. Kreier, vol. IV, pp. 137-161. Plenum Press, New York and London. .

Canning, E. U. 1990. Phylum Microspora. In *The Handbook of Protoctists*. Ed. by L. Margulis, D. Chapman, M. Melkonian and J. O. Corliss, pp. 53-72. Jones and Bartlett, Boston.

Canning, E. U. and Hazard, E. I. 1982. Genus *Pleistophora* Gurley, 1893. An assemblage of at least three genera. *Journal of Protozoology* 29, 39-49.

Canning, E. U., Hazard, E. I. and Nicholas, J. P. 1979. Light and electron microscopy of *Pleistophora* sp. from skeletal muscle of *Blennius pholis*. *Protistologica* 15, 317-332.

Canning, E. U., Lom, J. and Nicholas, J. P. 1982. Genus *Glugea* (Phylum Microspora): redescription of the type species, *Glugea anomala*, and recognition of its sporogonic development within sporophorous vesicles (pansporoblastic membranes). *Protistologica* 18, 193-210.

Canning, E. U. and Nicholas, J. P. 1980. Genus *Pleistophora* (Phylum Microspora): redescription of the type species *Pleistophora typicalis* Gurley, 1893 and ultrastructural characterization of the genus. *Journal of Fish Diseases* 3, 317-338

Chakravarty, M. 1939. Studies on Myxosporidia from the fishes of Bengal, with a note on the myxosporidian infection in aquaria fishes. *Archiv für Protistenkunde* 92, 169-178.

Chakravarty, M. 1943. Studies on Myxosporidia from the common food fishes of Bengal. *Proceedings of the Indian Academy of Sciences* 18, 21-35.

Chappell, L. H. 1969a. The parasites of the three-spined stickleback *Gasterosteus aculeatus* L. from a Yorkshire Pond. I. Seasonal variation of parasite fauna. *Journal of Fish Biology* 1, 137-152.

Chappell, L. H. 1969b. The parasites of the three-spined stickleback *Gasterosteus aculeatus* L. from a Yorkshire Pond. II. Variation of the parasite fauna with sex and size of fish. *Journal of Fish Biology* 1, 339-347.

Chatton, E. and Courrier, R. 1923. Formation d'un complexe xeno-parasitaire geant avec bordure en brosse, sous l'influence d'une microsporidie dans le testicule de *Cottus bubalis*. *Comptes rendus des seances de la Societe de biologie* 89, 579-583.

Chatton, E. and Lwoff, A. 1923. Un cas remarquable d'adaptation: *Ellobiophrya donacis*, n. g., n. sp.. peritriche inquilin des branchies de *Donax vitatus*. *Comptes rendus des seances de la Societe de biologie* 88, 749-752.

Chatton, E. and Lwoff, A. 1929. Contribution a l'etude de l'adaptation *Ellobiophrya donacis* Ch. et Lw., peritriche vivant sur les branchies de l'acephale Donax vittatus da Costa. *Bulletin biologique de la France et de la Belgique* 63, 321-349.

Cheissin, E. M., Shulman, S. S. and Vinnitchenko, L. P. 1961. Stroenie spor *Myxobolus*. *Citologija* 3, 662-667.

Chen, M. and Power, G. 1972. Infection of American smelt in Lake Ontario and Lake Erie with the microsporidian parasite *Glugea hertwigi* (Weissenberg). *Canadian Journla of Zoology* 50, 1183-1188.

Chilmonczyk, S., Cox, W. T. and Hedrick, R. P. 1991. *Enterocytozoon salmonis* n. sp.: an intranuclear microsporidium from salmonid fish. *Journal of Protozoology* 38, 264-269.

Ching, H. L. and Munday, D. R. 1984. Geographic and seasonal distribution of the

infectious stage of *Ceratomyxa shasta* Noble, 1950, a myxozoan salmonid pathogen in the Fraser River system. *Canadian Journal of Zoology* 62, 1075-1080.

Choudhury, A. and Nandi, N. C. 1973. Studies on myxosporidian parasites (Protozoa) from an estuarine gobiid fish of west Bengal. *Proceedings of the Zoological Society of Bengal* 26, 45-55.

Clamp, J. C. 1982. *Ellobiophrya conviva* n. sp., a commensal of marine ectoprocts, and a revision of the family Ellobiophryidae (Ciliophora, Peritricha). *Journal of Protozoology* 29, 149-156.

Clifton-Hadley, R. S., Burke, D. and Richards, R. H. 1984. Proliferative kidney diseases of salmonid fish: a review. *Journal of Fish Diseases* 10, 101-107.

Cloutman, D. G. 1975. Parasite community structure of largemouth bass, warmouth, and bluegill in Lake Fort Smith, Arkansas. *Transactions of the American Fisheries Society* 104, 277-283.

Cone, D. K. 1979. *Henneguya doori* Guilford 1963 (Myxosporidia) of *Perca flavescens* in New Brunswick. *Canadian Journal of Zoology* 57, 1758-1788.

Cone, D. K. and Anderson, R. C. 1977. Parasites of pumpkineseed (*Lepomis gibbosus* L.) from Ryan Lake, Algonquin Park, Ontario. *Canadian Journal of Zoology* 55, 1410-1423.

Copland, J. W. 1981. The occurrence and distribution of *Myxidium giardi* Cepede, 1906 in wild and cultured European eels, *Anguilla anguilla* L. in England. *Journal of Fish Diseases* 4, 231-242.

Copland, J. W. 1983. The pathology of *Myxidium giardi* Cepede, 1906 infections in wild and cultured eels, *Anguilla anguilla* L. *Journal of Fish Diseases* 6, 451-460.

Corliss, J. D. 1979. *The Ciliated Protozoa: characterization, classification and guide to the literature.* 2nd ed. Pergammon Press, New York.

Corliss, J. D. and Levine, N. D. 1963. Establishment of the Microsporidia as a new class in the protozoan subphylum Cnidospora. *Journal of Protozoology* (Suppl.) 26-27.

Cottrell, B. J. 1977. A trypanosome from the plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* 11, 35-47.

Crofton, H. D. 1971. A quantitative approach to parasitism. *Parasitology* 62, 179-193.

Csaba, G. 1976. An unidentifiable extracellular sporozoan parasite from the blood of the carp. *Parasitologia Hungarica* 9, 21-24.

Csaba, G., Jovacs-gayer, E., Bekesi, L., Bucsek, M., Szakolczia, J. and Molnar, K. 1984. Studies into the possible protozoan aetiology of swimbladder inflammation in carp fry. *Journal of Fish Diseases* 7, 39-56.

Current, W. L. 1979. *Henneguya adiposa* Minchew (Myxosporida) in the channel catfish: ultrastructure of the plasmodium wall and sporogenesis. *Journal of Protozoology* 26, 209-217.

Current, W. L. and Janovy, J. Jr. 1976. Ultrastructure of interlamellar *Henneguya exilis* in the channel catfish. *Journal of Parasitology* 62, 975-981.

Current, W. L. and Janovy, J. Jr. 1977. Sporogenesiss in *Henneguya exilis* infecting the channel catdish: an ultrastructural study. *Protistologica* 13, 157-168.

Current, W. L. and Janovy, J. Jr. 1978. Comparative study of ultrastructure of

interlamellar and intralamellar types of *Henneguya exilis* Kudo from channel catfish. *Journal of Protozoology* 25, 56-65.

Current, W. L., Janovy, J. Jr. and Knight, S. A. 1979. *Myxosoma funduli* Kudo (Myxosporida) in *Fundulus kansae*: Ultrastructure of the plasmodium wall and of sporogenesis. *Journal of Protozoology* 26, 574-583.

Davies, A. J. 1985. *Zschokkella russelli* Tripathi (Myxozoa: Myxosporea) from the five-bearded rockling, *Ciliata mustela* L., (Teleostei: Gadidae) in Wales. *Journal of Fish Diseases* 8, 299-308.

Davies, A. J. and Sienkowski, I. K. 1988. Further studies on *Zschokkella russelli* Tripathi (Myxozoa: Myxosporea) from *Ciliata mustela* L. (Teleostei: Gadidae), with emphasis on ultrastructural pathology and sporogenesis. *Journal of Fish Diseases* 11, 325-336.

Davis, H. S. 1924. A new myxosporidian parasite, the cause of "wormy" halibut. *Report of the United States Fish Commission.* 1923. Appendix 8, 15.

Delisle, C. 1969. Bimonthly progress of a non-lethal infection by *Glugea hertwigi* in young-of -the-year smelt, *Osmerus eperlanus mordax. Canadian Journal of Zoology* 47, 871-876.

Desportes-Livage, I. and Nicolas, G. 1990. The plasma membrane of myxosporidian valve cells: freeze fracture data. *Journal of Protozoology* 37, 243-249.

Desser, S. S., Molnar, K. and Horvath, I. 1983. An ultrastructural study of the myxosporeans *Sphaerospora angulata* and *Sphaerospora carassii*, in the common carp, *Cyprinus carpio* L. *Journal of Protozoology* 30, 415-422.

Desser, S. S., Molnar, K. and Weller, I. 1983. Ultrastructure of sporogenesis of *Thelohanellus nikolskii* Akhmerov, 1955 (Myxozoa: Myxosporea) from the common carp, *Cyprinus carpio. Journal of Parasitology* 69, 504-518.

Dessere, S. S. and Paterson, W. B. 1978a. Ultrastructural and cytochemical observations on sporogenesis of *Myxobolus* sp. (Myxosporida: Myxobolidae) from the common shiner *Notropis cornutus. Journal of Protozoology* 25, 314-326.

Desser, S. S. and Paterson, W. B. 1978b. Morphology of the spors of *Myxosoma notropis* Fanthem, Ponteri and Richardson, 1939. *Canadian Journal of Zoology* 56, 311-314.

Dickinson, A. B. and Threlfall, W. 1975. Metazoan parasites of *Fundulus heteroclitus* (Linnaeus, 1766) from insular Newfoundland. *Proceedings of the Helminthological Society of Washington* 42, 111-116.

Diamant, A. and Paperma, I. 1992. *Zschokkella icterica* sp. nov. (Myxosporea), a pathogen of wild rabbitfish *Signaus iuridus* (Ruppell, 1892) from the Red Sea. *European Journal of Protistology* 21, 71-78.

Doflein, F. 1899. Fortschritte auf dem Gebiete der Myxosporidienkunde, Zusammenfassende Uebersicht. *Zoologica Zentralblatt* 6, 361-379.

Dogiel, V. A. 1940. On the Classification of the genus *Trichodina*. *Trudy Leningradskoho Obshchestva Estestvoispytatelei* 68, 8-31.

Dogiel, V. A., Petrushevskii, G. K. and Polyanski, Y. I. 1958. *Parasitology of Fishes.* (In Russian). Leningrad University Press, Leningrad. (Transl. Z. Kabata, 1961. Oliver and Boyd, Edinburgh).

Dykova, I. and Lom, J. 1978. Histopathological changes in fish gills infected with

myxosporidian parasites of the genus *Henneguya*. *Journal of Fish Biology* 12, 197-202.

Dykova, I. and Lom, J. 1980. Tissue reactions to microspiridian infections in fish. *Journal of Fish Diseases* 3, 265-285.

Dykova, I. and Lom, J. 1982. *Sphaerospora renicola* n. sp., a myxosporean from carp kidney and its pathogenicity. *Zeitschrift für Parasitenkunde* 68, 259-268.

Dykova, I. and Lom, J. 1988. Review of pathogenic myxosporeans in intensive culture of carp (*Cyprinus carpio*) in Europe. *Folia Parasitologica (Praha)* 35, 289-307.

Dykova, I., Lom, J. and Grupcheva, G. 1987. Pathogenicity and some structural features of *Myxidium rhodi* (Myxozoa: Myxosporea) from the kidney of the roach *Rutilus rutilus*. *Diseases of Aquatic Organisms* 2, 109-115.

Ehrenberg, C. G. 1838. *Die Infusionstierchen als volkommene Organismes*. Leipzig.

Eiras, J. C. and Davies, A. J. 1991. *Haemogregarina bigemina* Laveran and Mesnil, 1901 (Protozoa, Apicomplexa) from *Blennius pholis* L. (Osteichthyes, Blenniidae): an investigation of seasonality in Portugal. *Journal of Fish Diseases* 14, 683-687.

Eller, L. L. 1975. Gill lesions of freshwater teleosts. In *The Pathology of Fishes*. Ed. by W. E. Ribelin and G. Migaki, pp.305-330. University of Wisconsin Press, Madison, Wisconsin.

El-Matbouli, M. and Hoffmann, R. 1989. Experimental transmission of two *Myxobolus* spp. developing bisporogeny bia tubificid worms. *Parasitology Research* 75, 461-464.

El-Tantawy S. 1989. Myxosporidian parasites of fishes in Lakes Dgal Wielki and Warniak (Mazurian Lakeland, Poland). II. Infection of fishes. *Acta Parasitologica Polonica* 34, 221-233.

Erickson, B. W., Bernick, S. H. and Sprague, V. 1968. Electron microscope study of the everted polar filament of *Glugea weissenbergi* (Microsporidia, Nosematidae). *Journal of Protozoology* 15, 758-761.

Evdokimova, E. b., Kuznetsova, I. G. and Stein, G. A. 1969. Parasitic ciliates of the family Urceolariidae (Peritricha, Mobilia) from some fish of the South-west Atlantic. *Zoologicheskii Zhurnal* 48, 1451-1455.

Fabre-Domergue, P. R. 1888. Etude sur l'organisation des Urceolaires et sur quelques genres d'infusoires voisins de cette famille. *Journal de l'Anatomie et de Physiologie Normales et Pathologiques de l'Homme et des Animaux* 23, 214-260.

Faisal, M. and Shalaby, S. I. 1987. *Myxosoma tilapiae* as a new species (Myxosoma: Myxosporea) in wild *Oreochromis niloticus* in lower Egypt. *Egyptain Journal of Veterniary Science* 24, 73-86.

Fantham, H. B. and Porter, A. 1943. On a myxosporidian *Sphaerospora periophthalmi*, sp. n. found in African and Indian mud-skippers (Pisces) and its possible significance. *Proceeding of the Zoological Society of London* 112, 113-119.

Faure-Fremiet, E. 1943. Etude biometrique de quelques trichodines. *Bulletin de la Societe Zoologique de France* 68, 158-169.

Faure-Fremiet, E., Rouilier, C. and Gauchery, M. 1956a. L'appareil squelettique et myoide des Urceolaires; etude au microscope electronique. *Bulletin de la Societe Zoologique de France* 81, 77-84.

Faure-Fremiet, E., Rouilier, C. and Gauchery, M. 1956b. Les structures myioides chez les cilies. Etude au microscope electronique. Archives d'anatomie microscopique et de morphologie experimentale. *Archives d'Anatomie Microscopique et de Morphologie Experimentale* 45, 139-161.

Favard, P., Carasso, N. and Faure-Fremiet, E. 1963. Ultrastructure de l'appareil adhesif des urceolaires cilies peritriches. *Journal de Microscopie* 2, 337-368.

Faye, N. and Toguebaye, B. S. 1990. Ultrastructure and development of *Pleistophora senegalensis* sp. nov. (Protozoa, Microspora) from the gilt-head sea bream, *Sparus aurata* L. (Teleost, Sparidae) from the coast of Senegal. *Journal of Fish Diseases* 13, 199-192.

Faye, N., Toguebaye, B. S. and Bouix, G. 1991. *Microfilum lutjani* n. gen. n. sp. (Protozoa, Microsporida), a gill parasite of the golden African snapper *Lutjanus fulgens* (Valenciennes, 1830) (Teleost Lutjanidae): developmenal cycle and ultrastructure. *Journal of Protozoology* 38, 30-40.

Feist, S. W., Chilmonczyk, S. and Pike, A. W. 1991. Structure and development of *Sphaerospora elegans* Thelohan, 1892 (Myxozoa: Myxosporea) in the sticklebacks *Gasterosteus aculeatus* L. and *Pungitius pungitius* L. (Gasterosteidae). *European Journal of Protistology* 27, 269-277.

Feng, S. J. 1985. A biological investigation of asexual reproduction of *Trichodina nobillis* Chen. *Acta Hydrobiologica Sinica* 9, 331-342.

Fisher, R. A. 1941. The negative binomal distribution. *Annals of Eugenics* 11, 182-187.

Fischer-Scherl, T., El-Matbouli, M. and Hoffmann, R. 1986. *Bulletin of European Association of Fish Pathology* 6, 16-19.

Foott, J. S. and Hedrick, R. P. 1987. Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. *Journal of Fish Biology* 30, 477-484.

Gajevskaja, A. V., Kovaljova, A. A. and Krasin, V. K. 1985. New species of myxosporidians (Protozoa, Myxosporidia) from urinary bladders of grenadiers in the Atlantic and Pacific. *Zoologicheskii Zhurnal* 64, 1576-1579.

Garden, O. 1992. The Myxosporea of fish: a review. *British Veterinary Journal* 148, 223-239.

Gassouma, M. S. S. and Ellis, D. S. 1973. The ultrastructure of sporogonic stages and spores of *Thelohania* and *Pleistophora* (Microsporida, Nosematae) from *Simulium ornatum* larvae. *Journal of General Microbiology* 74, 33-34.

Gonzalez-Lanza, M. C. and Alvarez-Pellitero, M. P. 1984. *Myxobolus farionis* n. sp. and *M. ibericus* n. sp. from *Salmo trutta f. fario* from the Duero basin (NW Spain). Description and population dynamics. *Angewandte Parasitologie* 25, 181-189.

Gorbunova, M. 1936. Variations of the parasitic fauna of the pike and the roach with their age. *Annls Leningrad State University* No.7, 5-30.

Gordon, B. 1962. Trichodinids from the gills of a new host, the lingcod *Ophiodon elongatus*. *Journal of the Fisheries Research Board of Canada* 19, 515-516.

Grabda, J. 1991. Marine Fish Parasitology, An Outline. PWN-Polish Scientific Publishers, Warszawa.

Grass, P. P. 1960. Les Myxosporidies sont des organismes pluricellulaires. *Compte rendu de l'Academie des sciences* 251, 2638-2640.

Grasse, P. P. and Lavette, A. 1978. The myxosporidia *Sphaeromyxa sabrazesi* and the new branch of myxozoans (Myxozoa): research on the primitive multicellular state and phylogenetic considerations. *Annales des sciences naturelles (Zoologie)* 20, 193-286.

Guilford, H. G. 1963. New species of myxosporidia found in percid fishes from Green Bay (Lake Michigan). *Journal of Parasitology* 49, 474-478.

Guilford, H. G. 1965. New species of myxosporidia from Green Bay (Lake Mucgugan). *Translatios of the American Microscopical Society* 84, 566-573.

Guilford, H. G. 1967. *Myxosoma pendula* n. sp. (Protozoa, Myxosporida) from the creek chub *Semotilus atromaculatus*. *Journal of Protozoology* 14, 196-198.

Gurley, R. R. 1893. On the classification of Myxosporidia, a group of Protozoan parasites infecting fish. *Bulletin of the United States Fish Commission* 2 (1891), 402-407.

Gurley, R. R. 1894. Myxosporidia or Psorosperms of fishes and the epidemics produced by them. *Report of the United States Fish Commission* 26 (1892), 65-304.

Haldar, D. P., Mukherjee, M. and Kundu, T. K. 1981. Observations on two new species of *Myxosoma* Thelohan, 1892 (Myxozoa: Myxosomatidae) from freshwater fishes. *Archiv für Protistenkunde* 124, 244-251.

Haley, A. J. 1954. Further observations on *Glugea hertwigi* Weissenberg 1911, 1913 (Microsporidia) in fresh water smelt in New Hampshire. *Journal of Parasitology* 40, 482- 483.

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: the causative organism of whirling disease of salmonids. *Journal of Fish Biology* 9, 339-357.

Hamilton, R. C. 1980. Ultrastructure of a *Sphaerospora* sp. (Myxosporida) in goldfish kidneys. *Micron* 11, 499-500.

Hanek, G. and Fernando, C. H. 1978. Spatial distribution of gill parasites of *Lepomis gibbosus* (L.) and *Ambloplites rupestri* (Raf.). *Canadian Journal of Zoology* 56, 1235-1240.

Harrison, G. E. and Hadley, W. F. 1977. The incidence of *Myxobolus dentium* (Protozoa: Myxosporida) in *Esox masquinongy* of the Upper Niagara River. *Transactions of the American Fisheries Society* 106, 476-480.

Hausmann, K. and Hausmann, E. 1981a. Structural studies on *Trichodina pediculus* (Ciliophora, Peritricha). I. The locomotor fringe and the oral apparatus. *Journal of Ultrastructure Research* 74, 131-143.

Hausmann, K. and Hausmann, E. 1981b. Structural studies on *Trichodina pediculus* (Ciliophora, Peritricha). II. The adhesive disc. *Journal of Ultrastructure Research* 74, 144-155.

Hedrick, R. P., Groff, J. M. and Mcdowell, T. S. 1990. Gill sphaerodporosisi in goldfish (*Carassius auratus*). *Journal of Wildlife Diseaseas* 26, 558-560.

Hedrick, R. P., Mcnowell, T. and Groff, J. M. 1990. *Sphaerospora ictaluri* n. sp. (Myxosporea: Sphaerosporidae) observed in the kidney of the channel catfish, *Ictalurus punctatus* Rafinesque. *Journal of Protozoology* 37, 107-112.

Hermann, H and Klinke, R. 1956. *Trichodina dohrni* n. sp., eine neue fischpathogene ciliatenart aus dem golf von neapel. *Zeischrift für Parasitenkunde* 17. 365-370.

Hermanns, W. and Korting, W. 1985. *Sphaerospora tincae* Plehn, 1925 in tench, *Tinca*

*tinca* L., fry. *Journal of Fish Diseases* 8, 281-288.

Hine, P. M. 1979. Factors affecting the size of spores of *Myxidium zealandicum* Hine, 1975 (Protozoa: Myxosporida). *New Zealand Journal of Marine and Freshwater Research* 13, 215-223.

Hine, P. M. 1980. A review of some species of *Myxidium* Butschli, 1882 (Myxosporea) from eels (*Anguilla* spp.). *Journal of Protozoology* 27, 260-267.

Hines, R. S. and Spira, D. T. 1973. *Ichthyophthirius multifiliis* (Fouquet) in the mirror carp, *Cyprinus carpio* L. I. Course of infection. *Journal of Fish Biology* 5, 385-392.

Hines, R. S. and Spira, D. T. 1974. Ichthyophthiriasis in the mirror carp *Cyprinus carpio* L. II. Pathology. *Journal of Fish Biology* 6, 189-196.

Hoffman, C. E. 1990. *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. *Journal of Aquatic Animal Health* 2, 32-37.

Hoffman, C. E., Putz, R. E. and Dunbar, C. E. 1965. Studies on *Myxosoma cartilageinis* n. sp. (Protozoa, Myxosporidea ) of centrarchid fish and a synopsis of the *Myxosoma* of North American freshwater fishes. *Journal of Protozoology* 12, 319-332.

Hoffman, O. L., Kazubski, S. L., Mitchell, A. J. and Smith, C. E. 1979. *Childonella hexasticha* (Krernik, 1909) Protozoa, ciliata from North American warmwater fish. *Journal of Fish Diseases* 2, 153-157.

Hopkins, C. A. 1959. Seasonal variations in the incidence and development of the cestode *Proteocephalus filicollils* (Rud. 1810) in *Gasterosteus aculeatus* (L. 1766). *Parasitology* 49, 529-542.

Hua, D. K. and Dong, H. J. 1983. A new species of *Pleistophora* from big-eyes, *Priacanthus tayenus* Richardson, and *P. macracanthus* Cuvier and Valenciennes, from the South China Sea. *Journal of Fish Diseases* 6, 293-301.

Hua, D. K. and Zhang, Z. Y. 1988. Light and electron microscopic observations on *Microsporidium zhanjiangensis* sp. nov. and *Pleistophora priacanthusis* (Hua, 1981) Hua and Dong, 1983 (Microsporidia). *Journal of the Zhanjiang Fishery College* 6, 1-18.

Hughes, G. M. and Morgan, M. 1973. The structure of fish gills in relation to their respiratory function. *Biological Reviews* 48, 419-475.

Hulbert, W.C., Komourdjian, M. P., Moon, T. W. and Fenwick, J. C. 1977. The fine structure of sporogenesis in *Myxidium zealandicum* (Protozoa: Myxosporidia). Canadian *Journal of Zoology* 55, 438-447.

Imai, S., Miyazaki, H. and Nomura, K. 1991. Trichodinid species from the gill of cultured Japanese eel, *Anguilla japonica,* with the description of a new species based on light and scanning electron microscopy. *European Journal of Protistology* 27, 79-84.

Inoue, K. and Hoshina, T. 1983. *Myxosoma gnathopogonae* sp. nov. (Myxosporea, Bivalvulida) found in the cutis of the roach gnathopogon, *Elongatus caerulescens. Japanese Journal of Parasitology,* 32, 371-404.

Institute of Hydrobiology Academia Sinica. 1973. *An Illustrated Guide to the Fish Disease and Causative Pathogenic Fauna and Flora in Hupei Province.* Scientific Publishing House, Bejing.

Ishihara, R. 1968. Some observations on the fine structure of sporoplasm discharged

from the spores of a microsporidan, *Nosema bombycis*. *Journal of Invertebrate Pathology* 12, 245-258.

Ivanova, N. S. 1970. The parasitic ciliates (Ulceolariidae, Peritricha) of pond fishes. *Acta Protozoologica* 8, 209-217.

Iversen, E. S. 1954. A new myxosporidian, *Myxosoma squamalis*, parasite of some salmonoid fishes. *The Journal of Parasitology* 40, 397-404.

Jameson, A. P. 1929. Myxosporidia from california fishes. *Journal of Parasitology* 16, 59-68.

Janovy, J. Jr. and Hardin, E. L. 1987. Population dynamics of the parasites in *Fundulus zebrinus* in the Platte River of Nebraska. *Journal of Parasitology* 73, 689-696.

Jirovec, O., Wening, K., Fott, B, Bartos, E., Weiser, J. and Sramek-Husek. 1953. *Protozoology*. Praha.

Johnston, T. H. 1916. A census of the endoparasites recorded as occurrung in Queensland, arranged under their hosts. *Proceedings of the Royal Society of Queensland* 28, 31-79.

Johnston, T. H. and Bancroft, M. J. 1918. Some new sporozoan parasites of Queensland freshwater fish. *Journal of Royal Society of New South Wales* 52, 520-528.

Johnston, T. H. and Cleland, J. B. 1909. Notes on some parasitic protozoa. *Proceedings of the Linnean Society of New South Wales* 34, 500-513.

Johnston, T. H. and Cleland, J. B. 1910. The haematozoa of Australian fish. *Proceedings of the Royal Society of New South Wales* 44, 406-415.

Joy, J. E., Tarter, D. C. and Franklin, H. 1978. Gill parasites *Octomacrum spinum* (Trematoda: Monogenea) and *Unicauda* sp. (Protozoa: Myxosporidea) from the stoneroller minnow *(Campostoma anomalum)* in West Virginia. *Transactions of the American Microscopical Society* 97, 100-104.

Jukhimenko, S. S. 1986. New species of Myxosporidia of the genus *Myxobolus* (Myxosporidia, Myxobolidae) from Cyprinidae of the Amur River. *Parazitologiya* 20, 416-422.

Jurine, L. L. 1825. Histoire des poissons du lec Leman. *Memoires de la Societe de Physique et d'Histoire Naturelle* vol. 3.

Kabata, Z. 1962. Five new species of Myxosporidia of marine fishes. *Parasitology* 52, 177-186.

Kabata, Z. and Whitaker, D. J. 1981. Two species of *Kudoa* (Myxosporea: Multivalvulida) parasiteic in the flesh of *Merluccius productus* (Ayres, 1855) (Pisces: Teleostei) in the Canadian Pacific. *Canadian Journal of Zoology* 59, 2085-2091.

Kalavati, C. and Narasimhamurti, C. C. 1985. Histopathological changes in the gills of *Channa punctatus* BL. infected with *Henneyguya waltairensis*. *Archive für Protistenkunde* 129, 199-202.

Kalavati, C., Sandeep, B. V. and Narasimhamurti, C. C. 1981. Two new species of myxosporidians, *Myxosoma channai* n. sp. and *Myxobolus tripathii* n. sp. from freshwater fishes of Andhra pradesh. *Proceedings of the Indian Academy of Sciences* 90, 61-78.

Kazubski, S. and El-Tantawy, S. 1989. *Myxobolus warniakiensis* sp. n. (Myxosporidia, Bivalvullea, Myxobolildae) new parasite of *Lota lota* from Lake

Warniak (Mazurian Lakeland, Poland). *Acta Parasitologica Polonica* 34, 199-202.

Kearn, G. C. 1967. Experiments on host-finding and host-specificity in the monogenean skin parasite *Entobdella soleae. Parasitology* 57, 585-605.

Kennedy, S. R. 1968. Population biology of the cestode *Caryophyllarus laticeps* (Pallas, 1781) in dace, *Leucisceus leuciscus* L. of the River Avon. *Parasitology* 59, 783-794.

Kennedy, C. R. and Hine, P. M. 1969. Population biology of the cestode *Proteocephalus torulosus* (Batsch) in Dace *Leuciscus Leuciscus* (L.) of the River Avon. *Journal of Fish Biology* 1, 209-219.

Kent, M. L. and Moser, M. 1990. *Ortholinea elata* n. sp. (Myxosporea: Ortholineidae) in the northern butterfly fish *Chaetodon rainfordi. Journal of Protozoology* 37, 49-51.

Kepr, T. 1991. Parasitic protozoa of cyprinid fishes: protozoa of the roach *Rutilus rutilus* (Linnaeus, 1758) in Czechoslovakia. *Folia Parasitologica* 38, 11-21.

Khan, R. A. 1972. Taxonomy, prevalence, and experimental transmission of a protozoan parasite, *Trichodina oviducti* Polyanski (Ciliata: Peritricha) of the thorny skate, *Raja radiata* Donovan. *Journal of Parasitology* 58, 680-686.

Khan, R. A. 1991. Mortality in Atlantic salmon *(Salmo salar)* associated with Trichodinid ciliates. *Journal of Wildlife Diseases* 27, 153-155.

Khan, R. A., Barber, V. C. and McCann, S. 1974. A scanning electron microscopical study on the surface topography of a Trichodinid ciliate. *Transactions of the American Microscopical Society* 93, 131-134.

Khan, R. A., Bowering, W. R., Burgeois, C., Lear, H. and Pippy, J. H. 1986. Myxosporean parasites of marine fish from the contental shelf off New Foundland and Labrador. *Canadian Journal of Zoology* 64, 2218-2226.

Kitchener, J. A. 1985. Life history and trophic ecology of four sympatric species of atherinid, *Atherinosoma microstoma, Atherinosoma presbyteroides, Atherinason esox* and *Atherinason* sp. (Pisces: Teleostei) in a seagrass meadow in the D'entrecasteaux channel, south-eastern Tasmania. Unpublished Honous Thesis, University of Tasmania.

Knight, S. A., Janovy, J. Jr. and Current, W. L. 1977. *Myxosoma funduli* Kudo, 1918 (Protozoa: Mysxosporida) in *Fundulus kansae:* summer epizootiology. *Journal of Parasitology* 63, 897-902.

Knight, S. A., Janovy, J. Jr. and Current, W. L. 1980. *Myxosoma funduli* Kudo, 1918 (Protozoa: Myxosporida) in *Fundulus kansae* (Pisces: Crprinodontidae): annual prevalence and geographic distribution. *Journal of Parasitology* 66, 806-810.

Komourdjian, M. P., Hulbert W. C., Fenwick, J. D. and Moon, T. W. 1977. Description and first occurrence of *Myxidium zealandicum* (Protozoa: Myxosporidia) in the North American eel *Anguilla rostrata* Le Sueur. *Canadian Journal of Zoology* 55, 52-59.

Konagaya, S. 1983. Protease responsible for jellification of mycosporidia - infected swordfish meat. *Bulletin of the Japanese Society of Scientific Fisheries* 49, 919-926.

Kovacs-Grayer, E. and Molnar, K. 1984. Studies on the biology and pathology of the common carp parasite *Myxobolus basilamellaris* Lom et Molnar, 1983

(Myxozoa: Myxosporea). *Acta Veterinaria Hungarica* 31, 91- 102.
Kudo, R. 1919. Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. *Illinois Biological Monograph* 5, 3-4; 1-26.
Kudo, R. 1920. Cnidosporidia in the vicinity of Urbana. *Transactions of the Illinois Academy of Sciences* 3, 298.
Kudo, R. 1921. On the nature of the structure characteristic of Cnidosporidian spores. *Transactions of the American Microscopical Society* 11, 59-74.
Kudo, R. 1924. A biologic and taxonomic study of the microsporidia. *Illinois Biological Monograph* 9, 83-344.
Kudo, R. 1933. A taxonomic consideration of Myxosporidia. *Transactions of the American Microscopical Society* 52, 195-216.
Kudo, R. R. 1966. *Protozoology*. 5th ed. Charles C. Thomas Publisher, Springfield, Illinois, USA.
Kumari Lalitha P. S. L. 1969. Studies on parasitic protozoan (Myxosporidia) of freshwater fishes of Andhra pradesh, Indian. *Rivista di Parassitologia* 30, 153-225.
Kundu, T. K. 1985. *Myxosoma indirae* sp. n. (Myxozoa: Myxosomatidae) from the head cartilage, scale and tail of *Cirrhina mrigala* (Hamilton). *Acta Protozoologica* 24, 333-337.
Labbe, A. 1899. Sporozoa. In *Das Tierreich*. Ed. by O. Butschli, vol. 5, pp.180. Friedlander U Sohn, Berlin.
Laird, M. 1953. The protozoa of New Zealand intertidal zone fishes. *Transactions of the Royal Society of New Zealand* 81, 79-143.
Laird, M. 1959. *Caliperia brevipes* n. sp. (Ciliata: Peritricha), epizoic on *Raja erinagea* Mitchill at Saint Andrews, New Brunswick. *Canadian Journal of Zoology* 37, 283-288.
Langdon, J. S. 1987. Spinal curvatures and an encephalotropic myxosporean, *Triangula percae* sp. nov. (Myxozoa: Ortholineidae), enzootic in redfin perch, *Perca fluviatilis* L., in Australia. *Journal of Fish Diseases* 10, 425-434.
Langdon, J. S. 1988. Diseases of introduced Australian fish. In *Fish Diseases*. Proceedings of the 106th Refresher Course for Veterinarians, Sydney NSW, May 1988. Ed. by D. I. Bryden, pp. 225-276. University of Sydeny, Sydney.
Langdon, J. S. 1990a. Observations on new *Myxobolus* species and *Kudoa* species infecting the nervous system of Australian fishes. *Journal of Applied Ichthyology* 6, 107-116.
Langdon, J. S. 1990b. Major protozoan and metazoan parasitic diseases of Australian finfish. In *Fin Fish Diseases*. Proceedings of the 128th Refresher Course for Veterinarians, Launceston Tas., January 1990. pp. 233- 255. University of Sydney, Sydney.
Langdon, J. S. 1991. Myoliquefaction post-mortem "milky flesh" due to *Kudoa thyrsites* (Gilchrist) (Myxosporea: Multivalvulida) in mahi mahi, *Coryphaena hippurus* L. *Journal of Fish Diseases* 14, 45-54.
Langdon, J. S., Gudkovs, N., Humphrey, J. D. and Saxon, E. C. 1985. Deaths in Australian freshwater fishes associated with *Chilodonella hexasticha* infection. *Australian Veterinary Journal* 62, 409-413.
Langdon, J. S. and Humphrey, J. D. 1985. A checklist of the disease status of

Australian fish and shellfish. In *Proceedings of the Workshop on Diseases of Australian Fish and Shellfish.* Ed. by J. D. Humphrey and J. S. Langdon , pp. 233-246. Australian Fish Health Reference Laboratory, Benalla.

Larsson, J. I. R. 1988. Identification of microsporidian genera (Protozoa: Microspora) - a guide with comments on the taxonomy. *Archive für Protistenkunde* 136, 1-37.

Larsson, R. 1986. Ultrastructure, Function, and Classification of Microsporidia. *Progress in Protistology* 1, 325-390.

Leger, L. and Hesse, E. 1922. Microsporidies bacteri-formes et essai de systematiqu du groupe. *Compte rendu de l'Academie des sciences* 174, 327-330.

Lester, R. J. G. 1979. Parasitic infection in *Seriola grandis* (abstract only). Australian Society of Fish Biology 6th Annual Conference, Port Stephens, New South Wales.

Lester, R. J. G. 1982. *Unicapsula seriolae* n. sp. (Myxosorea, Multivalvulida) from Australian yellowtail kingfish *Seriola lalandi. Journal of Protozoology* 29, 584-587.

Levine, N. D., Corliss, J. O., Cox, F. E. G., Deroux, G., Grain, J., Honigberg, B. M., Leedale, F. C., Loeblich, A. R. III, Lom, J., Lynn, D., Merinefeld, E. G., Page, F. C., Poljansky, G., Sprague, V., Vavra, J. and Wallace, F. G. 1980. A newly revised classification of the Protozoa. *Journal of Protozoology* 27, 37-58.

Li, L. X. and Desser, S. S. 1983. *Trichodina algonquinensis,* a new species of peritrich ciliate from Ontario freshwater fish, and observations on its transmission. *Canadian Journal of Zoology* 61, 1159-1164.

Lom, J. 1958. A contribution to the systematics and morphology of endoparasitic trichodinids from amphibians, with a proposal of uniform specific characteristics. *Journal of Protozoology* 5, 251-263.

Lom, J. 1959. On the systematics of the genus *Trichodinella* Sramek-Husek (= Brachyspira Raabe). *Acta Parasitologica Polonica* 7, 573-590.

Lom, J. 1960. On two endozoic trichodinids, *Trichodina urinaria* Dogiel, 1940 and *Trichodina polycirra* sp. n. *Acta Parasitologica Polonica* 8, 169-180.

Lom, J. 1961. Ectoparasitic trichodinids from freshwater fish in Czechoslovakia. *Vestnik Ceskoslovenske Spolecnosti Zoologicke* 30, 39-48.

Lom, J. 1962. Trichodinid ciliates from fishes of the Rumanian Black Sea Coast. *Parasitology* 52, 49-61.

Lom, J. 1963. The ciliates of the family Urceolariidae inhabiting gills of fishes (the *Trichodinella*-group). *Acta Societatis Zoologica Bohemoslov* 27, 7-19.

Lom, J. 1964. Notes of the extrusion and some other features of myxosporidian spores. *Acta Protozoologica* 2, 321-327.

Lom, J. 1965. Notes on the extrusion and some other features of myxosporidian *Myxobolus muelleri, Myxobolus elipsoideus* spores. *Acta Protozoologica* 2, 321-328.

Lom, J. 1969a. Notes on the ultrastructure and sporoblast development in fish parasitizing myxosporidian of the genus *Sphaeromyxa. Zeitschrift für Zellforschung und Mikroskopische Anatomie* 97, 416-437.

Lom, J. 1969b. On a new taxonomic character in myxosporidia as demonstrated in description of two new species of *Myxobolus. Folia Parasitologica (Praha)* 16,

97-103.
Lom, J. 1970a. Trichodinid ciliates (Peritricha, Urceolariidae) from some marine fishes. *Folia Parasitologica (Praha)* 17, 113-125.
Lom, J. 1970b. Some aspects of the host-parasite relation in myxosporidians of the genus *Henneguya*. *Journal of Parasitology* 56, 442.
Lom, J. 1973. The adhesive disc of *Trichodinella epizootica* - ultrastructure and injury to the host tissue. *Folia Parasitologica (Praha)* 20, 193-202.
Lom, J. 1986. Protozoan infection in fish. In *Pathology in Marine Aquaculture*, special publication of European Aquaculture Society. Ed. by C.P.Vivares, J.R.Bonami & E.Jaspers, 9, 95-104. Belgium.
Lom, J. 1987. Myxosporea: a new look at long-known parasites of fish. *Parasitology Today* 3, 327-332.
Lom, J. and Arthu, J. R. 1989. A guideline for the preparation of species description in Myxosporea. *Journal of Fish Diseases* 12, 151-156.
Lom, J., Canning, E. U. and Dykova, I. 1979. Cell periphery of *Glugea anomala* xenomas. *Journal of Protozoology* 36, 44A-45A (Abstract).
Lom, J. and Corliss, J. O. 1967. Ultrastructural observation on the development of the microsporidian protozoan *Plistophora hyphessobryconis* Schaperclaus. *Journal of Parasitology* 14, 141-152.
Lom, J. and de Puytorac, P. 1965a. Studies on the myxosporidian ultrastructure and polar capsule development. *Protistologica* 1, 53-65.
Lom, J. and de Puytorac, P. 1965b. Observations sur l'ultrastructure des trophozoites de myxosporidies. *Compte rendu de l'Academie des sciences* 260, 2588-2590.
Lom, J. and Dykova, I. 1988. Sporogenesis and spore structure in *Kudoa lunata* (Myxospore, Multivavulida). *Parasitology Research* 74, 521-530.
Lom, J. and Dykova, I 1992. *Protozoan Parasites of Fishes*. Elsevier Science Publishers, Netheriands.
Lom, J., Dykova, I. and Lhotakova, S. 1982. Fine structure of *Sphaerospora renicola* Dykova and Lom, 1982. A myxosporean from carp kidney and comments on the origin of pansporoblasts. *Protistologia* 18, 489-502.
Lom, J., Dykova, I. and Pavlaskova, M. 1983a. "Unidentified" mobile protozoans from the blood sample and some unsolved problems of myxosporean life cycle. *Journal of Protozoology* 30, 497-508.
Lom, J., Dykova, I. and Pavlaskova, M. 1983b. *Sphaerospora molnari* sp. nov. (Myxozoa: Myxosporea), an agent of gill, skin and blood sphaerosporosis of common carp in Europe. *Parasitology* 86, 529-535.
Lom, J., Dykova, I. and Shaharom, F. 1990. *Microsporidium arthuri* n. sp., parasite of *Pangasius sutchi* (Panhasiidae, Siluroidea) in South-East Asia. *Diseases of Aquatic Organisms* 8, 65-57.
Lom, J., Feist, S. W., Dykova, I. and Kepr, T. 1989. Brain myxoboliasis of bull head, *Cottus gobio* L., due to *Myxobolus jiroveci* sp. nov.: light and electron microscope observations. *Journal of Fish Diseases* 12, 15-27.
Lom, J., Golemansky, V and Grupcheva, G. 1976. Protozoan parasites of carp (*Cyprinus carpio* L.): a comparatice study of their occurrence in Bulgaria and Czechoslovakia, with the description of *Trichodina perforata* sp. n. *Folia Parasitologica (Praha)* 23, 289-300.

Lom, J. and Haldar, D. P. 1976. Observations on trichodinid endocommensals in fishes. *Transactions of the American Microscopical Society* 95, 527-541.

Lom, J. and Haldar, D. P. 1977. Ciliates of the genera *Trichodinella, Tripartiella* and *Paratrichodina* (Peritricha, Mobilina) invading fish gills. *Folia Parasitologica (Praha)* 24, 193-210.

Lom, J. and Hoffman, G. L. 1971. Morphology of the spores of *Myxosoma cerebralis* (Hofer, 1903) and *M. cartilagini* (Hoffman, Putz and Dunbar, 1965). *Journal of Parasitology* 57, 1302-1308.

Lom, J., Korting, W. and Dykova, I. 1985. Light and electron microscope redescription of *Sphaerospora tincae* Plehn, 1925 and *S. galinae* Evlanov, 1981 (Myxosporea) from the tench, *Tinca tinca* L. *Protistologica* 21, 487-497.

Lom, J. and Laird, M. 1969. Parasitic protozoa from marine and euryhaline fish of Newfoundland and New Brunswick. I. Peritrichous ciliates. *Canadian Journal of Zoology* 47, 1367-1380.

Lom, J. and Laird, M. 1976. Parasitic protozoa from marine and euryhaline fish of Newfoundland and New Brunswick. II. Microsporida. *Transactions of the American Microscopical Society* 95, 569-580.

Lom. J. and Lawler, A. R. 1973. An ultrastructural study on the attachment in dinoflagellates invading gills of Cyprinodontidae. *Protistologica* 9, 293-309.

Lom, J., Molnar, K. and Dykova, I. 1986. *Hoferellus gilsoni* (Debaissieux, 1925) comb. n. (Myxozoa, Myxosporea): redescription and mode of attachment to the epithelium of the urinary bladder of its host, the European eel. *Protistologica* 22, 405-413.

Lom, J. and Noble, E. R. 1984. Revised classification of the class Myxosporea Butschli, 1881. *Folia Parasitologica (Praha)* 31, 193-205.

Lom, J. Pavlovska, M. and Dykova, I. 1985. Notes on kidney-infecting species of the genus *Sphaerospora* Thelohan (Myxosporea), including a new species *S. gobinis* sp. nov. and on myxosporean life cycle stages in the blood of same freshwater fish. *Journal of Fish Diseases* 8, 221-232.

Lom, J., Rohde, K. and Dykova, I. 1992. Studies on protozoan parasites of Australian fishes. 1. New species of the genera *Coccomyxa* Leger et Hesse, 1907, *Ortholinea* Shulman, 1962 and *Kudoa* Meglitsch, 1947 (Myxozoa, Myxosporea). *Folia Parasitologica* 39, 289-306.

Lom, J., Rohde, K. and Dykova, I. 1993. *Crepidoodinum australe* n. sp., an ectocommensal dinoflagellate from the gills of *Sillago ciliata,* an estuarine fish from the New South Wales coast of Australia. *Diseases of Aquatic Organisms* 15, 63-72.

Lom, J. and Vavra, J. 1962. A proposal for the classification of subphylum Cnidospora. Systematic Zoology. 2, 172-175.

Lom, J. and Vavra, J. 1963a. Fine morphology of the spores in Microsporidia. *Acta Protozoologica* 1, 279-284.

Lom, J. and Vavra, J. 1963b. Mucous envelopes of spores of subphylum Cnidospora (Doflein, 1901). *Vestnik Ceskoslovenske Spolecnosti Zoologicke* 27. 4-6.

Lom, J. and Vavra, J. 1965. Notes on the morphologenesis of the polar filament in *Henneguya* (Protozoa, Cnidosporidia). *Acta Protozoologica* 3, 57-60.

Lom, J. and Weiser, 1972. Surface pattern of some microsporidian spores as seen in the

scanning electron microscope. *Folia Parasitologica (Prague)* 19, 359-363.

Loubes, C. and Maurand, J. 1975. Etude ultrastructurale de *Gurleya chironomi* n. sp. microsporidie parasite des larves d'Orthocladius (Diptera-Chironomidae). *Protistologica* 11, 233-244.

Loubes, C., Maurand, J. and Ormieres, R. 1979. Etude ultrastructurale de *Spraguea lophii* (Doflein, 1898), microsporidie parasite de la baudrole: eddai d'interpretation du dimorphisme sporal. *Protistologica* 15, 43-54.

Loubes, C., Maurand, J. and Walker, C. 1981. Developpement d'une microsporidie (*Glugea truttae* n. sp.) dans le sac vitellin de l'alevin de la truite *Salmo fario* L.: etude ultrastructurale. *Protistologica* 17, 177-184.

Loubes, C., Maurand, J., Gasc, C., Buron, I. de and Barral, J. 1984. Ultrastructural study of *Loma dimorpha* n. sp., a microsporidian parasite of fishes Gobiidae from the Languedoc. *Protistologica* 20, 579-589.

Lucky, A. 1978. Occurrence of *Myxobolus pavlovskii,* the parasite of *Hypophthalmichthys molitrix* and *Aristichthys nobilis* in the fish ponds in Czechoslovakia. *Acta Veterinaria (Brno)* 47, 203-207.

Mackerras, I. M. and Mackerras, M. J. 1925. The haematozoa of Australian marine Teleosteri. *Proceedings of the Linnean Society of New South Wales* 50, 359-366.

Mackerras, I. M. and Mackerras, M. J. 1961. The haematozoa of Australian frogs and fish. *Australian Journal of Zoology* 9, 123-139.

Margolis, L. and Evelyn, T. P. 1975. *Ceratomyxa shasta* (Myxosporida) disease in chum salmon *(Oncorhynchus keta)* in British Columbia. *Journal of the Fisheries Research Board of Canadia* 32, 1640-1643.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. and Schad, G. A. 1982. The use of ecological terms in parasitology (Report of AN AD HOC committee of the American Society of Parasitologists). *Journal of Parasitology* 68, 131-133.

Markiw, M. E. 1989. Portals of entry for salmonid whirling disease in rainbow trout. *Diseases of Aquatic Organisms* 6, 7-10.

Markiw, M. E. and Wolf, K. 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiologic agent of salmonid whirling diseases requires tubificid worm (Annelida: Oligochaeta) in its life cycle. *Journal of Protozoology* 30, 561-564.

Matthews, R. A. and Matthews, B. F. 1980. Cell and tissue reactions of turbot *Scephthalmis maxumus* (L) to *Tetramicra brevifilum* gen. n., sp. n. (Microspora). *Journal of Fish Diseases* 3, 495-515.

Maurand, J., Loubes, C., Gasc, C., Pelletier, J. and Barral, J. 1988. *Pleistophora mirandellae* Vaney and Conte 1901, a microsporidian parasite in cyprinid fish of river in Herault: taxonomy and histopathology. *Journal of Fish Diseases* 11, 251-258.

McCallum, H. L. 1982. Population dynamics of *Ichthyophthirius multifiliis*. *Parasitology* 85, 475-488.

McVicar, A. H. 1975. Infection of plaice *Pleuronectes platessa* L. with *Glugea (Nosema) stephani* (Hagenmuller, 1899) (Protozoa: Microsporidia) in a fish farm and under experimental conditions. *Journal of Fish Biology* 7, 611-619.

Meglitsch, P. A. 1942. *Myxosoma microthecum* n. sp., a myxosporidian inhibiting the mesenteries of *Minyterma melanops. Transectios of the American Microscopical*

*Society* 61, 33-35.

Meglitsch, P. A. 1960. Some coelozoic myxosporidia from New Zealand fishes. I. General and Family Ceratomyxidae. *Transactions of the Royal Society of New Zealand* 88, 265-356.

Meglitsch, P. A. 1970. Some coelozoic Myxosporida from New Zealand fishes: Family Sphaerosporidae. *Journal of Protozoology* 17, 112-115.

Mills, D. 1991. Habitat utilization by fish in a seagrass bed. Unpublished Honous Thesis, University of Tasmania.

Mitchell, L. G. 1977. Myxosporidia. In *Parasitic Protozoa*. Ed. by J. P. Kreier, vol. IV, pp.115-154. Plenum Press, New York and London.

Mitchell, L. G. 1989. Myxobolid parasite (Myxozoa: Myxobolidae) infecting fishes of western Montana, with note on histopathology, seasonality, and intraspecific variation. *Canadian Journal of Zoology* 67, 1915-1922.

Mitchell, L. G., Seymour, C. L. and Gamble, J. M. 1985. Light and electron microscopy of *Myxobolus hendricksoni* sp. nov. (Myxozoa: Myxobolidae) infecting the brain of the fathead minnow, *Pimephales promelas* Rafinesque. *Journal of Fish Diseases* 8, 75-89.

Modin, J. C. 1981. *Microsporidium rhabdophilia* n. sp. from rodlet cells of salmonid fishes. *Journal of Fish Diseases* 4, 203-211.

Molnar, K. 1982. Biology and histopathology of *Thelohanellus nikolskii* Achmerov, 1955 (Myxosporea, Myxozoa), a protozoan parasitic of the common carp (*Cyprinus carpio*). *Zeitschnift für Parasitenkunde* 68, 269-277.

Molnar, K and Kovacs-Gayer, E. 1986. Experimental induction of *Sphaerospora renicola* (Myxosporea) infection in common carp (*Cyprinus carpio*) by transmission of SB-protozoans. *Journal of Applied Ichthyology* 2, 86-94.

Molnar, K, and Rohde, K. 1988a. Seven new coccidian species from marine fishes in Australia. *Systematic Parasitology* 11, 19-29.

Molnar, K. and Rhode, K. 1988b. New coccidians from freshwater fishes of Australia. *Journal of Fish Diseases* 11, 161-169.

Moniez, R. 1887. Observations pour la revision des Microsporidies. *Compte rendu de l'Academie des sciences* 104, 1312-1314.

Morrison, C. M. 1983. The distribution of the microsporidian *Loma morhua* in tissue of the cod *Gadus morhua* L. *Canadian Journal of Zoology* 61, 2155-2161.

Morrison, C., Hoffman, G. L. and Sprague, V. 1985. *Glugea pimephales* Fantham, Porter and Richardson, 1941, n. comb. (Microsporidia, Glugeidae) in the fathead minnow, *Pimephales promelas*. *Canadian Journal of Zoology* 63, 380-391.

Morrison, C. M. and Marryatt, V. 1986. Further observations on *Loma morhua* Morrison and Sprague, 1981. *Journal of Fish Diseases* 9, 63-67.

Morrison, C. M., Marryatta, V. and Gray, B. 1984. Structure of *Pleistophora hippoglossoideos* Bosanquet in the American plaice *Hippoglossoides platessoides* (Fabricius). *Journal of Parasitology* 70, 412-421.

Morrison, N. D. and Pratt, I. 1973. *Sphaeromyxa maiyai* sp. n. (Protozoa: Myxosporidea), coelozoic parasite of the pacific tomcod, *Microgadus proximus*. *Journal of Protozoology* 20, 214-218.

Morrison, C. M. and Sprague, V. 1981a. Electron microscopical study of a new genus

and new species of microsporidia in the gills of Altantic cod *Gadus morha* L. *Journal of Fish Diseases* 4, 15-32.

Morrison, C. M. and Sprague, V. 1981b. Light and electron microscope study of microsporidia in the gill of haddock, *Melanogrammus aeglefinus* (L.). *Journal of Fish Diseases* 4, 179-184.

Morrison, C. M. and Sprague, V. 1981c. Microsporidian parasites in the gills of salmonid fishes. *Journal of Fish Diseases* 4, 371-386.

Morrison, C. M. and Sprague, V. 1983. *Loma salmonae* (Putz, Hoffman and Dunvar, 1965) in the rainbow trout, *Salmo gairdneri* Richardson, and *L. fontinalis* sp. nov. (Microsporida ) in the brook trout, *Salvelinus fontinalis* (Mitchill). *Journal of Fish Diseases* 6, 345-353.

Moser, M. 1976. *Ceratomyxa anoplopoma* sp. n. (Protozoa, Myxosporida) from the stablefish, *Anoplopoma fimbria. Canadian Journal of Zoology* 54, 1405-1406.

Moser, M. 1976. Inter-species associations of Myxosporida (Protozoa) in macrourid fishes. *Zeitschift für Parasitenkunde* 51, 133-135.

Moser, M. and Haldorson, L. 1976. *Zschokkella amibiotocidis* sp. n. (Protozoa, Myxosporida) from California pile perch, *Damalichthys vacca,* and atriped perch, *Embiotoca laterslis. Canadian Journal of Zoology* 54, 1403-1405.

Moser, M., Kent, M. L. and Dennis, D. 1989. Gall bladder Myxosporea in coral reef fishes from Heron Island, Australia. *Australian Journal of Zoology* 37, 1-13.

Moser, M., Love, M. S. and Jensen, L. A. 1976. Myxosporida (Protozoa) in California rockfish, *Sebastes* spp. *Journal of Parasitology* 62, 690-692.

Moser, M. and Noble, E. R. 1976. The genus *Ceratomyxa* (Protozoa: Myxosporida) in macrourid fishes. *Canadian Journal of Zoology* 54, 1535-1537.

Moser, M. and Noble, E. R. 1977a. Three genera of Myxosporida (Protozoa) in macrourid fishes. *International Journal for Parasitology* 7, 93-96.

Moser, M. and Noble, E. R. 1977b. *Zschokkella* (Protozoa, Myxosporida) in macrourid fishes. *Inrenational Journal for Parasitology* 7, 97-100.

Moser, M. and Noble, E. R. 1977c. Myxosporidan genera of *Auerbachia, Sphaerospora, Davisia* and *Chloromyxum* in macquird fishes and the sablefish, *Anoplopoma fumbria. Zeitschift für Parasitenkunde* 51, 159-163.

Mukherjee, M. and Haldar, D. P. 1982. Observations on the Urceolariid ciliates of the genera *Trichodina* and *Tripartiella* in freshwater teleosts. *Archiv fürr Protistenkunde* 126, 419-426.

Muller, J. 1843. Recherches sur une variete remarquable de production parasitique morbide avec des corpuscules seminaux specifiquement organises. *Archives de medecine comparee* 6, 226.

Muller, J. F. 1938. A new species of *Trichodina* (Ciliata) from the urinary tract of the muskalange, with a separation of the genus. *Journal of Parasitology* 24, 251-258.

Munday, B. L. 1985. Diseases of salmonids 1. In *Diseases of Australian Fish and Shellfish.* Ed. by J. D. Humphery and J. S. Langdon, pp. 127-141. Australian Fish Health Laboratory, Benalla, Australia.

Munday, B. L., Foster, C. K., Roubal, F. R. and Lester, R. J. G. 1990. Paramoebic gill infection and associated pathology of Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri* in Tasmania. In *Pathology in Marine Science.*

Ed. by F. O. Perkins and T. C. Cheng), pp. 215-222. Academic Press, Inc. Harcourt Brace Jovanovich, Publishers.

Munday, B. L., Lange, K., Foster, C., Lester, R. J. G. and Handlinger, J. 1993. Amoebic gill disease of sea-caged salmonids in Tasmanian waters.*Tasmanian Fisheries Research* 28, 14-19,

Naegeli, K. W. 1857. Uber die neue krankheit der Seidinraupe und Verwandte Organisem. *Botanische Zeitung* 15, 760-761.

Naidenova, N. N. and Zaika, W. E. 1969. Two new species of Protozoa from the fishes of the Black Sea. *Parazitologiya* 3, 97-101.

Narasimhamurti, C. 1970. *Myxosoma intestinalis* n. sp. (Protozoa, Myxosporidia) parasitic in the gut of the estury fish, *Liza macrolrpos* Smith. *Proceedings of the Indian Academy of Sciences* 81, 19-27.

Narasimhamurti, C. C. and Kalavati, C. 1979. *Myxosoma lairdi* n. sp. (Protozoa: Myxosporidia) parasitic in the gut of the estuarine fish, *Liza macrolepis* Smith. *Proceedings of the Indian Academy of Sciences* 88, 269-272.

Narasimhamurti, C. C. and Kalavati, C. 1984. Seasonal variation of the myxosporidian, *Henneguya waltairensis* parasitic in the gills of the fresh water fish, *Channa punctatus* BL. *Archiv für Protistenkunde* 128, 351-356.

Narasinhamurti, C., Kalavati, C. and Saratchandra, B. 1980. *Myxosoma microspora* n. sp. (Protozoa: Myxosporidia) parasitic in the gills of *Mugil cephalus* L. *Journal of Fish Biology* 16, 345-348.

Nemeczek, A. 1922. Uber *Zschokkella rovignensis* sp. nov. *Archiv für Protistnekunde* 45, 390-400.

Noble, E. R. 1943. Nuclear cycles in the protozoan parasite *M. gasterosteri* n. sp. *Journal of Morphology* 73, 281-292.

Noble, E. R. 1944. Life cycles in Myxosporidia. *Quarterly Review of Biology* 19, 213-235.

Noble, E. R. 1957. Seasonal variations in host-parasite relations between fish and their protozoa. *Journal of the Marine Biological Association of the United Kindom* 36, 143-155.

Noble, E. R. 1960. Fishes and their parasite-mix as objects for ecological studies. *Ecology* 41, 593-596.

Obiekezie, A. I., Moller, H. and Anders, K. 1988. Diseases of the African estuarine catfish *Chrysichthys nigrodigitatus* (Lacepede) from the cross river estuary, Nigeria. *Journal of Fish Biology* 32, 207-221.

Okada, M., Areche, N. and Ysikawa, E. 1981. Myxosporidian infestation of Peruvian Hake. *Bulletin of the Japanese Society of Scientific Fisheries* 47, 229-238.

Olson, R. E. 1976. Laboratory and field studies on *Glugea stephani* (Hagenmuller), a microsporidian parasite of pleuronectid flatfishes. *Journal of Protozoology* 23, 158-164.

Olson, R. E. 1978. Parasitology of the English sole, *Parophrys vetulus* in Oregon, U. S. A. *Journal of Fish Biology* 13, 237-248.

Owens, L., Glazebrook, J. S., Ladds, P. W. and Campbell, R. S. F. 1988. Diseases in tropical mariculture in Australia. In *Fish Diseases*. Proceedings of the 106th Refresher Course for Veterinarians, Sydney NSW, May 1988. Ed. by D. I. Bryden, pp. 375- 412. University of Sydeny, Sydney.

Padnos, M. and Nigrelli, R. F. 1942. *Trichodina spheroidesi* and *Trichodina halli* spp. nov., parasitic on the gills and skins of marine fishes, with special reference to the life history of *T. spheroidesi*. *Zoologica* 27, 65-72.

Paker, J. D., Spall, R. D. and Warner, M. C. 1971. Two new Myxosporida, *Henneguya gambusi* sp. n. and *Myxosoma pharyngeus* sp. n. in the mosquito fish *Gambusia affinis* (Barid and Girard). *Journal of Parasitology* 57, 1297-1301.

Paling, J. E. 1968. A method of estimating the relative volumns of water flowing over the different gills of freshwater fish. *Journal of Experimental Biology* 48, 785-802.

Paperna, I. 1980. *Amyloodinium ocellatum* (Brown, 1931) (Dinoflagellida) infestations in cultured marine fish at Eilat, Red Sea: epizootiology and pathology. *Journal of Fish Diseases* 3, 363-372.

Paperna, I., Hartley, A. H. and Cross, R. H. M. 1987. Ultrastructural studies on the plasmodium of *Myxidium glardi* (Myxosporea) and its attachment to the epithelium of the urinary bladder. *International Journal for Parasitology* 17, 813-819.

Paperna, I. and Van As. J. G. 1983. The pathology of *Chilodonella hexasticha* (Kiernik) infections in cichlid fishes. *Journal of Fish Biology* 23, 441-450.

Patashnik, M., Grominger, H. S., Barnett, J., Kudo, G. and Koury, B. 1982. Pacific whiting, *Merluccius productus*: I. Abnormal muscle texture caused by myxosporidian-induced proteolysis. *Marine Fisheries Review* 44, 1-12.

Pearse, L. 1972. A note on a marine trichodinid ciliate parasitic on the skin of captive flatfish. *Aquaculture* 1, 261-266.

Pellifero, M. P. A., Pereira, B. J. M. and Lanza, G. 1979. Myxosporidios de *Carassius carassius* y *Cyprinus carpio* en el rio Esla (Leon). *Revista iberica de Parasitologia* 39, 611-623.

Pennyciuck, L. 1971a. Frequency distributions of parasites in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L., with particular reference to the negative binomial distribution. *Parasitology* 63, 389-406.

Pennyciuck, L. 1971b. Differences in the parasite infections in three-spined sticklebacks (*Gasterosteus aculeatus* L.) of different sex, age and size. *Parasitology* 63, 407-418.

Perez, C. 1905. Microsporides parasites des crabes d'Archachon. *Travaux de la Societe Scientifique de'Archachon* 8, 15-36.

Peshkowsky, L. 1923. Biological and structural features of *Trichodina*. *Russkii Archiv Protistologica* 2, 249-279.

Petrushevskii, G. K. 1932. Zur systematic und Cytologie der Myxosporidia aus einigen Fischen des weissen Meers. *Archiv für Protistenkunde* 78, 543-556.

Pflugfelder, O. 1952. Ein neuer Fischparasit aus der Gruppe der Microsporidia. *Aquarien Terrarien* 5, 131-133.

Philbey, A. W. and Ingram, B. A. 1991. Coccidiosis due to *Goussia lomi* (Protista: Apicomplexa) in aquarium-reared murray cod, *Maccullochella peeli* (Mitchell), (Percichthyidae). *Journal of Fish Diseases* 14, 237-242.

Pickering, A. D. and Christie, P. 1980. Sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, *Salmo trutta* L. *Journal of Fish*

*Biology* 16, 669-683.

Podlipaev, S. A. 1974a. Polysaccharides in some myxosporidian species. Six all-union conference on disease and parasotes of fishes, Moscow. 185-187.

Podlipaev, S. A. 1974b. Polysaccharides in spores of mycosporidia of the family myxobolidae. *Parasitologia* 8, 535-542.

Podlipaev, S. A. and Shulman, S. S. 1978. The nature of the iodinophilous vacuole in myxosporidia. *Acta Protoaoologica* 17, 109-124.

Poisson, R. 1953. Ordre des Microsporidies. In *Traite de Zoologie 1*. Ed. by P. P. Grasse, pp. 1042-1070. Masson et Cie, Paris.

Poynto, S. L. and Lom, J. 1988. Some ectoparasitic trichodinids from Atlantic cod, *Gadus Morhua* L., with a description of *Trichodina cooperi* n. sp. *Canadian Journal Zoology* 67, 1793-1800.

Price, R. L. 1982. Incidence of *Pleistophora cepedianae* (Microsporida) in gizzard shad (*Dorosoma cepedianum*) of Carlyle Lake, Illinois. *Journal of Parasitology* 68, 1167-1168.

Price, R. L. 1983. Light and electron microscopic examination of *P. cepedianae* Putz, Hoffman, and Dunbar (Microsporida: Pleistophoridae) from gizzard shad, *Dorosoma cepedianum* Lesueur. *Transactions of the Illinois State Academy of Science* 76, 77-84.

Pulsford, A. and Matthews, R. A. 1982. An ultrastructural study of *Myxobolus exiguus* Thelohan, 1895 (Myxosporea ) from grey mullet, *Crenimugil labrosus* (Risso). *Journal of Fish Diseases* 5, 509-526.

Raabe, Z. 1950. Uwagi o Urceolariidae (Ciliata-Peritricha) skrzelryb. *Annales Universitatis Mariae Curie-Sklodowska* 5, 292-310.

Raabe, Z. 1958. On some species of *Trichodina* (Ciliata-Peritricha) of gills of Adriatic fishes. *Acta Parasitologica Polonica* 6, 355-362.

Raabe, Z. 1959. Urceolariidae of gills of Gobiidae and Cottidae from Baltic Sea. *Acta Parasitologica Polonica* 7, 441-452.

Raabe, Z. 1963. Systematics of the family Urceolariidae Dujardin, 1841. *Acta Protozoologica* 1, 121-138.

Ralphs, J. R. and Matthews, R. A. 1986. Hepatic microsporidiosis of juvenile grey mullet, *Chelon labrosus* (Risso), due to *Microgemma hepaticus* gen. nov. sp. nov. *Journal of Fish Diseases* 9, 225-242.

Ramasamy, P., Ramalingam, K., Hanna, R. E. B. and Halton, D. W. 1985. Microhabitats of gill parasites (Monogenea and Copepoda) of Teleosts (*Scomberoides* spp). International Journal of Parasitology 15, 385-397.

Rapacz, E., Iversen, E. S. and Feigenbaum, D. 1973. *Zschokkella floridanae* sp. n. (Myxosporidea ) from the goldspotted killifish, *Floridichthys carpio* (Gunther). *Journal of Protozoology* 20, 367-369.

Rawson, Mac V. Jr. 1976. Population biology of parasites of striped mullet, *Mugil cephalus* L. I. Monogenea. *Journal of Fish Diseases* 9, 185-194.

Reda, E. S. A. 1988. An analysis of parasite fauna of bream, *Abramis brama* (L.), in Vistula near Warsazawa in relation to the character of fish habitat. II. Seasonal dynamics of infestation. *Acta Parasitologica Polonica* 33, 35-58.

Roberts, R. J. and Bullock, A. M. 1976. The dermatology of marine teleost fish. II.

Dermatopathology of the integument. *Oceanographic Marine Biology, Annual Review* 14, 227-246.

Robertson, D. A., Roberts, R. J. and Bullock, A. M. 1981. Pathologenesis and autoradiographic studies of the epidermis of salmonids infested with *Ichthyobodo necator* (Henneguy, 1883). *Journal of Fish Diseases* 4, 113-125.

Robertson, O. H., Hane, S., Wexler, B. C. and Rinfret, A. P. 1963. The effect of hydrocortisone on immature rainbow trout (*Salmo gairdnerii*). *General and Comparative Endocrinology* 3, 422-436.

Rogers, W. A. and Gains, J. L. 1975. Lesions of protozoan diseases in fish. In *The Pathology of Fishes*. Ed. by W. E. Ribelin and G. Migaki, vol. 3, pp.117-150. The University of Wisconsin Press, Wisconsin.

Rohde, K. 1976. Marine parasitology in Australia. *Search* 7, 477-482

Rohde, K. 1982. *Ecology of Marine Parasites*. University of Queensland Press. St. Lucia. Londoan. New York.

Rohde, K. 1990. Marine parasites: an Australian perspective. *International Journal of Parasitology* 20, 565-575.

Romny, L. 1986. Ultrastructure, Function, and Classification of microsporidia. *Progression in Parasitology* 1, 325-390.

Roberts, R. J. 1989. *Fish Pathology*. 2nd ed. Bailliere Tindall, London.

Roth, R. R. 1972. Some factors contributing to the development of fungs infection in freshwater fish. *Journal of Wildlife Diseases* 8, 24-28.

Rothwell, J. T. and Langdon, J. S. 1990. Spinal curvature association with *Myxobolus* sp. cysts in the brain of sand-flathead (*Platycephalus bassensis* L). *Journal of Appllied Ichthyology* 6, 244-246.

Roubal, F. R. 1989. Comparative pathology of some monogenean and copepod ectoparasites on the gills of *Acanthopagrus australis* (Family Sparidae). *Journal of Fish Biology* 34, 503-514.

Roubal, F. R., Lester, R. J. G. and Foster, C. K. 1989. Studies on cultured and gill attached *Paramoeba* sp. (Gymnamoebae: Paramoebidae) and the cytopathology of amoebic gill disease in Atlantic salmon, *Salmon salar* L., from Tasmania. *Journal of Fish Disease* 12, 481-492.

Roughley, T. C. 1932. Note on white-spot disease of goldfish. *Proceedings of the Linnean Society of New South Wales* 57, 32-33.

Rowland, S. J. and Ingram, B. A. 1991. Diseases of Australian native freshwater fishes with particular emphasis on the ectoparasitic and fungal diseases of Murray cod (*Maccullochella peeli*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*). *Fisheries Bulletin 4, NSW Fisheries.*

Sanmartin Duran, M. L., Fernandez Casal, J., Tojo, J. L., Santamarina, M. T., Estevez, J. and Ubeira, F. 1991. Trichodina sp.: Effect on the growth of farmed turbot *(Scophthalmus maximus). Bulletin of the European Association of Pathology* 11, 89-91.

Sarkar, N. K. 1982. Myxosporidian *Myxidium haldari* sp. n. (Myxozoa: Myxidiidae) from Indian tree frog *Hyla arborea. Acta Protozoologica* 21, 197-199.

Sarkar, N. K. 1984. A new myxosporidan *Sphaeromyxa hareni* sp. n. (Myxozoa: Myxidiidae) from an Indian marine teleost *Tachysurus Platystomus* (Day). *Acta Protozoologica* 23, 183-186.

Sarkar, N. K. 1986. *Ceratomyxa tartoori* sp. nov. (Myxozoa) from the gall bladder of a marine telest (Clupeidae) of west Bengal, India. *Acta Protozoologica* 25, 351-354.

Sarkar, N. K., Haldar, B. and Chakraborti, R. 1982. *Myxosoma gangulli* sp. n. (Myxozoa: Myxosomatidae) from the head cartilage of *Sillago maculate* Quoy and Gaumard (Sillaginidae). *Acta Protozoologica* 21, 245-249.

Sarkar, N. K. and Mazumder, S. K. 1983. Myxosporidan *Sphaeromyxa dighae* sp. n. (Myxozoan: Myxidiidae) from the gall-bladder of *Hilsa ilisha* (Clupeidae). *Acta Protozoologica* 22, 257-260.

Sarkar, N. K., Mazumder, S. K. and Pramanik, A. 1985. Observations on 4 new species of Myxosporidia (Myxozoa) from channid (Ophicephalid) fishes of West Bengal, India. *Archiv für Protistenkunde* 130, 289-296.

Schafer, W. E. 1968. Studies on the epizootiology of the myxosporidian *Ceratomyxa shasta* Noble. *California Fish Game* 54, 90-99.

Schaudinn, F. 1899. Der Generationwechsel der Coccidien und Haemosporidien, ein Zusammenfassung der neuen Forschungsresultate. *Zoologica Zentrblatt* 6, 765-783.

Schaudinn, F. 1900. Untersuchungen uber den Generationwechsel der Coccidien. *Zoologische Jahrbucher (Morphologie)* 13, 197-292.

Schubert, G. 1966. Zur ultracytologie von *Costia necatrix* Leclerq, unter besonderer berucksichtigung des kinetoplast-mitochondrions. *Zeitschift für Parasitenkunde* 27, 271-286.

Schubert, G. 1968. Elektronenmikroskopische Untersuchungen zur sporenentwicklung von Henneguya pinnae Schubert (Sporozoa, Myxosporidea, Myxobolidae). *Zeitschift für Parasitenkunde* 30, 57-77.

Schubert, G., Sprague, V. and Reinboth, R. 1975. Observations on a new species of *Unicapsula* (Myxosporida ) in the fish *Maena smaris* (L.) by conventional and electronmicroscopy. *Zeitscarift für Parasitenkunde* 46, 245-252.

Shotter, R. A. 1973. A comparison of the parasite fauna of young whiting, *Odontogadus merlangus* (L.) (Gadidae) from an inshore and offshore location of the Isle of Man. *Journal of Fish Biology* 5, 185-195.

Shulman, S. S. 1966. *Myxosporidia of the USSR*. (In Russian). Nauka Publishers, Moscow-Leningrad. (Transl. S. Sharma, 1990. A. A. Balkema/Rotterdam).

Shulman, R. E. 1979. O zakonomernostjakh i faktorakh, obuslovlivajushhikh sezonnuju dinamiku zarazhenija ryb parazitai. In *Ehkologicheskaja i eksperimental'naja parazitologika*. 2, 117-136. Leningrad Byp.

Shulman, R. E., Donets, Z. S. and Podlipaev, S. A. 1978. The use of iodinophilous vacuole in taxonomy of Myxosporidia. *Acta Protozoologica* 17, 133-140.

Sindermanna, C. J. 1966. Disease of marine fishes. *Advanced Marine Biology* 4, 1-8

Sirgel, W. F. 1983. A new ciliate genus *Trichodoxa* n. g. (Ciliata, Peritricha, Mobilina, Trichodinidae) with two new species from the genital system of terrestrial pulmonates. *Journal of Protozoology* 30, 118-125.

Sitja-Bobadilla, A. and Alvarez-Pellitero, P. 1990. *Sphaerospora testicularis* sp. nov. (Myxosporea: Sphaerosporodae) in wild and cultural sea bass, *Dicentrarchus labrax* (L.), from the Spanish Mediterranean area. *Journal of Fish Diseases* 13, 193-203.

Sitja-Bobadilla, A. and Alvarez-Pellitero, P. 1992. Light and electron microscopic description of *Sphaerospora dicentrarchi* n. sp. (Myxosporea: Shaerosporidae) from wild and cultured sea bass, *Dicentrarchus labrax* L. *Journal of Protozoology* 39, 273-281.

Skidmore, J. F. and Tovell, P. W. A. 1972. Toxic effects of zinc sulphate on the gills of rainbow trout. *Water Research Pergamon Press* 6, 217-230.

Solangi, M. A., Overstreet, R. M. and Fournie, J. W. 1982. Effect of low temperature on development of the coccidium *Eimeria funduli* in the Gulf killifish. *Parasitology* 84, 31-39.

Spall, R. D. 1974. A new myxosporidan in red and golden shiners. *Journal of Parasitology* 57, 1302-1306.

Sprague, V. 1965. *Icthyosporidium* Callery and Mesnil, 1905, the name of a genus of fungi or a genus of sporozoans? *Systematic Zoology* 14, 110-114.

Sprague, V. 1970. Taxonomy of microsporidia. *Journal of Parasitology* 56, 327.

Sprague, V. 1977a. Annotated list of species of Microspridia. In *Comparative Patholobiology, vol. 2. Systematics of the Microsporidia.* Ed. by L. A. Bull and T. C. Cheng, pp. 31-334. Plenum Press, New York.

Sprague, V. 1977b. Classification and phylogeny of the Microsporidia. In *Comparative Pathobiology, vol. 2. Systematics of the Microsporidia.* Ed. by L. A. Bull and T. C. Cheng, pp.12-27. Plenum Press, New York.

Sprague, V. and Vernicke, S. H. 1966. Light and electron microscope study of developing stages of *Glugea* sp. (Microsporida) in the stickleback *Apeltes guadracus. American Zoologist* 6, 555-556.

Sprague, V. and Vernicke, S. H. 1968. Light and electron microscope study of a new species of *Glugea* (Microsporidia, Nosematidae) in the 4-spined stickleback *Apeltes quadracus. Journal of Protozoology* 15, 547-571.

Sprague, V. and Vernicke, S. H. 1969. Light and electron microscope observations on *Nosema nelsoni* Sprague, 1950 (Microsporida, Nosematidae) with particular reference to its Golgi complex. *Journal of Protozoology* 16, 264-271.

Sprague, V., Vernicke, S. H. and Lloyd, B. Jr. 1968. The fine structure of *Nosema* sp. Sprague, 1965. (Microsporida, Nosematidae) with particular reference to stages in sporogony. *Jouanal of Invertebrate Pathology* 12, 105-117.

Stehr, C. 1986. Sporogenesis of the myxosporean *Kudo paniformis* Kabata and Whitaker, 1981 infecting the muscle of the Pacific whiting, *Merluccius productus* (Ayres). *Journal of Fish Diseases* 8, 493-504.

Stehr, C. and Whitaker, D. J. 1986. Host-parasite interaction of the myxosporeans *Kudoa paniformis* Kabata and Whitaker, 1981 and *Kudoa thersites* (Gilchrist, 1942) in the mulscle of pacific whiting Merluccius productus (Ayres): an ultrastructural study. *Journal of Fish Diseases* 9, 505-517.

Stein, G. A. 1979. New data on parasitic ciliates (Peritricha, Urceolariidae) of fishes of the basins of Pacific ocean. *Acta Protozoologica* 18, 531-552.

Steinbruck, G. 1990. Recent advances in the study of ciliate genes. *European Journal of Protistology* 26, 2-24.

Stempell, W. 1909. Uber *Nosema bombycis* of microsporidian parasites with sodium dodecyl-sulphate polyacrylamide gel electrophoresis. XII th Annual Meeting of the Society of Invertebrate Pathology, University of Florida. *Abstracts* p. 9.

Stubbs, J. A. 1985. Ecological aspects of hepatic microsporidiosis in coastal waters with particular reference to the host *Taurulus bubalis*. Doctoral Dissertation from National Academic Awards, BLDSC Ref. No. D65503/86.

Stunkard, H. W. and Lux, F. E. 1965. A microsporidium infection of the digestive tract of the winter flounder, *Pseudopleuromectes americanus*. *Biololgy Bulletin* 129, 371-387.

Summerfelt, R. C. and Warner, M. C. 1970. Incidence and intensity of infection of *Plistophora ovariae*, a microsporidian parasite of the golden shiner, *Notemigonus crysoleucas*. Special publication No. 5., pp. 142-160. Washington, D. C., American Fisheries Society.

Supamattaya, K., Fishcher-Scheri, T., Hoffmann, R. W. and Boonyartpalin, S. 1991. *Sphaerospora epinephelli* n. sp. (Myxosporea: Sphaerosporidae) observed in grouper *(Epinephelus malabricus)*. *Journal of Protozoology* 38, 448-454.

Szololosi, D. 1971. Development of *Pleistophora* sp. (Microsporidian) in the eggs of the polychaete *Armandia brevis*. *Journal of Invertebrate Pathology* 18, 1-15.

Takeda, K., Nomura, T., Harada, M. and Sato, A. 1969. *Trichodina* found on reared chum salmon fry. *Fish and Eggs* 1390, 15-18.

Takvorian, P. M. and Cali, A. 1984. A. Seasonal prevalence of the microsporidan, *Glugea stephani* (Hagenmuller), in winter flounder, *Pseudopleuronectes americanus* (Walbaum), from the New York-New Jersey Lower Bay Complex. *Journal of Fish Biology* 24, 655-663.

Thelohan, P. 1889. Sur la constitution des Spores des Myxosporidies. *Compte rendu de l'Academie des sciences* 109, 919-922.

Thelohan, P. 1892. Observations sur les Myxosporidies et essai de classification de ces organismes. *Bulletin de la Societe Philomath que de Paris* 4, 173-174.

Thelohan, P. 1895. Recherches sur les Myxosporidies. *Bulletin Scientifique de la France et da la Belgique* 26, 100-394.

Theoforidea, J., Pujol, P., De Leffemberg, F. N. and Delsol, M. 1981. New cases of testicular prasitic infestation in Amphicians (Bufo, Ptychadena) by Myxosporidia of the Genus *Myxobolus*. *Annales Sciences Naturelles Zoologie et Biologie Animale* 3, 63-68.

Thomas, J. D. 1964a. Studies on populations of helminth parasites in brown trout (*Salmon trutta* L.). *Journal of Animal Ecology* 33, 83-95.

Thomas, J. D. 1964b. A comparison between the helminth burdens of male and female brown trout, *Salmo trutta* L., from a natural population in the River Teify, West Wales. *Parasitology* 54, 263-272.

Timothy, P. Y. and Noble, E. R. 1973. Myxosporida of macrourid fishes from southern California and Mexica. *Journal of Parasitology* 59, 844-850.

Tripathi, Y. R. 1948a. Some new Myxospodidia from Plymouth with a proposed new classification of the order. *Parasitology* 39, 110-118.

Tripathi, Y. R. 1948b. A new species of ciliate, *Trichodina branchicola*, from some fishes at Plymouth. *Journal of the Marine Biological Association of the United Kingdom* 27, 440-450.

T'sui, W. H., Wang, C. H. and Lo, C. F. 1988. On the *Plistophora (Pleistophora)* infection in eel II. The development of *Plistophora anguillarum* in experimentally infected elvers, *Anguilla japonica*. *Bulletin of Institute of Zoology, Academia*

*Sinica, Taiwan* 27, 24-258.

Tuzet, O., Maurand, J., Fize, A., Michel, R. and Fenwick, B. 1971. Proposition d'un nouveaux cadre systematique pour les genres de Microsporidies. *Compte rendu de l'Academie des sciences* 272, 1268-1271.

Udey, L. R., Fryer, J. L. and Pilcher, K. S. 1975. Relation of water temperature to Ceratomyxosis in rainbow trout *(Salmon gairdneri)* and coho salmon *(Oncorhynchus kisutch). Journal of the Fisheries Research Board of Canada* 32, 1545-1551.

Urawa, S. and Arthur, J. R. 1991. First record of the parasitic ciliate *Trichodina truttae* Muller, 1937 on chum salmon fry *(Oncorhynchus keta)* from Japan. *Gyobyo Kenkyu* 26, 83-89.

Urawa, S. 1992. *Trichodina truttae* Mueller, 1937 (Ciliophora: Peritrichida) on juvenile chum salmon (*Oncorhynchus keta*): pathogenicity and host-parasite interaction. *Gyobyo Kenkyu* 27, 29-37.

Uspenskeya, A. V. 1955. The biology and distribution of *Myxosoma cerebralis* (Hofer, 1903) Plehn, 1905 - agent of whirling diseases of trout. *Doklady AN SSSR.* 105, 1132-1135.

Uspenskeya, A. V. 1969. Ultrastructure of some stages of *Myxidium gasterostei. Acta Protozoologica* 7, 79-79.

Uspenskeya, A. V. 1982. New data on the life cycle and biology of Myxosporidia. *Archiv für Protistenkunde* 126, 309-338.

Uspenskaya, A. V. 1984. *Cytology of Myxosporidian.* Nauka, Leningrad.

Van As, J. G. and Basson, L. 1987. Host specificity of trichodinid ectoparasites of freshwater fish. *Parasitology Today* 3, 88-90.

Van As, J. G. and Basson, L. 1989. A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some ectoparasitic trichodinids. *Systematic Parasitology* 14, 157-179.

Van As, J. G. and Basson, L. 1992. Trichodinid ectoparasites (Ciliophora: Peritrichia) of freshwater fishes of the Zambesi River System, with a reappraisal of host specificity. *Systematic Parasitology* 22, 81-109.

Van den Broek, W. L. F. 1979a. Copepod ectoparasites of *Merlangius merlangus* and *Platichthys flesus. Journal of Fish Biology* 14, 371-380.

Van den Broek, W. L. F. 1979b. Infection of estuarine fish populations by *Cryptocotyle lingua* (Creplin). *Journal of Fish Biology* 14, 395-402.

Vavra, J. 1966. Some recent advances in the study of microsporidian spores. In *Proceedings of the First International Congress of Parasitology*. Ed. by A. Chorradetti, pp. 443-444. Pergamon Press, New York.

Vavra, J. 1968. Ultrastructural features of *Caudospora simuli* Weiser (Protozoa, Microsporidia). *Folia Parasitologica (Prague)* 15, 1-9.

Vavra, J., Canning, E. U. and Bauker, R. J. 1981. Characters of microsporidian genera (Workshop Proceedings, EMPO3). *Parasitology* 82, 131-142.

Ventura, M. P. and Paperna, I. 1985. Histopathology of *Myxidium giardi* Cepede, 1906 infection in european eels, *Anguilla anguilla* L., in Portugal. *Aquaculture* 43, 183-187.

Walker, T. M. 1979. A study of sympatry in two species of Palaemoninae. Unpublished Ph. D. Thesis, University of Tasmania. vol. 1

Walkey, M. 1967. The ecology of *Neoechinorhynchus rutili* (Muller). *Journal of Parasitology* 53, 795-804.

Wallengren, H. 1897. Zur Kenntnis der Gattung *Trichodina.* Biologisches *Zentralblatt* 17, 55-65.

Walliker, D. 1968a. Studies on *Myxidium oviforme,* a myxosporidan parasite of Irish salmon, *Salmo salar. Parasitology* 39, 110-118.

Walliker, D. 1968b. The nature of iodinophilous vacuole of myxosporidian spores and a proposal to synonymise the genus *Myxosoma* Thelohan 1892 with the genus *Myxobolus* Butschli, 1882. *Journal of Protozoology* 15, 571-575.

Weider, E. 1972. Ultrastructural study of microsporidian invasion into cells. *Zeitschrift für Parasitenkunde* 40, 227-242.

Weider, E. 1976. Ultrastructure of peripheral zone of a *Glugea* induced xenoma. *Journal of Prtozoology* 23, 234-238.

Weiser, J. 1977. Contributions to the classification of Microsporidia. *Vestnik Ceskoslovenske Spolecnosti Zoologicke* 61, 308-320.

Weiser, J. and Coluzzi, M. 1972. The microsporidian *Plistophora culicus* Weiser, 1946 in different mosquito hosts. *Folia Parasitologica (Prague)* 19, 197-202.

Weissenberg, R. 1911. Uber einige Microsporidian aus Fischen (*Nosema lophii* Dolflein, *Glugea anomala* Moniez, *Glugea hertwigi* nov. spe.). *Sitzunsberichte der Gesellschaft Naturforschender Freunde zu Berlin* 8, 344-351.

Wenyon, C. M. 1926. *Protozoology.* Vol. 1. Balliere, Thidall and Cox, London.

Wierzbicka, J. 1986. *Sphaerospora anguillae* sp. n. (Myxospora, Bivalvulida), a parasite of eel, *Anguilla anguilla* (marine). *Acta Protozoologica* 25, 119-121.

Wierzbicka, J. 1986. *Sphaeospora sphaerocapsularae* sp. n. (Myxospora, Bivalvulida) a parasite of eel, *Anguilla anguilla* (L.). *Acta Protozoologica* 25, 355-358.

Wierzbicka, J. 1987a. *Ceratomyxa americana* n. sp. (Myxospora, Bivalvulida), a parasite of mackerel (*Scomber scombrus* l ). *Acta Ichthyologica et Piscatoria* 17, 93-96.

Wierzbicka, J. 1987b. *Zschokkella stettinensis* sp. nov. (Myxospora: Bivalvulida) a parasite of eel, *Anguilla anguilla* (L). *Acta Protozoologica* 26, 79-82.

Wiles, M. 1968. The occurrence of *Diplozoon paradoxum* Nordmann, 1832. (Trematoda: Monogenea) in certain waters of northern England and its distribution on the gills of certain Cyprinidae. *Parasitology* 58, 71-70.

Williams, C. B. 1964. *Patterns in the Balance of Nature and Related Problems in Quantitative Ecology.* London, New York Academic Press.

Willis, A. G. 1949. On the vegetative forms and life history of *Cholomyxum thyrsites* Gilchrist and its doubtful systematic position. *Australian Journal of Science Research, Series B* 2, 379-398.

Willomitzer, I. 1980. Seasonal dynamics of parasitoses in grasscarp (Ctenopharyngodon idella) fry and fingerlings. *Acta Veterinary (Brno)* 49, 269-277.

Wolf, K. and Markiw, M. E. 1984. Biology controvenes taxonomy in the Myxozoa: new discoveries show alteration of invertebrate and vertebrate host. *Science* 225, 1449-1452.

Wolf, K., Markiw, M. E. and Hiltuen, J. K. 1986. Salmonid whirling disease: *Tubifex tubifex* (Muller) identified as the essential oligochaete in the protozoan life cycle. *Journal of Fish Diseases* 9, 83-85.

Wootten. R. 1974. The spatial distribution of *Dactylogyrus amphibothrium* on the gills of ruffe *Gymnocephalus cernua* and its relation to the relative amounts of water passing over the parts of the gills. *Journal of Helminthology* 48, 167-174.

Woolcock, V. 1936. *Cholomyxum peristiophori*, a new species of myxosporidid parasitic in the gall bladder of *Pristiophorus cirratus* (saw shark). *Parasitology* 28, 72-78.

Yamamoto, T. and Sanders, J. E. 1979. Light and electron microscopic observations of sporogenesis in the myxosporida, *Ceratomyxa shasta* (Noble, 1950). *Journal of Fish Diseases* 2, 411-428.

Zaika, V. E. 1966. On the fauna of protozoan parasites of fishes of the Black Sea. In *Gelmintofauna Shivot Zhivot. Yuzhn. Morey*. Ed. by V. O. Vodianyts'kyi, pp. 13-31. Publishing House of Naukai Dumka, Kiev.

**APPENDIX A Date of occurrence of *Zschokkella leptatherinae* and *Paratrichodina tasmaniensis* in bimonthly samples and in the host fishes of different lengths and sexes**

**Table A-1** Host-length related occurrence of *Zschokkella leptatherinae* in *Atherinosoma microstoma*.

| Range of length (mm) | Total no. fish examined | Observed no. fish infected | Observed no. fish uninfected | Expected no. fish infected | Expected no. fish uninfected | Standardised residuals $r_{sij}$ |
|---|---|---|---|---|---|---|
| 45-54 | 7 | 0 | 7 | 1.16 | 5.84 | -1.19 |
| 55-57 | 8 | 0 | 8 | 3.15 | 15.85 | -1.27 |
| 58-60 | 22 | 0 | 22 | 1.82 | 9.18 | -2.14 * |
| 61-63 | 46 | 5 | 41 | 7.62 | 38.38 | -1.09 |
| 64-66 | 42 | 3 | 39 | 6.96 | 35.04 | -1.72 |
| 67-69 | 69 | 9 | 60 | 11.43 | 57.57 | -0.85 |
| 70-72 | 63 | 9 | 54 | 10.44 | 52.56 | -0.52 |
| 73-75 | 73 | 15 | 58 | 12.09 | 60.91 | -0.99 |
| 76-78 | 68 | 13 | 55 | 11.26 | 56.74 | 0.61 |
| 79-81 | 37 | 8 | 29 | 6.13 | 30.87 | 0.86 |
| 82-84 | 39 | 12 | 27 | 6.46 | 32.54 | 2.49 ** |
| 85-87 | 13 | 6 | 7 | 2.15 | 10.85 | 2.91 ** |
| 88-93 | 8 | 2 | 6 | 1.33 | 6.67 | 0.65 |

* $r_{sij} < -2$, ** $r_{sij} > 2$.

**Table A-2** Host-length related occurrence of *Zschokkella leptatherinae* in *Leptatherina presbyteroides*.

| Range of length (mm) | Observed no. fish examined | Observed no. fish infected | Observed no. fish uninfected | Expected no. fish infected | Expected no. fish uninfected | Standardised residuals $r_{sij}$ |
|---|---|---|---|---|---|---|
| 44-54 | 12 | 0 | 12 | 3.83 | 8.17 | -2.40 * |
| 55-57 | 11 | 1 | 10 | 3.51 | 7.49 | -1.64 |
| 58-60 | 14 | 1 | 13 | 4.47 | 9.53 | -2.02 * |
| 61-63 | 30 | 5 | 25 | 9.57 | 20.43 | -1.85 |
| 64-66 | 54 | 12 | 42 | 17.23 | 36.77 | -1.63 |
| 67-69 | 54 | 12 | 42 | 17.23 | 36.77 | -1.63 |
| 70-72 | 49 | 13 | 36 | 15.64 | 33.36 | -0.86 |
| 73-75 | 47 | 15 | 32 | 15.00 | 32.00 | $7.44 \times 10^{-4}$ |
| 76-78 | 50 | 21 | 29 | 15.96 | 34.04 | 1.62 |
| 79-81 | 37 | 20 | 17 | 11.81 | 25.19 | 3.02 ** |
| 82-84 | 52 | 27 | 25 | 16.59 | 35.41 | 3.22 ** |
| 85-87 | 25 | 13 | 12 | 7.98 | 17.02 | 2.22 ** |
| 88-93 | 10 | 2 | 8 | 3.19 | 6.81 | -0.82 |

**Table A-3** Bimonthly occurrence of *Zschokkella leptatherinae* in female *Atherinosoma microstoma.*

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 91 | 7 | 84 | 14.13 | 76.87 | -2.38* |
| Mar.-Apr. | 111 | 9 | 102 | 17.24 | 93.76 | -2.59* |
| May-Jun. | 44 | 14 | 30 | 6.83 | 37.17 | 3.18** |
| Jul.-Aug. | 38 | 8 | 30 | 5.90 | 32.10 | 0.99 |
| Sep. -Oct. | 22 | 4 | 18 | 3.42 | 18.58 | 0.35 |
| Nov.Dec. | 8 | 2 | 6 | 1.24 | 6.76 | 0.75 |
| (1991) | | | | | | |
| Jan.-Feb. | 21 | 1 | 20 | 3.26 | 17.74 | -1.40 |
| Mar.-Apr. | 32 | 12 | 20 | 4.97 | 27.03 | 3.59** |
| Total | 367 | 57 | 310 | 57.00 | 310.00 | |

**Table A-4** Bimonthly occurrence of *Zschokkella leptatherinae* in male *Atherinosoma microstoma.*

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 16 | 0 | 16 | 3.2 | 12.8 | -2.15* |
| Mar.-Apr. | 5 | 0 | 5 | 1.0 | 4.0 | -1.14 |
| May-Jun. | 12 | 1 | 11 | 2.4 | 9.6 | -1.07 |
| Jul.-Aug. | 24 | 4 | 20 | 4.8 | 19.2 | -0.46 |
| Sep. -Oct. | 3 | 2 | 1 | 0.6 | 2.4 | 2.05** |
| Nov.Dec. | 28 | 4 | 24 | 5.6 | 22.4 | -0.86 |
| (1991) | | | | | | |
| Jan.-Feb. | 9 | 2 | 7 | 1.8 | 7.2 | 0.17 |
| Mar.-Apr. | 23 | 11 | 12 | 4.6 | 18.4 | 3.71** |
| Total | 120 | 24 | 96 | 24.00 | 96.00 | |

**Table A-5** Bimonthly occurrence of *Zschokkella leptatherinae* in female *Leptatherina presbyteroides*.

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 10 | 3 | 7 | 3.09 | 6.91 | -0.06 |
| Mar.-Apr. | 25 | 4 | 21 | 7.71 | 17.29 | -1.69 |
| May-Jun. | 36 | 12 | 24 | 11.11 | 24.89 | 0.35 |
| Jul.-Aug. | 32 | 10 | 22 | 9.88 | 22.12 | 0.05 |
| Sep. -Oct. | 52 | 16 | 36 | 16.05 | 35.95 | -0.02 |
| Nov.Dec. | 38 | 14 | 24 | 11.73 | 26.27 | 0.87 |
| (1991) | | | | | | |
| Jan.-Feb. | 24 | 4 | 20 | 7.41 | 16.59 | -1.58 |
| Mar.-Apr. | 39 | 16 | 23 | 12.04 | 26.96 | 1.49 |
| Total | 256 | 79 | 177 | 79.00 | 177.00 | |

**Table A-6** Bimonthly occurrence of *Zschokkella leptatherinae* in male *Leptatherina presbyteroides*.

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 15 | 3 | 12 | 5.20 | 9.80 | -1.25 |
| Mar.-Apr. | 3 | 3 | 0 | 1.04 | 1.96 | 2.40 ** |
| May-Jun. | 16 | 5 | 11 | 5.55 | 10.45 | -0.30 |
| Jul.-Aug. | 31 | 9 | 22 | 10.74 | 20.26 | -0.73 |
| Sep. -Oct. | 29 | 6 | 23 | 10.05 | 18.95 | -1.73 |
| Nov.Dec. | 29 | 16 | 13 | 10.05 | 18.95 | 2.54 ** |
| (1991) | | | | | | |
| Jan.-Feb. | 21 | 5 | 16 | 7.28 | 13.72 | -1.11 |
| Mar.-Apr. | 32 | 14 | 18 | 11.09 | 20.91 | 1.19 |
| Total | 176 | 61 | 115 | 61.00 | 115.00 | |

**Table A-7** Biomonthly samples of *Atherinosoma microstoma* with the infection of *Zschokkella leptatherinae*.

| Months | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no.fish infected | Expected no.fish uninfected | Standardised residuals $r_{sij}$ |
|---|---|---|---|---|---|---|
| Jan.-Feb.(1990) | 108 | 7 | 101 | 17.89 | 90.11 | -3.19 * |
| Mar.-Apr. | 117 | 9 | 108 | 19.38 | 97.62 | -2.95 * |
| May-Jun. | 56 | 15 | 41 | 9.28 | 46.72 | 2.18 ** |
| Jul.-Aug. | 63 | 12 | 51 | 10.44 | 52.56 | 0.57 |
| Sep.-Oct. | 26 | 6 | 20 | 4.31 | 21.69 | 0.92 |
| Nov.-Dec. | 36 | 6 | 30 | 5.96 | 30.04 | 0.02 |
| Jan.-Feb.(1991) | 31 | 4 | 27 | 5.14 | 25.86 | -0.57 |
| Mar.-Apr. | 57 | 23 | 35 | 9.61 | 48.39 | 5.03 ** |
| Total | 495 | 82 | 413 | 82.00 | 413.00 | |

**Table A-8** Biomonthly samples of *Leptatherina presbyteroides* with the infection of *Zschokkella leptatherinae*.

| Months | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no.fish infected | Expected no.fish uninfected | Standardised resudual $r_{sij}$ |
|---|---|---|---|---|---|---|
| Jan.-Feb.(1990) | 25 | 6 | 19 | 7.98 | 17.02 | -0.87 |
| Mar.-Apr. | 28 | 7 | 21 | 8.93 | 19.07 | -0.81 |
| May-Jun. | 53 | 17 | 36 | 16.91 | 36.09 | -0.03 |
| Jul.-Aug. | 65 | 19 | 46 | 20.74 | 44.26 | -0.50 |
| Sep.-Oct. | 81 | 22 | 59 | 25.85 | 55.15 | -1.01 |
| Nov.-Dec. | 67 | 30 | 37 | 21.38 | 45.62 | 2.45 ** |
| Jan.-Feb.(1991) | 45 | 9 | 36 | 14.36 | 30.64 | 1.81 |
| Mar.-Apr. | 81 | 32 | 49 | 25.85 | 55.15 | 1.62 |
| Total | 445 | 142 | 303 | 142.00 | 303.00 | |

**Table A-9** Host-length related occurrence of *Paratrichodina tasmaniensis* in *Atherinosoma microstoma.*

| Range of length (mm) | Total no. fish examined | Observed no. fish infected | Observed no. fish uninfected | Expected no. fish infected | Expected no. fish uninfected | Standardised residuals $r_{sij}$ |
|---|---|---|---|---|---|---|
| 45-54 | 7 | 1 | 6 | 2.49 | 4.51 | -1.18 |
| 55-57 | 8 | 3 | 5 | 2.84 | 5.16 | 0.12 |
| 58-60 | 22 | 14 | 8 | 7.82 | 14.18 | 2.81 ** |
| 61-63 | 46 | 22 | 24 | 16.36 | 29.64 | 1.83 |
| 64-66 | 42 | 27 | 15 | 14.93 | 27.07 | 4.07 ** |
| 67-69 | 69 | 31 | 38 | 24.53 | 44.47 | 1.75 |
| 70-72 | 63 | 25 | 38 | 22.40 | 40.60 | 0.73 |
| 73-75 | 73 | 20 | 53 | 25.96 | 47.04 | -1.58 |
| 76-78 | 68 | 10 | 58 | 24.18 | 43.82 | -3.87 * |
| 79-81 | 37 | 6 | 31 | 13.16 | 23.84 | -2.55 * |
| 82-84 | 39 | 6 | 33 | 13.87 | 25.13 | -2.74 * |
| 85-87 | 13 | 8 | 5 | 4.62 | 8.38 | 1.98 |
| 88-93 | 8 | 3 | 5 | 2.84 | 5.16 | 0.12 |

**Table A-10** Host-length related occurrence of *Paratrichodina tasmaniensis* in *Leptatherina presbyteroides.*

| Range of length (mm) | Observed no. fish examined | Observed no. fish infected | Observed no. fish uninfected | Expected no. fish infected | Expected no. fish uninfected | Standardised residuals rsij |
|---|---|---|---|---|---|---|
| 44-54 | 12 | 1 | 11 | 2.10 | 9.90 | -0.85 |
| 55-57 | 11 | 1 | 10 | 1.93 | 9.07 | -0.75 |
| 58-60 | 14 | 4 | 10 | 2.45 | 11.55 | 1.10 |
| 61-63 | 30 | 10 | 20 | 5.26 | 24.74 | 2.36 ** |
| 64-66 | 54 | 15 | 39 | 9.47 | 44.53 | 2.11 ** |
| 67-69 | 54 | 19 | 35 | 9.47 | 44.53 | 3.64 ** |
| 70-72 | 49 | 9 | 40 | 8.59 | 40.41 | 0.16 |
| 73-75 | 47 | 4 | 43 | 8.24 | 38.76 | -1.72 |
| 76-78 | 50 | 4 | 46 | 8.76 | 41.24 | -1.88 |
| 79-81 | 37 | 5 | 32 | 6.49 | 30.51 | -0.67 |
| 82-84 | 52 | 2 | 50 | 9.11 | 42.89 | -2.76 * |
| 85-87 | 25 | 3 | 22 | 4.38 | 20.62 | -0.75 |
| 88-93 | 10 | 1 | 9 | 1.75 | 8.25 | -0.63 |

**Table A-11** Biomonthly samples of *Atherinosoma microstoma* with the infestation of *Paratrichodina tasmaniensis.*

| Months | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no.fish infected | Expected no.fish uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| Jan.-Feb.(1990) | 108 | 80 | 28 | 38.40 | 69.60 | 9.46 ** |
| Mar.-Apr. | 117 | 19 | 98 | 41.60 | 75.40 | -4.99 * |
| May-Jun. | 56 | 4 | 52 | 19.91 | 36.09 | -4.72 * |
| Jul.-Aug. | 63 | 8 | 55 | 22.40 | 40.60 | -4.06 * |
| Sep.-Oct. | 26 | 10 | 16 | 9.24 | 16.76 | 0.32 |
| Nov.-Dec. | 36 | 27 | 9 | 12.80 | 23.20 | 5.13 ** |
| Jan.-Feb.(1991) | 31 | 24 | 7 | 11.02 | 19.98 | 5.03 ** |
| Mar.-Apr. | 57 | 4 | 54 | 20.62 | 37.38 | -4.85 * |
| Total | 495 | 176 | 319 | 176.00 | 319.00 | |

**Table A-12** Biomonthly samples of *Leptatherina presbyteroides* with the infestation of *Paratrichodina tasmaniensis.*

| Months | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no.fish infected | Expected no.fish uninfected | Standardised resudual $r_{sij}$ |
|---|---|---|---|---|---|---|
| Jan.-Feb.(1990) | 25 | 11 | 14 | 4.28 | 20.62 | 3.58** |
| Mar.-Apr. | 28 | 5 | 23 | 4.91 | 23.09 | 0.05 |
| May-Jun. | 53 | 1 | 52 | 9.29 | 43.71 | -3.19* |
| Jul.-Aug. | 65 | 2 | 63 | 11.39 | 53.61 | -3.32* |
| Sep.-Oct. | 81 | 10 | 71 | 14.20 | 66.80 | -1.36 |
| Nov.-Dec. | 67 | 29 | 38 | 11.74 | 55.26 | 6.02** |
| Jan.-Feb.(1991) | 45 | 14 | 31 | 7.89 | 37.11 | 2.53** |
| Mar.-Apr. | 81 | 6 | 75 | 14.20 | 66.80 | -2.65* |
| Total | 445 | 78 | 367 | 78.00 | 367.00 | |

**Table A-13** Bimonthly occurrence of *Paratrichodina tasmaniensis* in female *Atherinosoma microstoma*.

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 91 | 70 | 21 | 32.48 | 58.52 | 9.47 ** |
| Mar.-Apr. | 111 | 18 | 93 | 39.62 | 71.38 | -5.13 * |
| May-Jun. | 44 | 4 | 40 | 15.71 | 28.29 | -3.39 * |
| Jul.-Aug. | 38 | 5 | 33 | 13.56 | 24.44 | -3.06 * |
| Sep. -Oct. | 22 | 9 | 13 | 7.85 | 14.15 | 0.53 |
| Nov.Dec. | 8 | 5 | 3 | 2.86 | 5.14 | 1.60 |
| (1991) | | | | | | |
| Jan.-Feb. | 21 | 18 | 3 | 7.50 | 13.50 | 4.93 ** |
| Mar.-Apr. | 32 | 2 | 30 | 11.42 | 20.58 | -3.64 * |
| Total | 367 | 131 | 236 | 131.00 | 236.00 | |

**Table A-14** Bimonthly occurrence of *Paratrichodina tasmaniensis* in male *Atherinosoma microstoma*.

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 16 | 9 | 7 | 5.60 | 10.40 | 1.91 |
| Mar.-Apr. | 5 | 1 | 4 | 1.75 | 3.25 | -0.72 |
| May-Jun. | 12 | 0 | 12 | 4.20 | 7.80 | -2.68 * |
| Jul.-Aug. | 24 | 2 | 22 | 8.40 | 15.60 | -3.06 * |
| Sep. -Oct. | 3 | 1 | 2 | 1.05 | 1.95 | 0.06 |
| Nov.Dec. | 28 | 22 | 6 | 9.80 | 18.20 | 5.52 ** |
| (1991) | | | | | | |
| Jan.-Feb. | 9 | 5 | 4 | 3.15 | 5.85 | 1.34 |
| Mar.-Apr. | 23 | 2 | 21 | 5.05 | 14.95 | -2.94 * |
| Total | 120 | 42 | 78 | 42.00 | 78.00 | |

**Table A-15** Bimonthly occurrence of *Paratrichodina tasmaniensis* in female *Leptatherina presbyteorides*.

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 10 | 4 | 6 | 1.52 | 8.48 | 2.22 ** |
| Mar.-Apr. | 25 | 4 | 21 | 3.81 | 21.19 | 0.11 |
| May-Jun. | 36 | 1 | 35 | 5.48 | 30.52 | -2.24 * |
| Jul.-Aug. | 32 | 0 | 32 | 4.88 | 27.12 | -2.25 * |
| Sep. -Oct. | 52 | 6 | 46 | 7.92 | 44.08 | -0.83 |
| Nov.Dec. | 38 | 16 | 22 | 5.79 | 32.21 | 5.00 ** |
| (1991) | | | | | | |
| Jan.-Feb. | 24 | 7 | 17 | 3.66 | 20.34 | 2.00 ** |
| Mar.-Apr. | 39 | 1 | 38 | 5.94 | 33.06 | -2.39 * |
| Total | 256 | 39 | 217 | 39.00 | 217.00 | |

**Table A-16** Bimonthly occurrence of *Paratrichodina tasmaniensis* in male *Leptatherina presbyteorides*.

| Month | Total no.fish examined | Observed no.fish infected | Observed no.fish uninfected | Expected no. infected | Expected no. uninfected | Standardised residual $r_{sij}$ |
|---|---|---|---|---|---|---|
| (1990) | | | | | | |
| Jan.-Feb. | 15 | 7 | 8 | 3.15 | 11.85 | 2.55 ** |
| Mar.-Apr. | 3 | 1 | 2 | 0.63 | 2.37 | 0.53 |
| May-Jun. | 16 | 0 | 16 | 3.36 | 12.64 | -2.61 * |
| Jul.-Aug. | 31 | 2 | 29 | 6.52 | 24.48 | -2.19 * |
| Sep. -Oct. | 29 | 4 | 25 | 6.10 | 22.90 | -1.05 |
| Nov.Dec. | 29 | 13 | 16 | 6.10 | 22.90 | 3.44 ** |
| (1991) | | | | | | |
| Jan.-Feb. | 21 | 7 | 14 | 4.41 | 16.59 | 1.48 |
| Mar.-Apr. | 32 | 3 | 29 | 6.73 | 25.27 | -1.79 |
| Total | 176 | 37 | 139 | 37.00 | 139.00 | |

**APPENDIX B ANOVA of differences in mean fork length of bimonthly samples of fish infected and uninfected with *Zschokkella leptatherinae***

**Table B-1** Differences in mean fork lengths of infected and uninfected *Atherinosoma microstoma.*

| Month | No. infected fish | No.uninfected fish | DF | ANOVA | p |
|---|---|---|---|---|---|
| Jan.-Feb.1990 | 7 | 101 | 107 | 0.860 | 0.3559 |
| Mar.-Apr. | 9 | 108 | 116 | 4.236 | 0.0412* |
| May Jun. | 15 | 41 | 55 | 1.493 | 0.2270 |
| Jul.-Aug. | 12 | 51 | 62 | 2.967 | 0.0900 |
| Sep.-Oct. | 6 | 20 | 25 | 1.284 | 0.2683 |
| Nov.-Dec. | 6 | 30 | 35 | 3.401 | 0.0739 |
| Jan.-Feb.1991 | 4 | 27 | 30 | 0.126 | 0.7249 |
| Mar.-Apr. | 23 | 35 | 57 | 0.282 | 0.5972 |

**Table B-2** Differences in mean fork lengths of infected and uninfected *Leptatherina presbyteroides.*

| Month | No. infected fish | No.uninfected fish | DF | ANOVA | p |
|---|---|---|---|---|---|
| Jan.-Feb.1990 | 6 | 19 | 24 | 0.046 | 0.8312 |
| Mar.-Apr. | 7 | 21 | 27 | 0.118 | 0.7341 |
| May Jun. | 17 | 36 | 52 | 6.625 | 0.0130** |
| Jul.-Aug. | 19 | 46 | 64 | 12.724 | 0.0006** |
| Sep.-Oct. | 22 | 59 | 80 | 14.694 | 0.0003** |
| Nov.-Dec. | 30 | 37 | 66 | 9.342 | 0.0032** |
| Jan.-Feb.1991 | 9 | 36 | 44 | 0.681 | 0.4138 |
| Mar.-Apr. | 32 | 49 | 80 | 4.320 | 0.0409* |

**APPENDIX C Results of Fisher PLSD test of variations in the intensity of *Paratrichodina tasmaniensis* infestation**

**Table C-1** Variations in the intensity of *Paratrichodina tasmaniensis* infestation between different length groups of *Atherinosoma microstoma*.

| | 55-57 | 58-60 | 61-63 | 64-66 | 367-69 | 70-72 | 73-75 | 76-78 | 79-81 | 82-84 | 85-87 | 88-93 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 45-54 | 2.505 | 2.246 | 22.219 | 2.210 | 2.205 | 2.213 | 2.223 | 2.276 | 2.344 | 2.344 | 2.301 * | 1.505 * |
| 55-57 | | 1.380 | 11.335 | 11.321 | 11.312 | 1.326 | 1.343 | 1.428 | 2.534 | 1.534 | 1.469 * | 1.772 * |
| 58-60 | | | 0.742 | 0.715 | 0.699 | 0.724 | 0.766 | 0.898 | 1.059 | 1.059 | 0.962 * | 1.380 * |
| 61-63 | | | | 0.623 | 00.605 | 0.634 | 0.670 | 0.828 | 0.999 | 0.999 | 0.896 * | 1.335 * |
| 64-66 | | | | | 0.571 | 0.602 | 0.640 | 0.803 | 0.979 | 0.979 | 0.873 * | 1.321 * |
| 67-69 | | | | | | 0.583 | 0.622 | 0.789 | 0.968 | 0.968 | 0.860 * | 1.312 * |
| 70-72 | | | | | | | 0.651 | 0.312 | 0 986 | 0.986 * | 0.881 * | 1.326 * |
| 73-75 | | | | | | | | 0.840 | 1.010 | 1.010 * | 0.908 * | 1.343 * |
| 76-78 | | | | | | | | | 1.120 | 1.120 | 1.029 | 1.428 * |
| 79-81 | | | | | | | | | | 1.253 | 1.172 | 1.534 * |
| 82-84 | | | | | | | | | | | 1.172 | 1.534 * |
| 85-87 | | | | | | | | | | | | 1.469 |

*p < 0.05.

**Table C-2** Variations in the intensity of *Paratrichodina tasmaniensis* infestation between different length groups of *Leptatherina presbyterodies*.

| | 55-57 | 58-60 | 61-63 | 64-66 | 667-69 | 70-72 | 73-75 | 76-78 | 79-81 | 82-84 | 85-87 | 88-93 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 45-54 | 1.797 | 1.421 | 1.333 | 1.321 | 1.304 * | 1.339 | 1.421 | 1.421 | 1.392 * | 1.556 | 1.467 | 1.797 |
| 55-57 | | 1.421 | 1.333 | 1.321 | 1.304 | 1.339 | 1.421 | 1.421 | 1.392 | 1.556 | 1.467 | 1.797 |
| 58-60 | | | 0.752 | 0.715 | 0.699 | 0.764 | 0.899 | 0.899 | 0.852 * | 1.100 | 0.971 | 1.421 |
| 61-63 | | | | 0.519 | 0.496 * | 0.584 | 0.752 | 0.752 | 0.696 * | 0.984 | 0.836 | 1.333 |
| 64-66 | | | | | 0.439 | 0.536 * | 0.715 | 0.715 | 0.656 * | 0.975 | 0.804 | 1.312 |
| 67-69 | | | | | | 0.514 | 0.699 | 0.699 | 0.639 | 0.945 | 0.789 | 1.304 |
| 70-72 | | | | | | | 0.764 | 0.764 | 0.709 | 0.993 | 0.847 | 1.339 |
| 73-75 | | | | | | | | 0.899 | 0.852 | 1.100 | 0.971 | 1.421 |
| 76-78 | | | | | | | | | 0.852 | 1.100 | 0.971 | 1.421 |
| 79-81 | | | | | | | | | | 1.063 | 0.928 | 1.392 |
| 82-84 | | | | | | | | | | | 1.150 | 1.556 |
| 85-87 | | | | | | | | | | | | 1.467 |

**Table C-3** Variations in the intensity of *Paratrichodina tasmaniensis* infestation between bimonthly populations of *Atherinosoma microstoma*.

| | 1990 M.-A. | M-J. | J.-A. | S.-O. | N.-D. | 1991 J.-F. | M.-A. |
|---|---|---|---|---|---|---|---|
| 1990 J.-F. | 0.533* | 1.110* | 0.803* | 0.726 | 0.482 | 0.504* | 1.11* |
| M.-A. | | 1.191 | 0.913 | 0.846* | 0.649 | 0.665 | 1.191 |
| M-J. | | | 1.326 | 1.281* | 1.160* | 1.170 | 1.532 |
| J.-A. | | | | 1.027* | 0.872* | 0.884 | 1.326 |
| S.-O. | | | | | 0.802 | 0.815 | 1.281* |
| N.-D. | | | | | | 0.608 | 1.160 |
| 1991 J.-F. | | | | | | | 1.170 |

**Table C-4** Variations in the intensity of *Paratrichodina tasmaniensis* infestation between bimonthly populations of *Leptatherina presbyteroides*.

| | 1990 M.-A. | M-J. | J.-A. | S.-O. | N.-D. | 1991 J.-F. | M.-A. |
|---|---|---|---|---|---|---|---|
| 1990 J.-F. | 0.682* | 1.321 | 0.972* | 0.553* | 0.448 | 0.509 | 0.642* |
| M.-A. | | 1.385 | 1.058 | 0.693 | 0.612 | 0.659* | 0.766 |
| M-J. | | | 1.549 | 1.326 | 1.286 | 1.309 | 1.366 |
| J.-A. | | | | 0.979 | 0.924* | 0.956* | 1.032 |
| S.-O. | | | | | 0.464 | 0.524 | 0.653 |
| N.-D. | | | | | | 0.412 | 0.567* |
| 1991 J.-F. | | | | | | | 0.617* |