TAXONOMIC STUDIES OF STROPHARIACEAE (AGARICALES) IN SOUTH-EAST TASMANIA

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Declaration

This thesis contains no material which has been accepted for the award of any higher degree or graduate diploma in any tertiary institution and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

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Contents

Abstract

This project is a study of the family Strophariaceae utilizing comparative morphology, electrophoresis of extracellular enzymes and mating compatibility approaches. The study has found macromorphology to be effective in the delineation of taxa in the majority of cases.

Isozyme profiles of extracellular laccase, pectinesterase and polygalacturonase are found to be species distinctive in most species across the genera of this family. The one exception found is *Hypholoma* whose species show a certain degree of conservativeness in the enzymes examined in the study. This approach is found to be useful in establishing putative species groups which can then be further investigated through mating studies.

The multifaceted approach employed in the study has successfully resolved the relationships between *Psilocybe subaeruginosa* and *P. australiana*, *P. eucalypta* and *P. tasmaniana*, identified sibling species in both *Stropharia* and *Pholiota*, and established *Pholiota. squarrosipes* Clel. and *P. multicingulata* Horak as species showing a wide range of morphological variations. It is suggested from this study that *P. multicingulata* sensu lato may encompass *P. multicingulata* Horak and *P. austrospumosa* Hongo forming the southern hemisphere equivalent of *Pholiota spumosa* complex. In addition, a multifaceted approach such as that adopted in the study also lends support to the applicability of the common species concept proposed for Hymenomycetes in the family Strophariaceae.

Results gained have enabled a confident approach to the separation and enumeration of species within the family. Results from electrophoresis show that delineation of taxa can be achieved through distinctions in zymogram patterns. For example, the recently

described species *Psilocybe australiana* Guzmán & Watling, *P. eucalypta* Guzmán & Watling and *P. tasmaniana* Guzmán & Watling are shown to be synonymous with *P. subaeruginosa* Clel. In this particular case, the considerable degree of similarity in zymogram patterns is highly indicative of a single species and this conclusion is supported by mating studies. Diagnostic morphological characters used in previous studies to differentiate these taxa are shown to be invalid.

The phenomenon of sibling species is not uncommon in fungi. Electrophoretic analyses showed that within the genera of *Stropharia* and *Pholiota* sibling species were concealed within common morphological entities. This is exemplified in the *semiglobata*-like forms in *Stropharia*. Within this single morphological entity, two distinct species were identified by comparison of isozyme profiles and UPGMA cluster analysis using band frequencies, later the segregation was confirmed by mating compatibility studies.

The study has confirmed the use of enzyme electrophoresis as a useful technique when applied to a wide range of species across a family from the Agaricales. When this technique is used in conjunction with mating compatibility study, findings from electrophoresis have been complimented and confirmed by those from mating. Thus, the study has avoided some of the problems associated with a purely morphological approach, and as illustrated by the case of *Psilocybe subaeruginosa* the multifaceted approach employed here allows much more confidence to be placed on the described boundaries of the species studied.

31 taxa are delineated from the study, and of these, eight are previously undescribed species and one being a new variety.

Chapter 1

General Introduction

Taxonomy of Strophariaceae

The family Strophariaceae was validated by Singer & Smith (1946). Quélet's concept of the two giant genera *Geophila* and *Dryophila*, untenable under the present rules, has roughly formed the basis for the subfamilial/intergeneric relationships within the family Strophariaceae. Both Romagnesi and Singer (in Kühner 1984) have circumscribed the members of the family Strophariaceae based on the colour of spore print. This is also the basis for the division of the two subfamilies (Stropharioideae and Pholiotoideae) by Singer (1962). The spore print of species in Stropharioideae is predominantly "deep lilac, black lilac or deep fuscous sepia" and for Pholiotoideae "cinnamon brown or deep rusty brown" (*loc. cit.*). Hongo (1960) lowers Singer's subfamilies to the rank of tribus and does not consider the difference in spore print colour worthy of subfamily division.

Kühner (1980) has a much broader concept of this family than Singer. His genera span across Singer's families of Strophariaceae, Coprinaceae, Bolbitiaceae and Cortinariaceae. His giant genus *Psilocybe* is obviously a reflection of Quélet's *Geophila* (Table 1.1). The other black-spored members are from Panaeoleae (a tribe placed by Singer in Coprinaceae). There is a greater representation of brown-spored genera in Strophariaceae s. Kühner. Kühner (*loc. cit.*) has included in Strophariaceae Bolbitieae (Bolbitiaceae reduced to the rank of tribe), and set up new tribes to accommodate the remaining brown-spored genera (Table 1.1). Kühner (1984) also considers it appropriate that *Gymnopilus* (presently placed in Cortinariaceae by Singer because of the warty spores) be included within the Strophariaceae. He has based this conclusion on the presence of styrlpyrone pigments common within the Strophariaceae and also found in *Gymnopilus*. Similar consideration is also given to *Galerina* and

some related genera (*loc. cit.*).

There are divided opinions about the inclusion of brown-spored genera within this family. Watling & Gregory (1987) do not consider *Pholiota* in Strophariaceae or any other brown-spored genera of Kühner but prefer to treat this family in a narrower sense by accepting only the genera in Singer's Strophariodeae (Table 1.1). Jülich (1981) appears to accept a slightly broader concept of the family than Singer by recognizing two gasteroid genera, *Nivatogastrium* and *Weraroa*, the latter is considered a probable ancestor of Strophariaceae by Singer (1958). A synopsis of the various systematic treatments of Strophariaceae is given in Table 1.1.

Singer's concept of the family is followed throughout the study. While accepting Singer's family, concept and name of each genus in the family may not be the same as Singer. Each genus will be discussed accordingly in the appropriate chapter.

The family Strophariaceae has a cosmopolitan distribution, most genera are found in different parts of the world with the exception of *Melanotus* which tends to occur in the subtropical and tropical regions.

The members of this family produce brown to purple-brown to purple black spores. The fruit-bodies are often strongly coloured, mainly in the yellow and brown series, also with tints of red and olive, pholiotoid or crepidotoid. The former being centrally stipitate and the latter eccentric. The pileus may be moist, greasy, viscid to slimy viscid (with an obvious glutinous layer) or dry, hygrophanous or not hygrophanous. Both universal and partial veils may be present. The partial veil when present does not necessarily form a permanent ring but it may be membranous or cortinate. Lamellae (gills) may be adnate or adnexed, sometimes slightly emarginate. Lamellae trama of hyaline or slightly coloured hyphae usually regularly arranged, may become

disorganized with age.

Spores are smooth and often have a germ pore. Spore wall is complex and coloured. Cystidia are found on the face and edge or margin of lamellae. Cystidia of the type called chrysocystidia are found on the gill face, they are embedded and have amorphous contents when mounted in KOH or NH_4OH. Cystidia on the gill face which are not chrysocystidia are generally called pleurocystidia. The gill edge is usually sterile (=heteromorphous) and lined with cheilocystidia. Caulocystidia occur in the apical region of the stipe particularly above the veil line. They may be randomly distributed or in tufts and are quite variable in shape, size and content. Rhizomorphs below the base of stipe may be conspicuous or not and coloured whitish, yellow or brown. Cap cutis (pileipellis) is filamentous forming a trichoderm or with repent hyphae which may be gelatinised, encrusted and pigmented (usually yellow brown). The hypodermium (layer below pileipellis) may be well differentiated into a subcellular layer called the hypodermium (e.g. in *Hypholoma*) or less defined and consists of slightly broader hyphae. Clamp connections are almost always present in the cap cuticle.

Most members in this family are saprophytic. Many of these are lignicolous, and found on rotten logs or stumps or on damaged living tree. Some may also be found on ground litter (e.g. twigs, leafy debris, bark, etc.) whilst others are terrestrial (on dirt or buried wood) or coprophilous (directly on dung or in well manured areas). They can be found in a wide range of habitats, from temperate rainforest to mature mixed forest, wet sclerophyll and even dry sclerophyll. Some species are associated with pasture, burnt sites and others with frequent or occasional disturbance.

Criteria used in fungal taxonomy

Taxonomy of fungi is primarily based on morphological characters in particular the fruiting structures. To the early mycologists these were the only criteria available. In

fact, quite a few of the earlier records of fungi were represented by water colour paintings only. The morphological methods have made use of characters related to vegetative hyphae, sexual and asexual reproductive structures (such as spores, fruiting bodies, sclerotia, sporangia, conidia, etc.). These criteria have been very effective where the characters are distinct and consistent especially in majority of the agarics. The use of such criteria will continue to form the basis and be the major criterion in fungal taxonomy. However, despite the usefulness of morphological characters, there are limitations associated with them and other criteria have to be considered to supplement such inadequacy(ies). Hall (1973) points out that any character of an organism may prove to be useful in taxonomy and that "classification may be viewed as the hypotheses of biological relationships among organisms and may change as we accumulate more information".

There are several situations where morphological characters are inadequate and difficult to use, these include:

(a) where morphology is too simple to discern differences among genetically distinct groups and the few characters available would not allow one to use them with confidence. Or as Hall (1973) puts it "morphological simplicity masks genetic diversity within". This is particularly true in groups that do not produce fruiting structures and delineation of species is based on vegetative characters as in. *Rhizoctonia*, *Sclerotium* and *Penicillium*.

b) where similarities in morphology may not reflect genetic similarity, i.e. taxa which are indistinguishable morphologically may not possess similar genetic material that permits free exchange of genes. This is well illustrated in the phenomenon of sibling species e.g. *Laccaria laccata* complex (Mueller 1991).

c) where diversities in morphology may obscure genetic similarity, e.g. albino forms, colour variation in spore prints within genus or species such as *Camarophyllus* (Singer 1986). This is also illustrated in the phenomena of alternation of generations or pleomorphism, i.e., having more than one independent form or spore stage in the

life cycle.

d) where morphology is highly responsive to environmental conditions. This is particularly so in *Botrytis* (Backhouse *et al.* 1984).

The three problems that confront fungal taxonomists aptly summarized by Garber (1973) are still applicable today. They are:-

"1. the relative paucity of morphological characters which may be unduly plastic in response to environmental factors;

2. populations lacking a demonstrable sexual stage; and

3. a primal urge to lump or split."

This last point is a practice not freely admitted, but is nevertheless a truthful reflection. Differential weighting of characters by individual taxonomists creates confusion and controversy, and as a result the true genetic relationships may not be perceived. In the agarics there are nomenclatural complications that have stemmed from those early inadequate and imprecise descriptions. Also some of the concepts, in particular the species concept (Clémençon 1977), are not properly defined and differences in perception often leads to further confusion. Perhaps today an additional problem that confronts mycologists (or taxonomists) is the enormous build-up of information that has been achieved through the use of a wide array of techniques and the increase of mycological activity on all continents.

Some of the parameters that have been used in correlation to define or separate species in fungi will be discussed briefly below.

Morphology

This forms the basis of morphological species concept which has also been the general species concept for the agarics for many decades. With the advent of numerical taxonomy, multivariate analysis has been employed to aid species delineation in particular closely related species (Sneath & Sokal 1973; Mueller 1991).

Ability to interbreed

This forms the basis for the genetic species concept and when two members interbreed and produce viable offspring, they are referred to as the same "biological species". Incompatibility tests involving tester strains are used to establish the genetic relationships between the tested and tester strains. Many examples can be found in the Hymenomycetes (Macrae 1967; Hoffmann & Esser 1978; Anderson & Ullrich 1979; Eger *et al.* 1979; Boidin 1986; Kile & Watling 1988; Flynn & Miller 1990; Sen 1990 etc.). This criterion will be discussed in greater details in the section on mating incompatibility.

Host specificity

Host specificity is particularly in plant pathogens or other lower fungi.
The term "pathotype" is sometimes used to refer to the various strains (Bosland & Williams 1987) of the same or different taxa.

Ecological preference

This forms the basis for the ecological species concept and includes also host specificity and mycorrhizal associations. The implications from this are significant in the biogeography of the species and thus their evolutionary development.

Chemical or molecular data

Two kinds of molecular data are used, firstly those that are associated with secondary metabolites such as pigments and mycotoxins; and secondly those that are associated with proteins (e.g. total proteins and isozymes) and the more fundamental units of DNA and RNA. The data are obtained through various techniques such as TLC (Thin Layer Chromatography), electrophoresis, isoelectric focusing, serological/immunological responses, DNA/RNA sequencing and RFLP (Restriction Fragment Length Polymorphism) comparisons.

I Secondary metabolites

(a) Colour reaction

Macrochemical colour reaction has been used by agaricologists to differentiate the

various groups of agarics. This involves the simple colour reaction (easily observed with the naked eye) as a result of the application of certain chemicals such as Melzer's iodine to various parts of the carpophore. The main limitation of this technique is the lack of specificity since the chemical (from the fungi) that reacts with the test is often unknown (Benedict 1970).

(b) Pigments

Pigments are known to occur throughout the fungi and have been used as a taxonomic tool (colour) since the inception of mycology. Pigment analysis forms part of the rapidly growing field of chemotaxonomy. A very good review by Gill & Steglich (1987) has obviated the need to review this topic here. There is wide acceptance of this branch of fungal taxonomy since the late 1960s (Tyrrell 1969; Benedict 1970; Steglich 1980; Kühner 1984; Moser 1985). Knowledge about the identities of pigments and their biosynthetic pathways would no doubt help to make better taxonomic decisions in conjunction with other criteria.

(c) Mycotoxins

The screening of secondary metabolites such as mycotoxins through TLC has been proven to be effective in the separation of strains of *Aspergillus* and in particular *Penicillium* (Filtenborg & Frisvad 1980; Filtenborg *et al.* 1983; Frisvad & Filtenborg 1983).

Other compounds that have pharmaceutical and probably taxonomic importance are the indole compounds produced by hallucinogenic mushrooms such as *Psilocybe*, *Panaeolus*, *Conocybe* and *Stropharia* (Benedict *et al.* 1962). A high percentage of *Psilocybe* species, particularly the blueing species, have been reported to produce psilocybin and/or psilocin (Benedict 1970). Psilocybin has been detected in some species of *Conocybe* (Benedict *et al.* 1962) and allegedly *Panaeolus* (Benedict 1970). Baeocystin, a monomethyl analog of psilocybin, has also been detected in some *Psilocybe* species (Margot & Watling 1981).

II Isozymes and DNA/RNA data

(a) Isozymes

Isozyme data have been used by mycologists since the 1960s to complement morphology in species delineation. Most reviews have emphasised the effectiveness of this kind of data to address problems at the population, species and subspecies level (Gottlieb 1977; Crawford 1983; Buth 1984; Hillis 1987; Brown 1990). Other aspects of isozymes will be discussed in greater details in the next section on electrophoresis of isozymes.

(b) DNA/RNA data

DNA and RNA data from different organisms, in particular the comparisons of homologous sequences, provide the most basic genetic information which can be used to address the genetic relatedness and common evolutionary ancestry between organisms. Another advantage is that comparisons are applicable at any level of the taxonomic hierarchy (Hillis 1987). Despite these obvious advantages, the technique has proved too costly for extensive studies. Its application in fungi is still restricted though much work has been done on genera such as *Neurospora*, *Aspergillus*, some yeasts (e.g. in Rayner *et al.* 1987), pathogenic fungi (e.g. Manicom *et al.* 1987; Michelmore & Hulbert 1987; Smith & Anderson 1989; Carder & Barbara 1991), mycorrhizal fungi (e.g. Gardes *et al.* 1991; Marmisse *et al.* 1992) and some agarics (e.g. Pukkila & Cassidy 1987; Weber *et al.* 1986).

Electrophoresis of isozymes as a tool in Fungal Taxonomy

Two general forms of enzymatic proteins are frequently used to generate data through electrophoretic methods. They are firstly, isozymes which are functionally similar forms of enzymes coded by different genetic loci or different alleles at the same locus; and secondly, allozymes which are variant isozymes coded by different alleles at the same genetic locus (Murphy *et al.* 1990). When subjected to electrophoresis these proteins can then be visualized as bands on the supporting medium by using specific

staining techniques. The two supporting media most frequently used in electrophoretic methods are starch and polyacrylamide gels.

Recent reviews on various aspects of the technique and its applicability can be found in Shaw & Prasad (1970), Harris & Hopkinson (1976), Gottlieb (1977 & 1981), Bisby *et al.* (1980), Buth (1984), Brown (1990) and Hillis & Moritz (1990).

Both systematic and phylogenetic inferences made from observations and comparisons of phenotypes are based on the assumption that the phenotypic differences are genetically determined. Crawford (1983) points out that differences in the electrophoretic mobility of enzymes are the direct result of genetic differences. Isozymes can thus be treated as phenetic characters and this allows the data to be applicable in the phenotypic species concept. Furthermore, electrophoretic banding patterns or isozyme profiles are frequently predictable and reliable (Micales *et al.* 1986). Through direct comparison of banding patterns or other genetic analyses valuable evidence and useful information can be obtained to make systematic or phylogenetic inferences (Garber 1973; Cruickshank 1989; Stasz *et al.* 1989).

Electrophoretic data from allozyme studies provide information in the assessment of population genetics (e.g. gene flow) and also in the exploration of phylogenetic relationships at intrageneric or intergeneric level (Murphy *et al.* 1990). Isozyme studies are frequently used to clarify interspecific relationships especially in the clarification and delineation of species (Micales *et al.* 1986). Much work using isozyme electrophoresis has been done in human (Harris & Hopkinson 1976), animal (Avise 1974; Ayala 1983) and vascular plant genetics (Gottlieb 1977).

Electrophoresis as a tool in fungal taxonomy has expanded since the 1960s and its application is becoming more and more a routine practice. Due to practical importance

much work has been carried out on plant pathogens (e.g. Hall *et al.* 1969; Reddy & Stahmann 1972; Wong & Willets 1973 & 1975; Bonde *et al.* 1984;), medically important fungi (e.g. Schechter *et al.* 1972), commercially important fungi (e.g. Blaich & Esser 1975; Blaich 1977; Royse & May 1982a & 1982b; Kerrigan & Ross 1988 & 1989; Itävaara 1988) and mycorrhizal fungi (e.g. Seviour *et al.* 1972; Ho & Trappe 1987; Sen 1990). Relatively less work is done on the non-commercial saprophytic fungi which form an essential part in the process of energy cycling. As mentioned earlier, many workers have pointed out the usefulness of the technique in separating species and examining the genetic variations at population or subspecies level. Successful applications have been reported for various fungi (Chang *et al.* 1962; Clare *et al.* 1968; Wong & Willets 1973 & 1975; Wasfy *et al.* 1978; Cruickshank 1983; Cruickshank and Pitt 1987; Kaosiri & Zentmyer 1980; Backhouse *et al.* 1984; Sweetingham *et al.* 1986; Neate *et al.* 1988).

Mating Incompatibility as an aid in species delineation

The mating system of most species of Hymenomycetes has been found to be governed by homogenic or heterogenic incompatibility systems (Whitehouse 1949; Petersen 1992). Mating behaviour relating to such systems is found to be controlled by a single locus or two unlinked loci (Koltin *et al.* 1972), the former is called bipolar or unifactorial mating system and the latter tetrapolar or bifactorial. Comprehensive reviews of the sexual mechanism in fungi are given by Whitehouse (1949), Raper (1966), Esser (1966); Koltin *et al.* (1972) and Carlile (1987). Kniep in 1922 (in Whitehouse 1949) first demonstrated the presence of multiple allelomorphs in *Schizophyllum commune*. Later work (Mounce & Macrae 1938; Neuhauser & Gilbertson 1971) show that this phenomenon also occur in other bipolar and tetrapolar species.

Of the two systems mentioned above, the bifactorial or tetrapolar system is more

prevalent in the heterothallic forms studied (Whitehouse 1949). In theory, the unifactorial or bipolar system allows 50% inbreeding among siblings and only 25% in the bifactorial system. Koltin *et al.* (1972) illustrated through the sexual mechanism of *Schizophyllum commune* that bifactorial or tetrapolar system is more efficient in controlling inbreeding than the unifactorial or bipolar system. Whilst the unifactorial system can reach a higher outbreeding potential with a fewer number of alleles than the bifactorial system with single locus at each factor; the addition of an extra locus at each factor in this latter system would lead to a high outbreeding potential with a relatively small number of alleles (*loc. cit.*). Thus, both the unifactorial and two-locus bifactorial systems demonstrate a high efficiency in outbreeding whereas the bifactorial system is more efficient in the restriction of inbreeding. The breeding system is therefore a primary factor in determining the success and survival of a species.

The determination of compatibility in mating studies differs widely in the different groups of fungi (Esser & Blaich 1973). It is generally accepted for the Agaricales that the formation of clamp connections is a reliable indication of successful mating. However, Boidin (1986) points out that the presence of clamp connections "is not a sign of absolute interfertility". He argues that to be interfertile it is necessary to show that nuclear fusion, meiosis, basidiospore formation and basidiospore germination take place to demonstrate the viability of the progeny. One major problem in using mating incompatibility in speciation studies is the difficulty to induce basidiocarp formation in the laboratory. Therefore, there is much reliance on the nuclear state of the mated thalli and the presence of clamp connections. For this reason, Boidin prefers the term "intercompatible". Similarly, absence of clamps does not correspond to absolute intersterility and therefore it is more appropriate to use the term "interincompatible" (Boidin *loc. cit.*). However, terminology such as interfertility and intersterility is being widely used by workers in speciation studies. On a broader sense these two sets of terminology are interchangeable. Boidin's terminology however does seem to allow for more latitude in the understanding of these genetic systems.

To complicate matters further, partial or incomplete incompatibility has been noted in some groups of fungi (Hallenberg 1984; Brasier 1987). For example, in the Corticiaceae, partial incompatibility is observed between North American and European specimens of *Gloeocystidiellum porosum* as well as within the North American specimens (Hallenberg 1984). Mating studies have revealed seven intersterility groups within *Armillaria* in Europe, ten in North America (Korhonen 1987) and five in the Australasian region (Kile & Watling 1988). *Heterobasidion annosum* is another extensively studied taxon which has several intersterility groups as well as exhibiting partial compatibility (Korhonen 1987). The significance of these findings is that active speciation is in process in these taxa in particular *H. annosum*. They are therefore "ideal raw material for the study of speciation" (Brasier 1987).

The use of mating incompatibility to investigate species affinity has been carried out for various genera in the Hymenomycetes (Macrae 1967; Kemp 1975; Boidin 1977; Fries 1985; Hallenberg 1984 & 1985; Kile & Watling 1988; Petersen 1992). Findings from such studies have resulted in:

(a) the confirmation of conspecificity of the species concerned;

(b) the reduction to synonymy of species (e.g. Farr *et al.* 1977);

(c) the new placement of misplaced species (e.g. Ginns 1985); and

(d) the enrichment of information regarding the sexual mechanism of the taxa studied.

In addition to these, results from such studies also raise new queries which hopefully will inspire new studies to better understand the sexual mechanism and therefore speciation in higher fungi.

The utilization of a combination of different approaches to facilitate species delineation is becoming more frequent. Various studies have thus clarified or resolved some

confusion up till now exists in certain groups or 'species complex' (Edwards & Kennedy 1973; Farr *et al.* 1977; Eger *et al.* 1979; Bosland & Williams 1987; Flynn & Miller 1990; Chang & Mills 1992).

Species concepts in Agaricales

Various species concepts have been employed by mycologists to define species in the Agaricales. The two most common ones are morphological and biological species concepts. Debate about these species concepts has focused on their applicability (Clémençon 1977).

In the morphological species concept species are delimited by morphological characters descriptive of the basidiomes. This species concept has been the traditional approach to species delineation in the agarics. As mentioned earlier, limitations in the morphological approach prevent satisfactory application in some cases. Thus agaricologists have resorted to alternative phenotypic characters which may be cultural, isozymic, chemical, ecological, physiological or indeed any character of the phenotype (Jacobsson 1989; Micales *et al.* 1986; Moser 1985). The morphological species concept is often used in the documentation of flora (Kuyper 1988) and is synonymous with the term 'taxonomic species' (Radford 1986).

Mayr's (in Parmasto 1985) biological species concept defines species as "groups of interbreeding natural populations that are reproductively isolated from other such groups". Because of the exchange of genetic material it is also alternatively referred to as the 'genetic species concept' (Kuyper 1988). The application of this concept has been criticized as non-operational due to the difficulty in obtaining cultures in some and in others the difficulty in establishing genetic isolation as well as its inapplicability in uniparental organisms (*loc. cit.*).

Both concepts are widely applied in the studies of Agaricales. Discussions of the various relationships between morphological and biological species can be found in Burnett (1983), Boidin (1986), and Kuyper (1988). In some studies of agarics, there is complete congruence between the two concepts. While in others comparisons have resulted in the delineation of more than one intersterility groups. These morphologically similar but non-breeding populations are also referred to as "sibling species" (Burnett 1983).

The application of species concept becomes a problem when the criteria used to define it fail to separate or group the taxa in a convincing and meaningful manner. This leads to the search for alternative species concept, for example, Parmasto (1985) elected to use the "selectional species concept" proposed by Slobodchikoff in the taxonomy of Hymenochaetaceae. Or alternatively to develop and use new characters in systematics problem (Kohn 1992).

In applying a species concept, there is a need to first define the criteria used in a species concept before application. For the purpose of this study, the common species concept proposed in Kühner (1977) is adopted which encompass both the morphological and biological species concepts and is defined as follows:
"Populations belong to the same species when they are able to interbreed and produce viable offspring, provided that an absence of this interfertility is caused only by those genetic parameters operating in the entire sexual cycle".

A more practical definition of the common concept of species is also applicable:-
"A species is a population which possesses constant reproducible characters (morphological etc.) and for which a hiatus exists between this and other populations" (*loc. cit.*).

History of fungal taxonomy in Tasmania

There has been very little systematic study on the agaric flora in Tasmania. From mid 1830s to mid 1850s, resident collectors such as Gunn, Lawrence and Archer sent fungal material to M. J. Berkeley for study and identification (May 1990). Cooke's *Handbook of Australian fungi* published in 1892 was a comprehensive account, albeit somewhat unsatisfactory (Ainsworth 1976 & Reid 1980). Around the turn of the century, the government botanist, Leonard Rodway, sent much material to G. Massee of Kew who named and described many new species (Massee 1898 & 1899). Massee also coauthored with Rodway in the naming of new species (Massee 1901.), later Rodway named and described more new species, in particular Gasteromycetes and Ascomycetes (Rodway 1918; 1920; 1921). He also collaborated with J. B. Cleland in naming new species of *Poria* (Cleland & Rodway 1929 & Rodway & Cleland 1930).

Since Rodway very little work was done on the agaric flora in Tasmania. Pegler (1965) & Horak (1971 & 1983) have revised the earlier work on Australasian agarics. Guzmán & Watling (1978) review the genus *Psilocybe* in Australia and a recent study (Chang & Mills 1992) examines the relationships of *Psilocybe subaeruginosa* Clel. with closely related species. In his monograph of the New Zealand Hygrophoraceae, Horak (1990) includes a list of the Australian species as well. Other more recent studies include Kile's work on *Armillaria* (Kile & Watling 1983 & 1988), Honours projects at the University of Tasmania by Priest on *Agaricus* (1980), Browne on the genus *Hypholoma* (1983) and Monks on the Hygrophoraceae (1989; Monks & Mills 1991). Reid (1980) compiled a monograph of the genus *Amanita* in Australia which included some of the Tasmanian representatives. The field key of *Mycena* by Grgurinovic & Holland (1982) is also applicable to some Tasmanian species of that genus. Eygelsheim (1981), an amateur naturalist, has published a booklet on Tasmanian fungi which is mainly based on older European literature. Fuhrer's (1985) "A Field Companion to Australian Fungi" mentioned the occurrence of some of the agaric flora in Tasmania and a more recent

publication (Fuhrer & Robinson 1992) is a photographic record of some of the fungal flora in the rainforests of Tasmania and south-east Australia.

Scope of study

The strophariaceous flora of Tamania is largely unknown. Except for the more well known species the extent of species number in each genera of this family has not been documented for Tasmania and for Australia only a very rough estimate is given in Shepherd & Totterdell (1988).

The main objective of this study is therefore to find out which species belonging to Strophariaceae occur in south-east Tasmania and set up identification keys for each genera.

As a result of the paucity of information on the Tasmanian flora and the problems within morphological taxa in well studied genera of northern hemisphere (e.g. Farr *et al.* 1977), it is considered appropriate to initially delineate species in this family on morphological criteria. A multifaceted approach involving a synthesis of phenotypic and interbreeding relationships is adopted in cases which failed to be thus delineated in a convincing manner. This will be conducted in two stages. Firstly, to facilitate delineation in addition to the morphological parameters by the use of comparisons of isozyme data obtained from electrophoresis of extracellular enzymes. Secondly, to investigate the interbreeding relationships in the taxa concerned. This study also hopes to investigate how effective isozymes (in particular the comparison of isozyme profiles) are in supplementing the morphological criteria in species delineation.

Table 1.1. A synopsis of systematic treatments of Strophariaceae.

Singer (1986)

Strophariaceae

subfamily: Strophariodeae

genera: Stropharia, Hypholoma, Psilocybe, Melanotus

subfamily: Pholiotoideae

genera: Pholiota, Kuehneromyces, Pachylepyrium, Pleuroflammula & Phaeomarasmius

Kühner (1980)

Strophariaceae

tribus: Crepidoteae

genus: Crepidotus

tribus: Tubarieae

genera: Naucoria, Galerina, Phaeocollybia

tribus: Gymnopileae

genus: Gymnopilus

tribus: Pholioteae

genera: Pholiota (*subgenera* Kuehneromyces, Hemipholiota, Flavidula, Pholiota & Lubricula), Psilocybe (*subgenera* Psilocybe [including Deconica & Melanotus], Stropharia & Hypholoma)

tribus: Bolbitieae

genera: Agrocybe (*subgenera* Simocybe & Agrocybe), Conocybe (*subgenera* Pholiotina & Conocybe)

tribus: Panaeoleae.

genus: Panaeolus (*subgenera* Anellaria & Panaeolus)

Watling & Gregory (1987)

Strophariaceae

genera: Hypholoma, Melanotus, Psilocybe & Stropharia.

Chapter 2

Materials and Methods

Specimens were collected mainly from SE Tasmania with a few from NW Tasmania near Smithton, NE Tasmania near Scottsdale and from type localities whenever possible. Comparisons were made with reliably identified and/or type material (see under Specimens examined for citation of type specimens). Dried specimens were lodged at the Tasmanian Herbarium (HO), duplicates of some collections were lodged at Royal Botanic Garden, Edinburgh (E) and Biology Branch Herbarium of New South Wales Department of Agriculture and Fisheries, in Rydalmere, NSW (DAR). All cultures utilised in the study have been lodged at DAR. Abbreviations for herbaria followed the Index Herbariorum (Holmgren *et al.* 1981).

Pure cultures were obtained from fresh spore deposits or tissues of fresh specimens whenever possible. The non-quantitative dilution method used to obtain monosporous isolates was similar to that used by Farr *et al.* (1977). All isolates used in the study were checked microscopically for the absence of clamps. All stock cultures were maintained on 2% malt extract agar (MA) incubated at 20°C in the dark and then stored at 4°C. Wild isolates were obtained from various parts of the basidiome including the pileus and stipe tissues. An optimal number of 8-10 isolates would be desirable for each collection, however, this was not always possible. Thus, when spores of a particular collection germinated readily, a relatively large number of monosporous isolates were obtained.

Morphological studies

All specimens were identified to the genus and whenever possible species level using existing keys (Smith and Hesler 1968; Cleland 1934 [*Pholiota*]; Guzmán 1983; Watling & Gregory 1987; Cleland 1934 [*Psilocybe*]; Smith 1951; Cleland 1934 [*Hypholoma*];

Horak 1977 [*Melanotus*]; Watling 1973; Arora 1986 [general]). All specimens identified to the same species were referred to as a putative group.

Standard procedures (Singer 1986; Guzmán 1983; Smith & Hesler 1968) were followed for the examination of macroscopic and microscopic characters of both fresh and dried material. Fresh material was used in the examination of macroscopic characters, and collector's field notes (when present) were taken into consideration in the case of herbarium material. For each collection, all specimens were included in the observations of macroscopic characters such as colour and diameter of the pileus, length and width of the stipe etc. Fig 2.1 shows a diagramatic representation of the measurements included for the macroscopic characters. 10% ammonia or 5% potassium hydroxide (KOH) solution was used for the examination of microscopic characters of fresh and dried specimens, in particular, the cutis of the pileus. One basidiome from each collection was used for the measurement of microscopic characters and the four main microscopic characters were spores, basidia, pleurocystidia (when present), cheilocystidia and caulocystidia (when present). The usage of terminology of pleurocystidia, cheilocystidia and caulocystidia was based on Buller's (in Singer 1986) and were specified as follows:

a) pleurocystidia were cystidia that occurred on the gill face and at times were also referred to as facial cystidia;

b) cheilocystidia were cystidia that were found on the gill edge; and

c) caulocystidia were cystidia found on the stipe, in particular above the annulus or velar zone.

The distinction between these three kinds of cystidia was very clear throughout Strophariaceae. Chrysocystidia were one kind of pleurocystidia, usually embedded, fusoid ventricose in shape with contents varying from homogeneous pale or intense yellow to highly granular yellow brown or with a highly refractive amorphous body. These definitions were strictly adhered to when referring to such cell types.

Spore length was measured from the base of the apiculus (i.e. excluding the apiculus) to the apex, width in both face and side view was measured for the broadest part of the spore. Basidia length was measured from the base of the basidium to the base of the sterigmata and width was taken from the broadest part. Fig 2.2 shows a diagramatic representation of the measurements involved in these two microscopic characters. Similarly, length was measured from the apex to the base and width from the broadest part for both pleurocystidia and cheilocystidia. Spore measurements were taken from spore prints (air dried) or when this was not available, they were taken from tangential section of the stipe particularly near the velar zone. Mean values were based on measurements of 25 for spores and 10 each for pleurocystidia, cheilocystidia and basidia. In some cases, spore quotient, i.e. ratio of spore length to spore width, was used to compare the shapes of spores (both face and side view) between taxa. The range as specified by Bas (1969) was followed. The colour description and codes were according to Methuen (Kornerup & Wanscher 1978).

The resulting data matrices of spore and cystidia variables (all in the same units) were at first submitted to normality tests. After appropriate transformation they were then submitted to Canonical Discriminant Analysis (=CDA) using the CANDISC subprogramme of SAS (SAS Institue Inc 1988) separately to produce scattergrams based on the mean canonical variates (CV) generated from the analysis. The first axis (i.e. X-axis) in the scattergram accounted for the greatest variation and the second axis (i.e. Y-axis) explained the next largest portion of variation. All the mean canonical variates (MCV) were then used in an Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) (Sneath & Sokal 1973) and cluster analysis using the CLUSTER subprogramme of SAS (SAS Institue Inc 1988) to give a hierarchical cluster.

For groups which were not identified conclusively based on the morphological

characters, electrophoretic and mating compatibility studies were conducted. Results from these studies were used to confirm or revise the initial identification.

Electrophoretic studies

Preliminary trials were conducted to assist in the selection of extracellular enzyme systems for electrophoretic studies and to determine the effect of physiological age on the production of enzymes. The enzymes included in the preliminary trials included amylase, esterase, rRNase, laccase, peroxidase, pectinesterase, polygalacturonase and pectinlyase.

Cruickshank and Wade (1980) have associated yellow stained bands with pectinlyase activities. Karlsson and Stenlid (1991) also found these yellow bands of 'pectinlyase' but for reasons of variable behaviour excluded them from their final analysis. Further investigations into the enzymic nature of these bands revealed that they were non-enzymic and could be mimicked to a large extent by organic acids. Thus, extreme care should be exercised when interpreting these 'pectinlyase' activities. Cruickshank (per. comm.) has been consulted during the course of the investigation and agrees with this caution. For the present study, "pectinlyases" were excluded.

Four extracellular enzyme systems were initially selected based on results from preliminary trials. These were laccase , peroxidase, pectinesterase and polygalacturonase. In addition to these four enzyme systems , acid phosphatase was later included as an aid in the distinction of taxa in the genera *Psilocybe*and *Strophariaand Melanotus*.

For the production of enzymes, cultures were grown in loosely capped 5 ml Bijou bottles, each containing 2ml of growth medium autoclaved at 121°C for 15 min and incubated at 20°C stationary in the dark. To accomplish this, isolates from stock cultures were transferred onto fresh MA plates and incubated at 20°C for 5 -7 d or longer

in the case of slow growing strains. Discs of 8mm in diameter were cut from the actively growing edge of the colony and transferred to the selected growth medium.

For each putative group separated on the basis of morphological characters, the collection with the greatest number of isolates was used to establish a preliminary estimate of the range of internal variations for each enzyme system. Whenever possible, thereafter, all available isolates from other collections in the same or different putative group(s) were used when making comparisons.

For laccase production, the growth medium consisted of 0.05% gallic acid in malate buffer (pH 4.0) (Cruickshank per. comm.). Cultures were incubated at 20°C, stationary in the dark for 3 d. Because of the short time required laccase zymograms were used for the initial separation of species in addition to the morphological characters.

For pectic enzymes, a modified growth medium based on that used by Cruickshank and Pitt (1987) contained KH_2PO_4, 1.0g, $(NH_4)_2HPO_4$, 2.0g, NH_4NO_3, 0.6 g, $MgSO_4.7H_2O$, 0.1 g, KCl, 0.5 g, citrus pectin (Sigma P 9135), 10.0 g per litre of deionised water, with pH adjusted to 6.0 with 1N HCl. The cultures were incubated at 20°C, stationary in the dark for 12 d. for isolates in the genus *Pholiota*, and 14 d. for the other genera in the family.

For peroxidase and acid phosphatase production, the medium was a 20% potato decoction (20 g. chopped potato/100 ml deionised water, simmered for 1.5 h. and then sieved through 2 layers of muslin). The cultures were incubated at 20°C, stationary in the dark for 10 d.

A summary of the growth media and conditions for the production of these enzymes is presented in Table 2.1

Polyacrylamide electrophoresis was performed in horizontal slab gels after the system of Cruickshank and Pitt (1987).

Pectic enzymes were examined by the method of Cruickshank and Wade (1980). Laccase and peroxidase were examined by the method of Mills and Crowden (1968). In these two oxidase enzyme systems, to increase the resolution of the bands, gels were stained for 30 min. at room temperature. or with visual detection of bands and then a few hours to overnight at 4°C. Acid phosphatase was examined by the method of Ho and Trappe (1987). A summary of the extracellular enzyme systems and stain recipes used in the electrophoretic studies is listed in Table 2.2.

The bands were scored directly from the gel placed on a light table as well as from photo records. Photo records of gels were prepared by contact printing under water onto high contrast (No. 5) Ilfoprint paper.

Isozymes were analysed as phenetic characters. Bands showing similar mobilities within each enzyme system between different isolates were considered as the same character. UPGMA (Sneath & Sokal 1973) cluster analysis (using a SAS CLUSTER subprogram) was performed, based on the band frequency data for all the enzyme systems to produce a dendogram. Very faint bands were excluded and other exclusions might be decided in individual cases.

Mating compatibility studies

For each putative group (Refer to Morphological Studies) that was separated , whenever possible, at least eight isolates from one collection belonging to the group were paired in all possible combinations to determine the mating types. The mating types were used as tester strains to cross with monokaryons from other collections in the same putative group. In some cases, monokaryotic isolates from a clearly separate taxon in the same

genus were included in the crosses for comparison. The majority of crosses or confrontations were mon-mon pairings. Di-mon pairing was performed when wild isolate was the only available isolate of that collection. In relatively few cases, dikaryotic isolate resulted from multisporous germination was also used. The methodology described by Macrae (1967) was followed in the mating compatibility studies.

For each confrontation, two strains were scored as compatible when clamps were noted in the boundary zone between the two inocula and incompatible where clamps were absent. Observations on the reaction between the two inocula were also noted, such as barrage or pigment production. Boidin's (1986) terminology was used in results and discussion.

Fruiting trials

Of the morphologically distinct taxa, isolates from collections of *P. aurivella* were used to illustrate the species concept (Clémençon 1977) to the stage of producing viable offspring under more or less controlled laboratory conditions. The viability of offspring was judged by the ability of the synthesized dikaryotic mycelium to produce basidiospores which would germinate under the same conditions as the spores from the parental basidiome.

The compatible crosses from the mating studies between the tester strains of CYS159 and isolates of the remaining four collections were used to inoculate the sterilised fruiting medium (See Appendix I for components of the fruiting medium). After allowing the dikaryotic cultures to grow right through (~ 4 - 6 weeks) the medium at day temperature of 18°C and night temperature of 10°C, the medium was removed from the plastic bag and transferred to a humid chamber (R.H. = ~75 - 85%) with similar day/night temperature range under diffused fluorescent light (~8hr.).

Spores from the F1 basidiomes were collected and plated onto fresh MA plates and incubated at 20°C the same conditions the parental spores were subjected to.

In addtion to the above trial, any occasional fruiting in dikaryotic mycelia that happened under laboratory conditions would also be noted.

Results of the fruiting trials are given in Appendix II.

Table 2.1 Extracellular enzyme systems and stain recipes used in the electrophoretic study.

Enzyme	Gel pH	Stain recipe	Reference
Laccase	7.4	0.025 g O-dianisdine in 100 ml. acetate buffer pH 5.0.	Mills & Crowden 1969.
Peroxidase	8.7	0.025 g O-dianisdine in 100 ml. acetate buffer pH 5.0 and 3 drops of H_2O_2	Mills & Crowden 1969.
Acid Phosphatase	8.7	0.1 g each of ∂-naphthyl acid phosphate Na salt & fast garnet GBC salt in 100 ml. acetate buffer pH 4.0	Ho & Trappe 1987.
Pectinesterase & polygalacturonase	8.7	0.1M DL malic acid incubation buffer & 0.02% ruthenium red staining soln.	Cruickshank & Wade 1980 & Cruickshank per. comm.

Table 2.2 Summary of culture media, growth conditions, enzyme extract for the genera investigated in electrophoretic study.

Genus	Enzyme tested	Culture medium	Growth conditions
Pholiota *Hypholoma* *Stropharia* *Psilocybe* *Melanotus*	Laccase	0.05% gallic acid in malate buffer pH 4.0.	3 d, 20°C, stationary, dark.
Pholiota *Hypholoma* *Stropharia* *Psilocybe* *Melanotus*	Peroxidase, acid phosphatase	20% potato decoction	10 d, 20°C, stationary, dark.
Pholiota *Hypholoma* *Stropharia* *Psilocybe* *Melanotus*	Pectinesterase & polygalacturonase	1% pectin medium	12 d, 20°C, stationary, dark. 14 d, 20°C, stationary, dark.

The enzyme extract used was a slurry of 50 µl. of culture fluid mixed with approx. 2 mg. of sephadex G-150 superfine.

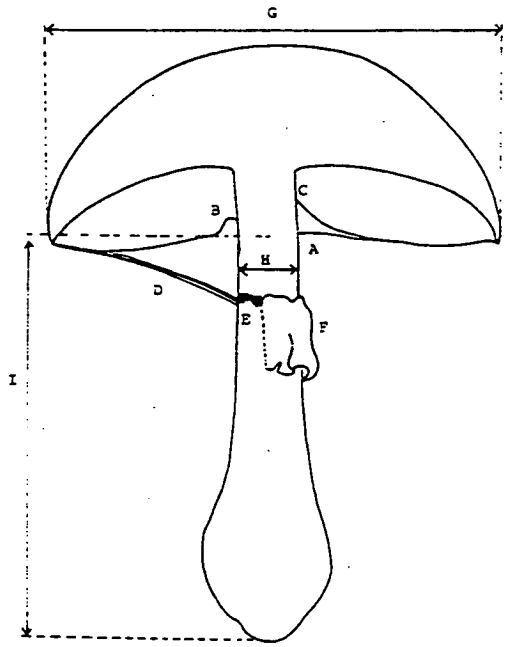

Fig. 2.1. Diagramatic representation of the macroscopic characters observed or measured for morphological studies. Legend: A - C, lamellae attachment; A= adnate, B= adnate with a tooth, C= adnexed. D - F, velar characters; D= cortina, E= velar remnants forming a fibrillose zone, F= membranous veil forming an annulus. G= diameter of pileus, H= width of stipe and I= length of stipe.

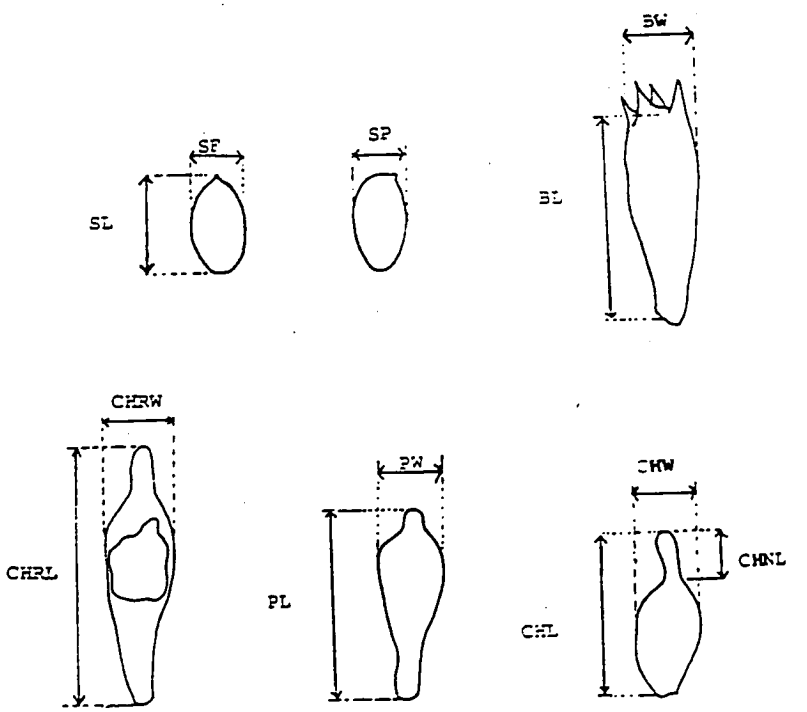

Fig. 2.2. Diagramatic representations of the microscopic characters measured for the morphological studies. Legend: SL= spore length, SF= spore width in face view, SP= spore width in profile, BL= basidium length, BW= basidium width, PL= pleurocystidium length, PW= pleurocystidium width, CHRL= chrysocystidium length, CHRW= chrysocystidium width, CHL= cheilocystidium length, and CHW= cheilocystidium width.

Chapter 3

Genus *Stropharia* (Fr.) Quél.

3.1 Introduction

The genus *Stropharia* forms a rather natural entity within the family Strophariaceae. Within the dark-spored (lilaceous black to fuscous purple spore deposit) genera in subfamily Stropharioideae, *Stropharia* is characterized by relatively fleshy basidiomes, central stipe, membranous or gelatinised annulus which is either persistent or evanescent, lamellae adnate or adnexed, spores smooth, often broad and distinctly truncate germ pore, and pleurocystidia present as chrysocystidia. Taking into consideration these key characters, the genus is well defined within the subfamily.

Other generally utilized characters of *Stropharia* are: the basidiocarps are often fleshy, medium to large, tall and slender or short and stocky. The shape of pileus is convex to semiglobate, then more or less expanding to plano-convex. The surface of pileus can be humid, greasy, tacky or strongly viscid. The stipe often ends with a sub-bulbous base. The shape of spores is ellipsoid; deep yellowish brown (5%KOH) or lilac in water (fresh); medium to large. Basidia are usually 4-spored. Cheilocystidia are well differentiated, crowded, forming a more or less sterile band (or heteromorphous edge). Gill trama is more or less regular becoming irregular with age. Epicutis consists of filamentous repent hyphae, may or may not gelatinised, clamped; hypodermium a layer of filamentous intertwined hyphae. The basal mycelium typically produces acanthocytes. Farr (1980) suggests that they may be of taxonomic significance. Most species can be found growing on soil, ground litter, bark mulch and dung.

Exannulate species of *Stropharia* differ from *Hypholoma* in not having a subcellular hypodermium. The presence of chrysocystidia in species of *Stropharia* separates them from *Psilocybe* and *Melanotus* though some species of *Psilocybe* with pleurocystidia

which are chrysocystidioid are placed in a separate section *Chrysocystidiatae* by Singer (1986).

The separation of most species of *Stropharia* from most species of *Pholiota* is by the colour of spore print. However, in some ambiguous species of *Pholiota* spore print colour alone is not enough to justify separation and other characters such as the narrow, non-truncate germ pore have to be considered to maintain a more natural grouping for the two genera. Singer (*loc. cit.*) uses the examples of *Pholiota albivelata* and *P. johnsoniana* to illustrate this. As for species with chrysocystidia and viscid stipe, as long as the characters do not conform to section *Stercophila* in *Stropharia*, they are included in *Pholiota*.

The genus is systematically arranged in three sections (Singer 1986; Watling & Gregory 1987), i.e. Section I. *Mundae* (Fr.) Konr. & Maubl., Section II. *Stropharia* and Section III. *Stercophila* (Romagnesi) Singer (Table 3.1). Species from each section have been reported from both northern (e.g. Dennis *et al.* 1960; Miller 1975; Imazeki & Hongo 1987) and southern hemispheres (e.g. Cooke 1892; McAlpine 1895; Singer 1969).

A survey of Australian records indicates that *Stropharia* is a small genus and includes species such as *S. aeruginosa* (Eygelsheim 1981), *S. aurantiaca* (Griffith 1985; Hilton 1988), *S. coronilla* (Cooke 1892 as *Agaricus coronillus*; McAlpine 1895; Cleland 1934 as *S. obturata*; Rodway 1900; Shepherd and Totterdell 1988), *S. luteonitens* (all records as *S. umbonatescens*) (Cleland 1934; Guzman & Watling 1978), *S. rugosoannulata* (Young 1986; Shepherd & Totterdell 1988 as *S. ferrii*), *S. semiglobata* (Saccardo 1887; Cooke 1892; McAlpine 1895; Rodway 1898; Eygelsheim 1981; Young 1982; Shepherd & Totterdell 1988) and *S. stercoraria* (Cleland 1934; Rodway 1900; Willis 1950). *S. semiglobata* appears to be a common and widespread

fungus in South America according to Dennis (1961) and Singer (1969), the latter also found *S. coronilla* to be a widespread fungus in that region.

Only four taxa of this genus were reported from Tasmania. In Rodway's collection of Tasmanian fungi, only two species (*S. semiglobata* and *S. coronilla*) are to be found. There are collections of *S. semiglobata* and *S. merdaria* from Tasmania amongst Cleland's fungal collections. Eygelsheim (1981) reported *S. aeruginosa* and *S. semiglobata* from Tasmania.

Many species within the genus *Stropharia* are relatively distinct and easily recognized. However, the separation of two taxa, *S. semiglobata* (Batsch ex Fr.) Quél. and *S. stercoraria* (Bull. ex Fr.) Quél., has proven to be problematic. There appears to be a very fine dividing line between these two taxa. Both taxa are associated with dung and almost indistinguishable in general morphology in the field. Some proposed differences in macroscopic characters include the persistent hemispherical cap and hollow stipe of *S. semiglobata* from the more expanded cap and stuffed stipe of *S. stercoraria* (Table 3.2). These are the original differences recognized by Fries (1821). Saccardo (1887) later included spore size as an additional difference between them. He considered *S. stercoraria* Fr. to have larger spores than *S. semiglobata* Batsch. However, the macroscopic distinctions do not always hold true (Cleland & Cheel 1918). Cleland (1934) referred to all Australian specimens that resemble *S. semiglobata* as *S. stercoraria* Fr. and that the latter has larger spores. *S. stercoraria* (Schum. ex Fr.) Quél. is an accepted synonym of *S. semiglobata* (Batsch ex Fr.) Quél. by British mycologists (Dennis *et al.* 1960; Watling & Gregory 1987) as well as *S. semiglobata* var. *stercoraria* (Schum. ex Fr.) J. Lange (*loc. cit.*). However, *Agaricus stercorarius* Schum. ex Fr. is not to be confused with *Agaricus stercorarius* Bull. ex St Amans whose identity is still a matter of dispute though this latter name has been accepted as a synonym of *Coprinus stercorarius* (Bull.) Fr. s. Ricken (Dennis *et al.* 1960).

The close affinity between *S. semiglobata* (Batsch ex Fr.) Quél. and *S. stercoraria* (Bull. ex Fr.) Quél. has been recognized by mycologists (Cleland 1934; Arora 1986). However, such recognition results in the lumping together of taxa to the extent of considering them forms of one species (Cleland 1934) and confusion arises regarding the finer dividing line between them. Much confusion arose from the large ranges of spore size reported for these two taxa (Table 3.3). However, the difference in spore size between the two taxa is not readily accepted by mycologists. It is readily seen in Table 3.3 that the epithet "*stercoraria*" has been little used in recent times and it would appear that *S. semiglobata* as a broad species may encompass a separate species, *S. stercoraria*, as well. What appears to be a mild confusion about the size range of spores of *S. semiglobata* has resulted in an apparent blurring of potential distinctions between the two separable taxa.

Kühner and Romagnesi (1978) have utilized the observations of Ricken to indicate a potential division between *S. semiglobata* and *S. stercoraria* by using the location of chrysocystidia as a discriminating feature. *S. semiglobata* is proposed to be without facial cystidia in the form of chrysocystidia and that chrysocystidia occur only on the gill edge with cheilocystidia; whereas chrysocystidia occur on the gill face in *S. stercoraria* as well as on the gill edge. Table 3.3 shows that this suggestion has not been taken up and tested by mycologists, instead fungi having facial cystidia have been included within the circumscription of *S. semiglobata* (Watling 1973; Watling & Gregory 1987; Lincoff 1981; and Arora 1986).

In this study all potential divisions within this *semiglobata - stercoraria* association are investigated. Investigations by Galland *et al.* (1979), Bas (1976), Flynn & Miller (1990) have shown that similar taxa may be grouped when a broad species concept is used.

The scope of study for this genus is to establish which species occur in SE Tasmania and using the three approaches of comparative morphology, isozyme analyses and biosystematics (mating compatibility tests) to resolve the problem of morphologically similar species in this genus, such as the *semiglobata*-like taxa. Preliminary delimitation of taxa is achieved using classical criteria such as morphological characters and habitat. For preliminary identification to species level, several keys (Cleland 1934; Watling & Gregory 1987; Moser 1983 and Arora 1986) were used.

3.2 Results

For the results of morphological studies, the Tasmanian specimens were divided into two groups based on habitat. One group was associated with ground litter (e.g. eucalypt bark mulch, cut grass, straw or ground litter associated with mature mixed forest) or just on ground, and the other with dung (cow, horse, or dung of native animals such as wallaby and wombat). Included in the morphological comparison of the coprophilous taxa was herbarium material from Tasmania (HO), mainland Australia (AD), in particular Cleland's material of *S. stercoraria*, and some material of *S. semiglobata* (Batsch ex Fr.) Quél. from the United Kingdom (K).

3.2.1 Morphological studies

Five groups were identified using morphological criteria. The first group consisted of two collections corresponding to *S. coronilla* (Bull. ex Fr.) Quél.; the second group consisted of four collections corresponding to *Stropharia aurantiaca* (Cooke) Orton; the third group consisted of 17 collections corresponding to *S. stercoraria* as understood by Cleland, the fourth and fifth groups were collections that fitted no known descriptions and were referred to as *Stropharia* sp. A and *Stropharia* sp. C. Details of locality , habitat, altitude and date of collection of the Tasmanian material are presented in Appendix IIIA.

Of the specimens found growing on ground or ground litter, three morphological species were identified. Two taxa found growing on ground amongst grass in suburban areas corresponded well to *S. coronilla* (Bull. ex Fr.) Quél. and *S. aurantiaca* (Cooke) Orton. The third taxon was found on ground litter in relatively undisturbed wet regions in temperate rainforests. It possessed characters that placed it in the genus *Stropharia* but morphologically very distinct from *S. aurantiaca* or *S. coronilla* and any other described species of *Stropharia*.

The three collections of *S. coronilla* were found at a very late stage in the project and since the specimens were morphologically distinct from the other taxa and fitted the current concept of *S. coronilla*, no electrophoretic or mating studies were undertaken. *S. coronilla* had been previously reported from Tasmania by L. Rodway (1898) and is a widely distributed species in South America and the northern hemisphere (Singer 1969; Miller 1975).

Both *Stropharia* sp. A and *S. aurantiaca* shared some similarity of habitats, though *S. aurantiaca* was found more frequently in lower rainfall regions than did *Stropharia* sp. A. *S. aurantiaca* was found particularly in disturbed habitats such as gardens and backyards of residential areas and was seldom seen in bushland or forest habitats. It was only seen once from a locality (Tahune Forest Reserve) where one collection (CYS341) of *Stropharia* sp. A was also collected. The macromorphological distinctions between these two taxa were very clear (Table 3.4). Specimens from four collections of *S. aurantiaca* (Appendix IIIA) agreed well with the published description (Orton 1960).

Specimens of *Stropharia* sp. A were all collected in the field (Appendix IIIA). The colour of the basidiocarps was a deep purplish brown (6D4 - 6E6) and the basidiomes were slimy viscid when fresh and moist. These three non-coprophilous taxa differed in

the stature of the basidiocarps, *Stropharia* sp. A was generally more robust than *S. aurantiaca* and very similar in stature to *S. hornemanii* as described by Aurora (1986) but differed in the colour of pileus.

Table 3.5 shows a summary of the mean measurements of the major microscopic characters of *S. coronilla*, *S. aurantiaca* and *Stropharia* sp. A. The three taxa were clearly separable on both length and width of spores.

The macroscopic characters of the coprophilous taxa of *Stropharia* included in the study are summarized in Table 3.6. Within the coprophilous specimens from Tasmania, macroscopic examination distinguished two forms which potentially indicated two species. Of the group collectively referred to as *S. stercoraria*, the colour of basidiocarps was corn, straw or pale yellow to olive brown (4B5, 4C4-6, 4E7-8), stipe varying from short to long and strongly viscid with a gelatinous covering or tacky below the ring. The position of the ring was sub-inferior, one-third or midway down the stipe. General stature of the basidiocarps varied greatly, from robust to slight. There were three collections (*Stropharia* sp. C) that did not fit the description of *S. stercoraria* but were definitely within the genus *Stropharia*. The colour of these basidiocarps was brownish orange (5C3) with a pinkish hue, pileus viscid to strongly viscid, stipe very long and slender, also viscid or tacky below the ring and not at all robust in stature. These basidiocarps were almost always found growing solitary on dung of native animals, in particular, wallaby dung. *S. stercoraria* which was generally associated with cow or horse dung, was also found growing on wallaby and wombat dung (CYS200 and 343), either solitary or gregarious. Specimens collected from an altitude range of ~75 - 1050 m. displayed no obvious clinal difference.

Table 3.7 shows a summary of the mean measurements of the major microscopic characters of the coprophilous species of *Stropharia*. Of the Tasmanian material collectively grouped as *S. stercoraria*, two size ranges of spore dimensions were

observed. Similar observations were made for the herbarium material of *S. stercoraria* and *S. semiglobata*. These two forms of *S. stercoraria* and *S. semiglobata* will be referred to from hereon as large- (LF) and medium-spored (MF) forms.

Chrysocystidia occurred on the gill face and gill edge of specimens of all the coprophilous taxa collected. Though *S. stercoraria* (LF) tended to have bigger chrysocystidia, there were also those that were within the range of *S. stercoraria* (MF). All these forms possessed similar shape of cheilocystidia. *Stropharia* sp. C appeared to be just separable from the two forms of *S. stercoraria* on the mean size of cheilocystidia while the latter were inseparable on this basis. There was considerable variation in the mean size of cheilocystidia in different basidiomes of the two forms of *S. stercoraria*.

Spore and cystidia variables were submitted to Canonical discriminant analysis (CDA) separately. The results for the analysis of spore data showed that the first two canonical variates (abbreviated MSCV1 and MSCV2) described 99.7% (96.8 and 2.9% respectively) of the total variation. Fig. 3.1 shows the scatter plot based on MSCV1 & MSCV2 generated from the analysis. Two distinct clusters are clearly resolved along the axis of MSCV1. The dispersion along this axis was due to spore profile width (MSCV1) with 96.8% of the total variation. The aixs of MSCV2 reflected primarily the contrasts between spore length and spore profile width (MSCV2). Cluster 1 includes mixed collections of the large-spored form of *S. stercoraria* (LF) and *S. semiglobata* and cluster 2 includes mixed collections of the medium-spored form of *S. stercoraria* (MF), *S. semiglobata* as well as *Stropharia* sp. C.

The results of CDA using cystidia variables showed that CV1, length of chrysocystidia, was responsible for 61.9% of the total variation and CV2 scored 23% which was attributed to the differences between width of cheilocystidia (with high positive values) and length of chrysocystidia (with negative values). Fig. 3.2 shows the scatter plot

based on the mean values of CV1 and CV2 (abbreviated as MCV1 & MCV2). There is no distinct cluster resolved along either of the axes. None of the three taxa could be separated based on cystidia variables. *Stropharia* sp. C showed considerable overlap in cystidia characters with collections of *S. stercoraria* (MF & LF).

Fig. 3.3 shows the dendogram constructed using all the mean values of CVs generated in CDA. It shows two clusters (Cluster 1 & Cluster 2) which are distinct segregation of the LF and MF forms. This cluster pattern indicated that spore variables were the dominant factors since *Stropharia* sp. C which appeared separable on cystidia variables did not form a cluster on its own. The two clusters consisted of mixed collections previously labelled as either *S. stercoraria* or *S. semiglobata* (MF) cluster also contained all collections of *Stropharia* sp. C.

Acanthocytes were consistently noted in the basal mycelium of fresh material in tissue cultures and monosporous isolates (Fig 3.4). They were quite visible even using a binocular dissecting microscope. This observation contradicted Singer's (1986) exclusion of this cell type from species in Section *Stercophila*. This cell type was noted in well preserved herbarium material with ample basal material, though careful scrutinizing may be necessary. Unfortunately, old herbarium material generally did not contain much of the basal mycelium.

3.2.2 Electrophoretic studies

In each enzyme system, there were five zymogram patterns equivalent to five zymogram groups produced by all the isolates included in the electrophoretic studies. However, due to the limited number of isolates available for this study, the results were interpreted with caution.

Lac (Laccase)

For laccase activities, a total of 18 bands were scored across the isolates of the five

putative groups. Five zymogram groups corresponding to *S. stercoraria* (MF), *S. stercoraria* (LF), *S. aurantiaca*, *Stropharia* sp. A and *Stropharia* sp. C were noted (Fig. 3.5). Within each putative group, there was intercollection variability. For example, in the group *S. stercoraria* (MF), Band 4 at R_f 0.16 was the common band across the collections within the group. Between R_f 0.24 and 0.56, the band patterns varied among isolates of the different collections in the group. Only three bands were detected in the isolates belonging to the group *S. stercoraria* (LF), thus, there appeared less variation. However, as a result of the limited number of available isolates, it was not clear of the extent of variation. Yet in the case of *Stropharia* sp. C, there was obvious variation between the two collections despite the small number of isolates. It appeared that Band 5 at R_f 0.18 was unique to the group. Little variability was noted among the non-coprophilous taxa.

Bands of the same R_f values were noted in isolates of different taxa (Table 3.8). Isolates of the two non-coprophilous taxa had the same band at R_f 0.52 which was also detected in some isolates of *S. stercoraria* (MF). Band 15 at R_f 0.46 was detected in isolates of *S. aurantiaca* and *S. stercoraria* (LF). Isolates of *Stropharia* sp. A and *S. stercoraria* (MF) had the same band at R_f 0.54. Of the coprophilous taxa, the two forms of *S. stercoraria* did not have any common bands whereas isolates of *Stropharia* sp. C showed similarity in a band at R_f 0.50 with isolates of *S. stercoraria* (MF) and another at R_f 0.42 with isolates of *S. aurantiaca*.

Per (Peroxidase)

A total of 20 bands were scored for the Per activities across the isolates of the five putative groups. Again five zymogram groups were noted (Fig. 3.6) corresponding to the five putative groups. Similarities in band activities were noted in isolates of different taxa (Table 3.8). For example, Bands 2 and 6 (R_f 0.07 & 0.18 respectively) were detected in isolates of *S. stercoraria* (MF), *S. stercoraria* (LF) and *Stropharia* sp. C.

Isolates of the two forms of *S. stercoraria* showed similarity in an additional band at R_f 0.52. This band was detected previously in the absence of H_2O_2 in isolates of *S. stercoraria* (MF). Its detection in the presence of H_2O_2 perhaps indicated its stronger activity in the presence of H_2O_2. The same was probably true for Band 15 at R_f 0.46 which was detected in isolates of both *S. aurantiaca* and *Stropharia* sp. C. Isolates of *Stropharia* sp. C also showed similarity in two additional bands at R_f 0.12 & 0.22 respectively with isolates of *S. stercoraria* (LF).

AcP (Acid phosphatase)

Both *S. aurantiaca* and *Stropharia* sp. A did not produce detectable activity of acid phosphatase. A total of 12 bands were scored across the isolates of the coprophilous taxa. One 'backrunner' (i.e. with negative R_f) each was noted in the isolates of *S. stercoraria* (MF) and *Stropharia* sp. C (Fig. 3.7). Band 11 at R_f 0.24 was detected in isolates of the three coprophilous taxa (Table 3.8). Isolates of the two forms of *S. stercoraria* showed similarity in Band 5 at R_f 0.11. Isolates of *Stropharia* sp. C showed similarity in two bands at R_f 0.08 & 0.14 respectively with isolates of *S. stercoraria* (LF).

PE (Pectinesterase) and PG (Polygalacturonase)

For PE activities, a total of 17 bands were scored across all the isolates. Five zymogram groups were noted corresponding to the five putative groups established so far (Fig 3.8). There was a certain degree of intercollection variability within the group *S. stercoraria* (MF). This was the only group with a 'backrunner' PE band detected and this band at R_f -.0.08 distinguished this group from the rest. Much variation was noted in the band patterns between R_f 0.30 - 0.48. Isolates of CYS200 showed similarity in only two bands (at R_f -0.08 & 0.42 respectively) with isolates of other collections in the group. One isolate had a unique band at R_f 0.48 not detected in any isolate of other collections in the group. Only a solitary PE band at R_f 0.26 was detected in the isolates

in the group *S. stercoraria* (LF). The absence of other bands seemed to have delimited this group. A lesser degree of variability was noted in the remaining three groups.

As in the previous enzyme systems, similarity in bands were noted between isolates of different groups (Table 3.8). Similarity was shown in Band 11 at R_f 0.34 by isolates of *S. stercoraria* (MF), *Stropharia* sp. C and *S. aurantiaca.* Isolates of *S. stercoraria* (MF) also had two other similar bands at R_f 0.44 & 0.48 respectively with isolates of *Stropharia* sp. A.

For PG activities, a total of 14 bands were scored across all the isolates. Much variability was again noted among isolates between collections in the group *S. stercoraria* (MF) and comparatively less variability was noted in the other groups. Band 6 at R_f 0.11 was the common band among the isolates in the group *S. stercoraria* (MF) and variations were mainly between R_f 0.16 - 0.26. Isolates of the two non-coprophilous taxa did not show similarity in any PG bands between them (Fig.3.9). However, isolates of *S. aurantiaca* had the same band at R_f 0.22 with isolates of the three coprophilous taxa. This was also the only similar band isolates of *S. stercoraria* (LF) had with the isolates of *S. stercoraria* (MF) (Table 3.8). Isolates of the former group, however, had another similar band at R_f 0.28 with isolates of *Stropharia* sp. C. Isolates of *Stropharia* sp. C, on the other hand, had three similar bands (at R_f 0.06, 0.11 & 0.16 respectively) with some isolates of *S. stercoraria* (MF).

Fig. 3.10 shows photograms representative of laccase and pectic zymograms of isolates of *S. stercoraria* (MF) and *S. stercoraria* (LF).

The isozyme data were analysed by UPGMA average linkage cluster analysis. Collections with only single isolate were excluded from the analysis. Fig 3.11 shows the dendogram constructed using band frequencies of Lac, AcP, PE and PG. At the five cluster level it shows the five clusters corresponding to the five taxa of *S. stercoraria*

(MF), *S. aurantiaca*, *S. stercoraria* (LF) *Stropharia* sp. A and *Stropharia* sp. C. CYS200 appeared quite different from the rest of the medium-spored form. The dendogram (Fig. 3.11) indicates that there is considerable distance between *S. stercoraria* (MF) and *S. stercoraria* (LF) with the remaining coprophilous taxon (*Stropharia* sp. C) being distinctly different from the two forms of *S. stercoraria*. *S. aurantiaca* seemed to show some affinity with the coprophilous taxa.

A second dendogram constructed form the cluster analysis based on the band frequencies of Per isozymes is given in Fig. 3.12. The separate analysis of Per data was as a result of the complexity associated with this group of enzymes (Gottlieb 1977). At the five clusters level it shows the five clusters corresponding to the five taxa of *S. stercoraria* (MF), *Stropharia* sp. A, *S. aurantiaca*, *S. stercoraria* (LF) and *Stropharia* sp. C. This cluster pattern reflects that based on the previous four enzyme systems.

3.2.3 Mating compatibility studies

As mentioned in Chapter 2, only collections/groups that were morphologically indistinct or where morphology failed to provide a satisfactory delineation were the isolates included in the mating incompatibility studies. In this case, the coprophilous taxa of *Stropharia* fell into this category.

Results from an 8 x 8 selfing mating matrix of CYS483 (medium-spored form of *S. stercoraria*) indicated a bipolar incompatibility system and the two mating types recovered were 'A' (01, 02, 06 & 08) and 'a' (03, 04, 05 & 07). Isolates of other collections of the medium-spored form were intercompatible with the tester strains of CYS483 except CYS200 (Table 3.9). In that confrontation, there was strong antagonistic interactions and the growth of isolates of CYS200 was greatly inhibited by the tester strains of CYS483. Similar results were obtained when this confrontation with CYS200 was repeated. Isolates of CYS200 were also interincompatible with

isolates of three other collections of the medium-spored form and the isolates of the large-spored form (CYS382). In all cases, strong antagonistic interactions and evident inhibition of growth of CYS200 was observed. The isolates of the two collections (CYS351 and 382) of the large-spored form of *S. stercoraria* were intercompatible between themselves but interincompatible with the tester strains of CYS483 (Table 3.9). However, pseudoclamp-connections were noted in two cases in the crosses with the tester strains of CYS483. These crosses were repeated and similar results were obtained. Isolates of CYS486 and 364 (*Stropharia* sp. C) were intercompatible between themselves but interincompatible with the tester strains of CYS483 and monokaryotic isolates of CYS343 and 351.

3.3 Discussion

The results of morphological studies indicate the presence of three morphological species occupying non-coprophilous habitats, i.e. *S. coronilla*, *S. aurantiaca* and *Stropharia* sp. A. Distinction between these three taxa is readily achievable by macromorphological comparison and to a lesser degree micromorphological characters. Results of electrophoresis show that *S. aurantiaca* and *Stropharia* sp. A are also separable at the isozymic level. The distinctions observed between the two taxa in the selected extracellular isozymes exemplify and strengthen the delineation based on morphology.

All Tasmanian material of *S. coronilla* and*S. aurantiaca* fits the current concept of *S. coronilla* (Bull. ex Fr.) Quél. and *S. aurantiaca* (Cooke) Orton respectively. A fungus previously known as *Psilocybe ceres* Clel. is believed to be the same as *S. aurantiaca* (Orton 1960; Guzmán and Watling 1978; Grgurinovic per. comm.). A description for the Tasmanian material of *S. aurantiaca* will be included in the Section Taxonomy at the end of this chapter.

The general habit of *Stropharia* sp. A is reminiscent of *S. rugosoannulata*, in particular the rich vinaceous brown or at times rich date brown pileus. However, the obvious absence of the thick, grooved annulus which gives the latter species its name quickly refutes this possibility. In fact, the annulus of sp. A is evanescent and usually little trace of the veil is visible on the stipe though it is more evident on the margin of pileus in the early stages. The strongly viscid pileus also differentiates it from *S. rugosoannulata.* In addition to this, the difference in habitats places them in separate sections, sect. *Mundae* for *S. rugosoannulata* and sect. *Stropharia* for *Stropharia* sp. A. This fungus also occurs in Victoria and probably other parts of south-eastern Australia (B. Fuhrer per comm.) and has been referred to simply as *Stropharia* sp. A name is definitely in need for this handsome fungus, hence, *S. formosa* sp. nov. will be formally described in the chapter on New Species.

For the coprophilous species, three biological species have been identified in this study. One of the biological species, *Stropharia* sp. C, appears to be a taxon endemic to Tasmania or possibly Australia. It is found on dung of native animals only. It differs from the *stercoraria*-like forms in gross morphology and stature. However, canonical discriminant analysis failed to separate this taxon from the other coprophilous taxa on microscopic characters. The isolates of this taxon produced their own distinct sets of isozyme patterns from the other two biological species. Furthermore, there is complete genetic isolation from the other two taxa as well. Thus, as a result of its delicate and petite habit, *S. parvula* sp. nov.,will be described formally in the chapter on New Species.

The remaining two biological species fit the concept of *S. semiglobata* (Bastch ex Fr.) Quél. s. Watling & Gregory and *S. stercoraria* (Bull. ex Fr.) Quél. s Kühner and Romagnesi. The former corresponds to the medium-spored form referred to earlier and the latter to the large-spored form. They are virtually indistinguishable in the field in

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stature, general macromorphology and habitat but separable on spore dimensions. These two species together may conform to the concept of sibling species as in the case of *Agrocybe praecox* (Flynn & Miller 1990) and *Psathyrella candolleana* (Galland *et al* 1979).

Canonical discriminant analyses (Figs 3.1, 2 and 3) fully resolved the two groups identified during electrophoretic and mating compatibility studies. However, they are very similar in most other morphological characters. It is evident that *S. semiglobata* is a separate taxon closely allied to *S. stercoraria*. It is possible that the two biological species in this study have separated relatively recently in evolutionary terms. It seems that isolation and differentiation may not be totally complete since pseudoclamps were noted in two of the crosses. The distinctions that separated them effected through genetic levels did not extend to gross phenotypic expressions (Galland *et al* 1979). According to Boidin (1986), this "... incomplete intercompatibility ... [is] best interpreted as representing taxa in the process of speciation".

Cleland's (1934) treatment of the Australian specimens as *S. stercoraria* seemed fitting at the time, but this previous lumping might have obscured the presence of two separate taxa. A close comparison with the early descriptions (Saccardo 1887 & Bresadola 1927) indicates that the the large-spored form would resemble closely the spore range of *S. stercoraria*. This means that collections CYS351 and 382 conform better to the concept of *S. stercoraria* Fr.(Saccardo 1887), and the presence of chrysocystidia on the gill face also fits the concept of *S. stercoraria* s. Kühner and Romagnesi (Kühner & Romagnesi 1978). As shown in Table 3.3, neither concept has been applied strictly to the *semiglobata*-like forms. The results show that none of the Tasmanian material of the medium-spored form included here fits the concept of *S. semiglobata* s. Kühner and Romagnesi, i.e. without any facial chrysocystidia. This raises doubt about Ricken's observations of the absence of facial chrysocystidia. Facial chrysocystidia are present in all three species, in this respect it conforms better to the concept of *S. semiglobata*

s.Watling and Gregory but there are obvious discrepancies where spore size is considered.

Two observations are noted within the medium-spored form, i.e. *S. semiglobata.* Firstly, the isolates of specimens with dark olive brown pileus were completely compatible with those with pale yellow pileus. The latter is the commonly encountered form of this species in northern hemisphere. The present study fails to establish any association with the substrate since specimens with olive brown pileus also occur on cow or horse dung as well as dung of native animals (e.g. wallaby or wombat).

Secondly, CYS200 appears to be an interesting collection by showing some unexpected mating behaviour. It resembles the other collections in both macroscopic and microscopic characters. Its isolates also produced similar isozyme patterns for the enzymes tested and were subsequently grouped together as one of the medium-spored form in the cluster analysis yet its isolates failed to mate with the tester strains of CYS483. This appears to be one instance where similarity in zymograms does not imply synonymy with CYS483. There may be a number of possibilities for the interincompatibility between the tester strains of CYS483 and monokaryons of CYS200. One possibility is the presence of various infraspecific factors that can impair intercompatibility (Perkins *et al* 1975). Another may be the deterioration of the mating ability of the isolates due to unknown causes (Boidin 1986). There is another possibility that this collection may represent an 'intersterility group' such as those encountered in *Heterobasidion annosum* (Korhonen 1987; Stenlid & Karlsson 1991)and *Armillaria mellea* (Kile & Watling 1988; Korhonen 1987). To conjecture that speciation may be in process could be overstating the significance of the interincompatibility noted between the isolates of these two collections. Furthermore, both CYS200 and CYS483 occur in the same geographical region. At this stage, there is insufficient evidence to support this concept of speciation. Therefore, CYS200 will

be considered as the same taxon as the rest of the medium-spored form.

In conclusion, Tasmanian specimens of the medium-spored form are considered to belong to *S. semiglobata* (Batsch ex Fr.) Quél. s. Watling & Gregory and the large-spored form, for the present, *S. stercoraria* Fr. s. Saccardo. By including specimens from different parts of the world in particular European material in cultural studies in the future would certainly help to elucidate the relationships between these two taxa. Descriptions of the Tasmanian representatives of both species are given in the section Taxonomy.

Acanthocytes are associated with the basal mycelium of all Tasmanian material of *Stropharia* and this implies a significance that has not been established before. However, this cell type is very different from the acanthocytes reported by Singer (1983) in *Amparoina* and *Mycena*, both in location and structure. Acanthocytes are observed in all cultures of *Stropharia* though similar cell types have also been noted in some cultures of *Pholiota* (see Chapter 7). Observations from this study suggest that acanthocytes (in the sense of Farr) can be used as a diagnostic character in the delimitation of *Stropharia* from other genera in the same family.

3.4 Taxonomy

This study of the genus *Stropharia* in Tasmania has resulted in the recognition of six distinct species, viz. *S. coronilla* (Bull. ex Fr.) Quél. *S. aurantiaca*, (Cooke) Orton, *S. formosa* sp. nov., *S. parvula* sp. nov, *S. semiglobata* (Batsch ex Fr.) Quél. and *S. stercoraria* Fr. s. Saccardo. A key is given below for these Tasmanian species.

Key to species of *Stropharia* in SE Tasmania

1 On ground or ground litter of woody or leafy debris 2

1' On dung (cow, horse or native animals) 4

2 Pileus brightly coloured, some shade of orange, red or date brown, exannulate 3

2' Pileus pale yellow or ochraceous brown, stipe with a short but distinct striate annulus ..... **1. coronilla**

3 Pileus orange red, greasy but not truly viscid ..... **2. aurantiaca**

3' Pileus date brown with a vinaceous tint, slimy viscid ..... **3. formosa sp. nov.**

4. Pileus convex to hemispherical, generally >10 mm. broad,straw yellow to olive brown, slimy viscid, on dung of both domesticated and native animals 5

4' Pileus subcampanulate to convex, generally <10 mm. broad, orange brown fading to pale buff, sometimes with a pinkish hue, viscid, on dung of native animals only ..... **4. parvula sp. nov.**

5 Spores >17µm in length and >10µm in width ..... **5. stercoraria**

5' Spores <17µm in length and <10µm in width ..... **6. semiglobata**

1. *S. coronilla* (Bull. ex Fr.) Quél., *Champ. Jura Vosg* , p.237, 1872.

Illustrations: Figs. 3.13-15.

Material studied:

TASMANIA, Hobart, near University of Tasmania, amongst grass, 24. xii. 1991, CYS556; 30. xii. 1991, CYS557; 3. i. 1992, CYS558.

This species is well described and illustrated in many current European works (e.g. Phillips 1981 & Watling & Gregory 1987). The Tasmanian specimens did not appear to deviate significantly from the published description. A few observations are noted from the study. The white margin of lamellae noted by Watling & Gregory (1987) is not evident in the Tasmanian specimens. On the other hand, the striate or grooved annulus is very distinct. The presence of acanthocytes (in the sense of Farr) in the basal mycelium is noted. This species appears to fruit in late December, early summer, as

opposed to the autumn fruiting indicated by Phillips (1981).

2. *S. aurantiaca* (Cooke) Orton, *Trans. Br. mycol. Soc.* **43**, 181, 381, 1960.
Syn.: *Agaricus squamosus* f. *aurantiacus* Cooke, Handbook of British Fungi, Ed. II, 199, 1883-91; *Stropharia percevalii* var. *aurantiaca* (Cooke) Sacc., Syll. Fung. 5, 1016, 1887; *S. squamosa* var. *aurantiaca* (Cooke) Mass., British Fungus Flora 1,402, 1892.
Illustrations: Figs. 3.16-17.

Pileus 20 - 42 mm., convex to plano-convex, copper or orange red (7C8) to paprika red (8B8), slightly greasy when moist, whitish veil remnants appendiculate on margin. *Lamellae* broadly adnate, sometimes seceding, pallid to greyish buff (5C2) at first, becoming fuscous black with spores, crowded, up to 5 mm. broad. *Stipe* 68 - 130 x 3.5 - 6 mm., ±equal, flexuose, stuffed then becoming hollow, apex pale yellow (2A3 - 3A3), orange brown towards base, also when bruised of handled, fibrillose below veil line. *Context* pallid to pale cream (2A3), thin, some hyphae turned yellow in 5%KOH. *Taste* mild, akin to pleasant.
Spores lilaceous black in mass, 10.4 - 12.5 x 6.2 - 7.5 x 6.2 - 7.5µm, smooth, ellipsoid, with broad germ pore, appearing truncate. *Basidia* 4-spored, more rarely 2- and 3-spored, 25.8 - 31.7 (-34.2) x 9.2 - 10.8µm, clavate or obovate. *Cheilocystidia* cylindric or lageniform, 24.6 - 32.5 x 5.8 - 10µm, thin-walled, forming ±sterile band. *Pleurocystidia* as chrysocystidia, clavate with or without apical appendage, with yellow inclusion or amorphous body (10%NH_3 and 5%KOH), 42.5 - 60 (-65) x 12.5 - 17.5 (-19.2)µm.
Subhymenium subcellular, narrow region but well defined. *Gill trama* regular, of broad, short, thin-walled hyphae, also refringent or oleiferous hyphae scattered. *Epicutis* repent hyphae, clamp connections present.
Habitat under cut grass, on lawn or eucalypt bark mulch, solitary, scattered or

gregarious.

Material examined:

AUSTRALIA Tasmania CYS202, on lawn solitary, Taroona, Hobart, v. 1989; CYS230, under cut grass, gregarious, Taroona, Hobart, vi. 1989; CYS281, on straw, gregarious, Taroona, Hobart, vii. 1989; CYS527, on eucalypt bark mulch under *Grevillea biternata* in garden, Cascades, Hobart, iv. 1991.

Observations

This fungus is easily recognized by its bright orange red colour. However, it is uncommon and rarely seen away from human habitation. When it does, it generally occurs solitary. It has been erroneously placed in *Naematoloma* by Guzmán (Singer 1986). *Psilocybe ceres* Cooke & Massee reported by Cleland (1918) is believed to be the same fungus. Judging from the habit of the basidiocarps of the Tasmanian material, it has all the distinctive characters of *Stropharia*. Though there may be certain difficulties in the delimitation of exannulate species between *Stropharia* and *Psilocybe* (Singer 1986), there is no doubt that *S. aurantiaca* belongs to the genus *Stropharia*.

3. *S. formosa* sp. nov.

This is a very elegant fungus on the forest floor. It is a rainforest species and has not been collected outside cool temperate rainforests (*Atherosperma moschatum* Labill./*Nothofagus cunninghamii* (Hook.) Oersted) and mature mixed forests (*Eucalyptus* spp/*Atherosperma*/*Nothofagus*). This species will be formally described and discussed in the chapter on New Species.

4. *S. parvula* sp. nov.

This fungus is easily overlooked as a result of its petite stature. It is always found growing on dung of native animals and likely to be an endemic species to Australia.

Rodway 19 (both as *Stropharia semiglobata*).

NEW SOUTH WALES, Milson Island, 1910-1920, AD22411; near Barellan, 12. viii. 1918; Pennshurst, DAR67067, 19. ix. 1909; DAR67065, 29. vi. 1907, AD5532; Hill top, ii. 1911, DAR67064; Centennial Park, Sydney, x. 1909, DAR67071; Leura, ii. 1911, DAR67069 (all DAR material as *S. semiglobata*).

SOUTH AUSTRALIA, Beaumont, 15. viii. 1920, AD22409; 6. iv. 1917, AD22401; ix. 1926, AD5536; Mt Lofty, 20. ix. 1913, AD22403; Adelaide, Fullerton, 11. ix. 1920, AD5534; Adelaide, Parkside Asylum, viii. 1926, AD5535.

WESTERN AUSTRALIA, Pemberton, 1926, AD22408.

UNITED KINGDOM, on cow dung,26. viii. 1874, K. Hargreaves & N. Sinnott 1429 (K); Surrey, Esher Common, on horse dung, 26. x. 1980 (day forey, K) (both as *S. semiglobata*).

Observations

Because the Tasmanian specimens fitted the description of *S. stercoraria* Fr. in Saccardo (1887), this reference is cited. As mentioned earlier, this fungus is indistinguishable from *S. semiglobata* in the field but they are separable on spore size. The stipe is viscid as compared to Saccardo's subviscid. This difference is probably as a result of the condition of the specimens since the viscid condition of the stipe can be affected by environmental factors. This fungus is less common than *S. semiglobata* in Tasmania.

6. *Stropharia semiglobata* (Batsch ex Fr.) Quél., *Memoires de la Societe d'emulation de Montbeliard ser* II, 5: 112, 1872.

Syn.: *S. stercoraria* (Schum. ex Fr.) Quél., *Mem. Soc. Emul. Montbeliard ser. II*, 5: 112, 1872; *S. semiglobata* var. *stercoraria* (Schum. ex Fr) J. Lange in *Flora Agaricina Danica* 4: 67, 1939.

Selected illustrations: Fuhrer & Robinson (1992), p. 58.

Illustrations: Figs. 3.22 - 26.

The following is a composite description of the Tasmanian specimens:

Pileus 10 - 28 mm. broad, convex to broadly convex, glabrous, striate at margin, approx. 1/5 to 1/4 up from margin, glutinous, hygrophanous, corn yellow (4B5) or olive brown (4E7) when moist, fading to golden yellow (4C6). *Lamellae* broad, adnate, yellowish grey (3D2) then becoming greyish brown (5F3) with spores, edges whitish. *Stipe* 56 - 92 x 1.5 - 2 mm., cylindric, broadening near base, straight or flexuose, ±smooth, pale yellow (3A3), becoming dingy coloured with age, velar zone indistinct, about 1/3 down from margin, glutinous covering on lower 2/3 of stipe, stuffed at first, then hollow. *Context* light yellow (2A4), thin.

Spores lilaceous black in mass, 13.3 - 15.8 (-16.7) x 7.9 - 9.6 (-10) x 7.5 - 9.6 µm [mean (25) = 14.53 x 8.82 x 8.53 µm], ellipsoid in both face and side view, smooth, thick-walled, fuscous brown (5%KOH), broad germ pore, apiculus not always evident. *Basidia* 24.2 - 31.7 x 10 - 12.5 µm, 4-spored, broadly cylindric or obovate, sometimes with constriction at waist or with narrow base. *Pleurocystidia* as chrysocystidia, 33.3 - 48.3 x 14.2 - 19.2 µm, yellow amorphous body (5%KOH) present, apical appendage prominent, ventricose. *Cheilocystidia* 32.5 - 45.8 x 5.8 - 10.8 µm, hyaline, thin-walled, clavate to ventricose-rostrate, also chrysocystidia but smaller in size than those on the gill face, forming a ±sterile band.

Subhymenium subcellular, defined region. *Trama* regular, hyphae with yellow brown pigment, 2 - 8 µm broad. *Epicutis* a thin gelatinised layer of repent, filamentous hyphae, 2 - 4 µm broad, clamped. *Hypodermium* encrusted hyphae, 3 - 6 µm broad, with yellow brown pigment.

Habitat gregarious on dung (cow, horse, or wallaby), in mature mixed forests (i.e. *Eucalyptus* spp/*Atherosperma moschatum*/*Nothofagus cunninghamii*) or wet sclerophyll (predominantly *Eucalyptus* spp).

Specimens examined:

See Appendix IIIA for Tasmanian collections.

NEW SOUTH WALES: Cowra, 23. vi. 1912, AD5526; Milson Island, 18. xi. 1914, AD5529; Narrabeen, 18. xii. 1918, AD22371; Orange, 24. xi. 1914, AD22402; Yanco, vii. 1915, AD22418.

SOUTH AUSTRALIA: Mt Lofty, 17. vii. 1914, AD22415 & 1. vii. 1922, AD22414; Belair, 26. vi. 1920, AD22416; Adelaide, Beaumont, 6. iv. 1917, AD22401.

Observations

This fungus is variable in the colour of pileus ranging from deep olive brown to pale corn yellow. Variations in size and general stature are probably due to the availability of nutrients in the substrate. The basidiocarps are generally smaller on dung of native animals (wombat or wallaby) and larger on dung of domesticated animals (cow or horse). It is more common than *S. stercoraria*.

Table 3.1 Systematic treatment of the genus *Stropharia* (Fr.) Quél.

Singer (1986)
Genus Stropharia
Section 1. Mundae (Fr.) Konr. & Maubl.
2. Stropharia
3. Stercophila (Rogmanesi) Sing.

Table 3.2 Recognized morphological distinctions between *Stropharia semiglobata* (Batsch ex Fr.) Quél. and *S. stercoraria* (Bull. ex Fr.) Quél.

	S. semiglobata	*S. stercoraria*
Pileus	hemispherical[1, 2]	hemispherical when young, plane at maturity[1]
	hemispherical[3, 4, 6]	hemispherical then expanded[3, 4, 6]
	persistently hemispherical[5]	at first hemispherical then expanding at maturity[5]
	hemispherical or campanulate, then convex umbonate[7]	conico-campanulate then plano-convex[7]
Stipe	hollow[1, 4, 6, 8]	stuffed with removable pith[4, 6, 8]
	hollow (fistulose)[3, 5]	with distinct pith[3, 5]
	hollow[7]	stuffed with pith then hollow[7]
Spores	13-14 x 8-9 µm.[4]	18-20 x 10-13 µm.[4]
	smaller, generally <16 µm.[5]	larger, generally >16 µm.[5]
	15-17 x 9-10 µm.[6]	18-20 x 8-10 µm.[6]
	15-18 x 9-10 µm.[7]	15-18 x 7-10 µm.[7]
Cystidia	only on edge of gill[6, 8]	on gill face and edge[6, 8]

[1] Fries (1821).
[2] Greville (1827).
[3] Berkeley (1860).
[4] Saccardo (1887).
[5] Cleland & Cheel (1918).
[6] Rea (1922).
[7] Bresadola (1927).
[8] Kühner & Romagnesi (1978).

Table 3.3 A comparison of the published spore data of *Stropharia semiglobata* (Batsch ex Fr.) Quél. and *S. stercoraria* (Bull. ex Fr.) Quél.

S. semiglobata	*S. stercoraria*	Source
13 - 14 x 8 -9 µm.	18 - 20 x 10 -13 µm.	Saccardo 1887.
15 - 18 x 9 - 10 µm.	15 - 18 x 7 - 10 µm.*	Bresadola 1927.
also var. *radicata*		
16 - 20 x 8 -10 µm.		Bresadola 1927.
15 - 17 x 9 - 10 µm.	18 - 20 x 8 - 10 µm.*	Rea 1922.
	16 - 19 x 9 - 10 µm. &	Cleland 1934.
	14 - 24 x 7 - 12 µm.	
(15-)17 - 20 x 8 - 10 µm.*		Watling 1973 & Watling & Gregory 1987.
15 - 20 x 8 - 10 µm.		Lange & Hora 1975.
15 - 22 x 8.5 - 11 µm.		Miller 1975.
16 - 21(-24) x 8 -10 (-11) µm.		Kuhner & Romagnesi 1978.
15 - 20 x 8.5 - 11 µm.		Groves 1979.
15 - 19 x 7.5 - 10 µm.		Lincoff 1981.
15 - 19 x 8 - 10 µm.†		Moser 1983.
15 - 19 x 7.5 - 10 µm.*		Aurora 1986.
15 - 20 x 8 - 10 µm.		Young 1986.

* Facial cystidia (as chrysocystidia) present.

† Cystidia present but did not specify whether on gill face or edge.

Table 3.4 A summary of the macroscopic characters and habitats of the non-coprophilous species of *Stropharia* in SE Tasmania.

	S. coronilla	*S. aurantiaca*	*Stropharia* sp. A
Pileus			
colour	pale yellow to ochraceous brown	reddish orange	date or vinaceous brown
surface	greasy, glabrous	greasy, glabrous	slimy viscid, glabrous or with fine whitish appressed squamules
Annulus	striate, semi-persistent	evanescent	evanescent
Lamellae	grey	pallid to greyish buff	yellowish grey
Stipe	±glabrous	fibrillose	flocculose
Habitat	amongst grass on ground	on lawn, ground litter or eucalypt wood chips	on ground litter

Table 3.5 Summary of microscopic characters of non-coprophilous taxa of *Stropharia* in Tasmania. Mean values and standard deviation (s.d.) are given in µm, the number in parentheses is the number of collections used to calculate the mean. Legend: SL = length of spore, SF = facial width of spore, SP = profile width of spore, BL = length of basidium, BW = broadest part of basidium, CHRL = length of chrysocystidium, CHRW = broadest part of chrysocystiodium, CHL = length of cheilocystidium, CHW = broadest part of cheilocystidium.

	S. aurantiaca (n=4)	**Stropharia sp A** (n=5)	**S. coronilla** (n=3)
SL (±s.d.)	10.71 ±0.50	12.00 ±1.22	8.31 ±0.40
SF (±s.d.)	6.83 ±0.34	7.30 ±0.72	5.55 ±0.25
SP (±s.d.)	6.65 ±0.36	7.12 ±0.65	5.33 ±0.26
BL (±s.d.)	25.39 ±2.01	27.74 ±2.83	25.57 ±1.80
BW (±s.d.)	9.04 ±1.26	9.50±0.71	8.49 ±0.95
CHRL (±s.d.)	42.93 ±4.66	49.4752±6.18	38.26 ±5.018
CHRW (±s.d.)	13.77±1.58	14.19 ±1.87	11.423 ±1.15
CHL (±s.d.)	27.62±2.74	29.24 ±3.06	34.67 ±4.06
CHW (±s.d.)	10.21 ±1.91	7.01 ±1.36	12.55 ±1.95

Table 3.6 Summary of macroscopic characters of the coprophilous taxa of *Stropharia* included in the morphological study where T = fresh collections of Tasmanian material, l = length, w = width and N/A= not availble.

	S. stercoraria (T)	*S. stercoraria*†	*S. semiglobata**	*Stropharia* sp C
Pileus				
colour	corn, straw or pale yellow to olive brown (4B5, 4C4 -6 or 4E7-8)	yellow	yellow ochre shaded with grey	brownish orange (5C3) with a pinkish hue
surface	slimy viscid	viscid	very viscid	slimy viscid
diam. (mm.)	7-27	25		4 -10
Lamellae				
colour	yellowish grey (3D2 - 4B3)	whitish	grey	yellowish grey (4B3)
attachment	adnate	adnate	deep acute adnate	subdecurrent
Stipe				
surface	slimy viscid with a layer of gluten	viscid, smeared with glutinous veil	viscid	slimy viscid with a layer of gluten
l x w (mm.)	28-96 x 1-3	37-100 W(N/A)	N/A	18-52 x 0.5-1.5
Spore print	violaceous black	violaceous black	violaceous black	violaceous black
Habitat	Dung of cow, horse, wallaby or wombat	Cow and horse dung	Cow dung	Wallaby dung

† Taken from Cleland & Cheel (1918) & Cleland(1934).

* Taken from collector's notes.

Table 3.7 Summary of major microscopic characters of coprophilous taxa of *Stropharia* included in the study. Mean values and standard deviations are given in µm. For legend see Table 3.5.

	S. stercoraria (MF) (n=13)	S. stercoraria (LF) (n=6)	S. semiglobata (MF) (n=1)	S. semiglobata (LF) (n=2)	Stropharia sp C (n=3)
SL (± s.d.)	13.59 ± 0.94	19.33 ± 1.24	13.42 ± 0.77	18.15 ±1.11	14.33 ± 0.66
SF (± s.d.)	8.16 ± 0.64	11.66 ± 0.68	7.88 ± 0.40	11.05 ± 0.66	8.60 ± 0.43
SP (± s.d.)	8.01 ± 0.63	11.54 ± 0.66	7.65 ± 0.38	10.85 ± 0.75	8.52 ± 0.43
BL (± s.d.)	30.46 ± 2.97	34.21 ± 4.15	28.25 ± 2.13	36.35 ± 3.71	26.94 ± 4.61
BW (± s.d.)	12.11 ± 1.36	14.42 ± 2.05	10.54 ± 0.76	14.92 ± 2.03	12.75 ± 2.24
CHRL (± s.d.)	45.55 ± 7.09	52.19 ± 7.58	48.58 ± 7.05	49.78 ± 7.84	41.69 ± 3.88
CHRW (± s.d.)	15.80 ± 1.96	16.21 ± 2.33	15.00 ± 1.53	15.22 ± 2.08	14.31 ± 1.88
CHL (± s.d.)	34.45 ± 6.33	38.14 ± 7.52	36.87 ± 4.34	35.33 ± 5.25	31.49 ± 3.84
CHW (± s.d.)	9.01 ± 1.49	8.03 ± 1.52	8.25 ± 0.78	7.80 ± 1.41	6.76 ± 1.18

Table 3.8 A summary of similarity in band activity (of same R_f) in the five enzyme systems (Lac, AcP, Per, PE & PG) between isolates of the five putative groups of *Stropharia.*

Band No	R_f	Taxa showing similarities in band activities
Lac		
13	0.46	*S. stercoraria* (LF) & *S. aurantiaca*
14	0.50	*S. stercoraria* (MF) & *Stropharia* sp C
15	0.52	*S. stercoraria* (MF), *S. aurantiaca* & *Stropharia* sp A
AcP		
4	0.08	*S. stercoraria* (LF) & *Stropharia* sp C
5	0.11	*S. atercoraria* (MF) & *S. stercoraria* (LF)
6	0.14	*S. stercoraria* (LF) & *Stropharia* sp C
11	0.24	*S. stercoraria* (MF), *S. stercoraria* (LF) & *Stropharia* sp C
Per		
2	0.07	*S. stercoraria* (MF), *S. stercoraria* (LF) & *Stropharia* sp C
4	0.12	*S. stercoraria* (LF) & *Stropharia* sp C
6	0.18	*S. stercoraria* (MF), *S. stercoraria* (LF) & *Stropharia* sp C
8	0.22	*S. stercoraria* (LF), *Stropharia* sp A & *Stropharia* sp C
13	0.38	*S. aurantiaca* & *Stropharia* sp A
15	0.46	*Stropharia* sp C & *S. aurantiaca*
18	0.52	*S. stercoraria* (MF) & *S. stercoraria* (LF)
PE		
12	0.34	*S. stercoraria* (MF), *Stropharia* sp C & *S. aurantiaca*
13	0.37	*S. stercoraria* (MF) & *S. aurantiaca*
16	0.46	*S. stercoraria* (MF) & *Stropharia* sp A
17	0.49	*S. stercoraria* (MF), *S. aurantiaca* & *Stropharia* sp A
PG		
4	0.06	*S. stercoraria* (MF) & *Stropharia* sp C
6	0.11	*S. stercoraria* (MF) & *Stropharia* sp C
7	0.16	*S. stercoraria* (MF) & *Stropharia* sp C
9	0.22	*S. stercoraria* (MF), *S. stercoraria* (LF) & *Stropharia* sp C
12	0.28	*S. stercoraria* (LF) & *Stropharia* sp C

Table 3.9. Crosses between the known mating types of CYS483 and isolates of other collections of the coprophilous taxa of *Stropharia*. MF = medium-spored form, LF = large-spored form, MK = monokaryon, W = wild type & * =pseudoclamps noted in one confrontation.

Species & strain no.	No. of isolates & type	Species & strain no.	No. of isolates & type	Total no. pairings	No. of positive pairings	No. of negative pairings
S.stercoraria **(MF)**		***S. stercoraria*** **(MF)**				
CYS483	4 (MK)	x CYS437	2 (MK)	8	8	0
(01, 06, 03 & 05)		(01, 02 & 03)				
		x CYS386	1 (MK)	4	4	0
		(01)				
		x CYS431	1 (W)	4	4	0
		x CYS443	1 (W)	4	4	0
		x CYS390	1 (MK)	4	2	2
		(01)				
		x CYS343	1 (MK)	4	4	0
		(01)				
		x CYS200	2 (MK)	8	0	8
		(01 & 02)				
		x CYS444	1 (W)	4	4	0
		x CYS447	1 (W)	4	4	0
		S. stercoraria **(LF)**				
		x CYS351	2 (MK)	8	0	8
		(04 & 05)				
		x CYS382	3 (MK)	12	0	12*
		(01, 02 & 03)				
		Stropharia **sp. C**				
		x CYS486	4 (MK)	16	0	16
		(06, 07, 12 & 15)				
		x CYS364	1 (MK)	4	0	4
		(01)				
CYS200	2 (MK)	x CYS382	2 (MK)	4	0	4
		(01 & 02)				
		S.stercoraria **(MF)**				
		x CYS437	2 (MK)	4	0	4
		x CYS390	1 (MK)	2	0	2
		x CYS386	1 (MK)	2	0	2
S.stercoraria **(LF)**		***S.stercoraria*** **(LF)**				
CYS351	2 (MK)	x CYS382	2 (MK)	4	4	0
Stropharia **sp. C**		***Stropharia*** **sp. C**				
CYS486	4 (MK)	x CYS364	1 (MK)	4	4	0
		S. stercoraria **(MF)**				
		x CYS343	1 (MK)	4	0	4
		S. stercoraria **(LF)**				
		x CYS351	2 (MK)	8	0	8

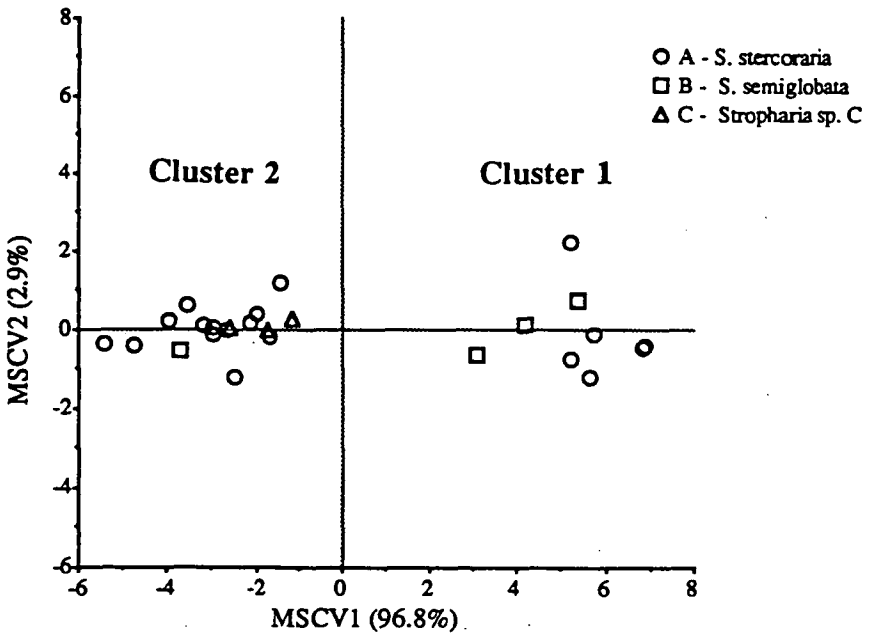


Fig. 3.1. Scatter plot of mean canonical variates (MSCV1 & MSCV2) generated from canonical discriminant analysis of spore variables from collections of coprophilous taxa of *Stropharia*.

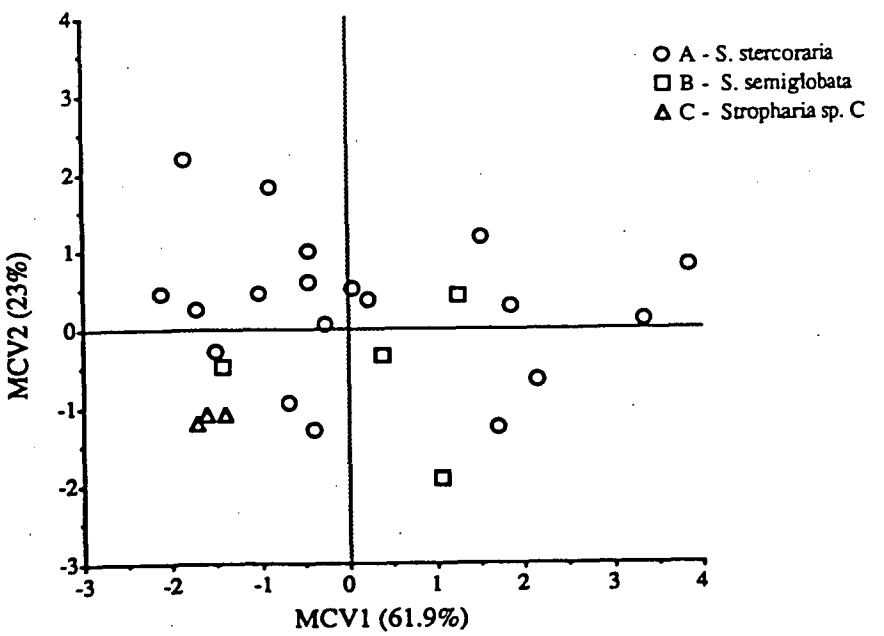


Fig. 3.2. Scatter plot of mean canonical variates (MCV1 & MCV2) generated from CDA of cystidia variables from collections of the coprophilous taxa of *Stropharia*.

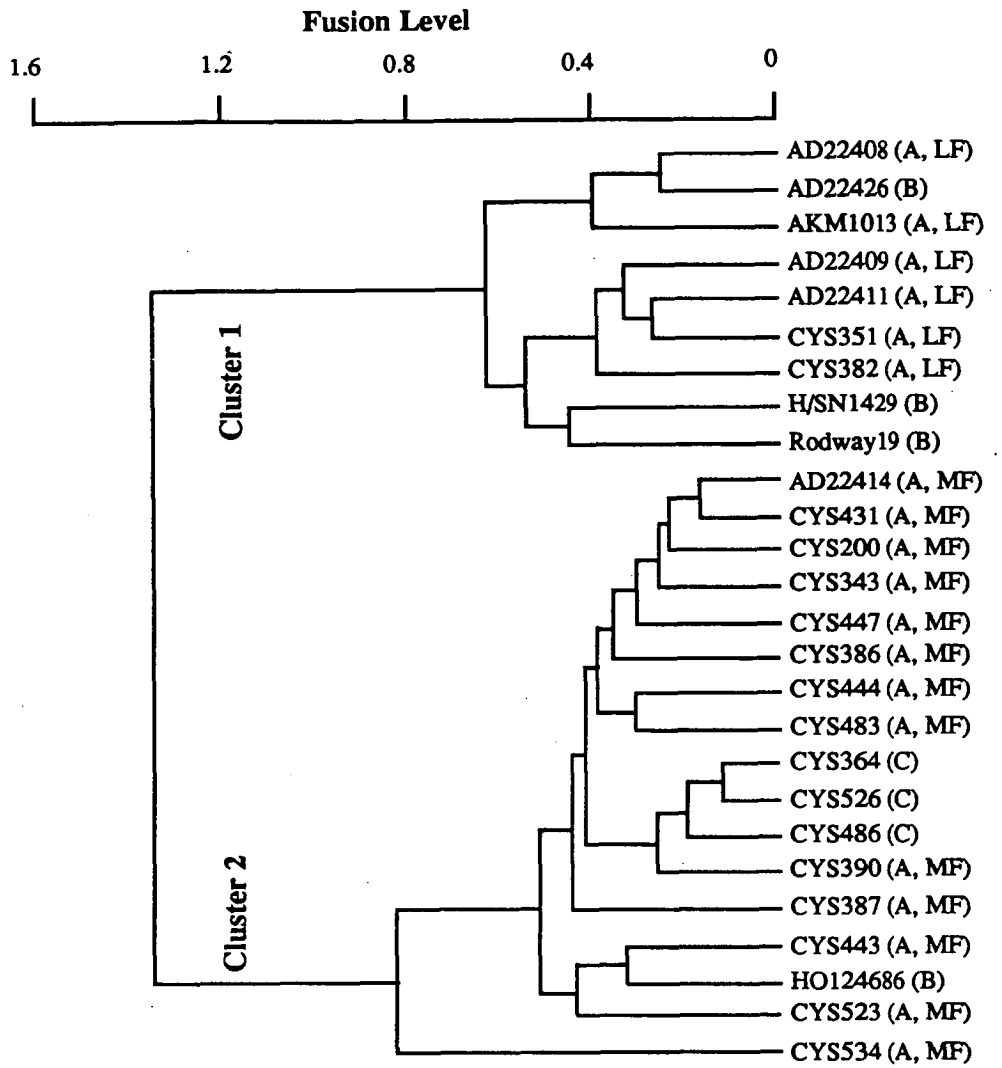
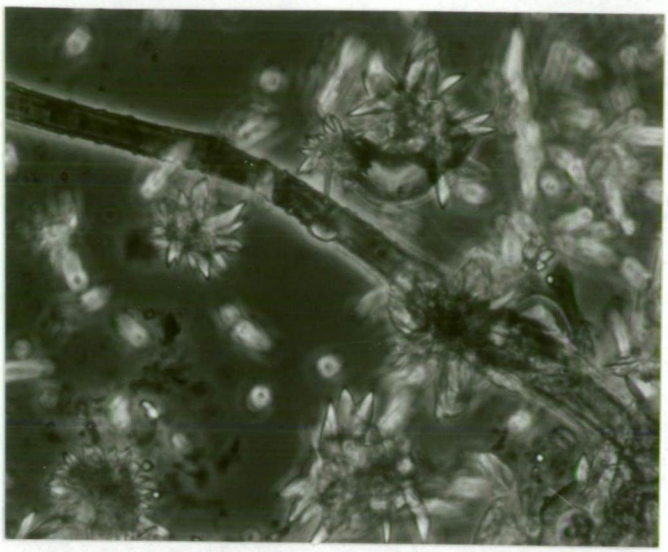
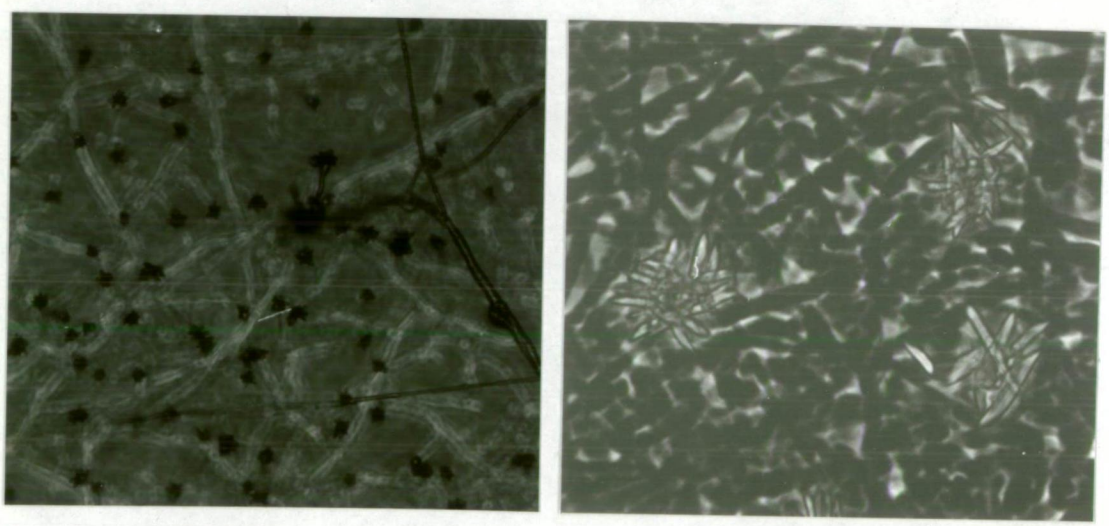


Fig. 3.3. Dendogram constructed from UPGMA cluster analysis based on all the mean canonical variates showing two distinct clusters, cluster 1=collections of *S. stercoraria* (LF) & *S. semiglobata* and cluster 2=collections of *S. stercoraria* (MF), *S. semiglobata* & *Stropharia* sp. C.

a



b



CYS447 CYS483

Fig. 3.4. Acanthocytes observed in species of *Stropharia*. (a) lightly squashed preparation of basal mycelium of CYS386, and (b) cultures of CYS447 and CYS483, all *S. stercoraria* (MF).

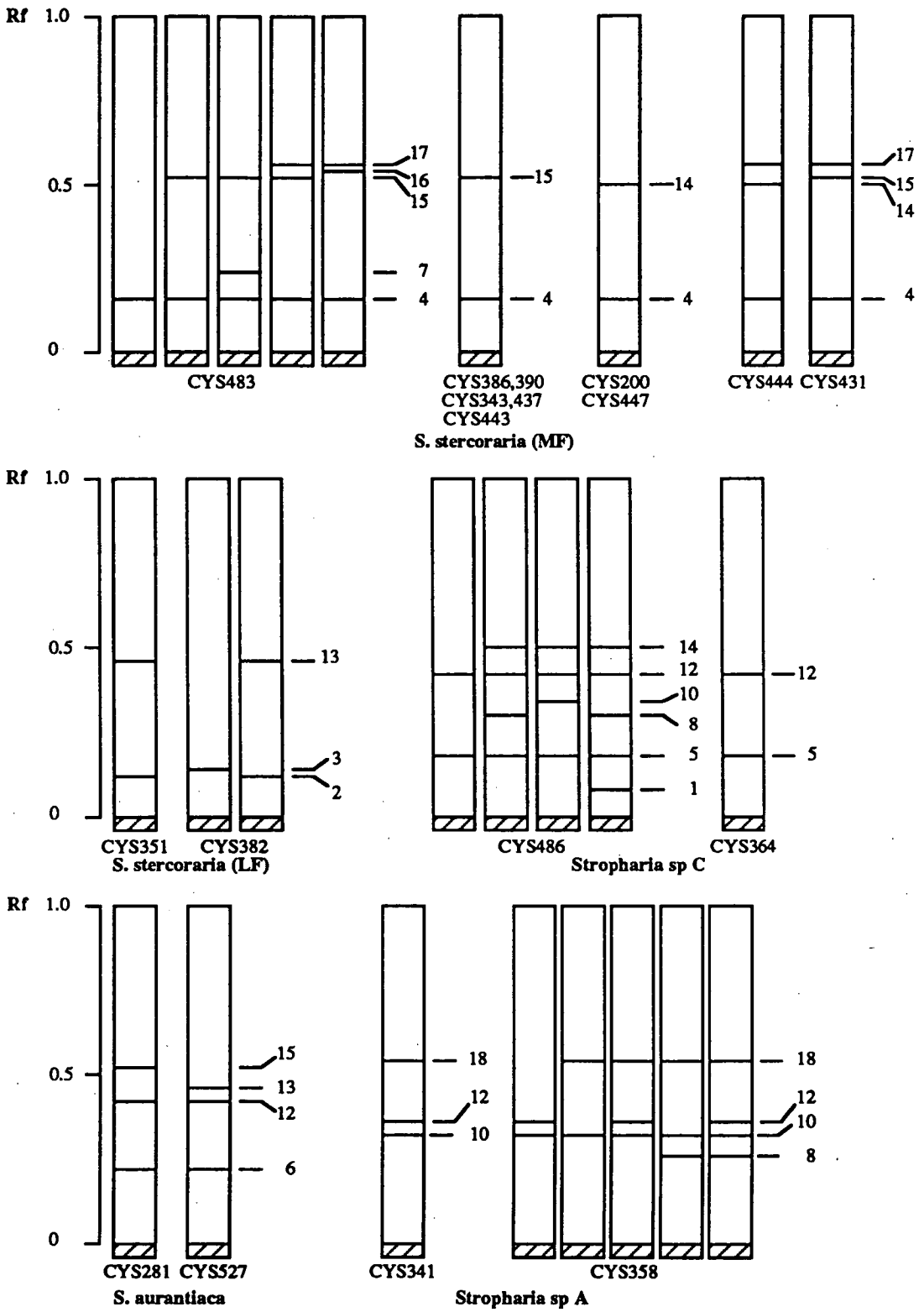
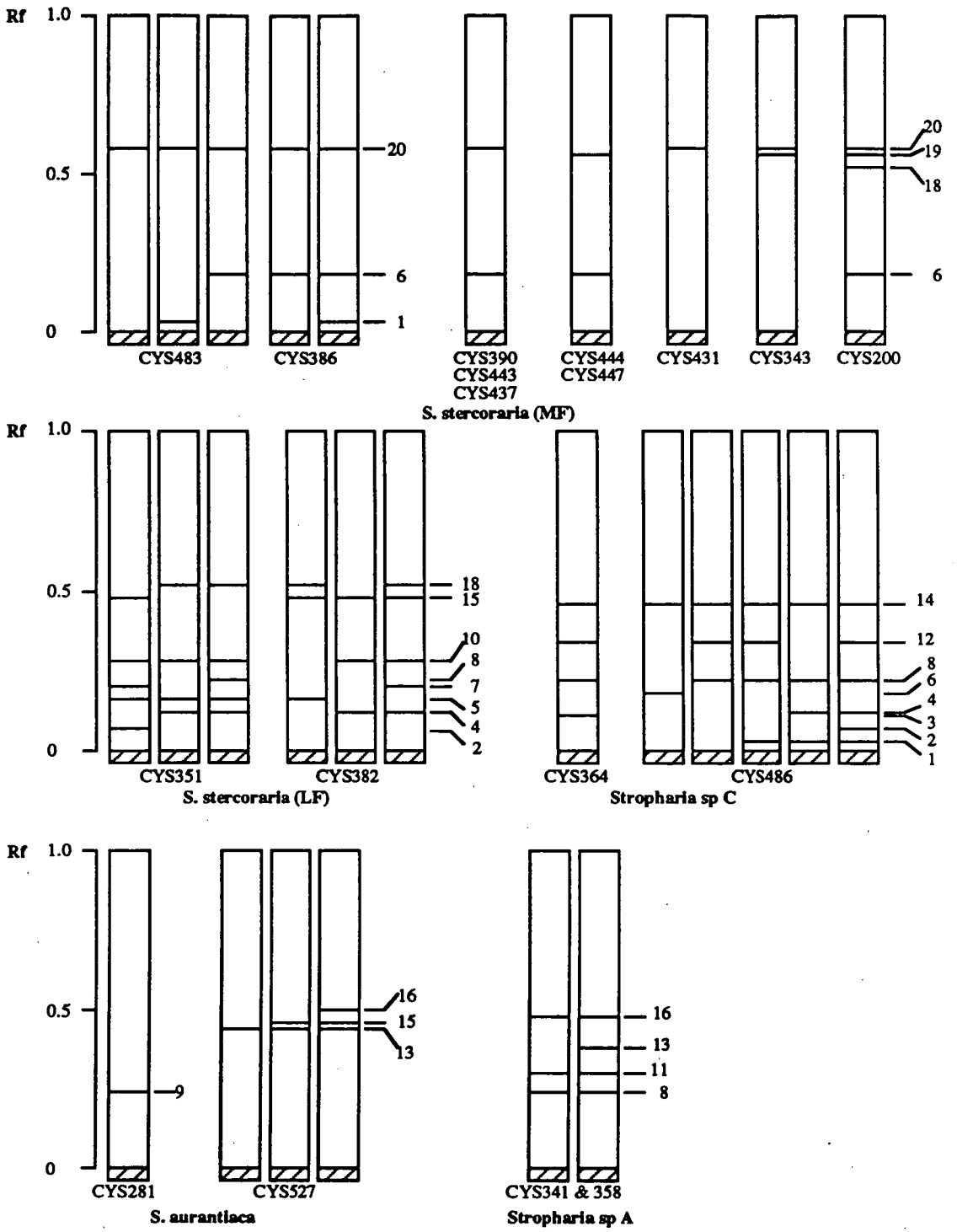


Fig 3.5. Schematic representations of Lac isozymes of isolates of taxa of *Stropharia* included in the study. Band numbers start from the cathodic end. Rf values: 1=0.08, 2=0.12, 3=0.14, 4=0.16, 5=0.18, 6=0.22, 7=0.24, 8=0.30, 9=0.32, 10=0.34, 11=0.36, 12=0.42, 13=0.46, 14=0.50, 15=0.52, 16=0.54, 17=0.56, 18=0.58 & 19=0.62.



Flg. 3.6. Schematic representations of Per isozymes of isolates of taxa of *Stropharia* included in the study. Band numbers start from the cathodic end. Rf values: 1=0.03, 2=0.07, 3=0.11, 4=0.13, 5=0.16, 6=0.18, 7=0.20, 8=0.22, 9=0.24, 10=0.28, 11=0.30, 12=0.34,13=0.38, 14=0.44, 15=0.46, 16=0.48, 17=0.50,18=0.52, 19=0.56 & 20=0.58.

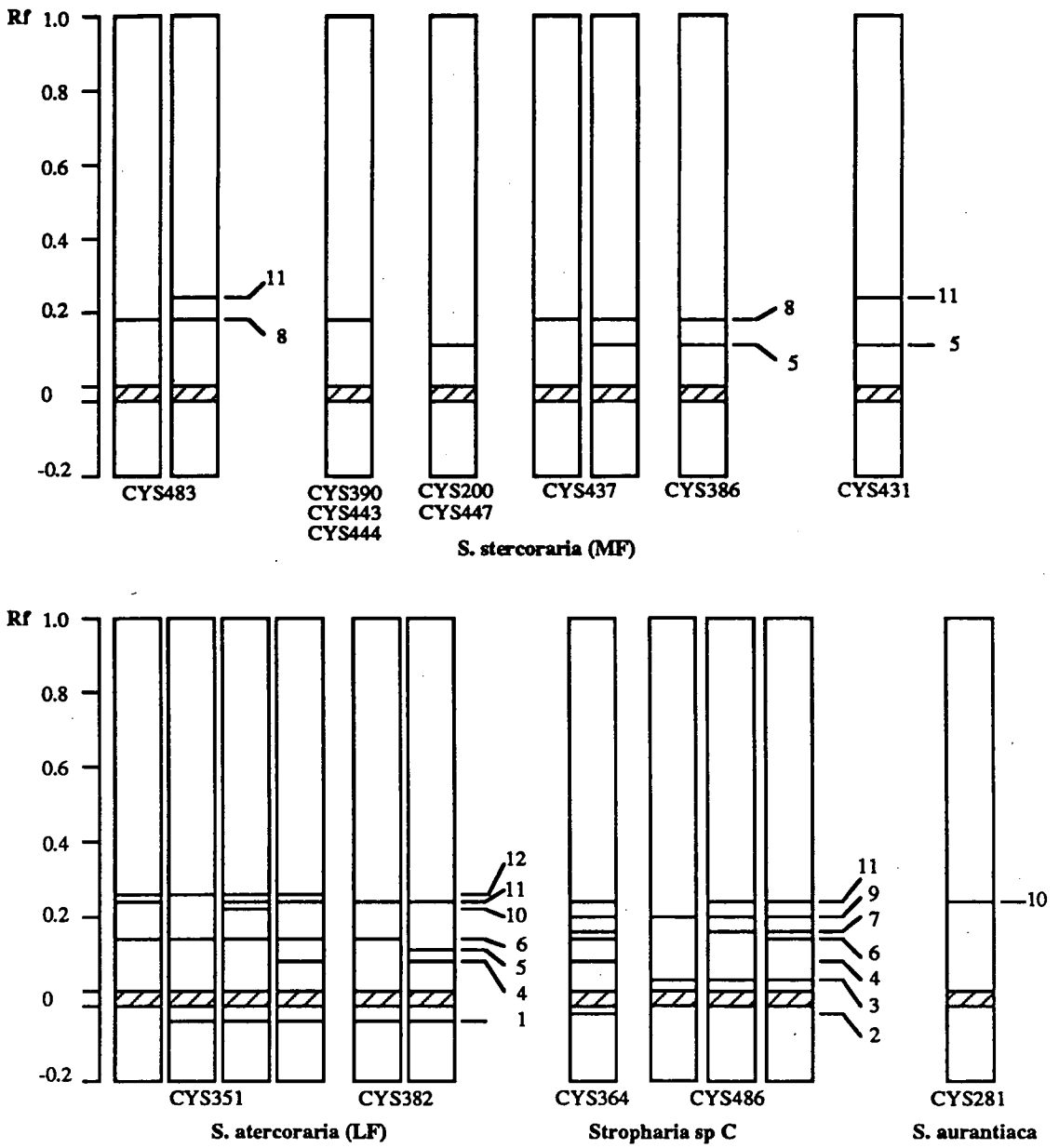


Fig. 3.7. Schematic representations of AcP zymograms of isolates of taxa of *Stropharia* included in the study. Band numbers are given from the cathodic end. Rf values : 1=-0.04, 2=-0.02, 3=0.03, 4=0.08, 5=0.11, 6=0.14, 7=0.16, 8=0.18, 9=0.20, 10=0.22, 11=0.24 and 12=0.26.

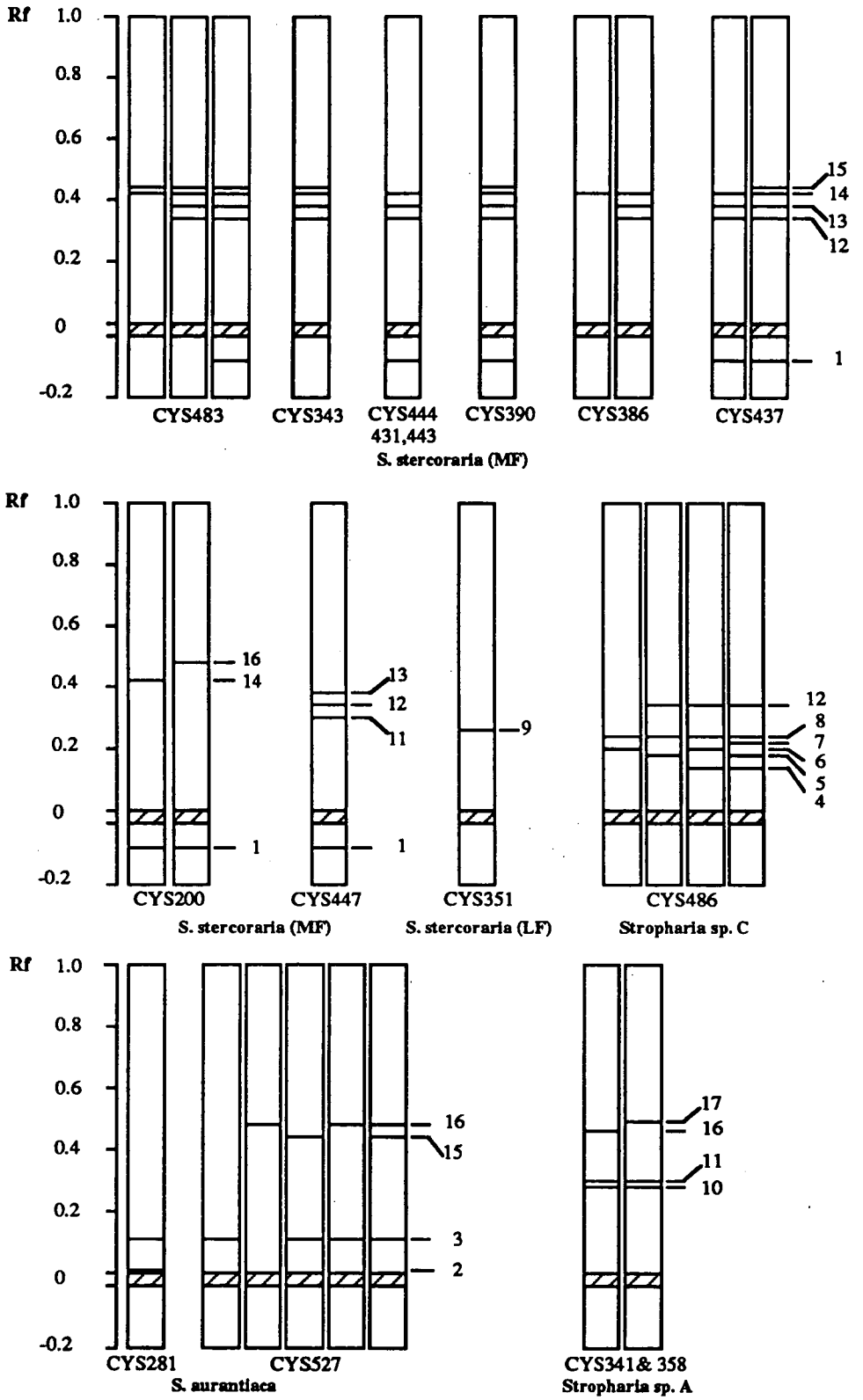


Fig. 3.8. Schematic representations of PE zymograms of isolates of species of *Stropharia*. Band numbers start from the cathodic end. Rf values: 1=-0.07, 2=0.01, 3=0.11, 4=0.14, 5=0.18, 6=0.20, 7=0.22, 8=0.24, 9=0.26, 10=0.28, 11=0.30, 12=0.34, 13=0.37, 14=0.41, 15=0.44, 16=0.46 &17=0.49.

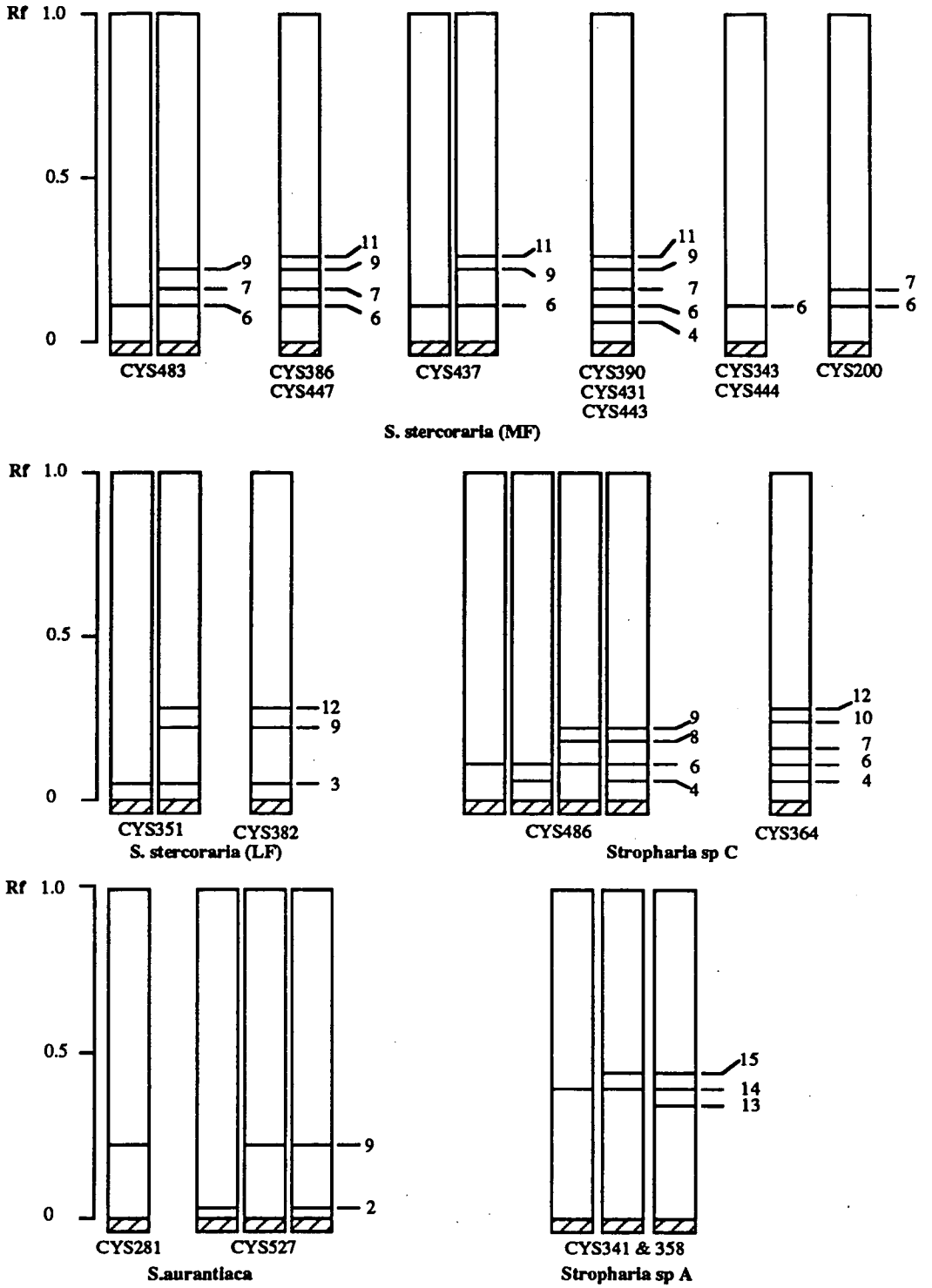


Fig. 3.9. Schematic representations of PG isozymes of isolates of taxa of *Stropharia* included in the study. Band numbers start from the cathodic end. Rf values: 1=0.01, 2=0.03, 3=0.05, 4=0.06, 5=0.08, 6=0.11, 7=0.16, 8=0.18, 9=0.22, 10=0.24, 11=0.26,12=0.28, 13=0.34,14=0.40 & 15=0.44.

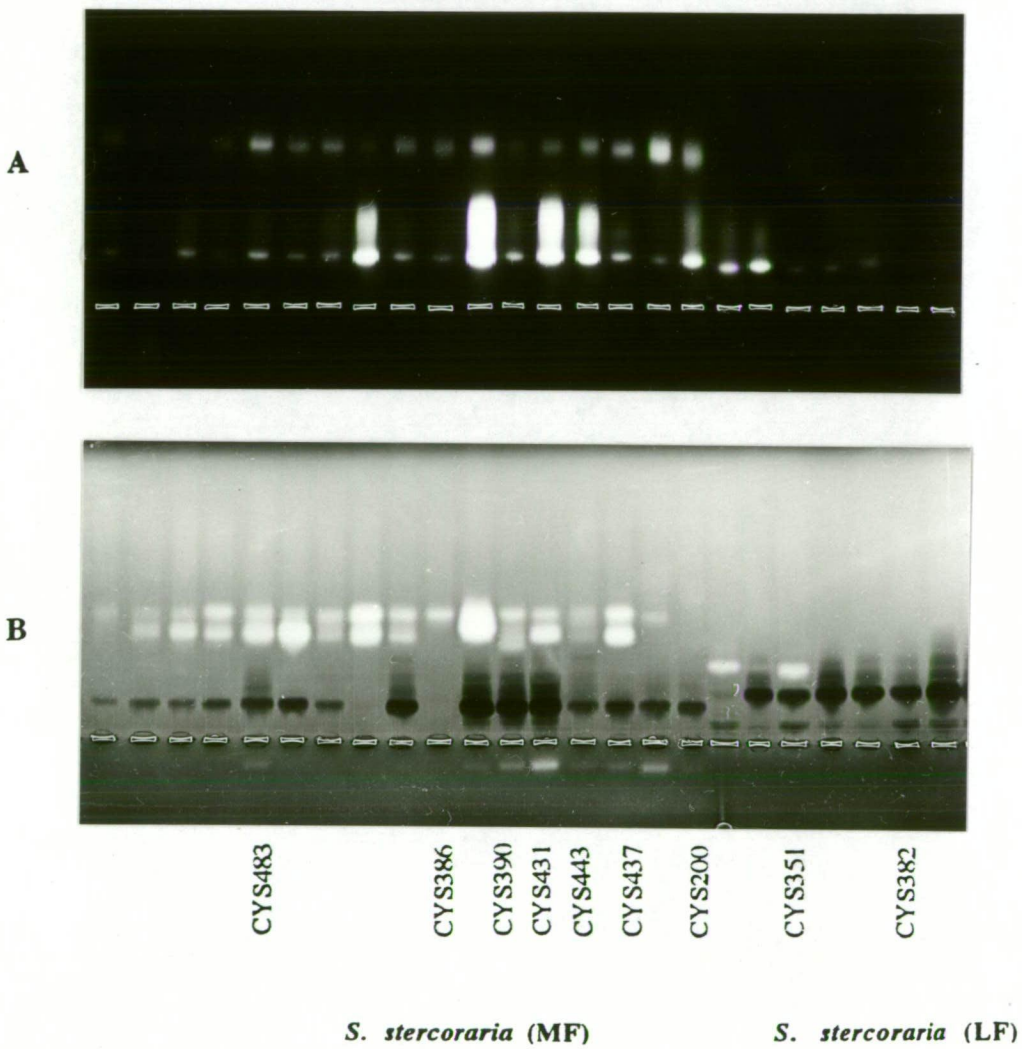


Fig. 3.10. Photograms of laccase (A) and pectic (B) zymograms of isolates of *Stropharia stercoraria* (MF) and *S. stercoraria* (LF). In the pectic zymogram, dark bands represent activities of polygalacturonase (PG) and white bands those of pectinesterase (PE). (x 0.8)

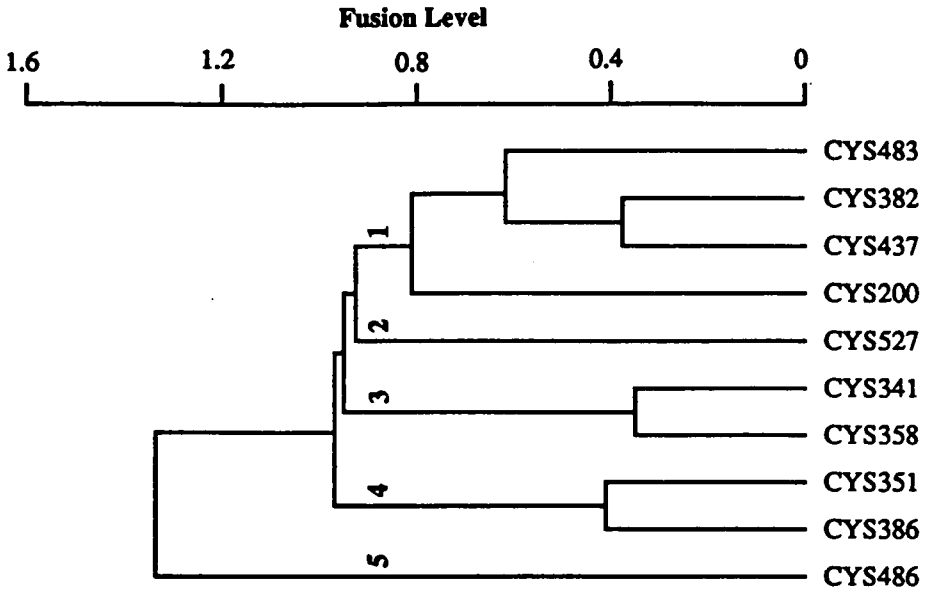


Fig. 3.11. Dendogram constructed from UPGMA cluster analysis based on band frequencies of Lac, AcP, PE and PG of isolates of the five putative taxa of *Stropharia*. 1=*S. stercoraria* (MF), 2=*S. aurantiaca*, 3=*S. stercoraria* (LF), 4= *Stropharia* sp. A and 5=*Stropharia* sp. C.

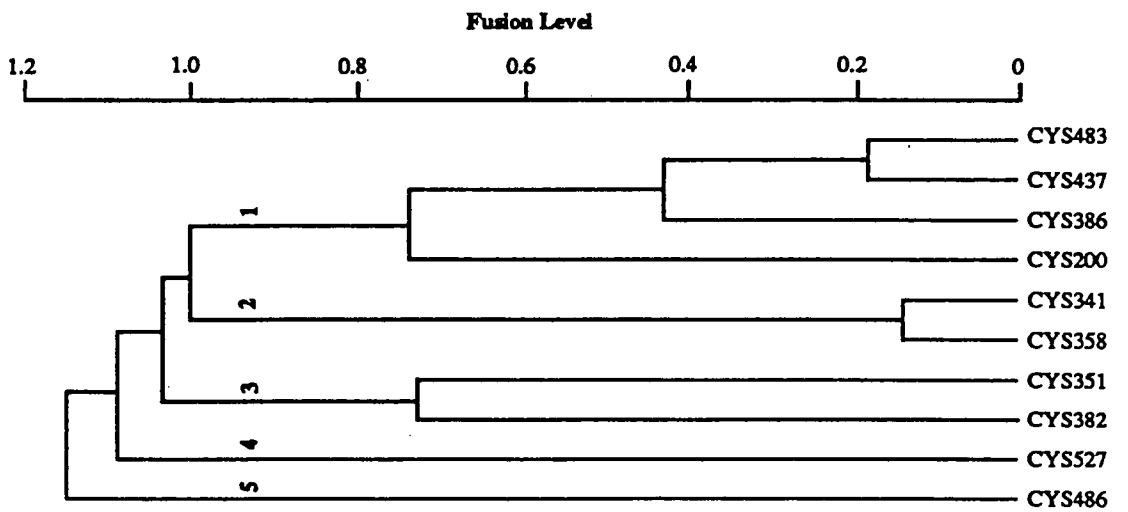
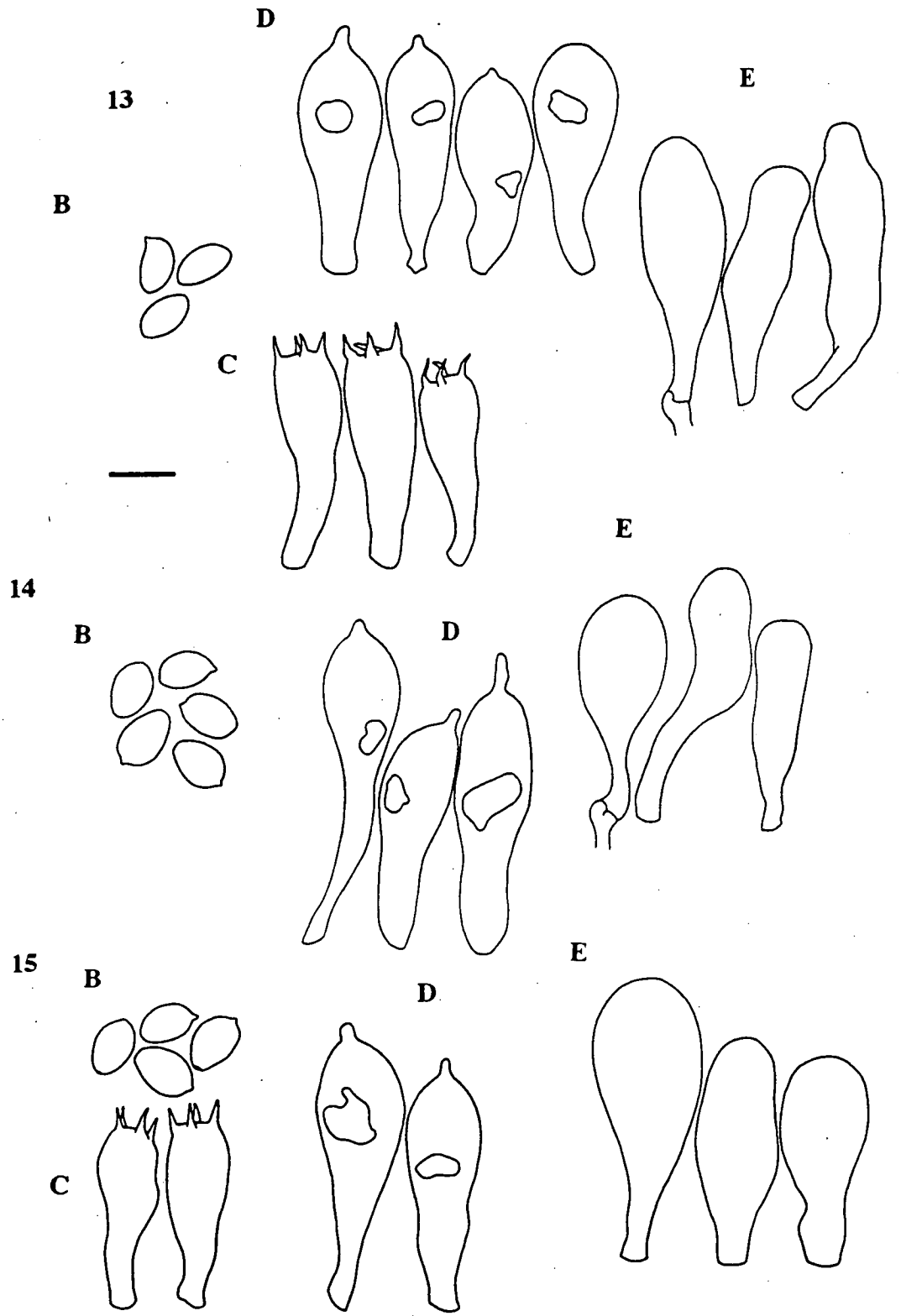
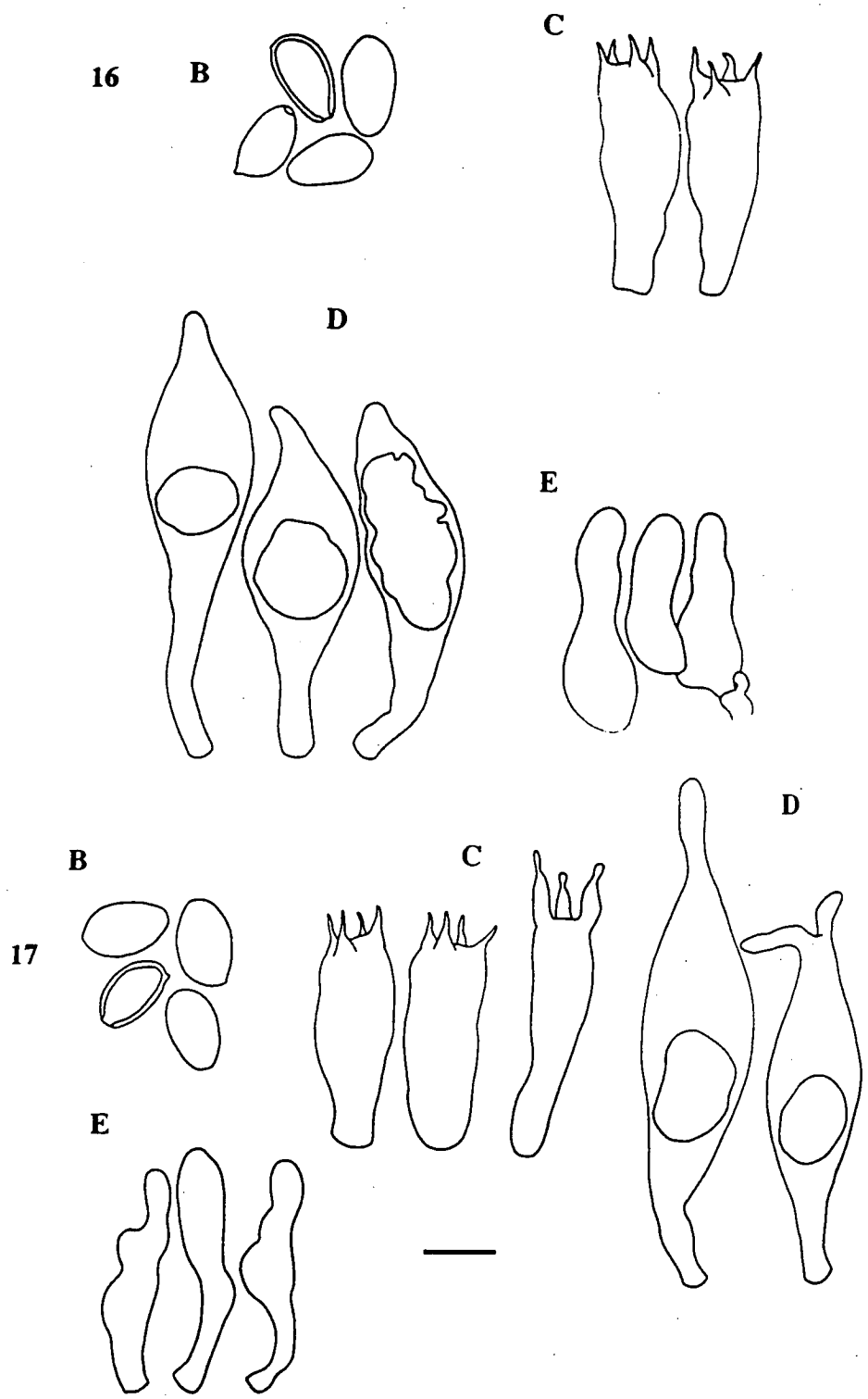


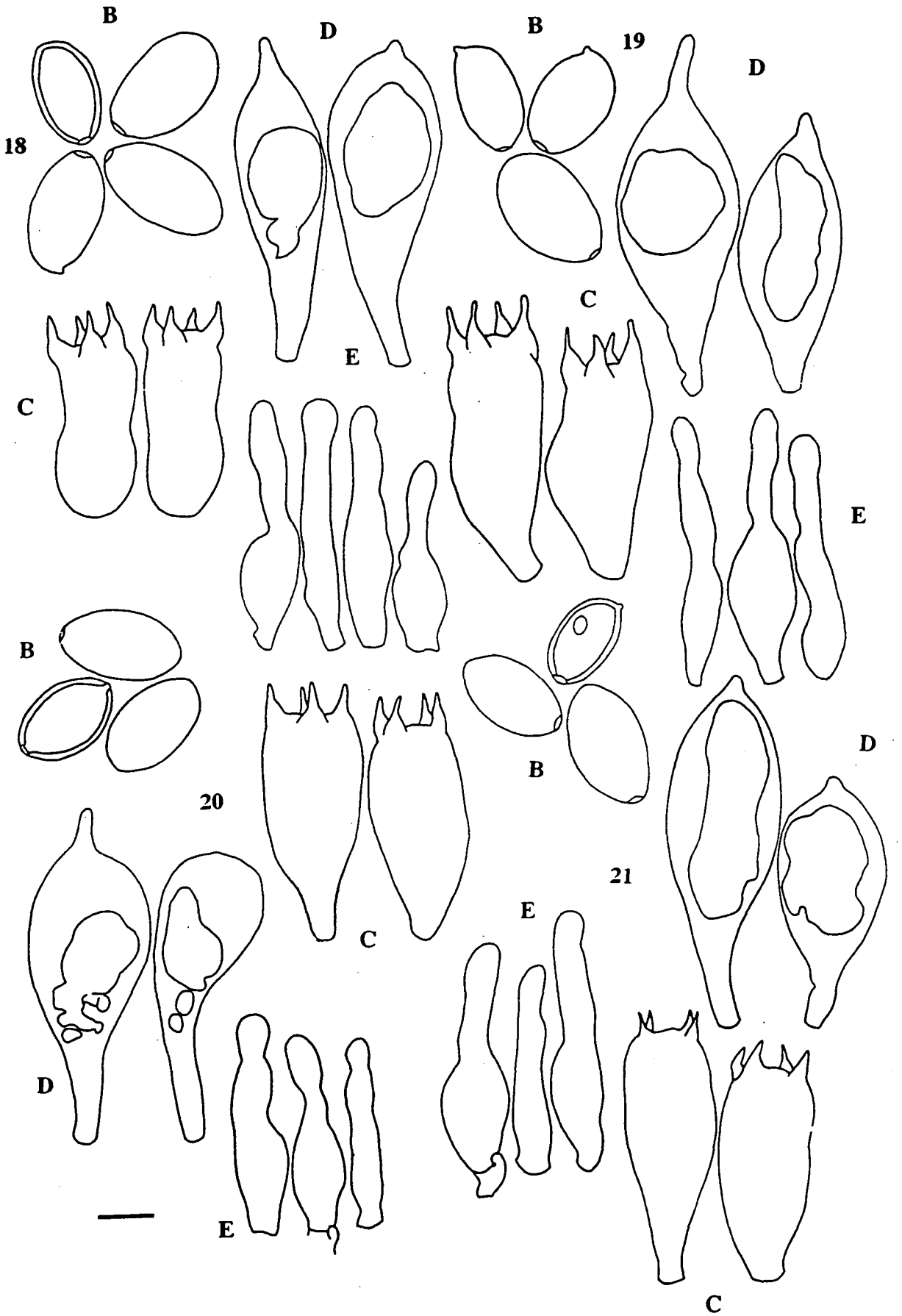
Fig. 3.12. Dendogram constructed from UPGMA cluster analysis based on band frequencies of Per isozymes of isolates of the five putative taxa of *Stropharia* where 1=*S. stercoraria* (MF), 2=*Stropharia* sp. A, 3=*S. stercoraria* (LF), 4=*S. aurantiaca* and 5=*Stropharia* sp. C.



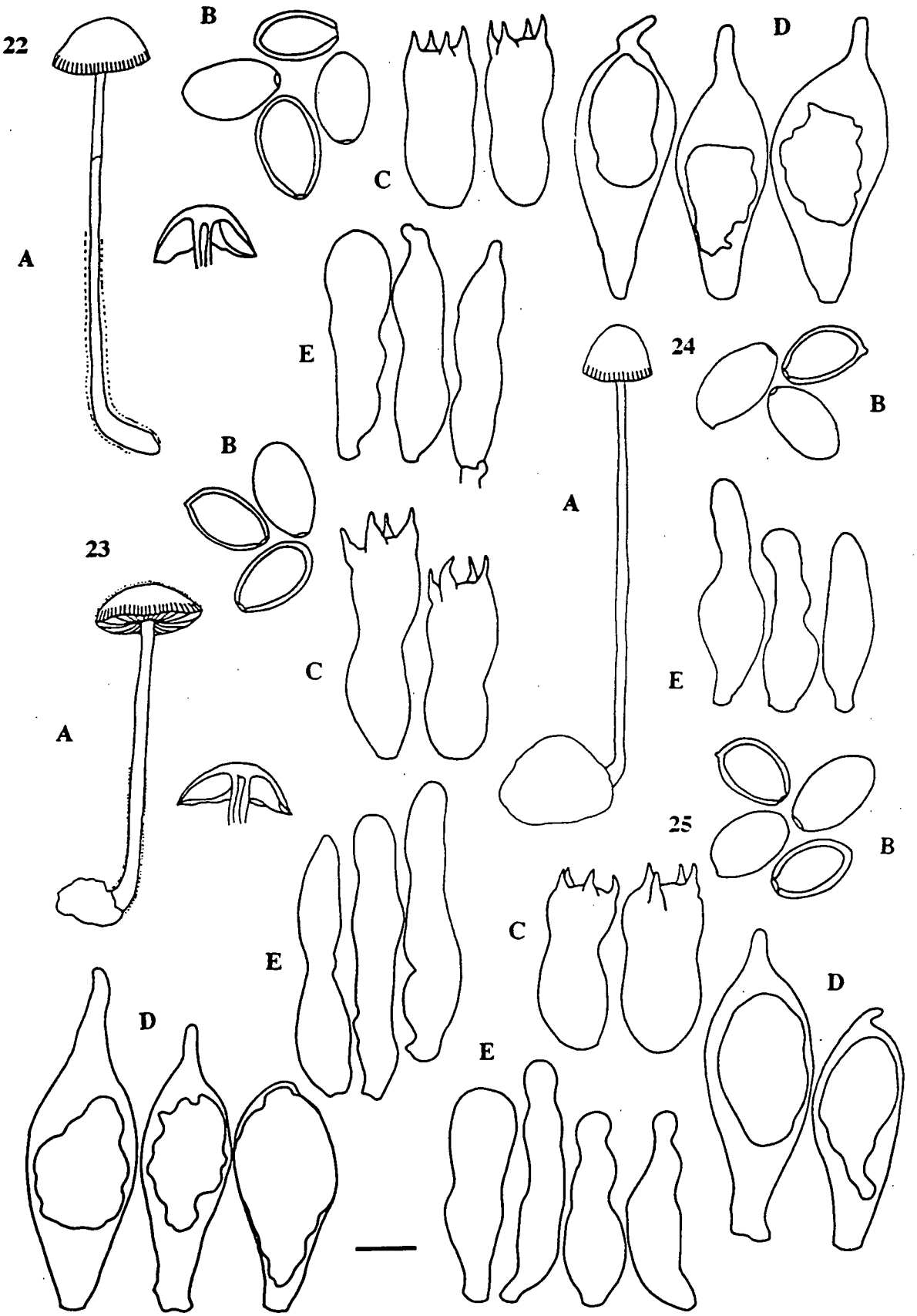
Figs. 3.13 - 15. *Stropharia coronilla.* B: spores, C: basidia, D: chrysocystidia and E: cheilocystidia. 13. CYS 556. 14. CYS 557. 15. CYS 558.



Figs. 3.16 - 17. *Stropharia aurantiaca.* B: spores, C: basidia, D: chrysocystidia and E: cheilocystidia. 16. CYS 230. 17. CYS 527.



Figs. 3.18 - 21. *Stropharia stercoraria*. B: spores, C: basidia, D: chrysocystidia and E: cheilocystidia. 18. CYS351. 19. CYS382. 20. AD22409. 21. *Hargreaves & Sinnott* 1429 (as *S. semiglobata*, K).



Figs. 3.22 - 25. *Stropharia semiglobata.* A: habit, B: spores, C: basidia, D: chrysocystidia and E: cheilocystidia. 22. CYS483. 23. CYS270. 24. CYS200. 25. AD22414 (as *S. stercoraria*).

Fig. 3.26. Habit of Tasmanian specimens of *Stropharia semiglobata* (CYS269, 270 and 273) showing variations in pileus colour.

Chapter 4

Genus *Hypholoma* (Fr.) Kummer

4.1. Introduction

The first taxonomically important description of the genus *Hypholoma* was that of Fries in 1821 (Parker 1933). He subsequently regarded *Hypholoma* as a subgenus of the composite genus *Agaricus*. He at first divided the species in *Hypholoma* according to the hygrophanous nature of the pileus. In his Hymenomycetes Europaei of 1874 (see Parker 1933), Fries further divided the species in *Hypholoma* into five sections (Table 4.1).

Kummer and Quélet expanded the Friesian concept of *Hypholoma* by the inclusion of *Flammula*-like species by the former and the 'fragile' species such as *H. candolleanum* (now *Psathyrella candolliana*) by the latter. Smith (1951) considered Karsten to be the first person to make the distinction between *Flammula* -like and fragile species and preferred the genus *Naematoloma* Karst. to include the *Flammula*-like purple-brown-spored species which previously formed part of the genus *Hypholoma*. Like Smith, Singer (1986) preferred the use of *Naematoloma*. This acceptance of Karsten's genus is not echoed by Donk (1962) and various other mycologists (Kühner & Romagnesi 1978; Kühner 1980; Watling 1973; Watling & Gregory 1987). Authors such as Donk (1962) and Orton (1960) commented on their preference for the generic name of *Hypholoma*(Fr.) Kummer. They are joined by modern mycologists such as Kühner, Maublanc and Konrad & Maublanc (see Donk 1962) while others acknowledge both as synonyms. As a result of this difference in the acceptance of the generic name of *Hypholoma*, the type species for the genus has been changed several times. Starting from *H. velutinum* which is rejected by Donk because it is not an original species of Fries, to *H. lacrymabundum* which found a place in *Psathyra* (Fr.) Kummer, now *Psathyrella* (Fr.) Quél. *Agaricus fascicularis* is selected by Donk (1962) to be the type

species of the genus on the basis that it is an original species of Fries and that both Fries and Kummer emphasised the important characters that typify the genus in their descriptions. However, the type species of *Naematoloma* is *A. sublaterius* Fr.

In his treatment of the North American species, Smith (1951) established two sections (Tenacia and Fascicularia) for *Naematoloma* (Table 4.1). Singer (1986) recognizes four sections (Table 4.1) of which section *Naematoloma* is equivalent to Smith's *Fascicularia*, and *Psilocyboides* to *Tenacia*. Two of the species in Singer's section *Stropholoma* are better known as species of *Stropharia*. *N. squamosum* (Pers. ex Fr.) Singer is better known as *S. squamosa* (Pers. ex Fr.) Quél. and *N. aurantiacum* (Cooke) Guzmán ex Singer as *S. aurantiaca* (Cooke) Orton (Orton 1960; Dennis *et al.* 1960). While *N. elongatipes* (Peck) Singer in Singer's section *Psilocyboides* has been transferred to *Pholiota* by Smith & Hesler (1968) along with several species of Naematoloma in Smith's section *Tenacia*.

There is not much doubt as to the affinity of genera *Stropharia*, *Hypholoma*, *Psilocybe* and *Melanotus*. Species of *Hypholoma* differ from *Psilocybe* and *Melanotus* in the presence of chrysocystidia. At the macroscopic level, *Hypholoma* frequently differs from *Stropharia* in the absence of an annulus. This distinction breaks down when comparing the exannulate species of *Stropharia*. However, since most of the exannulate species of *Stropharia* are coprophilous, their habitat is distinctive. The subcellular hypodermium is by far most effective in the separation of *Hypholoma* from these three purple brown-spored genera.

Distinction of *Hypholoma* from *Pholiota* is at times not very obvious and even difficult. Spore print colour is only decisive when the difference is obvious (i.e. violaceous black or fuscous black for majority of *Hypholoma* species and the predominantly brown colour of *Pholiota* species) and less so in species with

intermediate colours. Both genera have species with chrysocystidia thus the presence of this character is not a very useful distinction between the two. The surface characters of the pileus appears a better distinction, since none of the species of *Hypholoma* is scaly. However, this distinction tends to break down when comparing the glabrous species of *Pholiota* with *Hypholoma.* The scaly surface is quite evident in the young carpophores of many of these glabrous species of *Pholiota*, thus comparison at the same stage of development may assist in the separation of these two genera. In addition, characters other than surface features such as the subcellular hypodermium and the usually non-viscid pileus become more important in these cases.

The pileus is variously coloured but usually in the yellows and browns or bright orange red. Shape is generally collybioid or convex at first and later expanding to plano-convex to almost plane. Surface of pileus is usually glabrous, only fine squamules noted in some when young, rarely viscid, at most greasy, hygrophanous or not and rarely striate. Veil appendiculate on margin of pileus, evanescent. Lamellae adnexed, adnate or decurrent with a tooth. Stipe central, long and tough, becoming hollow with age, joined at base to form caespitose clusters.

Spore print from violaceous black or fuscous black to dull cinnamon brown. Individual spore dull yellow brown in 5%KOH, thick-walled, germ pore evident to pronounced causing the apex to appear truncate. Basidia generally 4-spored. Pleurocystidia as chrysocystidia, mucronate, with amorphous body easily detected in KOH. Cheilocystidia forming a ±sterile band, hyaline or intermixed with chrysocystidioid forms.

Epicutis consists typically of a non-gelatinised filamentous layer over a subcellular hypodermium. Trama regular. Clamp connections present.

Lignicolous habitat, usually on dead and living or buried wood, also in boggy areas of deep moss (*Sphagnum* and *Polytrichum*).

H. fasciculare and *H. dispersus* have been reported from Tasmania (Cooke 1892). There is a collection of *H. flexipes* Massee & Rodway amongst Rodway's fungal collections held in HO, this name appears to be a *nomen nudum* (Rodway 1900). Reid (1955) describes *H. brunnea* from Tasmanian specimens. Browne (1983) describes two forms of *H. fasciculare* (Hudson ex Fr.) Kummer, *H. sublaterium* (Fr.) Quél.and *H. epixanthum* (Fr.) Quél. (tentative placement).

The name *Hypholoma* (Fr.) Kummer is used throughout this study and is taken to be synonymous with *Naematoloma* (Fr.) Karst. Also any brown-spored species with appropriate characters will be treated as *Hypholoma*. This is a relatively well-studied genus when compared with other genera in the family. Species such as *H. fasciculare* and *H. sublaterium* are well documented (Bresadola 1927; Cleland 1934; Phillips 1981; Watling 1973, Arora 1986 etc.). Consequently much attention is focused on detailing the morphological characters of the Tasmanian material. Electrophoresis of isozymes will be undertaken to see if distinctions in morphology would be extended to zymograms.

4.2. Results

The studies of species of this genus were concentrated chiefly on morphological comparisons because all the taxa collected were morphologically distinct from each other.

4.2.1. Morphological studies

A total of six taxa were separated based on morphological characters. For convenience, all collections corresponding to a particularly taxon were referred to collectively. These taxa corresponded to *Hypholoma fasciculare*, *H. sublaterium*, *H. brunnea* and three

taxa which did not immediately fit any published description. The latter three taxa were from hereon referred to as *Hypholoma* sp. A, *Hypholoma* sp. B and *Hypholoma* sp. C. Two forms were noted in *H. fasciculare* and were referred to as *H. fasciculare* (Y) and *H. fasciculare* (AP). Details of all the collections included in the present study are given in Appendix IIIB.

Tables 4.2 and 4.3 show summaries of the macroscopic characters of the purplish-brown spored and brown-spored taxa of *Hypholoma* respectively. *H. fasciculare* (Y) and *H. fasciculre* (AP) differed only in the colour of pileus and lamellae otherwise they were similar in every aspect. This observation was first noted by Browne (1983) in her studies of *Hypholoma* in Tasmania. The remaining four taxa could be segregated quite easily on the colour of pileus. The non-lignicolous habitat and association with *Sphagnum* and *Polytrichum* separated *Hypholoma* sp. A from the others which were lignicolous. *Hypholoma* sp. C appeared to be less homogenous (Table 4.4). There appeared to be some variations in the colour of the pileus and lamellae. However, due to the limited material it was not possible to establish the range of variation.

Tables 4.5 and 4.6 show summaries of the major microscopic characters of the purplish brown-spored and brown-spored taxa respectivley. Two subgroups could be noted based on spore print colour corresponding approximately to purple brown-spored species and brown-spored species respectively. No spore print was obtained from *Hypholoma* sp. A. However, small patches of brown spore deposit in the apical region of the stipe and the brownish colour of lamellae in mature basidiomes indicate that the spore mass could be predominantly brown. Thus, it was regarded as in the same subgroup as *Hypholoma* sp. B and C. *Hypholoma* sp. B differed from *Hypholoma* sp. A in spore size (Table 4.6) while the other microscopic characters were less distinctive. Collections of *Hypholoma* sp. C showed a notable degree of variation in terms of mean spore length ranging from 5.91 to 6.87 μm. (Table 4.6). However,

4.6). However, the spore range of each collection within this group was very similar.

CDA (Canonical Discriminant Analysis) was performed using both spore and cystidia characters. Fig 4.1a shows the scatter plot of the mean canonical variates generated form the spore characters (MSCV). Two clusters are evident. The large cluster appears more or less uniform and encompasses collections of six taxa while the three collections of E (*Hypholoma* sp. A) form the smaller separate cluster. 94.5% of the total variation was due to SCV1 and only 5% was attributed to SCV2. The two clusters are resolved along the axis of MSCV1, i.e. the greater value of profile width of spores of E contributed largely to this resolution. Taxa in the large cluster are not resolved along either of the axes (Fig. 4.1b), though approximately 50% of the collections of C (*Hypholoma sublaterium*) tend to shift slightly away from the main cluster as a result of the contrast between length and profile width of spores (SCV2). F (*Hypholoma* sp. B) appears to be outside the main cluster in Fig. 4.1b. However, since only a single collection was available for this analysis this result must be treated with caution, consequently, F is considered as part of the main cluster.

Fig. 4.2 shows the scatter plot of the first two mean canonical variates generated from cystidia variables (MCV). 59% of the total variation was attributed to CV1 and 19.8% to CV2. Length of chrysocystidia contributed largely to the variation along the first canonical axis, whereas the variation along the second canonical axis was due mainly to the width of cheilocystidia. Two broad clusters were resolved along the axis of MCV1 (Fig. 4.2). Cluster 1 consisted of collections of the common taxa of *H. fasciculare* (Y), *H. fasciculare* (AP), *H. sublaterium* and *H. brunnea* and none could be resolved along either of the axes. Cluster 2 consisted of collections of the brown-spored species (*Hypholoma* sp. A, sp. B & sp. C) with slight overlap with Cluster 1. There was no clear resolution within this group. Collections of *Hypholoma* sp. C showed a considerable degree of variation, thus supporting the earlier view that it was less

homogeneous.

Fig. 4.3 shows a dendogram from the UPGMA cluster analysis using the mean CVs generated from the spore and cystidia variables in CDA. Two clusters are evident corresponding to *Hypholoma* sp. A (E) and the remaining taxa. The remaining taxa showed a high degree of similarity in both spore and cystidia variables. At the two subcluster level, *Hypholoma* sp. B and sp. C were separated out as a single group whereas the other subcluster consisted of mixed collections of *H. fasciculare*, *H. sublaterium* and *H. brunnea.*

4.2.2. Electrophoretic studies

There was an insufficient number of isolates for this part of the study. Spores of the common taxa such as *H fasciculare* germinated readily but it was the lack of cultures of the problematic species such as *Hypholoma* sp. C which posed the problem. Consequently caution was exercised in the interpretation of the results and average linkage cluster analysis was not performed because the results would not be representative.

Lac (Laccase)

For Lac activities, a total of eight bands were scored across all the available isolates. Fig 4.4 shows a schematic representation of band patterns of isolates in each collection included in this part of the study. It shows that Band 4 is present in majority (~ 57%) of the isolates across the various taxa. Bands 2 & 3 occurred in isolates of the three taxa previously identified on morphological criteria as *H. fasciculare* (CYS333 & 344), *H. sublaterium* (CYS337 & 408) and *H. brunnea* (CYS373) (Table 4.6). Only four bands (i.e. Bands 5, 6, 7 & 8) were noted not to be detected in isolates of these three taxa. Of these, Band 7 occurred in the wild isolate of both CYS427 (*Hypholoma* sp. C) and CYS491 (*Hypholoma* sp. B). Band 8 which was detected only in the isolates of CYS373 (*H. brunnea*) could be important in the separation of this taxon from other

taxa. However, due to the limited number of available isolates and the overlap shown in the possession of other bands, it was difficult to attach any significance to this observation. Isolates of *Hypholoma* sp. A did not produce any detectable laccase activity. On the whole, no distinct patterns could be associated with individual taxa.

Per (Peroxidase)

For Per activities, a total of 12 bands were scored across the isolates. Of the isolates of the three common taxa of *H. fasciculare*, *H. sublaterium* and *H. brunnea*, there was a certain degree of similarity in band mobilities (Table 4.6). However, within each of these taxa the variability was such that the arrangement of bands did not produce any unique pattern (Fig. 4.5). This absence of unique band patterns appear to coincide with the trend observed in Lac activities for these three taxa Isolates of *Hypholoma* sp. B and sp. C showed similar activity in Band 12 at R_f 0.51 which also occurred in the wild isolate of CYS337, *H. sublateritium* (Fig. 4.5). Isolates of *Hypholoma* sp. A did not produce any detectable Per activity.

PE (Pectinesterase) & PG (Polygalacturonase)

For PE activities, a total of 15 bands were scored across all the isolates. Again similarities in band activities (e.g., Bands 2, 13 and 14) were noted between the isolates of *H. fasciculare*, *H. sublaterium* and *H. brunnea* (Fig. 4.6, Table 4.6). Within each of these three groups, variations were noted in the fast moving bands. As in the previous two enzyme systems, no unique banding pattern could be associated with any one of these three taxa. Isolates of Hypholoma sp. A and sp. B appeared to produce unique banding pattern for each taxon (Fig. 4.6). However, due the limited number of available isolates, it was not possible to determine whether the banding patterns were unique to each of these two taxa. No detectable PE activity was noted for the isolate of CYS427.

For PG activities, a total of eight bands were scored across the isolates. As in the previous cases, similarities in band activities were again noted in the isolates of the three lignicolous species of *H. fasciculare*, *H. sublaterium* and *H. brunnea* (Table 4.6) Similarity in band activities were noted at Band 3 (R_f 0.37) and 4 (R_f 0.41) in three taxa (Fig. 4.7). Band 5 which was detected in the wild isolate of CYS337 (*H. sublaterium*) also occurred in the wild isolate of CYS491 (*Hypholoma* sp. B) (Fig. 4.7). The isolates of CYS491 and CYS543 (*Hypholoma* sp. A) showed similar band activity at R_f 0.33 (Band 2). Isolates of *Hypholoma* sp. A appeared to be separated from the other isolates by the presence of Band 1 (at R_f 0.29) (Fig. 4.7). However, as in the case with Lac zymograms the significance of this band should not be exaggerated. But unlike the Lac zymograms, the pectic zymograms appeared to provide some distinctions for *Hypholoma* sp. A, sp. B and sp. C when compared with *H. fasciculare*, *H. sublaterium* and *H. brunnea* but not so between the latter three taxa.

4.3. Discussion

The results show that the delineation of the Tasmanian species of *Hypholoma* can be achieved confidently based on morphological criteria in particular the macroscopic characters. However, Parker's (1933) observation that the microscopic characters are less distinct is still applicable in the case of *H. fasciculare*, *H. sublateritium* and *H. brunnea*, the three common taxa occurring in Tasmania. This lack of distinction in micro-characters is evident in the scatter plots and dendogram (Figs. 4.1a & b, 4.2 and 4.3) of mean canonical variates generated from both the spore and cystidia characters as well as the high degree of overlap in size range (Tables 4.3 & 4.4) of these characters.

A relatively interesting situation has occurred in *Hypholoma*. Electrophoresis of extracellular enzymes as a taxonomic tool appears to be less effective in species delineation within *Hypholoma* than within other genera of Strophariaceae. This may be attributed to the conservativeness of that part of the total genome which controls the

production of the enzyme systems examined. However, this is not seen in the previous genus *Stropharia*. The potential of this approach has not been fully explored as a result of the unavailability of sufficient number of isolates, in particular from the brown-spored species, to conduct the study. It is especially unfortunate in the case of *Hypholoma* sp. C where such an approach, supplemented by mating compatibility tests, may help to sort out the nature of this group. For other taxa such as *H. brunnea*, *H. fasciculare*, *H. sublaterium* and *Hypholoma* sp. A, delineation based on morphological criteria still appears to be the more effective means.

The six taxa thus delineated are recognized as forming the bulk of this genus in south-east Tasmania. This study has confirmed the work of Browne (1983) i.e. the two forms of *H. fasciculare* are conspecific. The second form, i.e. apricot orange pileus and lamellae, will be given a variety status.

The rediscovery of *H. brunnea* and assessment of its range in south-east Tasmania shows that this species is still a common fungus in the temperate rainforests of Tasmania. It seems to be a fairly uniform species in terms of variation in both macro- and micro-characters. It is distinct from *H. fasciculare* and *H. sublaterium* in the colour of the pileus and lamellae.

After careful comparison with other described species of *Hypholoma* of similar habitats, e.g. *H. elongatipes* and *H. polytrichum*, *Hypholoma* sp. A is considered a new taxon and given a specific epithet. Despite the limited number of collections the taxon is distinct and consistent enough in morphology to warrant a name of its own.

Hypholoma sp. B has only been collected from a single locality which is a fairly unstable environment (a cultivated patch on the campus of University of Tasmania). This taxon is considered to be in the brown-spored species of *Hypholoma*, and more

material is needed to establish its variations and range of distribution before considering its species status.

Hypholoma sp. C on the other hand appears to be a less homogeneous group. There is a possibility that this is a variable species like *Psilocybe subaeruginosa* (see Chapter 5) or *Pholiota multicingulata* (see Chapter 7). Since most of the specimens occurred solitarily, it has not been possible to establish the morphological variation within a population. However, the collections are treated as a single taxon until more information is available. Spore germination in this taxon is difficult and unpredictable. Results from relatively few possible studies of electrophoresis are possibly non-representative for this taxon. Therefore, with the information available much evidence for a separate species from the known species of *Hypholoma* is from morphological data. This taxon certainly requires further work to establish its species status, thus, no specific epithet is given.

4.4. Taxonomy

Key to species of *Hypholoma* in SE Tasmania

1. Solitary or caespitose on woody litter, rotten or buried wood or dead tree stump 2

1'. Solitary or subgregarious in boggy areas, amongst *Sphagnum* or *Polytrichum* ..... **5. Hypholoma sp. nov.**

2. Pileus some shade of yellow, brown or red 3

2'. Pileus uniformly bay or dark brown, lamellae pale or greyish yellow, caespitose on wood ..... **4. brunnea**

3. Spore mass violaceous black 4

3'. Spore mass predominantly brown 6

4. Pileus pale yellow, often with a reddish brown disc, lamellae olivaceous yellow becoming blackish with spores, caespitose on rotten wood ..... **1. fasciculare**

4'. Pileus apricot orange or brick red to reddish brown, caespitose on rotten wood or

buried wood 5

5. Pileus apricot orange throughout, lamellae concolorous with pileus with bright orange margin, caespitose on rotten wood .... **2. fasciculare var. armeniacum var. nov.**

5'. Pileus brick red or reddish brown, lamellae greenish yellow, caespitose on wood or ground, also in disturbed areas . ... **3. sublaterium**

6. Pileus orange brown, lamellae light yellow becoming browner with spores, gregarious to subcaespitose on ground litter ..... **6. Hypholoma taxon 1**

6'. Pileus yellowish brown, with reddish brown disc, concentric rings of whitish squamules on surface, lamellae sulphur or pale yellow, solitary to subgregarious on wood or ground litter ..... **7. Hypholoma taxon 2**

1. *H. fasciculare* (Huds. ex Fr.) Kummer, *Der Führer in die Pilzkunde* 72 (1871).

Syn.: *Naematoloma fasciculare* (Huds. ex Fr.) Karst. in *Bidr. Finl. Nat. Folk* 32: 496 (1879).

Selected illustrations: Cole *et al.* (1984), plate 3; and Shepherd & Totterdell (1988), p. 65.

Illustrations: Figs.4.8 - 11.

Material examined: See Appendix IIIB.

Observations

This is a very well described species, good descriptions can be found in many modern mycological works (Smith 1951; Watling 1973; Watling & Gregory 1987; Aurora 1986; Phillips 1981). The Tasmanian specimens generally lack the intense sulphur yellow colour so pronounced in both European and North American representatives of this species. It fruits very early (April) in the season and lasts till about mid-winter (August)

if conditions are favorable. It is very common on rotten wood or fallen logs in temperate rainforests. *H. acutum* from New Zealand is believed to be the same fungus (Horak 1971).

2. *H. fasciculare* var. *armeniacum* Chang & Mills var. nov.

Until Browne's (1983) work, this form of *H. fasciculare* has been erroneously referred to as a species of *Pholiota* (Cole *et al* 1978). It resembles *H. fasciculare* in every respects except for the colour of pileus and lamellae. A full description of this taxon will be included in the chapter on New Species.

3. *H. sublaterium* (Fr.) Quélet in *Mémoires de la société d'Emulation de Montbéliard sér. II*, 5:112 (1872).

Selected illustrations: Cole *et al* (1984), plate 3 [young carpophores], Fuhrer (1985), p.49 [young carpophores]; Shepherd & Totterdell (1988), p. 66 and Fuhrer & Robinson (1992), p.36 [mature carpophores].

Illustrations: Figs. 4.12 - 15.

Material examined: See Appendix IIIB.

Observations

This is another well documented species (Parker 1933; Smith 1951; Watling 1973; Watling & Gregory 1987; Phillips 1981). Its most distinctive features are the brick-red pileus and greenish yellow gills. Its habitat is usually associated with wood, also with buried wood in lawns or bare rocky ground and appears to have some association with disturbance as well.

4. *H. brunnea* (Massee) Reid, *Kew Bull.* 10: 644 (1955).

Syn.: *Flammula brunnea* Massee, *Trans. Proc. New Zeal. Inst.* 31: 300 (1898).

Selected illustration: Fuhrer & Robinson (1992), p. 36.

Illustrations: Figs. 4.16 - 19.

Pileus 18-53 mm. in diam., convex to plano-convex when expanded, generally glabrous, occasionally fine whitish appressed squamules visible on surface, greasy to tacky, uniform bay or dark brown (7F6-8 to 8F6-7), margin only slightly striate, floccose veil remnants along the margin.

Lamellae adnexed or adnate with a tooth,to slightly decurrent when fully expanded, pale yellow (3A3) to greyish yellow (2B4, 2C2-4 or 3C4) with slight olive tint becoming browner (5E5) with spores. *Stipe* 25 - 76 (-80) x 2 - 7 mm., hollow, fibrillose, pale buff near apex, becoming tawny brown or concolorous with pileus towards base, fuzzy velar zone at superior position, equal, base slightly attenuated when joined and sub-bulbous when not. *Context* pallid (4A4-5) to pale watery greyish yellow (3C3) or dull watery orange, moderately thin. *Veil* cortinoid, whitish, evanescent.

Spores violaceous black in mass, (5.6-) 6.25 - 7.5 x 3.75 - 4.6 (-5) x 3.75 - 4.6 (-5) µm., smooth, thick-walled, pale yellowish brown or melleous (in 5% KOH), ellipsoid in face view, slightly inequilateral in profile, germ pore broad and distinct. *Basidia* (17.5-) 18.3 - 23.3 (-28.3) x (5-) 5.4 - 6.7 (-7.1) µm., majority 4-spored, more rarely 2-spored, clavate or cylindric. *Pleurocystidia* as chrysocystidia, (29.2-) 30 - 49.8 (-51.7) x 7.9 - 15 (-22.1!) µm., mucronate clavate, apical protuberance prominent, hyaline with amorphous body or yellowish brown throughout, abundant.

Cheilocystidia (16.7- 0 17.5 - 32.9 (39.2) x (5.8-) 6.7 - 11.2 µm., generally numerous, forming a more or less sterile band, hyaline, utriform or obpyriform.

Subhymenium cellular. *Trama* parallel, hyphae up to 12 µm. broad. *Epicutis* non-gelatinised repent hyphae, loosely intertwined, incrusted with brown pigments.

Hypodermium subcellular layer, also with brown pigments. Clamp connections present in all tissues.

Habit & habitat caespitose on rotten wood (probably hard wood) or moss-covered

wood in mature mixed forest or wetter regions of temperate rainforest.

Specimens examined: Hobart, 1953, Herb. F.P.S.M. No.3818 (DFP) (this collection was cited by Reid in 1955, specimens are in very poor condition), see Appendix IIIB for other Tasmanian collections.

Observations

This is the same fungus that Browne (1983) erroneously referred to as *H. epixanthum*. The pileus colour of *H. epixantum* in Bresadola (1927) is honey brown and this obvious difference in the colour makes it highly unlikely that the Tasmanian specimens would be the same species. Reid's (1955) description of this species is based on specimens from Hobart, Tasmania. It is a relatively common fungus in the forests of Tasmania.

5. *Hypholoma paludicolum* sp. nov.

This fungus is uncommon and is found associated with boggy areas sometimes growing in *Sphagnum* or *Polytrichum* communities. It will be formally described in the chapter on New Species.

6. *Hypholoma* taxon 1

Illustrations: Fig. 4.20-21.

This fungus has only been collected from a single locality. Despite the limited material the browner spore print colour implies deviation from the purple brown-spored species of *Hypholoma* and a description is given below.

Pileus 31 - 37 mm. in diam., convex or plano-convex to almost plane when fully expanded, whitish squamules at disc, silky covering of outer veil in young carpophores,

greasy to slightly tacky, dull raw sienna (6D7). *Lamellae* adnexed, light yellow (4A6) when veil breaks, becoming browner (5D6) with spores, crowded. *Stipe* 27 - 38 x 5 - 9 mm., equal, cylindrical, hollow, more or less smooth, white mycelium at base and white rhizomorph below, pale pastel yellow(1A4) near apex becoming dingy brown towards base. *Context* whitish, thick. *Veil* cortinate, whitish, evanescent.

Spores cocoa brown (6E6) in mass, 5.8 - 6.7 x 3.7 - 5 x 3.7 - 4.6 µm., ovate elongate in face view, slightly inequilateral in profile, smooth, yellowish brown (in 5%KOH), germ pore minute. *Basidia* 22.5 - 27.5 x 5.8 - 7.1 µm., 4-spored. *Pleurocystidia* as chrysocystidia, 35.8 - 61.2 x (66.7) x 10.8 - 18.7 µm., fusoid ventricose, yellow amorphous body noted in majority, apex pronounced. *Cheilocystidia* 27.5 - 37.5 x 5.4 - 10.2 µm., not forming an obvious sterile band, hyaline,.utriform or subutriform, pyriform or obpyriform or elongate clavate.

Subhymenium subcellular. *Trama* regular, hyphae 8 - 36 µm. in width. *Epicutis* filamentous, repent, non-gelatinised hyphae. *Hypodermium* subcellular. Clamp connections present in all tissues.

Habit & habitat Scattered to subcaespitose on eucalypt wood chips.

Observations

This fungus resembles closely *H. sublateritium* in appearance. It differs from *H. sublaterium* in the colour of the spore deposit and lamellae. The spore deposit is predominantly brown as compared to violaceous black, the typical spore mass colour of the subfamily Strophariodeae. In terms of spore print colour, this fungus may represent an intermediate species between Strophariodeae and Pholiotoideae. However, its other morphological characters, such as the subcellular hypodermium, dry stipe, chrysocystidia and the distinct apical germ pore show greater affinity with species of *Hypholoma*.

7. *Hypholoma* taxon 2

Illustrations: Fig. 4.22 - 27.

The following is a composite description of this taxon based on the Tasmanian collections.

Pileus 24 - 38 mm. in diam., subumbonate or plano-convex to almost plane with margin slightly outcurled and centre slightly depressed, whitish veil remnants scattered near disc, margin striate when fresh, greasy to tacky or viscid when moist, hygrophanous, wheat to orange yellow (4B5-7) to blond (4C4) throughout, near apricot (5B6) or brown (7E7) at disc. *Lamellae* broadly adnate or adnexed, greyish yellow to wheat yellow (3C4 to 4B5) becoming browner with spores (4D5 to 6E5). *Stipe* 32 - 60 x 3 - 5 mm., ±equal, base slightly attenuate or broaden, ±glabrous more fibrillose towards base, pastel yellow near apex becoming dingy brownish yellow near base, hollow. *Context* pale yellow (3A3 to 4A4), thickest below disc, generally thin. *Veil* cortinate, whitish, evanescent.

Spores cocoa to dark brown (6E6 to 6F6) in mass, 5.8 - 7.5 x 3.7 - 4.6 (-5) x 3.5 - 4.6 µm., melleous, ovate to elliptic in face view, slightly bean-shaped to inequilateral in profile, germ pore distinct but minute. *Basidia* 18.3 - 28.3 (-31.5) x 5.8 - 9.2 µm., 4-spored, obovate to elongate obovate, occasionally with constriction at waist. *Pleurocystidia* as chrysocystidia, 35.4 - 67.5 (-88.3!) x 10.4 - 19.2 µm., mucronate ventricose with a prominent apical protuberance, yellow brown throughout or with amorphous body. Cheilocystidia 22.5 - 43.7 x 5.8 - 13.3 µm., hyaline, utriform to obpyriform, or chrysocystidioid, similar in shape to those on gill face but smaller. *Subhymenium* subcellular. *Trama* ±regular, intermixed with long and short but broad hyphae, 48 - 60 mm. long and up to 28 µm. broad. *Epicutis* filamentous, repent hyphae. *Hypodermium* subcellular to cellular. Clamp connections present.

Habit & habitat solitary or scattered on ground litter.

Specimen examined: See Appendix IIIB.

Observations

Specimens in this taxon differ from *Hypholoma* taxon 1 in the colour and surface features of the pileus. There is generally a stronger yellow tint in the pileus. Fine appressed whitish squamules are noted in some carpophores. This fungus appears to occur almost always solitarily.

Table 4.1 Systematic treatments of the genus *Hypholoma*.

Fries (1874) (From Parker 1933)

Subgenus Hypholoma

Sections 1. Fasciculares
2. Viscidi
3. Velutini
4. Flocculosi
5. Appendiculati

Smith (1951)

Genus Naematoloma Karst.

Sections 1. Tenacia
2. Fascicularia

Singer (1986)

Genus Naematoloma Karst.

Sections 1. Cyanoloma Singer
2. Stropholoma Singer
3. Naematoloma (=Fascicularia Smith 1951)
4. Psilocyboides Singer (=Tenacia Smith 1951)

Watling & Gregory (1987)

Genus Hypholoma (Fr.) Kummer

Sections 1. Hypholoma
2. Psilocyboides

Table 4.2. A summary of the macroscopic characters and habitats of purplish brown-spored taxa of *Hypholoma*. Colour of lamellae is from young carpophores unless specified otherwise.

	H. fasciculare (Y)	H. fasciculare (AP)	H. sublateritium	H.brunnea
Pileus				
colour	pale sulphur yellow or pale yellow, brick red at disc	apricot or reddish orange throughout	brick red to dull orange brown	uniform bay or dark brown
surface	silky covering of outer veil when young, otherwise glabrous hygrophanous	silky covering of outer veil when young, otherwise glabrous hygrophanous	whitish veil remnants floccose along margin	silky covering of outer veil when young, otherwise glabrous
diameter	16 -40 mm.	10 -37 mm.	16 - 77 mm.	18 - 52mm.
Lamellae				
attachment	adnate, slightly emarginate or adnexed	adnate with a tooth, or adnexed	adnexed or adnate with a tooth	adnexed or adnate with a tooth
colour	olivaceous yellow	apricot orange	greenish yellow	greysih yellow
Stipe				
colour	pale straw near apex	pale orange brown	pallid or pallid yellow	pale cinnamon brown
surface	fibrillose below veil line	whitish fibrillose below veil line	whitish recurved scales below veil line	whitish fibrillose
l x w (mm.)	24-52 x 2-6	27-72 x 2-5	29-94 (103!) x 2-8 (15!)	22-80 x 2.5-5
Habitat	on rotten wood	on rotten wood	on or near stumps, on buried wood in lawn	on rotten wood

Table 4.3. A summary of the macroscopic characters and habitats of the brown-spored taxa of *Hypholoma* included in the study. Colour of lamellae is from young carpophores unless specified otherwise.

	Hypholoma sp A	**Hypholoma sp B**	**Hypholoma sp C**
Pileus			
colour	greyish orange	orange brown	yellowish brown, brick red at disc
surface	glabrous, margin deeply striate	silky covering of outer veil when young otherwise glabrous	rings of whitish squamules near disc
	hygrophanous	greasy when moist	greasy when moist, slightly hygrophanous
shape	mycenoid to subcam-panulate	convex to plano-convex	convex then plano-convex to almost plane
diameter	-> 15 mm. across	24-37 mm.	17-42 mm.
Lamellae			
attachment	subdecurrent	adnexed	adnexed or adnate to slightly decurrent
colour	brownish (6E4) with spores, white margin	light yellow (4A6)	pale yellow (3C4)
Stipe			
colour	transluscent orange brown	whitish near apex, becoming dingy brown towards base	sulphur yellow to pale yellow
surface	smooth	fibrillose	coarsely fibrillose otherwise glabrous
l x w (mm.)	52-60 x 1-2	27-38 x 5-9	32-65 x 3-5
Habitat	in wet, boggy areas on mud or amongst *Sphagnum* or *Polytrichum*	on eucalypt wood chips	on ground litter or near stumps or on wood or manfern frond

Table 4.4 A summary of the major macroscopic characters and habitats of the collections collectively grouped as *Hypholoma* sp C.

Collection	Pileus	Lamellae	Stipe	Habitat
CYS259	orange yellow (5A6) throughout, darker at disc paler squamules over surface	adnate yellowish brown	sulphur yellow near apex becoming browner towards base	solitary on fallen manfern frond
CYS262	yellowish brown orange brown at disc whitish squamules veil remnants floccose along margin, deeply striate	broadly adnate light yellow (4B5) becoming browner with spores	pale yellow (2A3) near apex, brownish orange (5C6) towards base	solitary on ground litter
CYS287	yellowish brown throughout whitish squamules near margin	broadly adnate to slightly decurrent yellowish brown with spores	sulphur yellow	solitary on ground
CYS292	yellowish orange (4B7) throughout brown (7E7) at disc whitish squamules over surface margin outcurled	adnexed olive brown (4D5)	brown (6E6) darkening towards base	solitary on ground
CYS332	greyish yellow (4C4) throughout brick red at disc whitish squamules over surface	broadly adnate brown (6E5) with spores	light yellow (1A4) near apex becoming dingy brown towards base	solitary on dead tree stump
CYS365	light yellow (4B5) throughout, dull apricot yellow (5B6) at disc, concentric rings of whitish squamules	adnate greyish yellow (3C4) becoming browner with spores	light yellow (1A4) near apex becoming dingy pale yellow (2A4) towards base	subgregarious on ground

Table 4.5. Summary of the major microscopic characters of the purplish brown-spored taxa of *Hypholoma* included in the study. Colour is that observed in 5%KOH and measurements are of mean values (±standard deviation). Legend: Y = yellow-gilled, AP = apricot-gilled, l = length, w = width at the broadest part, f = width of spore in face view, p = width of spore in profile.

	H. fasciculare (Y)	**H. fasciculare (AP)**	**H. sublateritium**	**H. brunnea**
Spore				
germ pore	distinct & broad	distinct & broad	distinct & broad	distinct & broad
shape	elliptic in face view, slightly inequilateral in profile	elliptic in face view, slightly inequilateral in profile	elliptic in face view, slightly inequilateral in profile	elliptic in face view, slightly inequilateral in profile
l x f x p µm.	6.5 x 4.33 x 4.23	7.08 x 4.56 x 4.46	7.28 x 4.36 x 4.27	6.66 x 4.19 x 4.10
	±0.44 ±0.30 ±0.24	±0.47 ±0.31 ±0.28	±0.50 ±0.26 ±0.24	±0.40 ±0.23 ±0.20
Basidia	4-spored	4-spored	4-spored	4-spored
l x w µm.	20.94 x 6.77	22.07 x 7.24	22.06 x 6.53	20.84 x 5.91
	±1.98 ±0.86	±2.00 ±0.71	±2.47 ±0.72	±1.81 ±0.45
Chrysocystidia				
l x w µm.	38.69 x 11.75	38.53 x 11.90	40.92 x 11.16	38.05 x 11.46
	±5.09 ±1.65	±4.41 ±1.35	±5.84 ±1.60	±4.69 ±1.89
Cheilocystidia				
l x w µm.	24.16 x 8.22	26.34 x 8.77	26.80 x 7.92	23.71 x 8.11
	±3.53 ±1.53	±3.01 ±1.65	±4.09 ±1.36	±3.08 ±1.20

Table 4.6. Mean measurements (in µm) of microscopic characters of collections of *Hypholoma* sp. A, sp. B and sp. C showing range, mean and standard deviation. Legend as in Table 4.5.

Collections	Spores (l x f x p)	Basidia (l x w)	Chrysocystidia (l x w)	Cheilocystidia (l x w)
Hypholoma sp. A				
CYS531	10-11 x 6.2-7.1 x 5.8-6.7	26.2-31.7 (-35.8) x 6.7-8.3	54.2-74.2 x 10.8-15.4	30-44.8 x 6.2-7.9
	10.66 x 6.63 x 6.52 ±0.29 ±0.22 ±0.23	28.58 x 7.62 ±1.92 ±0.59	65.87 x 12.66 ±7.51 ±1.36	35.04 x 7.39 ±5.34 ±0.75
CYS534	(9.6-) 10-11.7 x 6.2-7.1 x 5.6-7.1	23.7-30.8 x 7.5-10	(40.8-) 45-77.5 x 11.7-15	28.3-36.2 x 6.7 -9.2
	10.72 x 6.63 x 6.52 ±0.52 ±0.26 ±0.28	27.96 x 8.43 ±2.14 ±0.74	60.73 x 13.35 ±10.35 ±1.21	33.43 x 7.42 ±3.43 ±0.81
AKM1001	(9.6-) 9.8-11.1 x 5.8-6.7 x 5.8-6.7	27.5-30.8 x 8.7-10.8	48.3-64.2 x 10.8-17.5	26.7-38.7 x 6.1-8.3
	10.56 x 6.40 x 6.28 ±0.41 ±0.25 ±0.26	29.58 x 9.73 ±1.50 ±0.62	55.12 x 14.33 ±5.29 ±2.51	31.12 x 7.17 ±3.61 ±0.68
Hypholoma sp. B				
CYS491	5.8-6.7 x 3.7-5 x 3.7-4.6	22.5-27.5 x 5.8-7.1	35.8-61.2 (-66.7) x 10.8-18.7	27.5-37.5 x 5.4-10.2
	6.10 x 4.42 x 4.17 ±0.34 ±0.29 ±0.26	25.13 x 6.48 ±1.39 ±0.40	51.41 x 14.25 ±7.03 ±2.25	30.83 x 7.02 ±3.21 ±1.40
Hypholoma sp. C				
CYS259	5.8-6.7 x 4.0-4.6 x 3.7-4.6	18.3-25.4 x 5.8-6.7	46.7-67.5 x 12.1-15.4	27.9-34.2 x 5.6-12.5
	6.11 x 4.21 x 4.09 ±0.30 ±0.15 ±0.21	22.04 x 6.21 ±1.90 ±0.40	58.24 x 13.5 ±6.65 ±1.30	32.12 x 9.31 ±2.14 ±2.41
CYS262	5.8-6.7 x 4.0-5 x 3.7-4.2	21.7-27.5 x 5.8-7.1	43.3-67.1 x 11.7-15.8	25.8-30.6 x 7.5-13.3
	5.91 x 4.13 x 4.04 ±0.30 ±0.25 ±0.19	24.5 x 6.42 ±1.93 ±0.45	51.96 x 13.39 ±8.75 ±1.45	29.10x 11.58 ±2.06 ±1.65
CYS287	5.8-7.1 x 3.7-4.2 x 3.7-4.4	24.2-35.8 x 5.8-6.7	35.4-60.8 x 12.3-15.4	24.6-43.7 x 6.2-11.0
	6.25 x 4.05 x 3.95 ±0.38 ±0.16 ±0.20	28.19 x 6.08 ±4.01 ±0.31	50.35 x 13.85 ±7.97 ±1.56	35.16 x 7.87 ±6.04 ±1.68
CYS292	6.5-7.3 x 3.7-4.2 x 3.6-4.2	18.7-23.3 x 5.8-6.7	40.4-62.5 (-88.3!) x 10.8-18.7	22.9-29.6 (30) x 5.4-10.2
	6.87 x 4.07 x 3.97 ±0.36 ±0.18 ±0.19	20.95 x 6.31 ±1.65 ±0.37	46.83 x 11.48 ±6.81 ±0.74	25.6 x 7.60 ±2.08 ±0.77
CYS332	5.8-7.1 x 3.7-4.2 x 3.3-4.2	20.8-28.3 (-30) x 5.8-7.1 (-7.7)	48.3-61.6 x 10.8-15.4	22.5-30 x 6.7-9.2
	6.59 x 3.99 x 3.81 ±0.37 ±0.21 ±0.19	24.87 x 6.75 ±2.49 ±0.43	53.61 x 13.47 ±4.39 ±1.73	26.29 x 7.96 ±2.04 ±0.80
CYS365	5.8-7.1 x 3.7-4.2 x 3.5-4.2	21.7-26.7 x 6.2-7.5 x 12.5-21.7	53.3-65	30-40 x 6.2-8.3
	6.48 x 4.08 x 3.93 ±0.29 ±0.15 ±0.18	23.96 x 7.33 ±2.01 ±0.79	59.92 x 16.27 ±4.54 ±2.63	35.21 x 7.87 ±3.35 ±0.75
CYS427	5.8-7.1 x 3.7-4.2 x 3.7-4.3	23.3-26.7 x 6.2-7.5 x 14.2-17.5	45.8-64.2 (65.8)	29.2-40 (43.3) x 5-8.3
	6.59 x 3.97 x 3.91 ±0.33 ±0.15 ±0.15	24.96 x 6.99 ±1.46 ±0.35	57.03 x 15.25 ±5.32 ±1.18	33.71 x 6.46 ±3.91 ±0.88

Table 4.7 Similarities in band activities in laccase (Lac), peroxidase (Per), pectinesterase (PE) and polygalacturonase (PG) between isolates of the six taxa of *Hypholoma* included in the electrophoretic studies.

Enzyme	Band No.	R_f	Taxa showing similarity in band activity
Lac	2	0.27	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
	3	0.29	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
	4	0.31	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
Per	6	0.32	*H. fasciculare* & *H. sublateritium*
	7	0.34	*H. fasciculare*, *H. sublateritium* & *Hypholoma* sp. B
	8	0.35	*H. fasciculare* & *H. brunnea*
	9	0.36	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
	12	0.51	*H. sublateritium*, *Hypholoma* sp. B & *Hypholoma* sp. C
PE	2	0.07	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
	8	0.52	*H. fasciculare* & *H. sublateritium*
	13	0.65	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
	14	0.68	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
PG	2	0.33	*Hypholoma* sp. A & *Hypholoma* sp. B
	3	0.37	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
	4	0.41	*H. fasciculare*, *H. sublateritium* & *H. brunnea*
	5	0.45	*H. fasciculare*, *H. sublateritium* & *Hypholoma* sp. B

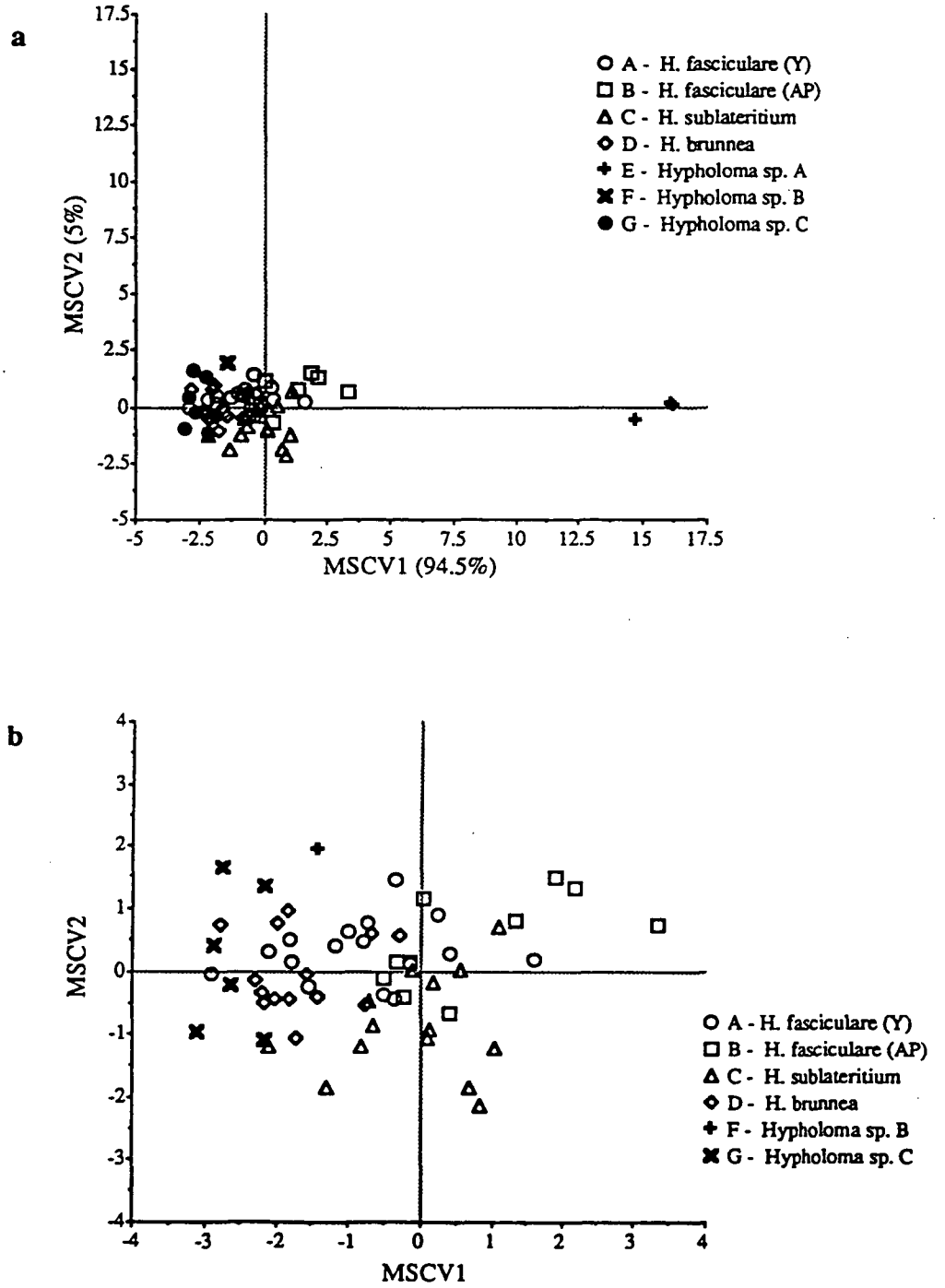


Fig. 4.1. Scatter plot of mean canonical variates (MSCV1 & MSCV2) generated from CDA of spore variables for collections of taxa of *Hypholoma* included in the morphological study. a) Plot of all the collections, and b) plot of collections in the large cluster.

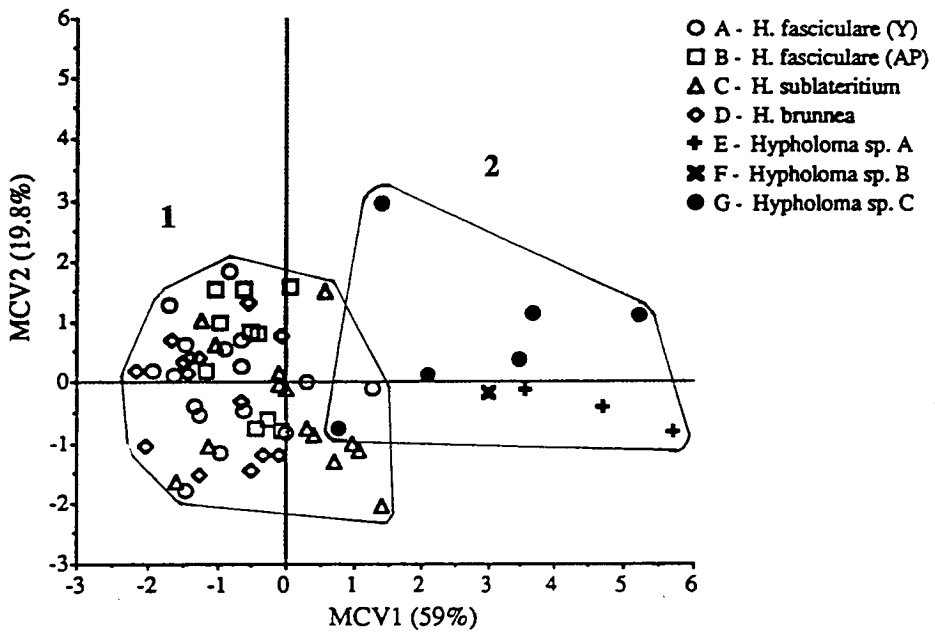


Fig. 4.2. Scatter plot of mean canonical variates (MCV1 & MCV2) generated from CDA of cystidia variables for collections of taxa of *Hypholoma* showing two clusters.

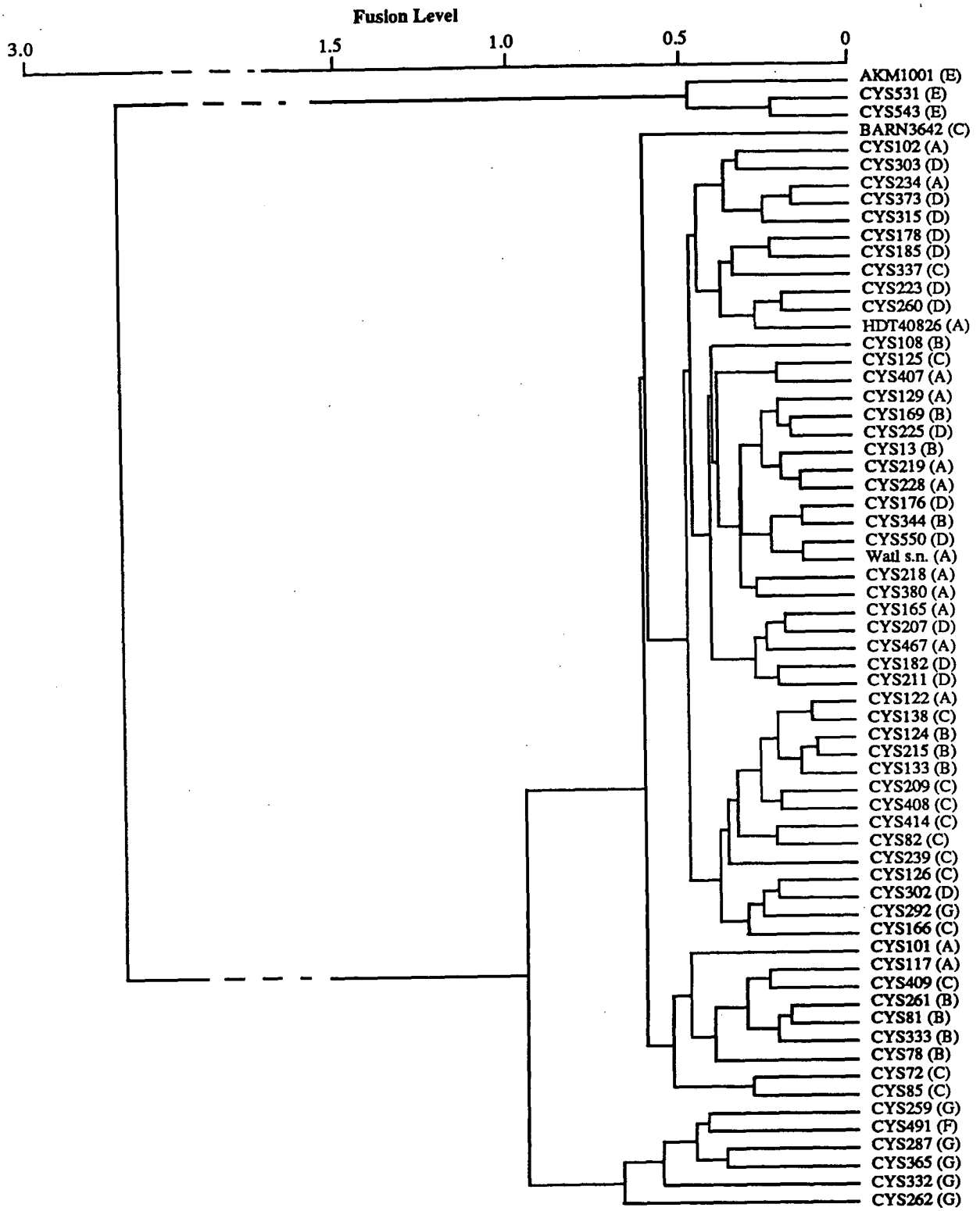


Fig. 4.3. Dendogram constructed based on the mean canonical variates generated from CDA for the collections of *Hypholoma* taxa included in the morphological study.

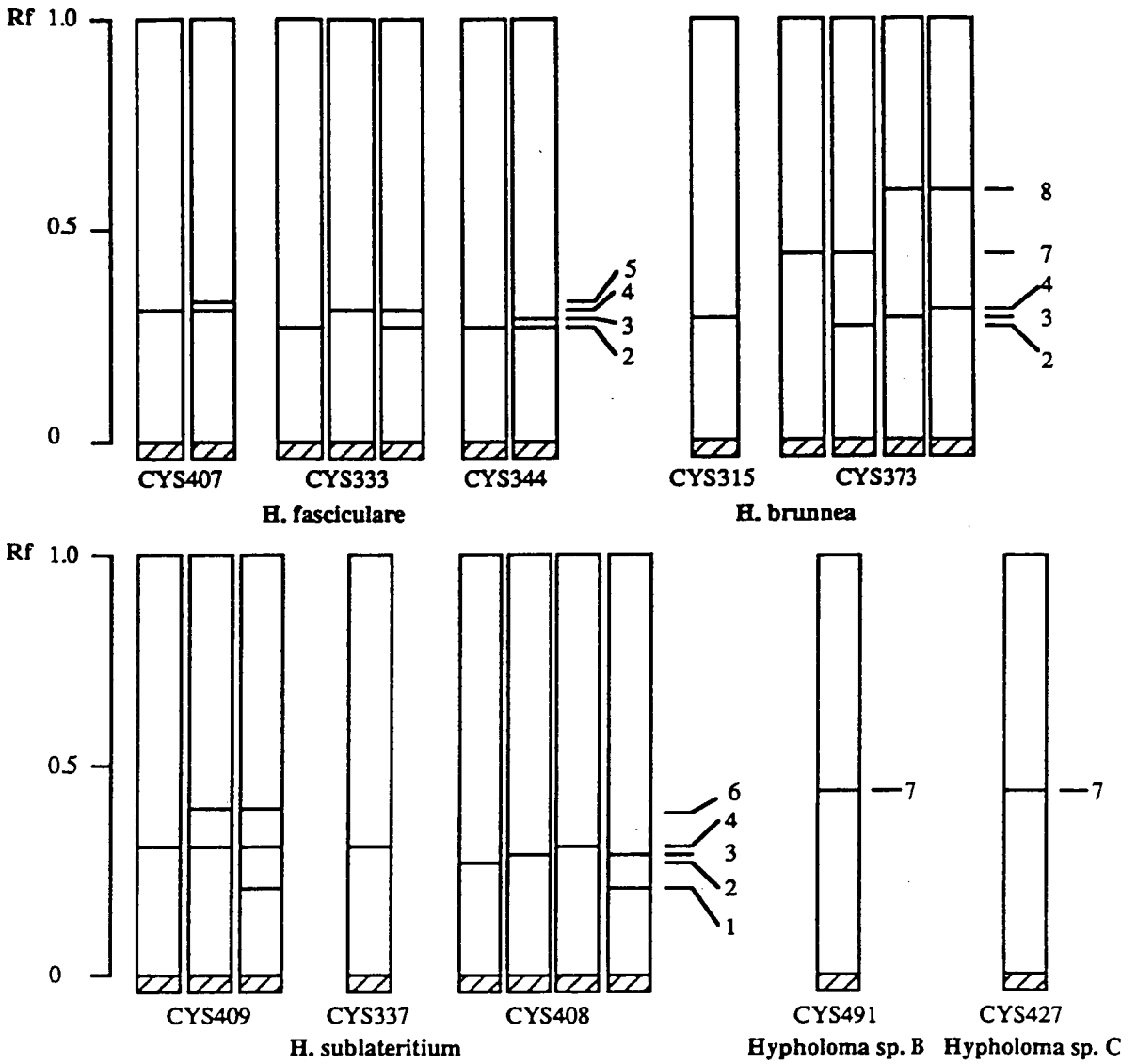


Fig 4.4. Schematic representations of Lac zymograms of isolates of *Hypholoma* species included in the electrophoretic studies. Band numbers start from the cathodic end. Rf values: 1=0.21, 2=0.27, 3=0.29, 4=0.31, 5=0.33, 6=0.40, 7=0.44 & 8=0.59.

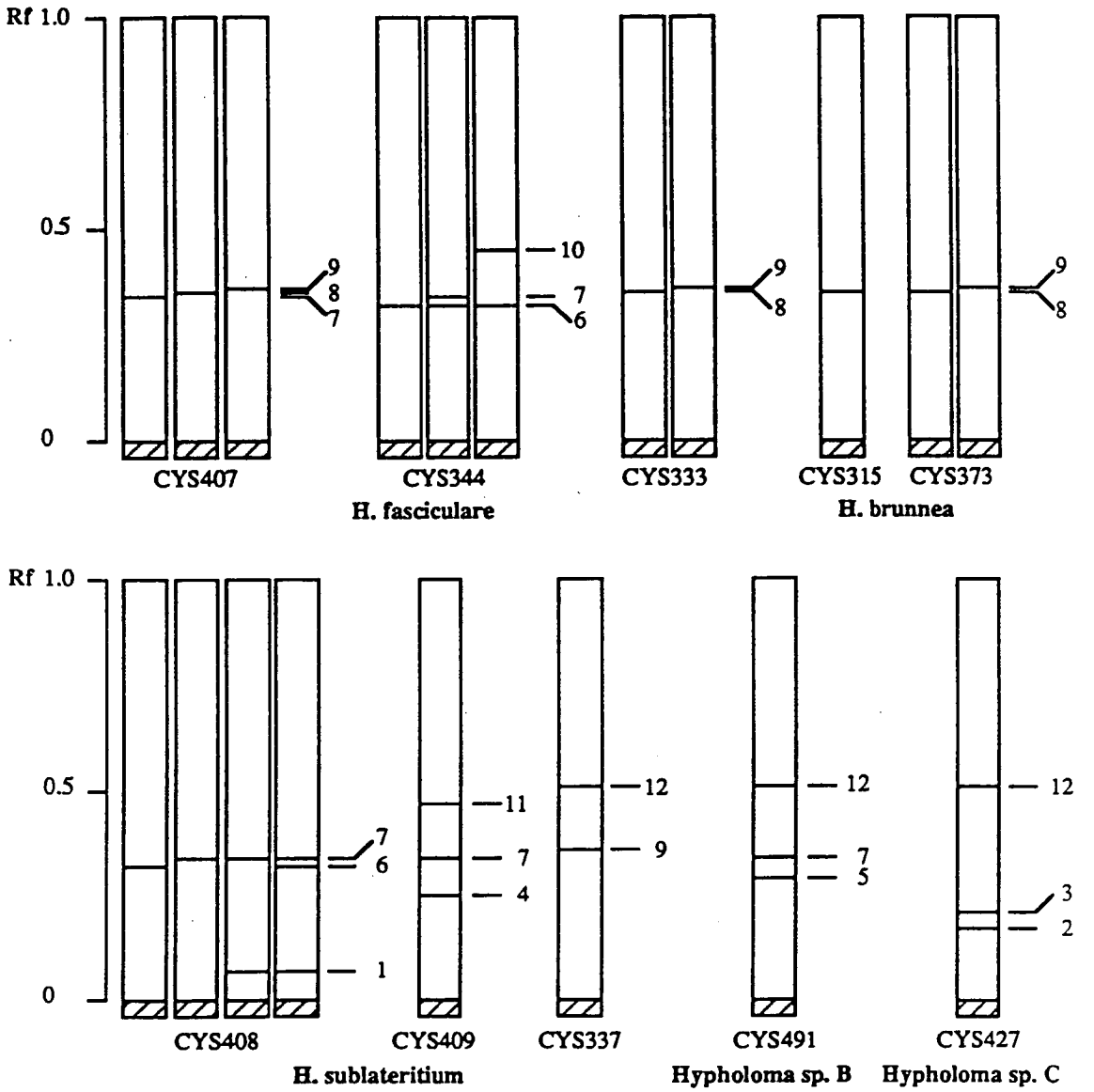


Fig. 4.5. Schematic representations of Per zymograms of isolates of collections of the different taxa of *Hypholoma*. Band numbers start from the cathodic end. Rf values: 1=0.07, 2=0.17, 3=0.21, 4=0.25, 5=0.29, 6=0.32, 7=0.34, 8=0.35, 9=0.36, 10=0.45, 11=0.47 and 12=0.51.

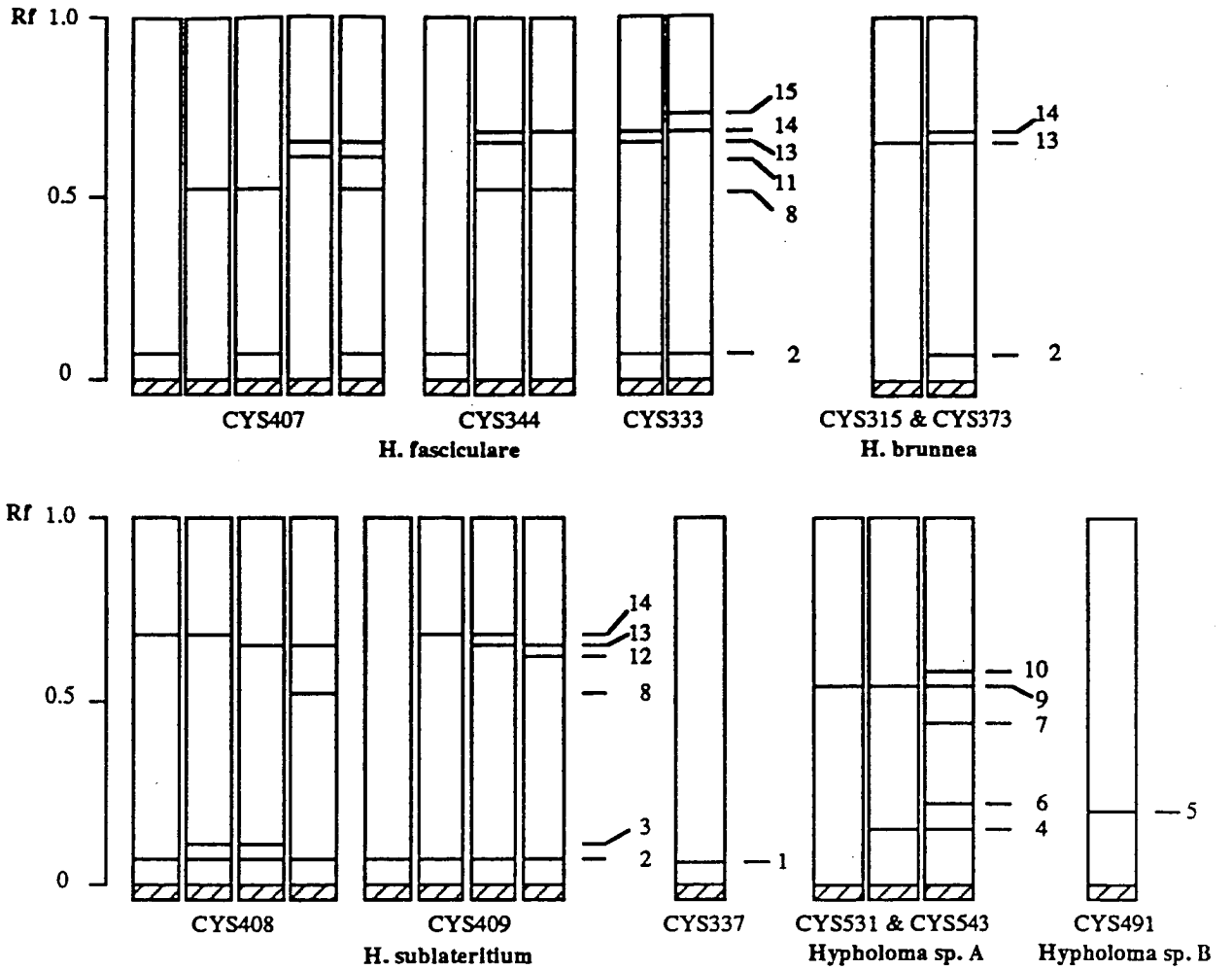


Fig. 4.6. Schematic representation of PE zymograms of isolates of *Hypholoma* species. Band numbers start from the cathodic end. Rf values: 1=0.06, 2=0.07, 3=0.11, 4=0.15, 5=0.20, 6=0.22, 7=0.44, 8=0.52, 9=0.54, 10=0.58, 12=0.62, 13=0.65, 14=0.68 & 15=0.73.

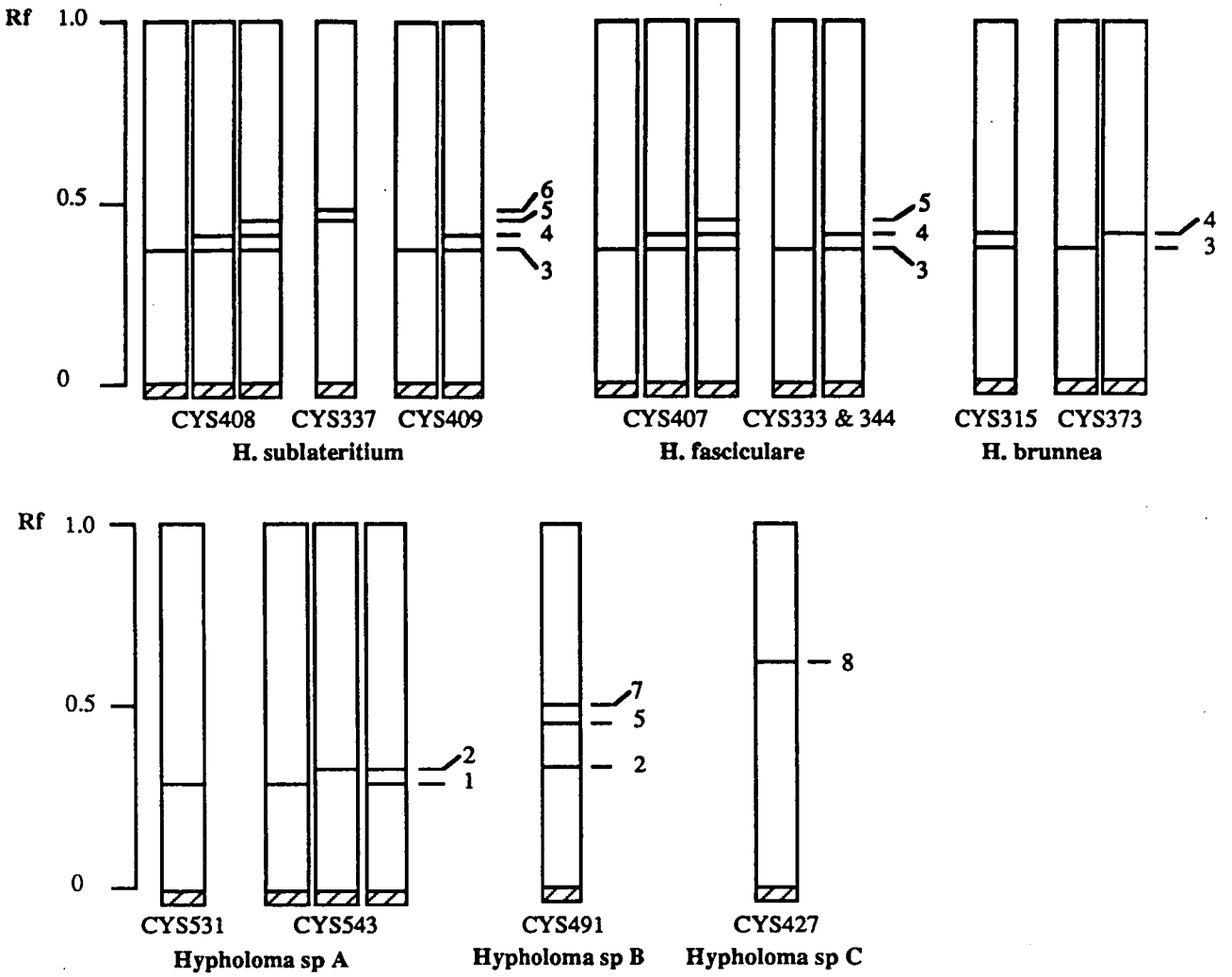
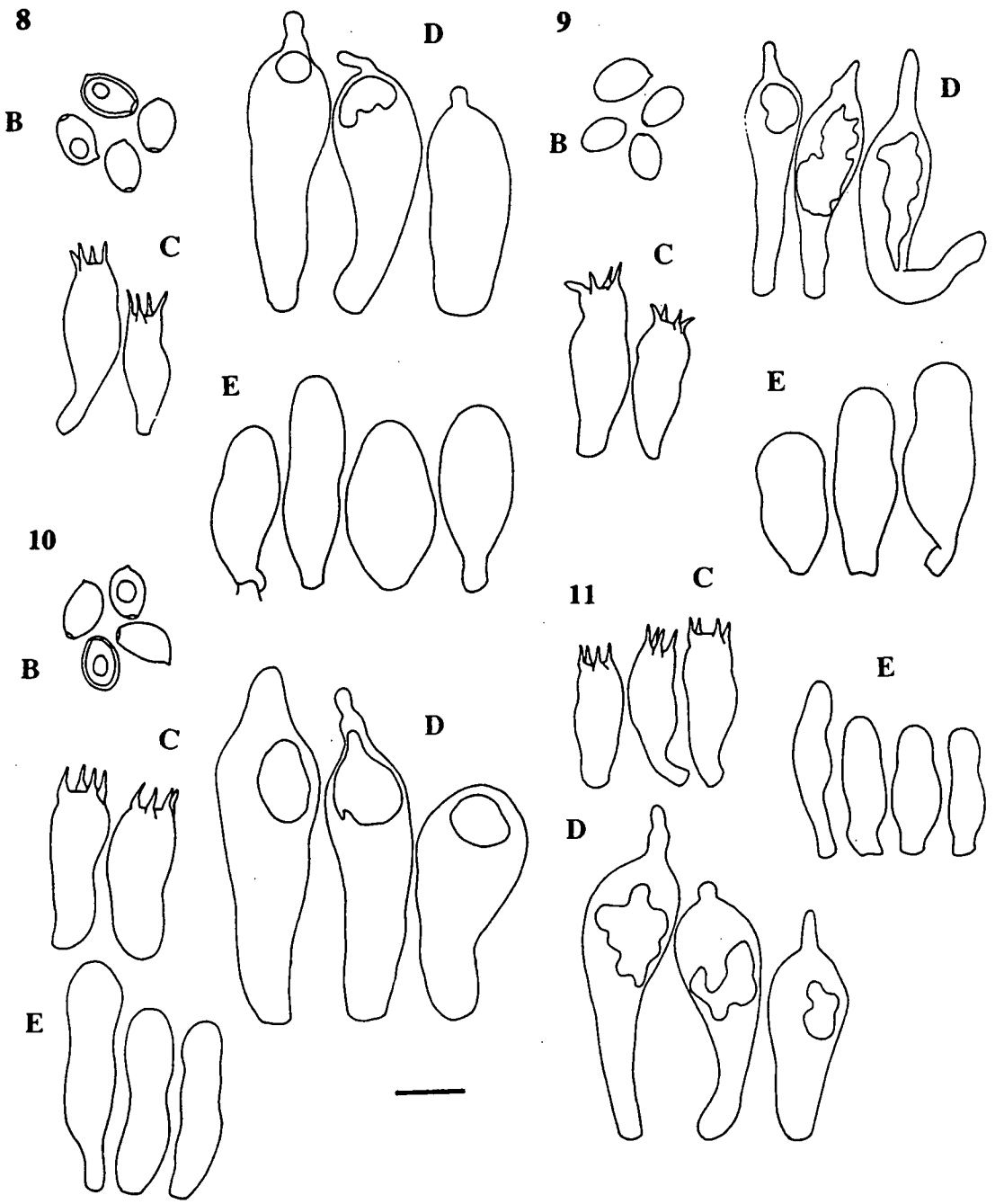
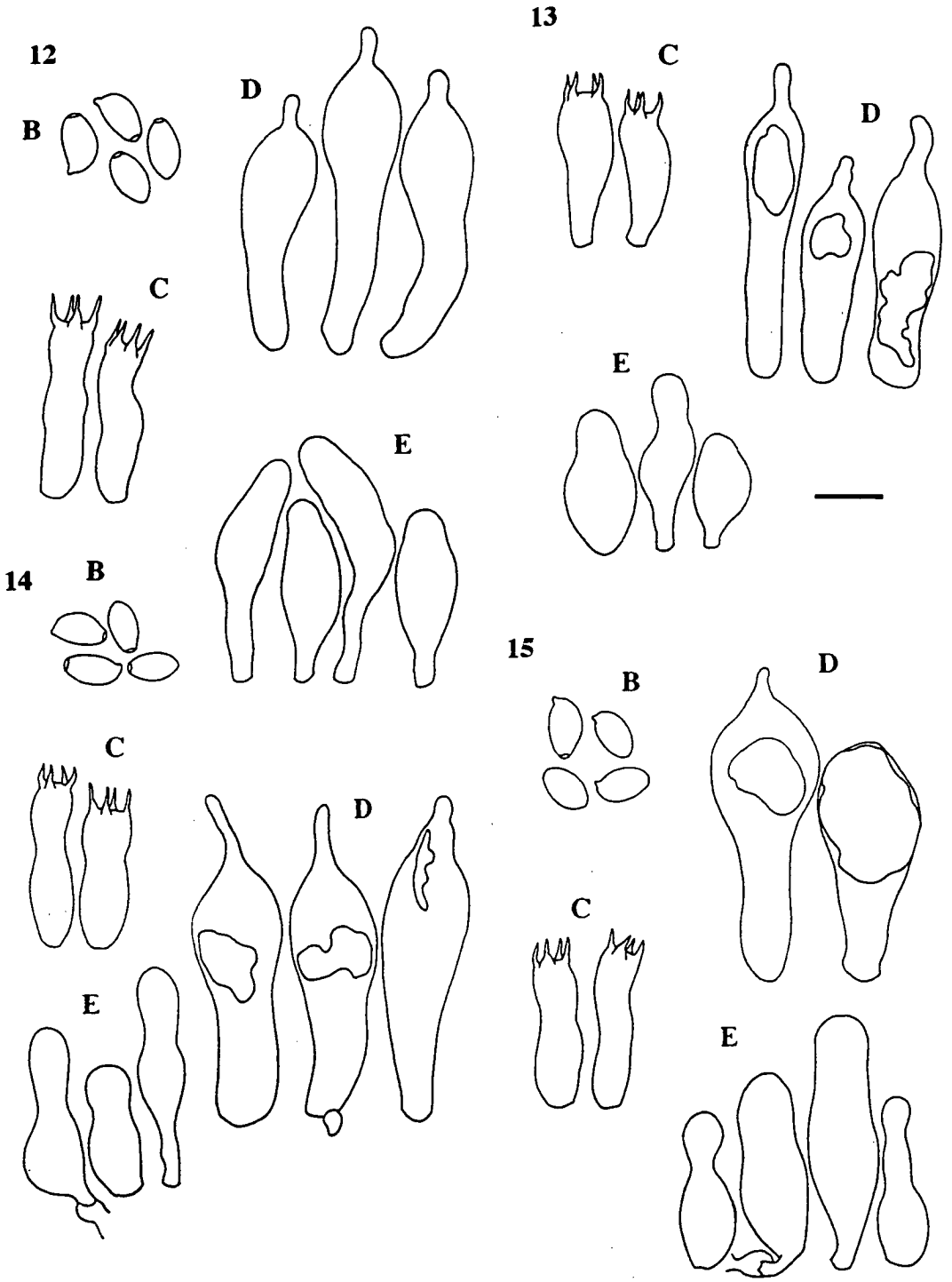


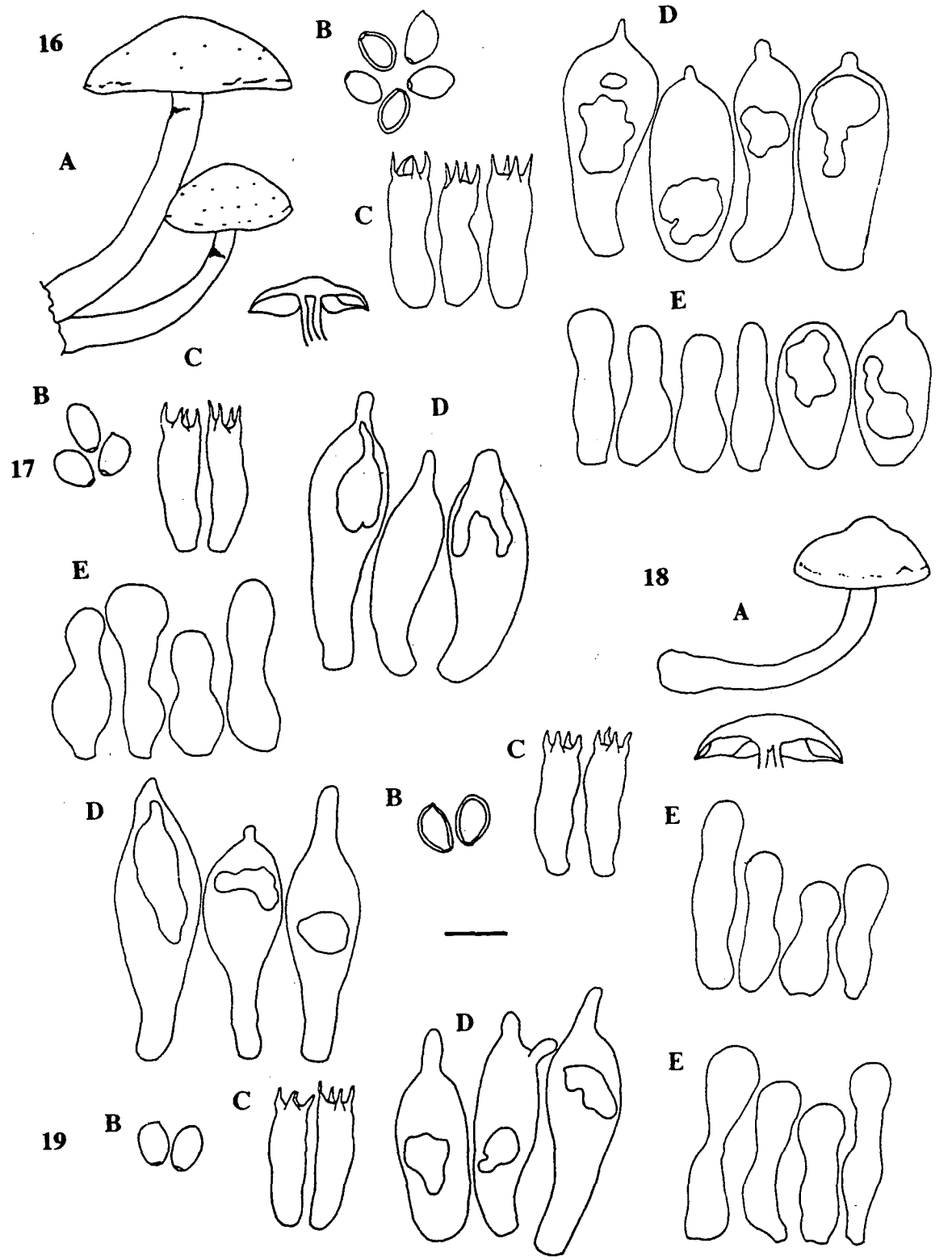
Fig.4.7. Schematic representation of PG zymograms of isolates of *Hypholoma* species.
Band numbers start from the cathodic end. Rf values: 1=0.29, 2=0.33, 3=0.37, 4=0.41, 5=0.45, 6=0.48, 7=0.50 & 8=0.62.



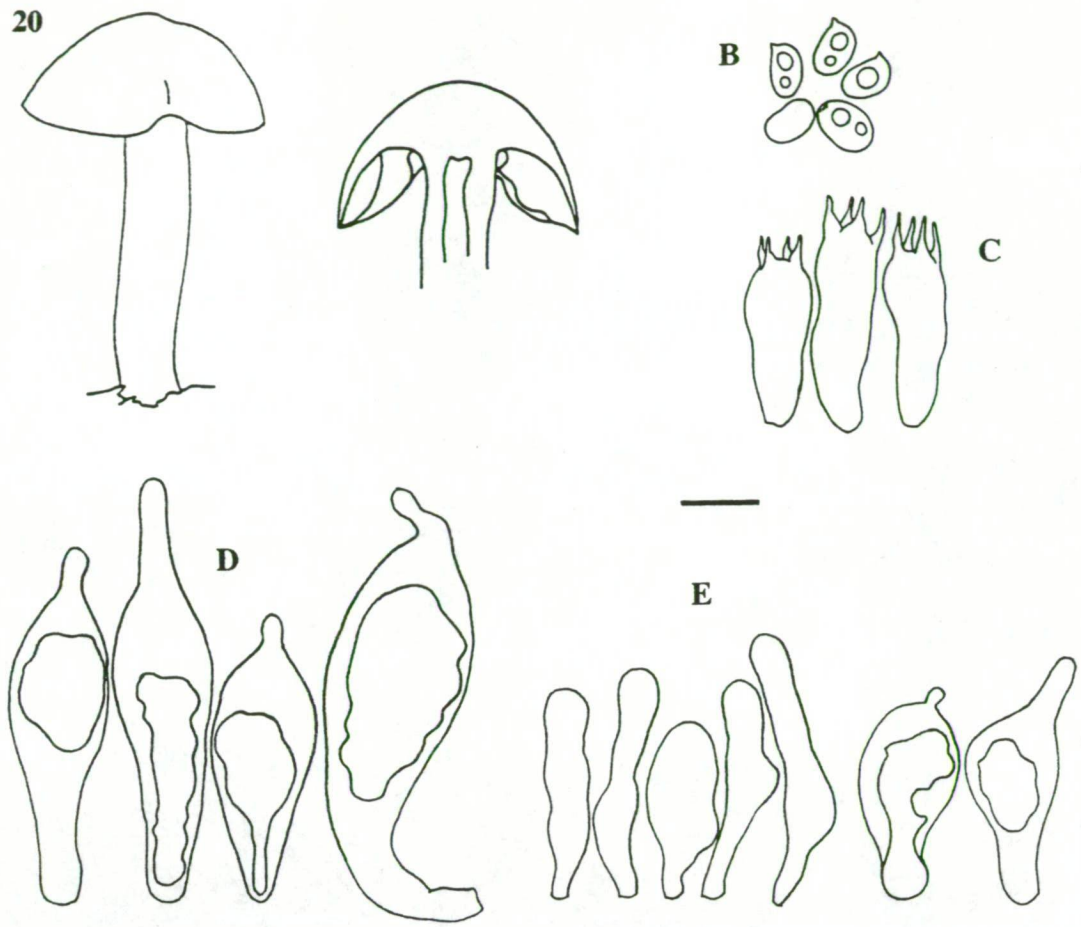
Figs. 4.8 - 11. *Hypholoma fasciculare*. B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 8. CYS219. 9. HDT40826 (from U.S.A.). 10. CYS101. 11. CYS102.



Figs. 4.12 - 15. *Hypholoma sublateritium.* B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 12. CYS166. 13. CYS337. CYS414. 15. Baroni3642 (from U.S.A.).



Figs. 4.16 - 19. *Hypholoma brunnea.* A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 16. CYS207. 17. CYS223. 18. CYS303. 19. CYS315.



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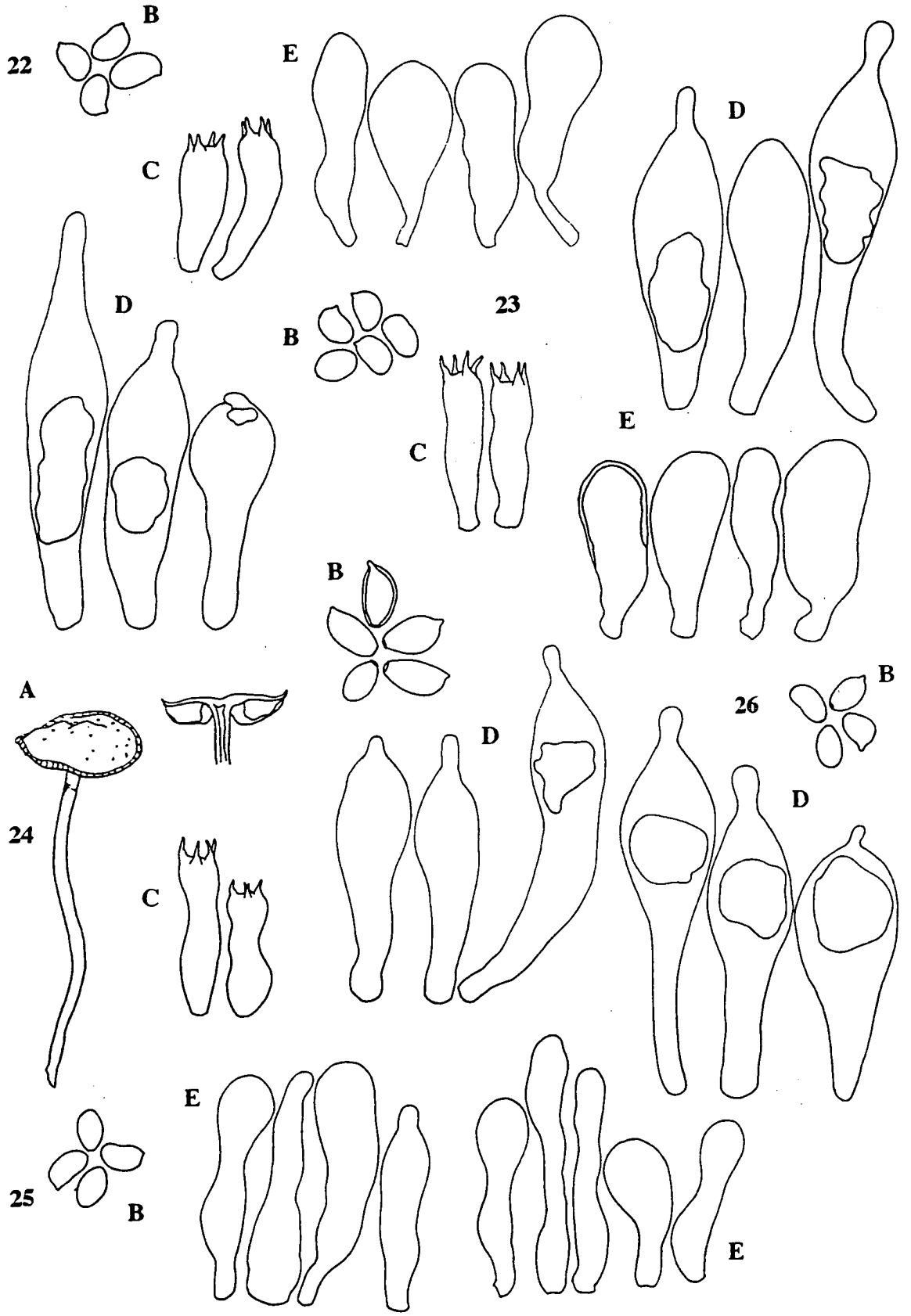
Figs. 4.20 - 21. *Hypholoma* taxon 1. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 20. CYS491. 21. Photo of habit of CYS491.



Figs. 4.22 - 26. *Hypholoma* taxon 2. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 22. CYS259. 23. CYS262. 24. CYS292. 25. CYS365. 26. CYS427.

Fig. 4.27. Habit of CYS262, *Hypholoma* taxon 2.

Chapter 5

Genus *Psilocybe* (Fr.) Kummer

5.1 Introduction

The genus *Psilocybe* is best known for its hallucinogenic species. However, not all species of *Psilocybe* are hallucinogenic and only some blueing species are known to be hallucinogenic. These hallucinogenic species (e.g. *P. mexicana*, *P.cubensis* & *P.aztecorum*) usually contain one or several indole compounds known as psilocybin, psilocin, baeocystin or nor-baeocystin. The hallucinogenic species of *Psilocybe* are found in various parts of the world notably more records are from the American continents (Guzmán 1983).

The main characters for the genus are as follows:
Pileus conic, semiglobate, convex then expanding to plano-convex, or campanulate; subviscid, or viscid, hygrophanous or dry; papillate or non-papillate; usually in the yellow and brown series with reddish, or olivaceous or greyish tints. Lamellae broad, adnexed to adnate, or subdecurrent. Stipe central, generally long and slender, dry, glabrous or fibrillose, straight or at times somewhat flexuose. Partial veil evanescent, rarely forming an annulus. Spore mass lilaceous black or dull purplish brown; spores with truncate germ pore, melleous brown to deep chestnut brown in KOH. Pleurocystidia present or absent; cheilocystidia present and abundant usually forming a heteromorphous edge. Epicutis a layer of filamentous, repent hyphae which may or may not gelatinized; hypodermium usually a layer of broader hyphae; hymenophoral trama regular. Occurring on ground litter (leafy or woody), on rotten wood, on soil in pasture or on dung.

In his treatment of the family Strophariaceae, Kühner has relegated *Psilocybe* as a subgenus in his giant genus *Psilocybe* (see Table 1.1). But Singer (1986) recognized

Psilocybe as an autonomous genus which also includes another smaller genus *Deconica*, now reduced to a synonym of *Psilocybe*. Singer's concept of *Psilocybe* is adopted here in this study.

Guzmán (1983) in his world monograph of the genus proposed 18 sections to illustrate the phylogenetic relationships between the species. Though this may not be a very satisfactory arrangement, some Australasian species have been placed in Guzmán's sections, as a result an adoption of this systematic arrangement, albeit tentatively would seem appropriate. Singer and Smith (1958) treated the majority of blueing species in Section *Caerulescentes* Singer, which may seem a more practical treatment of the blueing species. While Guzmán had spread the blueing species into seven different new sections apparently by raising certain stirps to the level of section. A synopsis of three systematic treatments of the genus *Psilocybe* is shown in Table 5.1.

There has been close to 30 species of *Psilocybe* identified or described in Australia (Cooke 1892; McAlpine 1895; Cleland 1934; Pegler 1967; Horak 1971; Guzmán and Watling 1978 and Guzmán and Vergreer 1978). Shepherd and Totterdell (1988) suggested 15 Australian representatives. Many of the previously described species of *Psilocybe* have since been placed accordingly in their appropriate genera.

A survey of Rodway's collection of Tasmanian fungi showed only four collections of *Psilocybe*, one of which was a collection of *Deconica* sp. and the remaining three collections consisted of *P. spadiceus*, *P. oedipus* and *Psilocybe* sp. Later reports included *Stropharia merdaria*(syn. *Psilocybe merdaria*), *P. semilanceata*, *P. subaeruginosa* and *P. tasmaniana*. A total of less than ten species has so far been reported for Tasmania. Every effort has been made to collect these previously reported species from a wide range of habitats including the published locations.

Guzmán and Watling (1978) recognized the morphological affinities between *P. subaeruginosa* and *P. australiana*, *P. eucalypta* and *P. tasmaniana*. *P. semilanceata* is known to be a variable species (Singer & Moser 1965).

5.2. Results

Initially four groups were identified based on morphological characters. Group I consisted of (A) 16 collections identified most closely to *P. australiana*; (B) one collection reliably identified as *P. eucalypta* from the type locality (Tidbinbilla Nature Reserve) of the species and was supplied by M. Priest of DAR; (C) one collection which appeared closer to *P. tasmaniana* based on the neck length of cheilocystidia (This collection was supplied as dried material by a zoologist, hence no viable spores were available) and (D) one collection reliably identified as *P. subaeruginosa*, from Warandyte, Victoria, supplied by B. Fuhrer. Appendix IIIC gives details for all the collections included in the study. Collections from (A) to (D) were tentatively grouped together as Group I and collectively referred to as the "*subaeruginosa* complex". All results from this study regarding the complex have been published (Chang & Mills 1992) and will not be repeated here but will be included in Appendix IV.

Group II consisted of a total of six collections, one (CYS451) of which had been positively identified as *P. semilanceata* (Fr.) Kummer while the other five collections bore affinities to CYS451 but with some notable differences. These five collections were collectively referred to as *Psilocybe* sp. A and were tentatively grouped together with CYS451 on the previously mentioned affinity. Details of these six collections on localities, habitats and date of collection is given in Appendix IIIC.

Group III consisted of a single collection found growing on dung, referred to as *Psilocybe* sp. B.

Group IV consisted of three collections from a lignicolous habitat and were from referred to as *Psilocybe* sp. C. Details on collections is given in Appendix IIIC. Groups III and IV were morphologically distinct from each other and from Groups I and II. All four groups possessed distinctive characters that placed them in the genus *Psilocybe*. For convenience, results of groups II to IV will be given under the heading 'other *Psilocybe* taxa'.

4.2.1. Morphological studies

Other *Psilocybe* taxa

The morphological characters of CYS451 agreed well with the description of *P. semilanceata* (Guzmán 1983; Singer & Moser 1965; Watling & Gregory 1978) and the herbarium material (HO and PDD) of the species. Pleurocystidia are generally regarded as absent in *P. semilanceata*, thorough examination of the specimens (both fresh and herbarium material) indicated that they were not entirely absent but very, very rare.

Collections of *Psilocybe* sp. A were morphologically indistinguishable from each other. The specimens of *Psilocybe* sp. A resembled closely to CYS451 (*P. semilanceata*) in microscopic characters, but with some notable differences in macroscopic characters (Table 5.2). The main differences noted in the macroscopic characters were the shape and colour of the basidiomes. CYS451 possessed the typical half-pear shape associated with *P. semilanceata* and remained campanulate even in the mature stage. The shape of the basidiomes of specimens of *Psilocybe* sp. A was more mycenoid, i.e., conical and tended to expand more when mature, though never plane. The basidiomes were generally not acutely papillate though occasional acute papilla could be observed. The colour difference was more obvious when comparing fresh specimens. CYS451 was clayey buff colour (close to 5C5) when fresh whereas specimens of sp. A were almost always dark leathery or dark reddish

brown (7E5-6) when fresh and drying to a dull brownish yellow. Both CYS451 and sp. A did not blue readily, though some blueing was noted near the base of stipe of specimen of CYS451 and on the gill edge in one specimen of sp. A. There was also a slight difference in habitats. CYS451 was collected from rich or well-manured pasture, whereas specimens of *Psilocybe* sp. A were always on dung.

The shape of the pileus of *Psilocybe* sp. B was convex or more or less hemispherical and expanded slightly with maturity. The basidiomes were very small with the pileus up to only 10 mm. in diameter and could be easily overlooked in the field. It lacked the reddish tint in the pileus as in sp. A. It was found growing on wallaby dung.

Specimens of *Psilocybe* sp. C were morphologically distinct from the above taxa. The colour of the pileus was close to chestnut brown but being strongly hygrophanous, it faded to whitish. The pileus was umbonate with an acute umbo. These two features as well as the obviously lignicolous habitat separated this taxon from the four taxa mentioned above. Table 5. 2 summarizes the main macrocharacters of these four taxa.

Table 5.3 gives a summary of the microcharacters of these four taxa of *Psilocybe*. There was an overlap in the size range of spores, basidia, and cheilocystidia in *P. semilanceata* and sp. A. The shape of spores in both taxa were ellipsoid, with a broad germ pore and the colour of spore wall was dark yellowish brown in 5% KOH. *Psilocybe* sp. B differed from the other taxa by its subhexagonal spores. *Psilocybe* sp. C was separated from the others because it possessed the smaller and narrower spores.

No obvious distinction could be discerned from the shape and size of basidia and cheilocystidia between CYS451 and sp. A. In both taxa, cheilocystidia showed

varying degree of branching, from simple, bi- and tri-furcate to multiple branching. The more common presence of mucronate pleurocystidia separated *Psilocybe* sp. B from *P. semilanceata* and sp. A. It also differed from sp. C in the shape of both pleurocystidia and cheilocystidia. The microscopic characters of *Psilocybe* sp. C was very distinctive from the other taxa. The most obvious distinction was in the shape of both the pleurocystidia and cheilocystidia. The lobed apex of the pleurocystidia was unusual in *Psilocybe* and the inflated cheilocystidia were variable and of a shape seldom seen in this genus.

5.2.2 Electrophoretic studies

In each enzyme system, allelic designations were not assigned but observations regarding recognizable loci were noted. Each band was scored as an independent phenetic character and numbered from the cathodic end.

Other *Psilocybe* taxa

Lac (Laccase)

When comparing the activities of laccase across all the isolates of the four *Psilocybe* taxa, nine regions of activities (or nine bands) were scored (Fig. 5.1). The number of isolates available for comparison was rather low due to the unpredictable behaviour in spore germination in most taxa. As a result of this, loci designation was not attempted. The overall comparison resulted in four zymogram patterns with no similarity in any region of activity (Fig. 5.1) between the isolates of the four putative groups.

For the reason mentioned above it was not possible to establish any reasonable range of variation within each group. The only group where some idea of variation could be discerned was *Psilocybe* sp. A. Table 5.4 shows a summary of the percentage occurrence of similar bands across the isolates in this group. The two dominant

regions of activities (Bands 5 and 7 at R_f 0.36 & 0.42 respectively) occurred in 60 and 80% of the isolates respectively followed by Band 9 at R_f 0.66 with 53.3%.

Of the other groups, for example in *P. semilanceata*, Band 6 at R_f 0.38 occurred in all the four isolates of CYS451 and Band 2 at R_f 0.22 recorded a 100% occurrence in the isolates (total of nine) of CYS381.

Per (Peroxidase)

A total of six bands of Per isozyme activities were scored across the isolates. Again as a result of the small number of isolates, it was not certain of the range of variability within each group. However, a certain degree of similarity in band activities was noted (Fig. 5. 2, Table 5.5) between isolates of different groups. Isolates of CYS451 (*P. semilanceata*) & *Psilocybe* sp.A showed similarity in Band 5 at R_f 0.44, while similar activity at R_f 0.38 (Band 3) was noted between isolates of sp. A, sp. B and sp. C.

AcP (Acid Phosphatase)

There was no AcP activity detected in isolates of CYS451 while isolates of sp. C showed a solitary band at R_f 0.13 (Fig. 5.3). The band at R_f 0.14 appeared to be the dominant band (100% occurrence) in isolates of *Psilocybe* sp. A (Table 5.4). A small degree of similarity was noted in isolates of *Psilocybe* sp. A and sp. B (Fig. 5.3). Three regions of similar activities corresponding to R_f 0.11, 0.14 and 0.24 respectively were noted in these isolates. Despite the similarities between isolates of different groups, the overall band pattern appeared to be distinct for the group concerned.

PE (Pectinesterase) & PG (Polygalacturonase)

A total of 13 bands of PE isozyme activities were noted across the isolates including three 'backrunners' (i.e. bands that moved towards the cathode). The various

combinations of bands resulted in four zymogram groups corresponding to the four putative groups (Fig. 5.4). Again it was not possible to establish any reasonable range of variability within each group. For the isolates of *Psilocybe* sp. A, three bands appeared to be subdominant since they occurred in only close to or just over half the total number of isolates (Table 5.4). In the case of *P. semilanceata* (CYS451), the two 'backrunners' at R_f -0.08 and -0.04 were detected in all the isolates of this group whereas Band 4 at R_f 0.17 was detected in all isolates of *Psilocybe* sp. B. Similarity in some PE activities was noted between the isolates of *P. semilanceata* and *Psilocybe* sp. B at R_f 0.28 (Table 5.5). Band 9 at R_f 0.36 was noted in isolates of both *Psilocybe* sp. A and *P. semilanceata.*

No PG activity was detected in isolates of *P. semilanceata.* In one repeated run, only very faint bands were noted. As a result of its inconsistency, they were excluded. Isolates of the other three groups showed activities with various mobilities. The various combinations of bands gave rise to three zymogram groups corresponding to these three taxa (Fig. 5.5). Isolates of *Psilocybe* sp. A were unusual in the activities of the 'backrunner' bands at R_f -0.08 and -0.04 respectively. These bands appeared to be unique to isolates of this taxon occurring at 66.7 and 46.7% respectively of the isolates (Table 5.4). Only one band of similar activity, Band 6, at R_f 0.17 was noted in isolates of both *Psilocybe* sp. B and sp. C.

To better envisage the relationships between the four groups, isolates of an outgroup species, *Melanotus hepatochrous*, were included for the UPGMA cluster analysis based on the band frequencies of the five enzyme systems. *M. hepatochrous* was chosen because of the recognized affinity with the genus *Psilocybe.* The results of cluster analysis are presented in a dendogram in Fig. 5.6. Five distinct clusters are evident corresponding to the five taxa included.

4.2.3. Mating compatibility studies

Other *Psilocybe* taxa

All the monokaryotic isolates of collections in *Psilocybe* sp. A were intercompatible with isolates of each collection referred to as *Psilocybe* sp. A but interincompatible with the isolates of CYS451 (Table 5.6). Since the mating system of *P. semilanceata* was not established as a result of insufficient number of monokaryotic isolates, the interincompatibility noted in the crosses with isolates of *Psilocybe* sp. A was treated with caution. However, this incompatibility was consistent with the morphological and electrophoretic distinctions noted between these two taxa.

5.3. Discussion

Results from this study have resolved the relationships between *P. subaeruginosa* and the three closely allied species *P. australiana*, *P. eucalypta* and *P. tasmaniana* (Chang & Mills 1992, also see Appendix IV); as well as confirming the existence of four distinct and separate species.

Within the "*subaeruginosa* complex", only one morphological species was identified and this corresponded to a single biological species from the results of morphological, electrophoretic and mating compatibility studies. This has consequently resulted in the emendation and lectotypification of the species *Psilocybe subaeruginosa* Clel. (Chang & Mills 1992).

Of the other *Psilocybe* taxa, two biological species have been identified in Group II, one corresponds to *P. semilanceata* and other *Psilocybe* sp. A. *P. semilanceata* is recognised to be a variable species in particular in spore size (Singer & Moser 1965). The high degree of overlap in micromorphology may be an indication of close affinity between these two taxa. Macromorphology , on the other hand, provide some distinctions between the two taxa (See Table 5.2). However, the use of isozyme

analyses (both isozyme profiles and average linkage clustering analysis) provides a more solid ground for delineation and further negates the possibility that *Psilocybe* sp. A may be a variant of *P. semilanceata*. This has also been supported in part by the results from mating compatibility tests. Though the mating results were treated with caution, the coincidence of interincompatibility with the distinct isozyme profiles for the tested enzymes supported the conclusion that two separate species were within this group.

As a result of the morphological affinity to *P. semilanceata*, *Psilocybe* sp. A is best placed in section *Semilanceatae* Guzmán and a species epithet is given. *P. alutacea* sp. nov. will be described formally in the chapter on New Species.

The remaining two taxa of *Psilocybe* sp. B and sp. C are morphologically distinct from each other and from *P. subaeruginosa*, *P. semilanceata* and *Psilocybe* sp. A. Isolates of these two taxa also showed distinctive isozyme profiles associated with the respective taxon despite the similarity noted in some Per and PE band activities.

The coprophilous habitat, subhexagonal spores and presence of pleurocystidia are some characters that affiliate *Psilocybe* sp. B to *P. coprophila*. *P. coprophila* is known to be widespread in tropical, subtropical and subtemperate regions (Guzmán 1983). However, Pegler *et al.* (1981) has reported *P. coprophila* from subantarctic habitats. The Tasmanian material agrees well with the description of *P. coprophila* except for its occurrence in a cool temperate region. Since this species is not restricted in its distribution, the Tasmanian material is considered to belong to *P. coprophila* (Bull. ex Fr.) Kummer.

Psilocybe sp. C does not fit any description of the currently known species in this genus. It is close to species in section *Aztecorum* Guzmán in the strongly hygrophanous nature of the pileus with the colour change from brown to whitish. The

only obvious difference from species within the section is that it is not blueing at all. There are also other distinctive morphological characters such as the lobed apex of pleurocystidia and the greatly inflated cheilocystidia. All these characters are diagnostic enough to grant species status to this fungus. *P. brunneo-albescens* sp. nov. will be described formally in the chapter on New Species.

Taxonomy

A key is given below for all the taxa delineated in the above study. A synopsis of the taxa delineated is given in Table 5.7.

Key to species of *Psilocybe* in SE Tasmania

1. Growing on rotten wood or ground litter of woody or leafy debris 2
1'. Growing in pasture or on dung 3
2. Basidiomes blueing when damaged, pileus pale or dull brown to ochraceous buff ..... **1. subaeruginosa**
2'. Basidiomes not blueing at all, pileus brown then fading to whitish from the disc and spreading outwards ..... **2. brunneo-albescens sp. nov.**
3. Pileus campanulate and not expanding to convex, papillate, growing in pasture ..... **3. semilanceata**
3'. Pileus convex or plano-convex, on dung 4
4. Pileus convex, not >10 mm. in diameter, spores up to 14 µm. long, subhexagonal in face view, ellipsoid in profile, on wallaby dung ..... **4. coprophila**
4'. Pileus at first conical then convex, non- to slightly papillate, >10 mm. in diameter, tawny brown to ochre brown, spores ellipsoid oblong in face view, up to 17 µm. long ..... **5. alutacea sp. nov.**

1. *Psilocybe subaeruginosa* Clel. emend Chang & Mills, *Mycol. Res.* 96: p.438. *Lectotype*: SOUTH AUSTRALIA, National Park, AD5603!

Psilocybe subaeruginosa Clel. *Trans. & Proc. Roy. Soc. South Australia* 51: 305,

1927.

Psilocybe australiana Guzmán & Watling, *Notes Roy. Bot. Gard. Edinb.* 36: 206, 1978. Holotype: New South Wales, near Canberra, Cotter Dam, Blue Range, Watling10617 (E!).

Psilocybe eucalypta Guzmán & Watling, *Notes Roy. Bot. Gard. Edinb.* 36: 204, 1978. Holotype: A. C. T., near Canberra, Tidbinbilla Nature reserve, Watling 10656 (E!).

Psilocybe tasmaniana Guzmán & Watling, *Notes Roy. Bot. Gard. Edinb.* 36: 207, 1978. Holotype: Tasmania, NE of Hobart, Nugent, Buckland, Watling 10393 (E!).

Selected illustrations: Cleland (1934),fig. 25, p.141; Cole *et al.* (1978), pl.5; Fuhrer (1985), p.75 (as *Psilocybe* sp.); Shepherd & Totterdell (1988), p.93 (as *Psilocybe* sp.).

Illustrations: Figs. 5.7 -12.

Obervations

This is the only known blueing species of *Psilocybe* in Tasmania and has been shown from the present study to be a variable species. Like *P. semilanceata*, it has been sought after for recreational use. It is very common and widespread in Tasmania. See Appendix IV for more comments and specimens examined.

2. *Psilocybe* brunneo-albescens sp. nov.

The most easily recognized field character for this fungus is the strongly hygrophanous pileus and this is one character that relates it to the species in section *Aztecorum*. The other known species of this section blue to varying degrees, however, this Tasmanian species is non-blueing and therefore it is uncertain whether it will be hallucinogenic. This fungus will be formally described in the chapter on New Species (See there for further discussion).

3. ***Psilocybe semilanceata*** (Fr. ex Secr.) Kummer *Führ. Pilzk.*, p 71, 1871.
Illustrations: Figs. 5.13 - 15.

Material examined:
TASMANIA, Neika, 19. vi. 1990, CYS451; police exhibit, HO31610.
UNITED KINGDOM, PDD56613 (identified by R. Watling).

This fungus is well documented and is one of the most common hallucinogenic species in Australia, and has been used for recreational purposes.

4. ***Psilocybe alutacea*** **sp nov.**
This is a weakly blueing species close to *P. semilanceata* and resembles other species in various aspects in section *Semilanceatae*. It is a species with small, inconspicuous carpophores. This species will be described formally in the chapter on New Species.

5. ***Psilocybe*** **coprophila (Bull. ex Fr.) Kummer**, *Der Führer in die Pilzkunde*, p. 71, 1871.
Illustrations: Fig. 5.16.

Pileus 8 - 10 mm. in diam, convex, glabrous, viscid, margin slightly striate, brown (6E5) when fresh, drying to brownish orange or topaz (5C5) and slightly shiny. *Lamellae* broadly adnate, subdistant, cocoa brown (6D4) then darken (6F4) further with spores, white margin. *Stipe* 20 - 28 x 1 - 2 mm., equal, delicate, dull congnac brown (6E7). *Annulus* absent. *Context* thin, concolorous with pileus.
Spores (10-) 10.8 - 13.3 (-14.2) x (7.5-) 7.9 - 10 x 6.7 - 8.7 µm., subhexagonal in face view, subelliptic in profile, yellowish brown with a broad germ pore, thick-walled. *Basidia* (18.3-) 19.2 - 31.7 (-32.5) x 7.5 - 11.7 µm., 4-spored, hyaline, sterigmata up to 5.8 µm. long. *Pleurocystidia* very pale yellow (5% KOH), mucronate or with a

short neck, different in shape from cheilocystidia. *Cheilocystidia* 21.7 - 40 x 5.8 - 9.2 µm., abundant, lageniform, thin-walled, long-necked, generally >5 µm. long. *Subhymenium* subcellular. *Trama* regular. *Epicutis* a thin layer of gelatinised hyphae. Clamp connections present.

Habitat on dung (wallaby).

Material studied:

TASMANIA, Geeveston, 23. v. 1990, *Y. S. Chang*, CYS 381; QUEENSLAND, 17. viii. 1961, *B. Brown*, BRIP 10238; New South Wales, Quakers Hill, 3. i. 1977, *A. Young*, DAR30679.

Observations

P. coprophila is usually reported from tropical, subtropical and warm temperate habitats. It is close to *P. argentina* which occurs in the temperate region but differs from it by the smaller spores as well as the more commonly occurred pleurocystidia. Cleland (1934) reported *P. coprophila* for South Australia but later examination of Cleland's specimens by Grgurinovic (per. comm.) has transferred them to *P. argentina*. *P. coprophila* is very rare in Tasmania, there is no previous record of this fungus from Tasmania.

Table 5.1. Systematic treatments of the genus *Psilocybe*.

Singer (1986)

Genus Psilocybe (Fr.) Kummer

Section 1. Merdariae (Fr.) Singer

. 2. Caerulescentes Singer

Stirps 1. Cubensis; 2. Yungensis;

3. Mexicana; 4. Silvatica;

5. Cyanescens; 6. Zapotecorum;

7. Caerulipes

Section 3. Tenaces (Fr.) Sacc.

4. Atrobrunneae Singer

5. Septembres Singer

6. Psilocybe Singer

7. Chrysocystidiatae Singer

Watling & Gregory (1987)

Genus Psilocybe (Fr.) Kummer

Section 1. Caerulescentes

Stirps 1. Cubensis; 2. Cyanescens

Section 2. Semilanceatae

Stirps 3. Semilanceata; 4. Fimetaria.

Section 3. Merdariae

Stirps 5. Coprophila

Section 4. Psilocybe

Stirps 6. Montana; 7. Inquilina; 8. Bullacea.

Section 5. Atrobrunneae

Stirps 9. Atrobrunnea

Section 6. Squamosae

Stirps 10. Luteonitens; 11. Squamosa.

Guzmán (1983)

Genus Psilocybe (Fr.) Kummer

Section 1. Brunneocystidiatae Guzmán

2. Blattariopsidae Guzmán

3. Subaeruginosae Guzmán

4. Cordisporae Guzmán

5. Psilocybe

6. Mexicanae Guzmán

. 7. Stunzae Guzmán

8. Coprophilae

9. Merdariae

10. Cubensae Guzmán

11. Zepotecorum Guzmán

12. Singerianae Guzmán

13. Pratensae Guzmán

14. Atrobrunneae

15. Squamosae

16. Aztecorum Guzmán

17. Cyanescens Guzmán

18. Semilanceatae Guzmán

Table 5.2. Differences in macrocharacters and habitat preferences noted in *Psilocybe semilanceata*, *Psilocybe* sp. A, *Psilocybe* sp. B and *Psilocybe* sp. C.

Characters	*P. semilanceata*	*Psilocybe* sp A	*Psilocybe* sp. B	*Psilocybe* sp. C
Pileus				
colour	pale ochraceous	dark leathery to soft leathery brown drying to ochraceous	brown or brownish orange to topaz	chestnut brown turning whitish on drying
shape	subcampanulate, papillate	conic or semiglobate slightly papillate	convex	acute umbonate
surface	glabrous, viscid hygrophanous slightly striate	glabrous, viscid, hygrophanous, deeply striate	viscid margin striate	glabrous, greasy to tacky, strongly hygrophanous, deeply striate
diam (mm.)	10 - 18	9 - 13	up to 10 mm.	7 -15
Lamellae				
attachment	adnate	adnate	broadly adnate	adnate to slightly adnexed
colour	fuscous brown with spores white margin	greyish brown with spores margin whitish	cocoa brown with spores white margin	pale brown
Stipe				
colour	concolorous with pileus, slightly blued near base	yellowish brown	dull brown	whitish
surface	dry, ±glabrous	dry	dry	dry, fibrillose
others		stuffed, ±equal, flexuose		stuffed, then hollow equal
l x w (mm.)	75 x 1	25 - 57 x 1-2.5	20 - 28 x 1 -2	20 - 31 x 1-2
Habitat	in rich or well-manured pastures	on dung of cow, horse & wallaby	on wallaby dung	gregarious on rotten wood

Table 5.3. Differences in microcharacters noted in *Psilocybe semilanceata*, *Psilocybe* sp. A, *Psilocybe* sp. B and *Psilocybe* sp. C. Measurement are given in mean and standard deviation, n is the number of collections used to calculate the mean.

Characters	*P. semilanceata* (n=2)	*Psilocybe* sp A (n=5)	*Psilocybe* sp. B (n=1)	*Psilocybe* sp. C (n=3)
Spore				
spore print	purple brown*	violaceous black	violaceous black	fuscous brown
germ pore	distinct & broad	distinct & broad	distinct & broad	distinct
shape	elliptic to slightly inequilateral	elliptic to slightly inequilateral	subhexagonal in face view, sub-elliptic in profile	elliptic to slightly inequilateral
l x f x p (µm.)	14.02 ±0.89 x 8.45 ±0.58 x 8.37 ±0.61	13.88 ±0.94 x 8.47 ±0.42 x 8.43 ±0.48	11.82 ±0.93 x 8.31 ±0.36 x 7.40 ±0.49	6.89 ±0.31 x 4.18 ±0.16 x 4.27 ±0.20
Basidia	4-spored	4-spored 2-spored	4-spored, rarely-occasionally 2-spored	4-spored
	sterigmata up to 6 µm. long	sterigmata up to 6 µm. long	sterigmata up to 8 µm. long	
l x w (µm.)	30.08 ±3.48 x 9.89 ±1.58	30.12 ±2.37 x 10.77 ±0.92	25.92 ±3.50 x 10.17 ±0.65	24.08 ±2.99 x 6.04 ±0.71
Pleurocystidia	hyaline	hyaline	hyaline or very pale yellow	hyaline
	very rare	rare to infrequent	short-necked lageniform	apex obtuse or lobed
				lageniform
l x w (µm.)	20.51 ±2.60 x 7.32 ±1.89	23.40 ±3.05 x 7.23 ±1.46	33.42 ±3.92 x 12.25 ±1.83	37.62 ±6.85 x 7.03 ±1.22
Cheilocystidia	hyaline	hyaline	hyaline	hyaline
	long-necked	long-necked	long-necked,	variable in shape
	simple or bi-furcate	simple, bi- or tri-furcate	simple	usually inflated
	elongate lageniform		lageniform	
l x w (µm.)	29.23 ±4.88 x 6.25 ±1.11	27.76 ±3.29 x 6.64 ±0.92	28.04 ±2.72 x 7.54 ±0.60	32.04 ±5.46 x 17.25 ±4.96

* From Watling (1973), no spore print was obtained for the fresh collection of CYS451.

Table 5.4. Percentage occurrence of similar bands in isolates of *Psilocybe* sp. A in the five enzyme systems of Lac, Per, AcP, PE and PG (n=15).

Enzyme	Band No.	R_f	% occurrence
Lac	5	0.36	60
	7	0.42	80
	8	0.64	6.7
	9	0.66	53.5
Per	3	0.38	6.7
	5	0.44	40
	6	0.50	93.
AcP	1	0.06	40.
	3	0.11	26.7
	5	0.14	100
	6	0.19	60
	9	0.46	33.3
PE	1	-0.18	46.7
	9	0.36	13.3
	10	0.40	53.5
	11	0.42	53.3
	13	0.48	26.7
PG	1	-0.08	66.7
	2	-0.04	46.7
	3	0.02	20
	11	0.44	20
	12	0.50	6.7

Table 5.5. Similarity in PE and PG isozyme activities between isolates of the four taxa of *Psilocybe*.

Enzyme	Band	Rf	Isolates of taxa showing similarity
Per	3	0.38	*Psilocybe* sp. A, *Psilocybe* sp. C & *Psilocybe* sp. B
	5	0.44	*P. semilanceata* & *Psilocybe* sp. A
PE	6	0.28	*P. semilanceata* & *Psilocybe* sp. B
	9	0.36	*P. semilanceata* & *Psilocybe* sp. A
PG	6	0.17	*Psilocybe* sp. B & sp. C

Table 5.6 Results of mating crosses between isolates of *Psilocybe semilanceata* and *Psilocybe* sp. A. All the isolates were monokaryons except W448, a dikaryotic wild isolate.

Species & isolate No.	No. of isolates		Species & isolate No.	No. of isolates	Total No. of pairings	No. of positive pairings	No of negative pairings
P. semilanceata		x	***Psilocybe* sp. A**				
CYS451 (01, 03 & 04)	3	x	CYS389 (01, 02 & 04)	3	9	0	9
		x	CYS391 (02 & 08)	2	6	0	6
		x	W448	1	3	0	3
***Psilocybe* sp. A**		x	***Psilocybe* sp. A**				
CYS389	3	x	CYS391	2	6	6	0
		x	W448	1	3	3	0
CYS391	2	x	W448	1	2	2	0

Table 5.7. Synopsis of Tasmanian species of *Psilocybe* delineated from the study.

Section Cyanescens	*Section* Coprophilae
Species 1. subaeruginosa	*Species* 1. coprophila
Section Semilanceatae	*Section* Aztecorum
Species 1. semilanceata	*Species* 1. brunneo-albescens sp. nov.
2. alutacea sp. nov.	

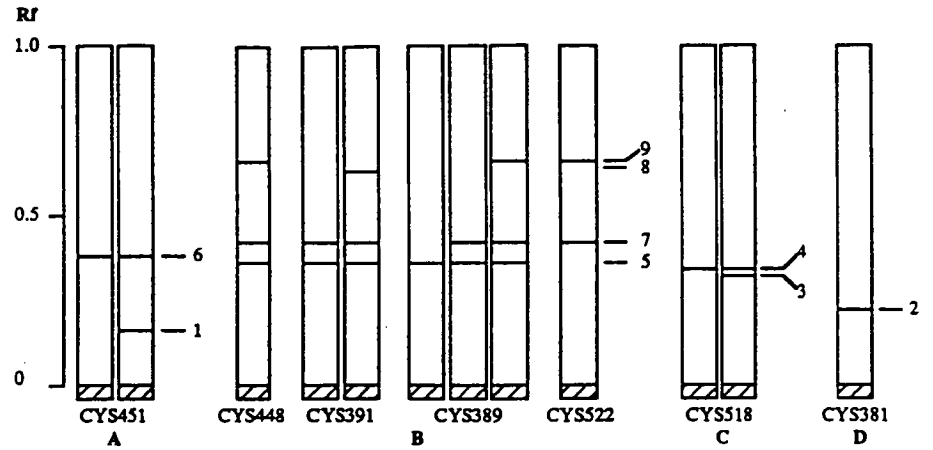


Fig. 5.1. Schematic representations of Lac zymograms of isolates of taxa of *Psilocybe*. Band numbers start from the cathodic end. Rf values: 1=0.16, 2=0.22, 3=0.32, 4=0.34, 5=0.36, 6=0.38, 7=0.42, 8=0.64 & 9=0.66. Legend: A= *P. semilanceata*, B= *Psilocybe* sp A, C= *Psilocybe* sp C and D= *Psilocybe* sp B.

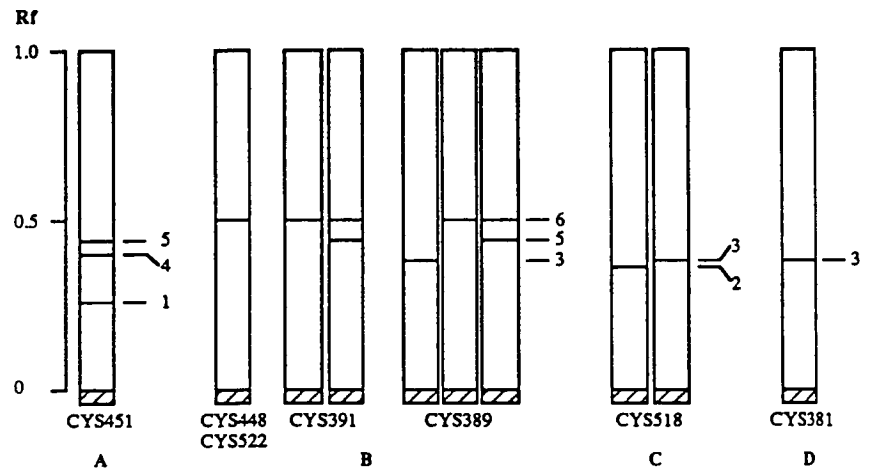


Fig. 5.2. Schematic representations of Per zymograms of isolates of taxa of *Psilocybe*. Band numbers start from the cathodic end. Rf values: 1=0.26, 2=0.36, 3=0.38, 4=0.40, 5=0.44 & 6=0.50. Legend: A= *P. semilanceata*, B= *Psilocybe* sp A, C= *Psilocybe* sp C and D= *Psilocybe* sp B.

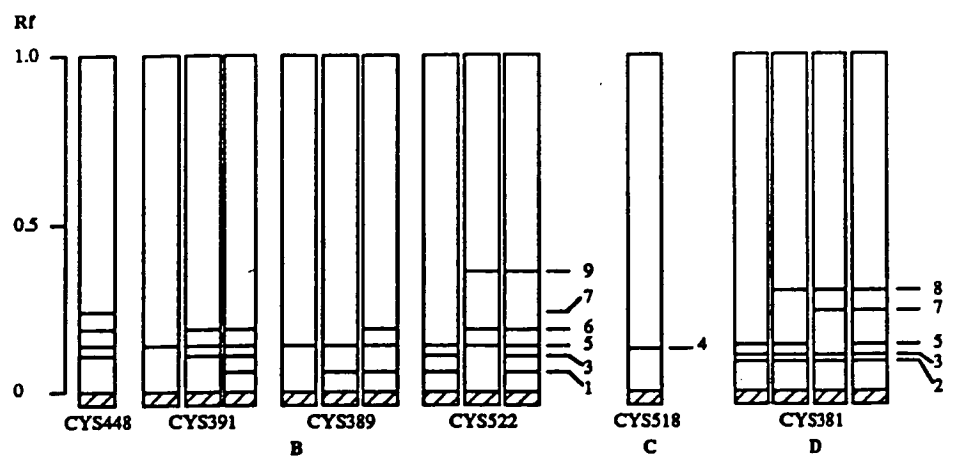


Fig. 5.3. Schematic representations of AcP zymograms of isolates of taxa of *Psilocybe*. Band numbers start from the cathodic end. Rf values: 1=0.06, 2=0.09, 3=0.11, 4=0.13, 5=0.14, 6=0.19,7=0.24, 8=0.30 & 9=0.46. Legend: B= *Psilocybe* sp A, C=*Psilocybe* sp C and D= *Psilocybe* sp B.

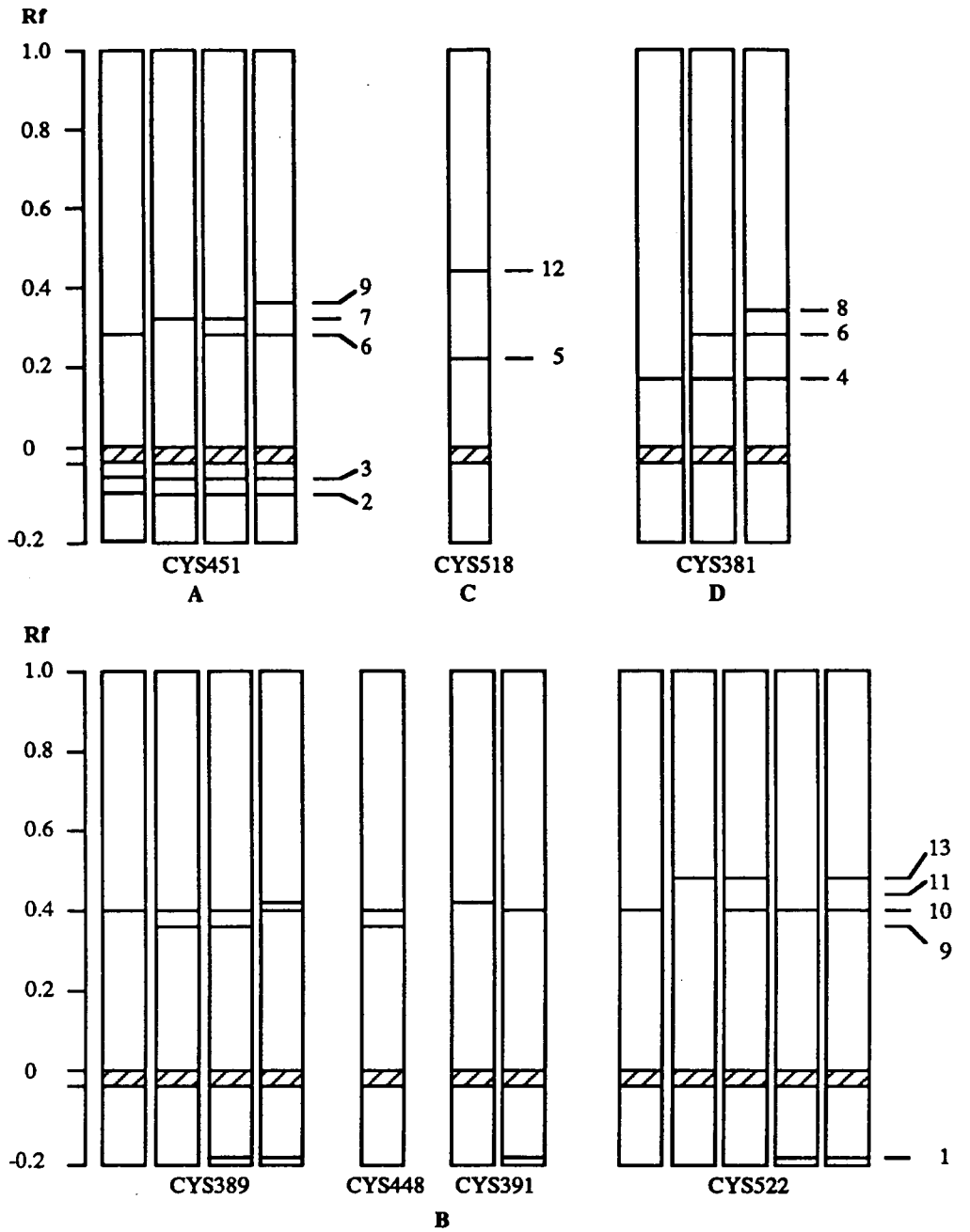


Fig. 5.4. Schematic representations of PE zymograms of isolates of taxa of *Psilocybe*. Band numbers start from the cathodic end. Rf values: 1=-0.18, 2=-0.08, 3=-0.04, 4=0.17, 5=0.22, 6=0.28, 7=0.32, 8=0.34, 9=0.36, 10=0.40, 11=0.42, 12=0.44 & 13=0.48. Legend: A= *P. semilanceata*, B= *Psilocybe* sp A, C=*Psilocybe* sp C and D= *Psilocybe* sp B.

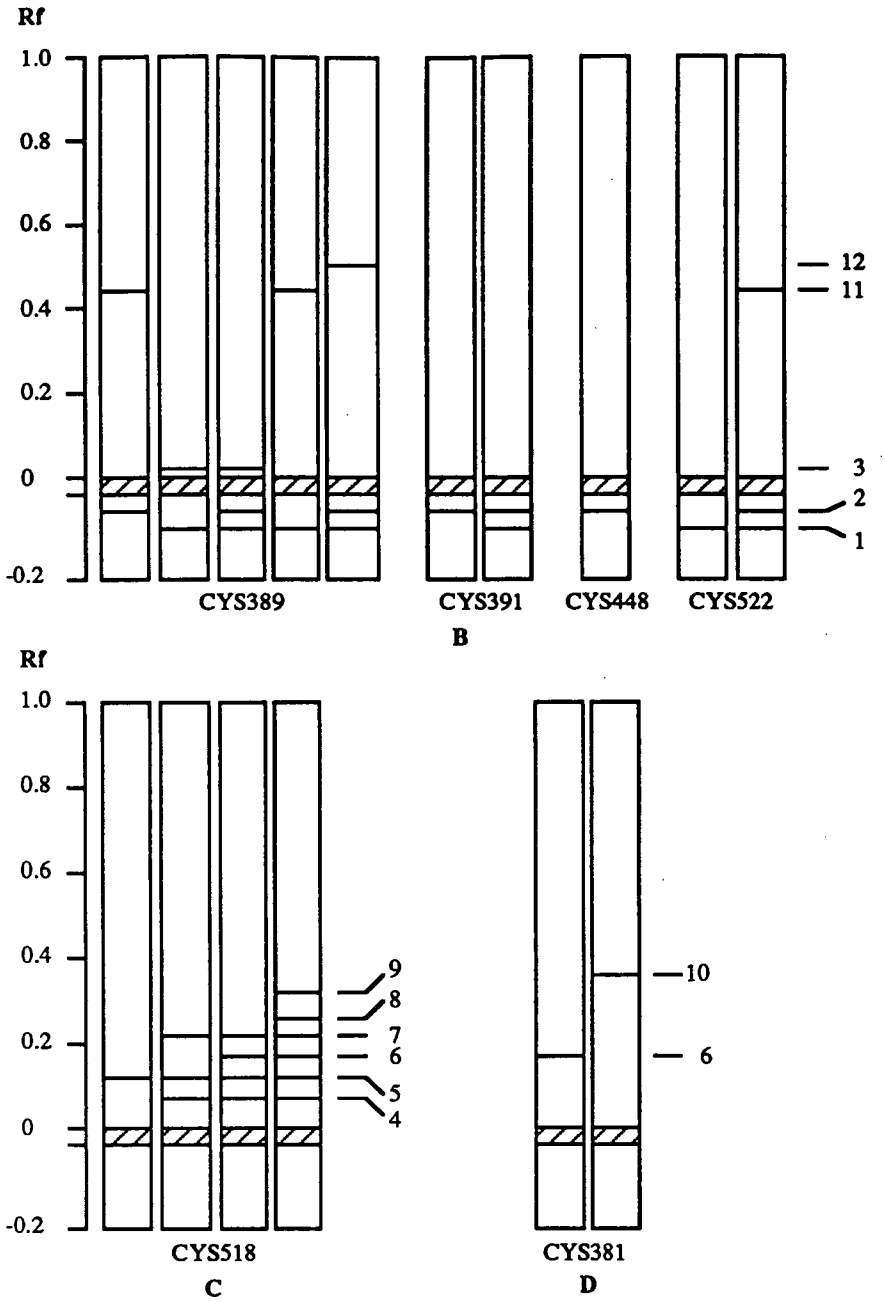


Fig. 5.5. Schematic representations of PG zymograms of isolates of taxa of *Psilocybe* . Band numbers start from the cathodic end. Rf values: 1=-0.08, 2=-0.04,3=0.02, 4=0.07, 5=0.12, 6=0.17, 7=0.22, 8=0.26, 9=0.32, 10=0.36, 11=0.44 & 12=0.50. Legend: B= *Psilocybe* sp A, C= *Psilocybe* sp C and D= *Psilocybe* sp B.

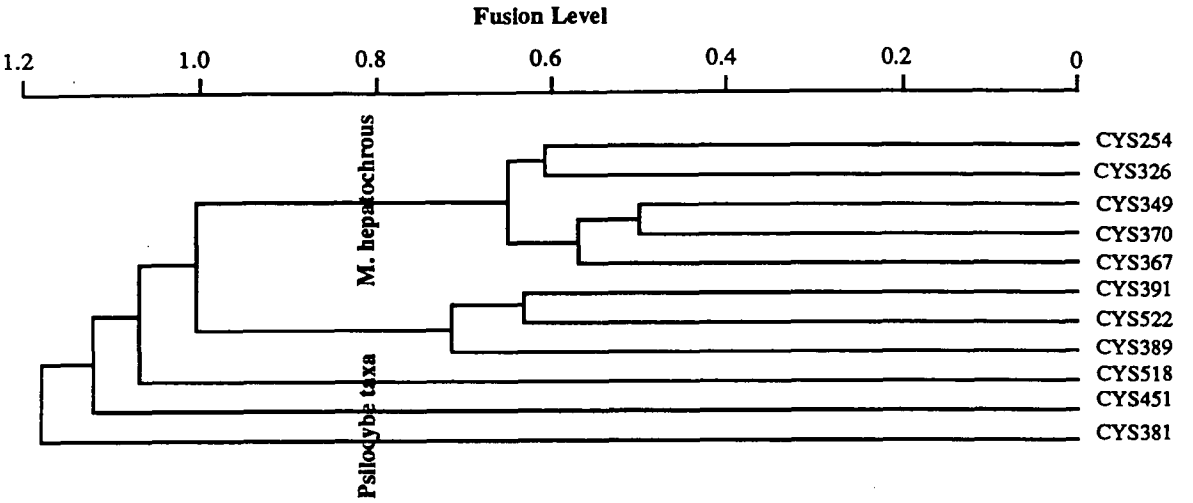
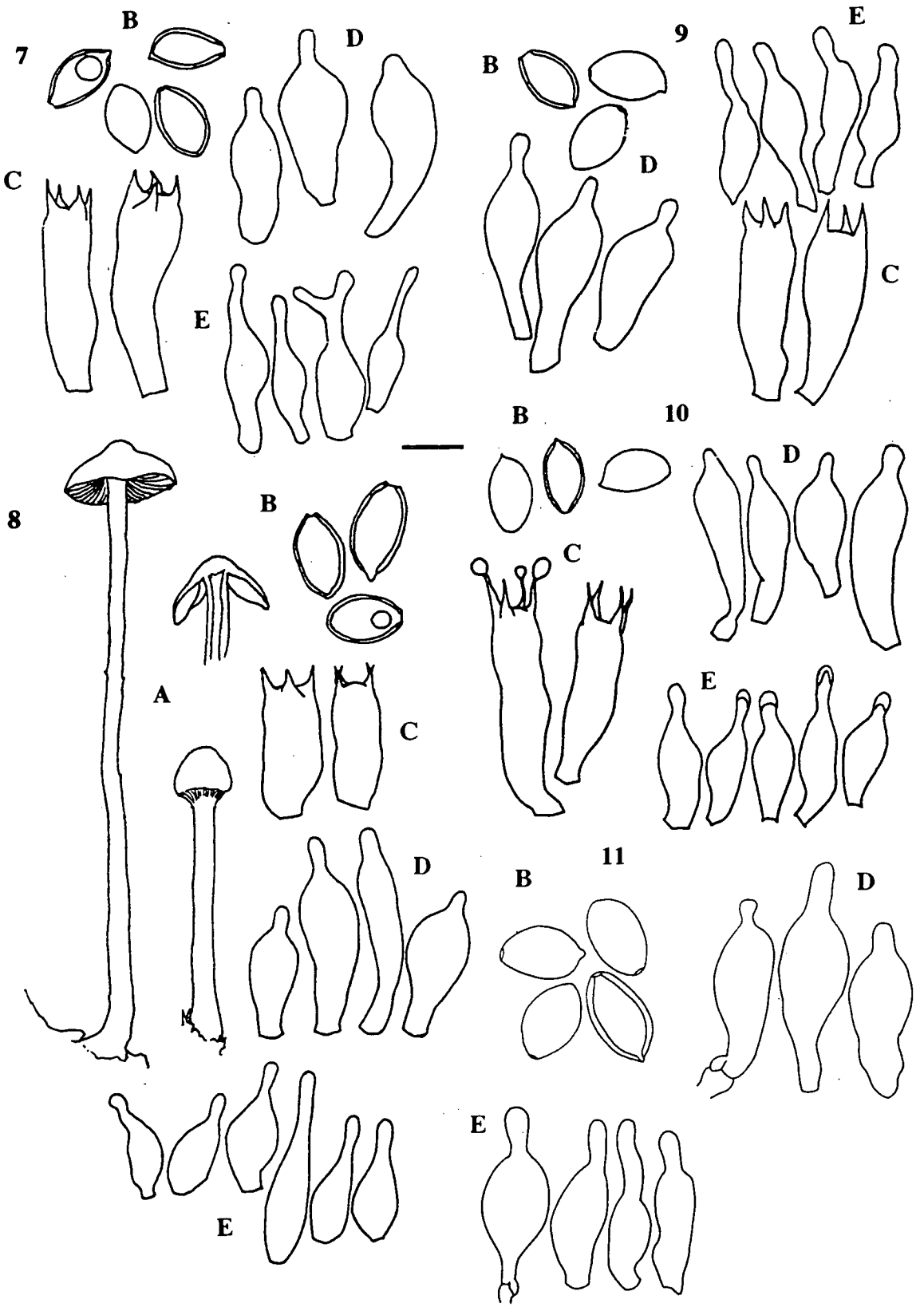
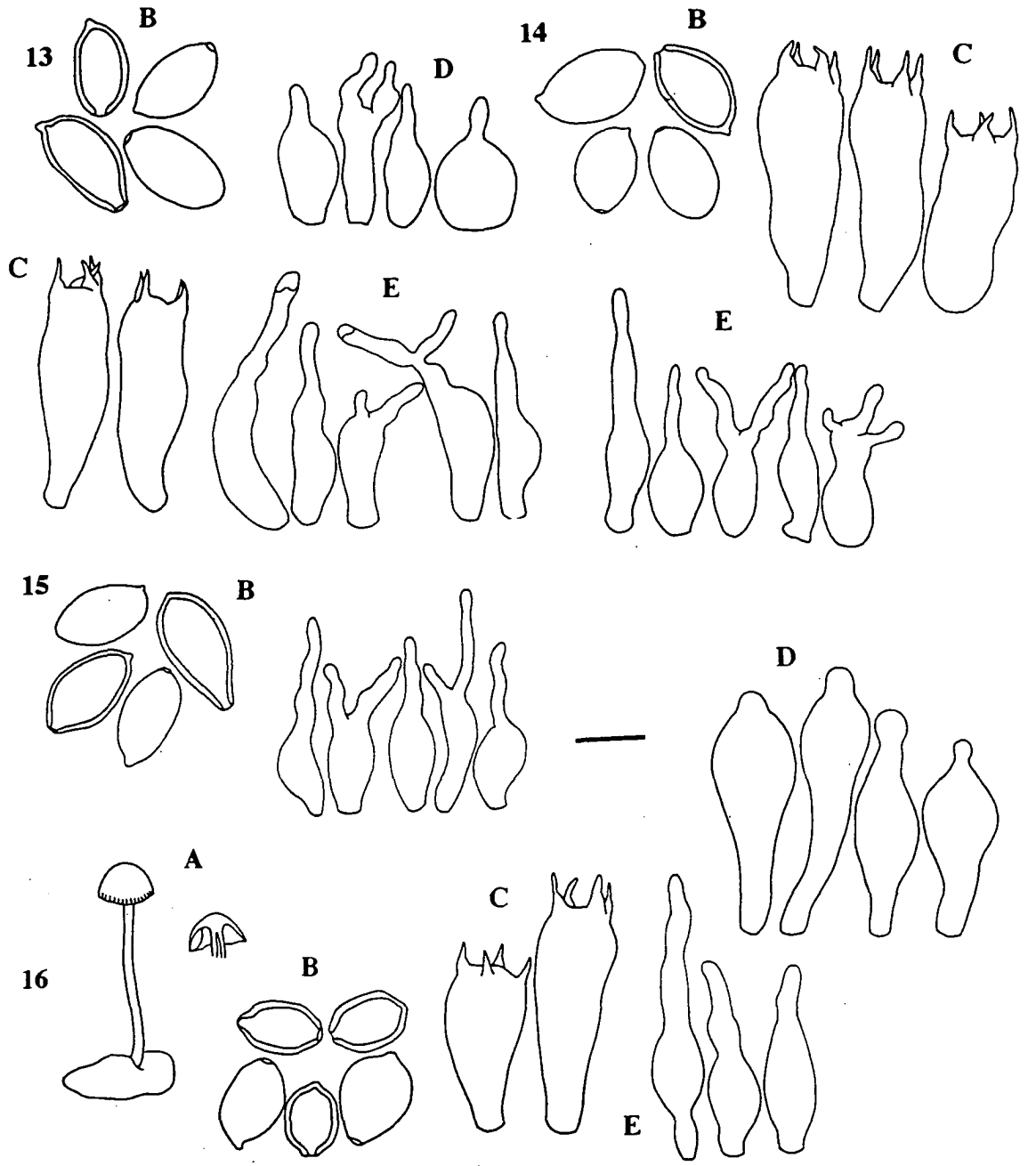


Fig. 5.6. Dendogram from UPGMA cluster analysis based on the band frequencies of all the enzymes examined for isolates of *Psilocybe* taxa with isolates of *Melanotus hepatochrous* included for comparison.



Figs. 5.7 -11. *Psilocybe subaeruginosa.* A: habit, B: spores, C: basidia, D: pleurocystidia and E: cheilocystidia. 7. CYS95. 8. CYS112. 9. CYS161. 10. CYS170. 11. AD5603 (lectotype).

Fig. 5.12. Habit of CYS132, *Psilocybe subaeruginosa*.



Figs. 5.13 - 16. A: habit, B: spores, C: basidia, D: pleurocystidia, and E: cheilocystidia. 13 - 15. *Psilocybe semilanceata*. 13. CYS451. 14. HO31610. 15. PDD56613. 16. *Psilocybe coprophila*, CYS381.

Chapter 6

Genus *Melanotus* Pat.

6.1 Introduction

Melanotus Pat. is one of the smaller genera in Strophariaceae. The genus is easily recognized, but, due to the small size of most carpophores, determination to species level is difficult. Most species are limited to the warmer subtropical and tropical regions with a few found in the cooler temperate regions of Europe, North and South America and Australasia.

The genus *Melanotus* Pat. is characterized by small, dull-coloured carpophores, eccentric or with reduced, lateral stipes and lacking pleurocystidia. The shape of pileus is convex then expanding, fan or scallop-shaped in the crepidotoid forms; surface hoary then smooth. Basidia are 4-spored, spores elliptic and rarely lentiform, distinct apical germ pore and cheilocystidia numerous forming a heteromorphous edge. Species of this genus are found on decaying plant debris, wood and also on manufactured material such as fabrics.

Close affinity of this genus with *Psilocybe* has been widely acknowledged (Singer 1986; Horak 1977; Orton 1984). The main difference is in the eccentric or lateral, reduced stipe. However, the significance of this character has been questioned (Horak 1977). For example, carpophores of *M. patagonicus* Sing. are predominantly centrally stipitate (*loc. cit.*). The crepidotoid habit has often been the source for earlier confusion of this genus with *Crepidotus*, *Claudopus* (Singer 1986; Horak 1977) or *Pleuroflammula* (Singer 1986), however, microscopic characters of *Melanotus* bear no taxonomic relationship to these genera (Horak 1977). Horak (*loc. cit.*) points out that separation from the eccentric species of *Phaeomaramius* and *Tubaria*, in particular those with apical germ pore, could be difficult.

Singer's (1986) taxonomic concept of *Melanotus* is followed in this study. Delineation of species within the genus is according to substrates (or host plants), size and shape of spores. These criteria will be considered in this study.

Rodway (1898) reported *Crepidotus cassiaecolor* Berk., *C. hepatochrous* Berk. and *C. insidiosus* Berk. from Tasmania. They are found to be conspecific by Horak (1977) and synonymised under *Melanotus hepatochrous* (Berk.) Singer and this is the only reported species of *Melanotus* in Tasmania. The scope of study for this genus is therefore to find out if any other taxa occur in SE Tasmania and by using the three approaches to investigate the degree of variation in the known species of *M. hepatochrous*.

6.2 Results

6.2.1 Morphological studies

Morphological comparisons were based on both macro- and micro-characters. The following were included: colour and shape of pileus, position of stipe, general growth habit, cuticle structure, spore length and width, spore quotient and cheilocystidia length and width. Habitat was also included in the comparisons. Collections included in the comparisons were all the Tasmanian material of *M. hepatochrous* collected from 1989 to 1991 as well as Rodway's collections of *C. hepatochrous* and *C. cassiaecolor* (Appendix IIID).

Within the fresh collections, the colour of the pileus ranged from dark brown to reddish brown (liver brown) when moist, fading to pale cinnamon brown or whitish. The shape of pileus ranged from orbicular, fan or scallop-shaped to reniform. Stipe was generally eccentric to lateral but occasionally centrally stipitate forms intermingled with the eccentric forms in a single collection. A circular pad or disc was apparent in most of the specimens at the base of the stipe where the carpophore was attached to the substratum.

The carpophores generally appeared gregarious on twigs, dead wood or stumps, *Eucalyptus* logs or branches or the rachis of manfern (*Dicksonia antarctica* Labill.) fronds, from sheltered to more or less exposed areas.

The epicutis was noted to consist of filamentous, repent hyphae, often encrusted with yellow brown pigments and with occasional branching tips.

Fig. 6.1 illustrates the variations noted in spores based on mean spore length. *Rodway*670 (as *Agaricus hepatochrous*) was the collection with the greatest mean spore length, but was still within the range for *M. hepatochrous*,i.e. 5.5 - 7.5 µm. (Horak 1977). Variations in both facial and profile width of spores were noted to be within the range of 4-5µm (3.5-5 µm. [*loc. cit.*]) (Fig. 6.1a). Consequently, shape of spores varied from broadly ellipsoid (Q= 1.15-1.30 [Bas 1969]), ellipsoid (Q=1.30-1.60 [*loc. cit.*]) to elongate (Q=1.60-2.00 [*loc. cit.*]) in both face view and profile (Fig. 6.2b & c). The shape in face view in majority of the spores was ellipsoid (Fig. 6.3a) whereas the shape in profile was more elongate ellipsoid than the former (Fig. 6.3b).

There was considerable variation noted in both mean length and width of basidia (Fig. 6.4). Rodway 166 (*Crepidotus cassiaecolor* Berk.) was noted to have a greater range of mean basidia length than other collections whilst CYS425 showed greater variations in the mean width of basidia.

The mean length of cheilocystidia (Fig. 6.5a) of the Tasmanian material was within the published size range (20 -30 x 3 - 6 µm. [Horak 1977]). *Rodway*670 was noted to show greater variation in the mean length of cheilocystidia whereas variation in its mean width of cheilocystidia was negligible (Fig. 6.5b) compared to the other collections. The differences noted in both the mean length of spores and cheilocystidia of

*Rodway*670 was considered to be well within the limits of intraspecific variation for *M. hepatochrous*.

6.2.2 Electrophoretic studies

For each enzyme system, each band was scored as an independent phenetic character and numbered from the cathodic end. As a result of the smaller number of bands, allelic designation was attempted but band frequencies were used in the UPGMA cluster analysis.

Lac (Laccase)

Only two dominant bands, Bands 1 & 2, were scored across the isolates (Fig. 6.6) and occurred in 87.5% and 81.25% of the isolates respectively (Table 6.1). These two bands corresponded to two monomorphic loci ($LacI_A$ & $LacII_A$).

Per (Peroxidase)

As mentioned in previous chapters, due to the behaviour of certain laccases with hydrogen peroxide, two bands (Band 1 & 2 at R_f 0.23 & 0.27 respectively) were excluded from comparisons. Thus, only two Per bands (Band 3 & Band 4 at R_f 0.30 & 0.36 respectively) were included and each was noted in approximately 25% of the isolates. These latter two bands corresponded to two monomorphic loci ($PerI_A$ & $PerII_A$ respectively).(Fig. 6.7)

AcP (Acid phosphatase)

During the staining of AcP, yellow-orange bands stained actively at sites corresponding to peroxidase activities. These paler bands were treated as artifacts and only the dark bands were scored as AcP isozymes.

There was much variation in the activities of AcP across the isolates. Five bands were scored. Band 2 was the dominant band (occurring in 25% of isolates) followed by

Band 5 (18.75%) (Table 6.1). Band 1 appeared to be alternating with Band 2 (Fig. 6.8) while the remaining bands appeared to be associated with monomorphic loci.

PE (Pectinesterase) and PG (Polygalacturonase)

Five bands were scored for PE activities. Of these, four were 'backrunners', i.e. moving towards the cathodic end. There were three bands (Bands 1, 3 & 4) which occurred in at least 25% of the isolates. All five bands were probably of monomorphic loci (Fig. 6.9).

A total of five bands were scored for PG activities. Of these, Bands 1 to 3 were dominant occurring in 79%, 90% & 81% of isolates respectively. These five bands appeared to correspond to five monomorphic loci (Fig. 6.10).

There was a high degree of uniformity in both Lac and PG systems. Much variation was noted in Per, AcP and PE systems, and this probably accounted for much of the intercollection variation. To better assess the variation within this group, it was included in a cluster analysis together with four *Psilocybe* species acting as outgroup taxa.

The dendogram (Fig. 6.11) shows five clusters corresponding to *M. hepatochrous* and the four outgroup taxa of *Psilocybe*. This hierarchical clustering further illustrated the close affinity between the collections within *M. hepatochrous* and indicated that they all belonged to the same species.

6.2.3 Mating compatibility studies

Nine isolates of CYS349 were paired in all possible combinations to determine the mating system. The results indicated a bipolar (or unifactorial) incompatibility system and two mating types (A = CYS34901, 03, 04, 05, 06, 09 & 13 and a = 08 & 11) were recovered from the polarity matrix. The results from the confrontation between isolates

from these two mating types and monokaryons from other collections showed compatibility in all isolates tested (Table 6.2). This indicated that mating in Tasmanian *M. hepatochrous* was under the control of a multiple allelic bipolar incompatibility system.

6.3 Discussion

It is established from the results of the three separate studies that all the Tasmanian fresh collections belong to a single species and they correspond well to the circumscription of *Melanotus hepatochrous* (Berk.) Sing.(Horak, 1977). This conclusion also applies to the two Rodway collections of *C. hepatochrous* and *C. cassiaecolor*. The latter in particular conforms better to *M. cassiaecolor* sensu Horak (=*M. hepatochrous*) than to *M. cassiaecolor* sensu Singer. The number of collections available for comparisons is small, hence the variation existing within this taxon may be underestimated. However, the results showed a certain degree of morphological variation in the Tasmanian collections.

There are no obvious variations in macrophology and the few variations noted could be attributed as responses to environmental factors. For example, the colour change in pileus from brownish to drying whitish is a response to the changes in humidity in the immediate surrounding of the carpophore. Though the genus is typified by the eccentric to laterally stipitate forms, centrally stipitate forms occur occasionally. Horak (1977) has attributed the position of stipe as a "direct response to microtopography at the point of attachment to substrate". This study shows that at least for *M. hepatochrous* the majority of the basidiomes are of the eccentric stipitate forms. Unlike other species in the genus, *M. hepatochrous* does not appear to be host specific since it is found growing on a variety of substrate.

Variation in micromorphology is more noticeable in the size and shape of spores and to a certain degree size of cheilocystidia within this taxon. The shape of spores in

Melanotus is generally ellipsoid. This study shows that the shape in face view is ellipsoid but there is a tendency towards elongate ellipsoid in profile.

Both the results of electrophoretic and mating incompatibility studies provided convincing support to the morphological finding. Laccases and peroxidases show considerable uniformity across the isolates. Isolates of this taxon produce more 'backrunners' in PE activities than isolates in the previous three genera. Despite the variations, in particular variations in band combinations, noted in acid phosphatases and pectic isozymes the resulting dendogram indicates clearly the close affinity of the Tasmanian collections. This is further supported by the successful mating between the isolates of different collections. Extracellular laccases, thus, appear to be effective in species delineation especially in preliminary sreening.

6.4 Taxonomy

Melanotus hepatochrous (Berk.) Singer in *Sydowia* 5; 472, 1951.

Basionym: *Agaricus* (*Crepidotus*) *hepatochrous* Berk. in Hook., *J. Bot.* 7: 574, 1848.

Synonyms: *Agaricus* (*Crepidotus*) *insidiosus* Berk. in Hook., *J. Bot.* 7: 574, 1848 – *M. insidiosus* (Berk.) Pegler in Aust. J. Bot. 13: 336, 1965.

Agaricus (*Crepidotus*) *cassiaecolor* Berk. in Hook., *Fl. Tasm.* 2: 246, 1860 – *M. cassiaecolor* (Berk.) Singer in Sydowia 15: 70, 1950.

Agaricus (*Crepidotus*) *turbidulus* Berk. apud Saccardo, *Syll. Fung.* 5: 889, 1887.

Crepidotus subhaustellaris Cleland, *Toadstools& Mushrooms and other larger fungi of South Australia*, Pt. I: 131, 1934.

Selected illustrations: Horak (1977), p.322, Figs. 61 - 76.

Illustrations: Figs. 6.12 - 19.

Pileus 9 - 28 mm. in diam., orbicular to reniform, scallop-shaped in profile, flabellate convex to plane, dull reddish brown (6F5, 6E7 to 7D7)) paling towards the periphery, drying whitish, hoary at first then appearing more glabrous, non-viscid. *Lamellae* adnate, greyish yellow (4D5) becoming browner (5D5 to 6E5) with spores, margin whitish, fimbriate. *Stipe* 2 -7 x 1.5 -3.5 mm., eccentric to lateral, also centrally inserted, attenuate towards base ending in a disc or attach directly to substrate, pallid or concolorous with pileus. *Context* thin, light orange (5B5).

Spores dark brown (7F6) in mass, (5.4-) 5.8 - 7.5 x 4.2 - 5 x 3.7 - 4.6 (-5) µm., ovate to elliptic, germ pore distinct. Basidia (15.8-) 17.5 - 22.5 x 5.4 - 8.3 µm., 4-spored. *Pleurocystidia* absent. *Cheilocystidia* (18.3-) 20 - 31.7 (-34.2) x 4.6 - 6.7 µm., lanceolate or fusoid with an elongate neck, generally simple, sometimes bifurcate, hyaline, thin-walled, numerous, forming a heteromorphous edge.

Subhymenium subcellular. *Trama* parallel tending to irregular, incrusted hyphae with brown pigments, 2.5 - 10 µm. broad. *Epicutis* interwoven repent hyphae, terminal cells often branched, heavily encrusted with yellow brown pigments, 3.3-6.7 µm. broad. Clamp connections present.

Habitat gregarious on twigs, rachis of manfern fronds, *Eucalyptus* log or dead tree stumps.

Material examined: See Appendix IIID.

Observations

This species is recognized by its thin-walled spores, lanceolate cheilocystidia and dull brown to reddish brown pileus. It is noted that some cheilocystidia extend some way up the gill face but decreasing in size. An additional observation is the circular disc at the base of stipe where the basidiome is attached to the substrate. Its occurrence is common enough to warrant a note of mention.

Table 6.1. Percentage occurrence of dominant bands in laccase, acid phosphatase and pectic enzymes from isolates of *Melanotus hepatochrous*.

Enzyme	Band No.	R_f	% occurrence
Lac	1	0.24	87.5
	2	0.27	81.25
AcP	2	0.05	25
	5	18.75	

Enzyme	Band No.	R_f	% occurrence
PG	1	0.03	79
	2	0.08	90
	3	0.12	81

Table 6.2. Results of pairing between the mating types of CYS349 and monokaryotic isolates of other collections of *M. hepatochrous*.

Isolate No.	No. of monokaryons	Isolate No.	No. of monokaryons	No.of pairings	Total no. of positive pairings	Total no. of negative pairings
CYS349 (03 & 11)	2	X CYS254 (01, 06 & 08)	3	6	6	0
		X CYS326 (01 & 07)	2	4	2	2
		X CYS367 (01 - 04)	4	8	6	2
		X CYS370 (01 - 04)	4	8	6	2
		X CYS425 (02 - 05)	4	8	8	0

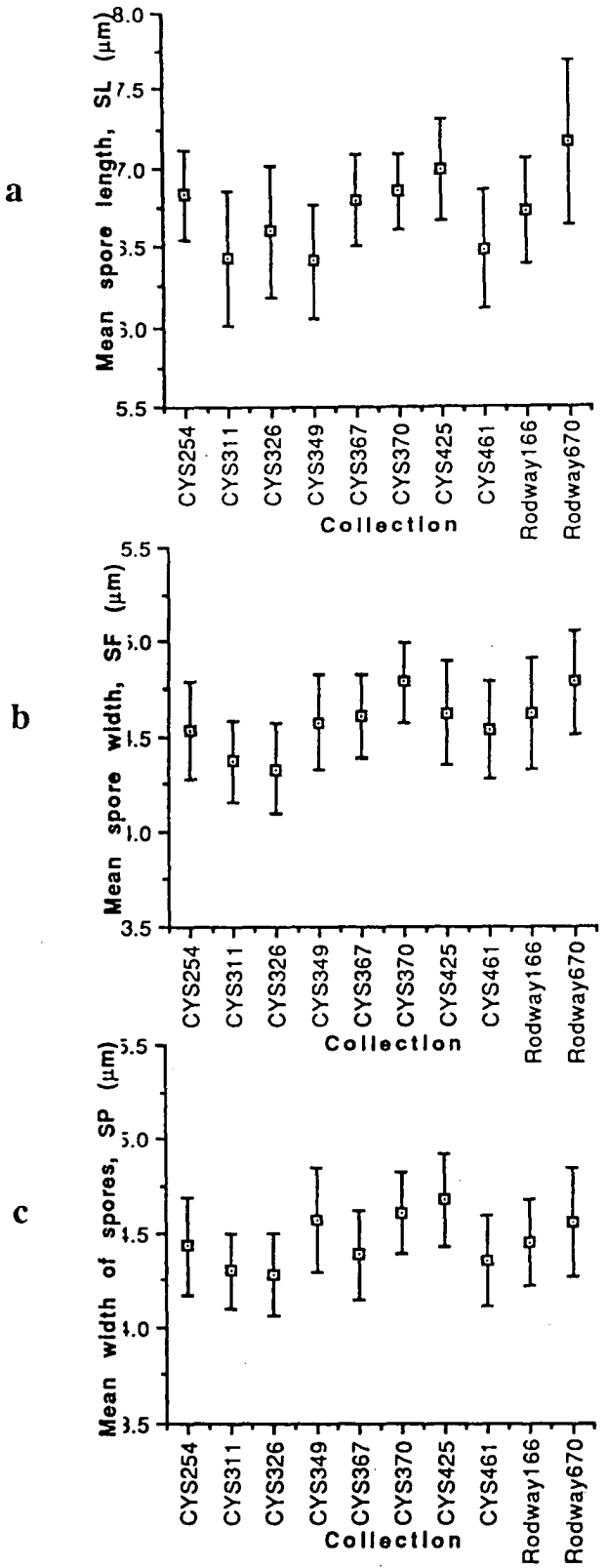


Fig. 6.1. Mean measurements of spore characters of Tasmanian material of *Melanotus hepatochrous*. a) length of spore, SL, b) width of spore in face view, SF, and c) width of spore in profile, SP. Standard deviations are shown as vertical bars.

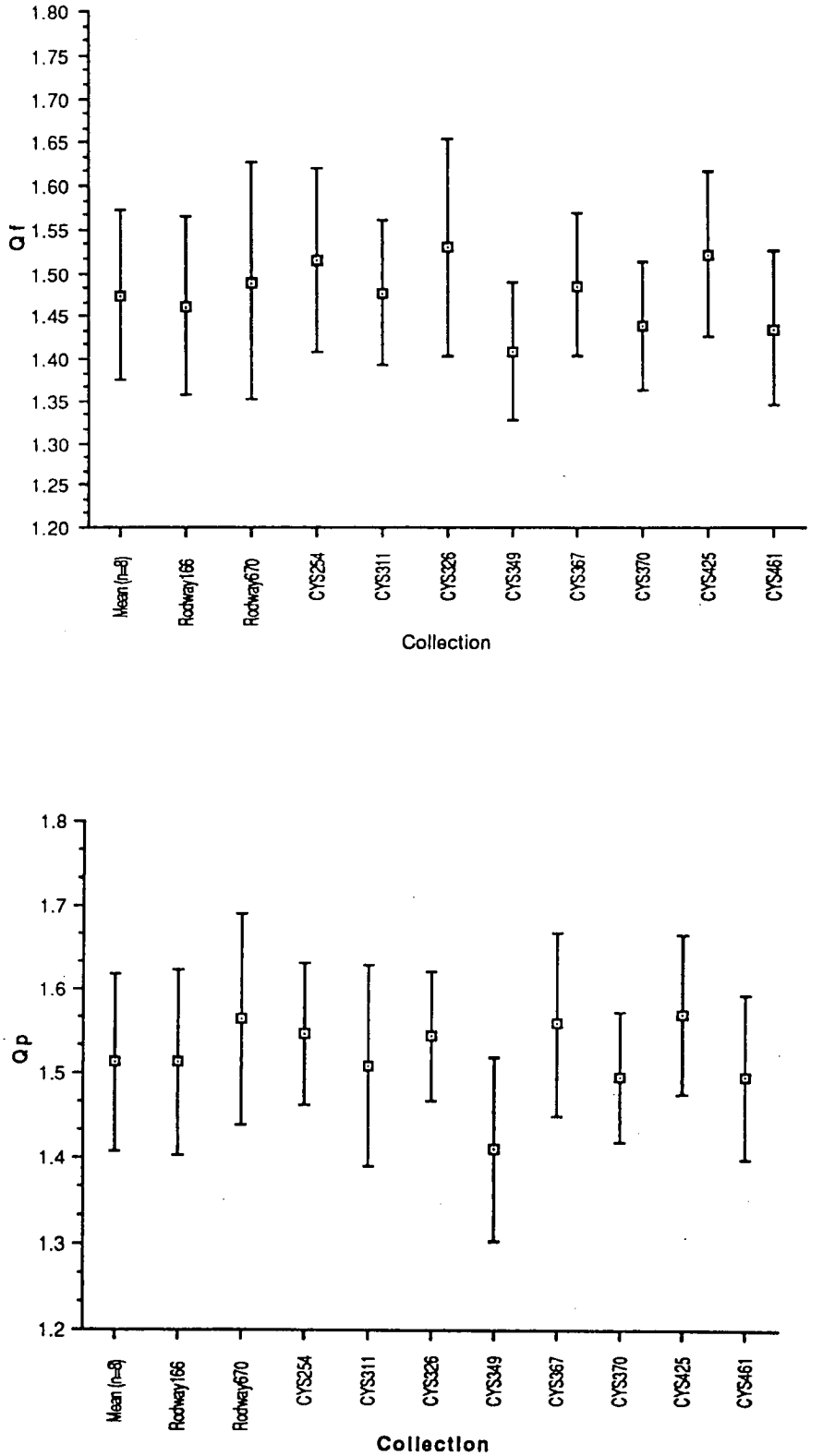


Fig. 6.2. Mean values of spore quotients of Tasmanian material of *Melanotus hepatochrous* where Q_f=SL/SF and Q_p=SL/SP.

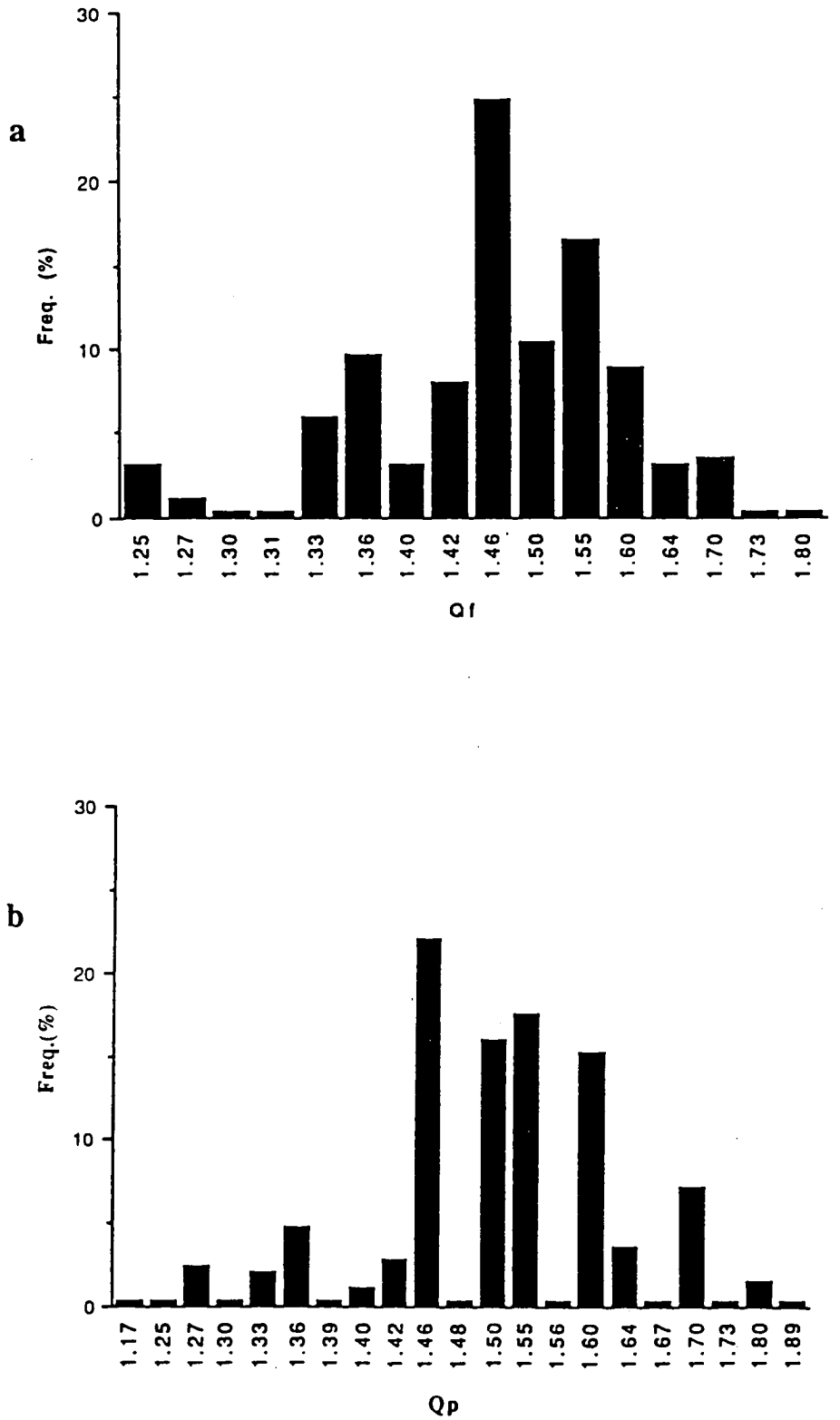


Fig. 6.3. Percentage distribution of the range of spore quotients across the Tasmanian material of *Melanotus hepatochrous*. a) Q_f and b) Q_p.

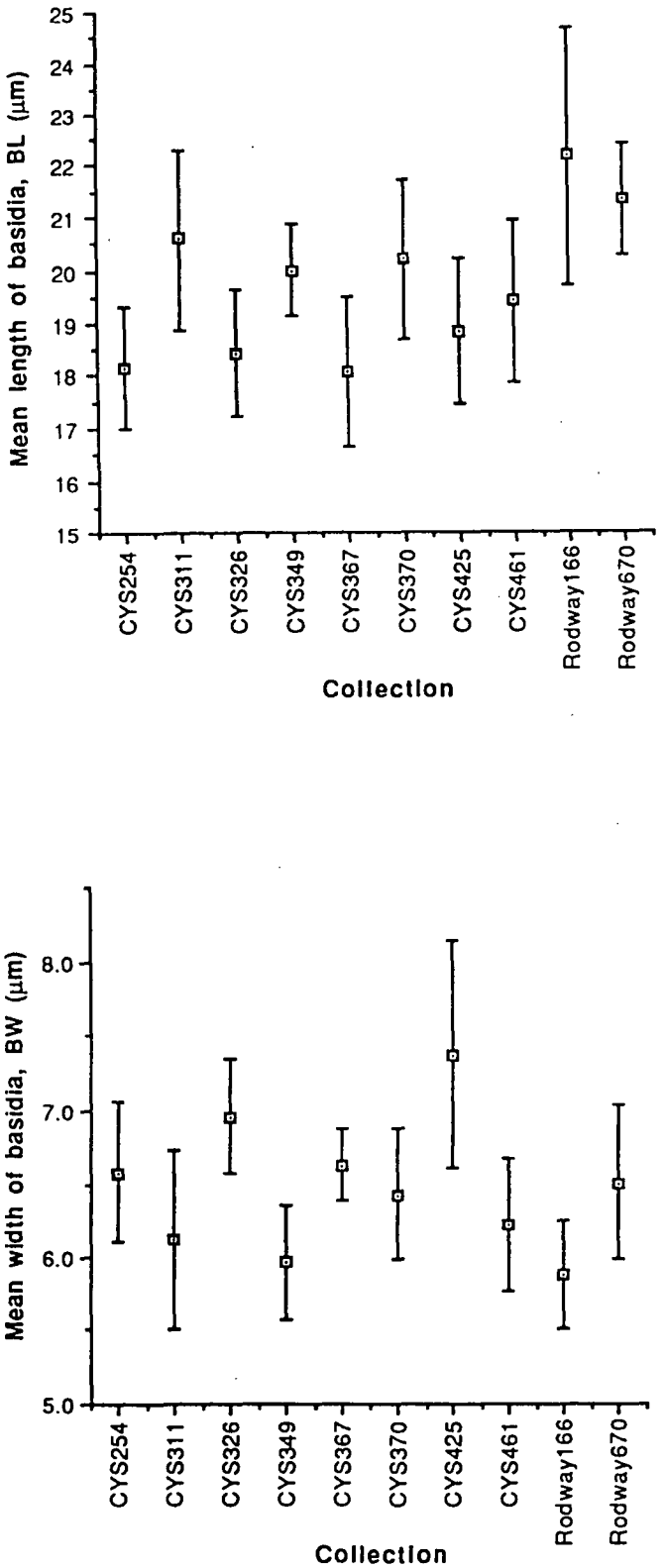
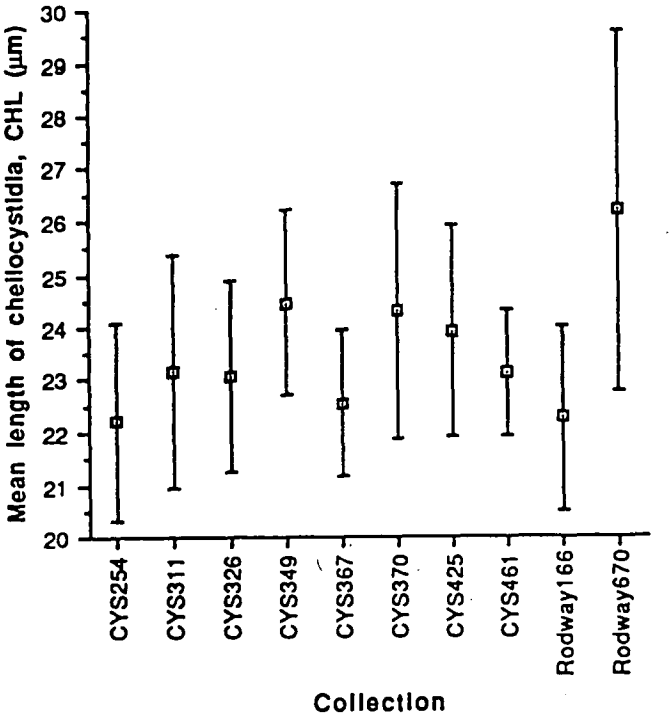


Fig. 6.4. Mean measurements of basidia of Tasmanian material of *Melanotus hepatochrous*, standard deviations are shown as vertical bars.

a



b

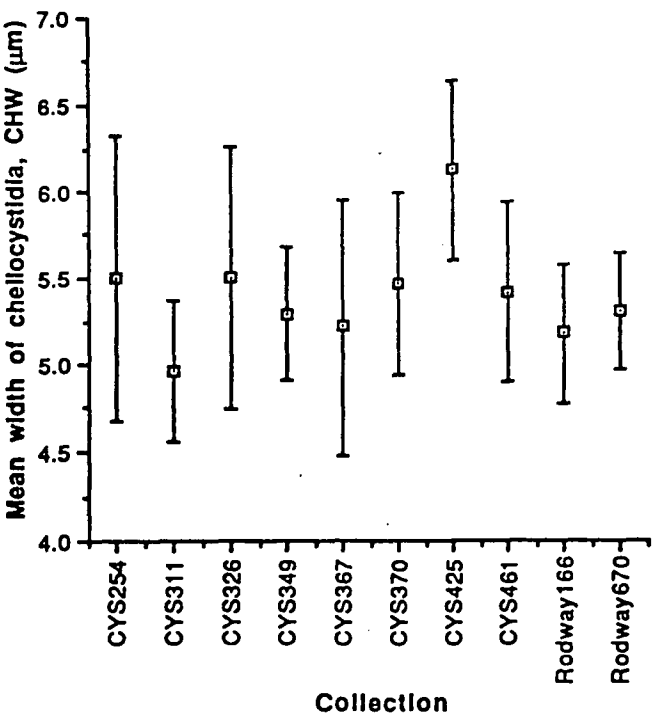


Fig. 6.5. Mean measurements of cheilocystidia of Tasmanian material of *Melanotus hepatochrous*, standard deviations are as vertical bars, a) CHL and b) CHW.

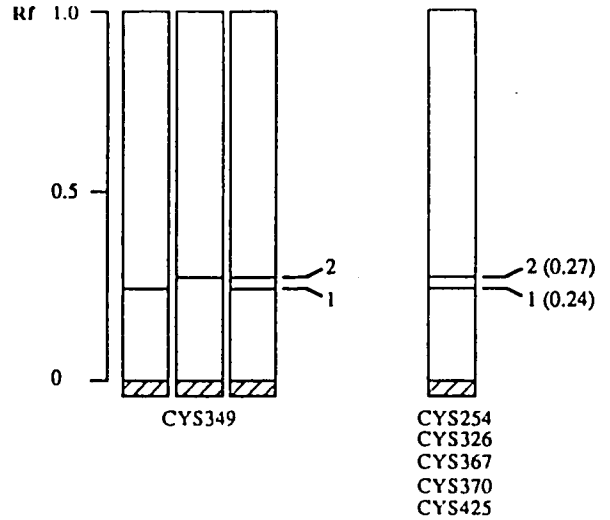


Fig. 6.6. Schematic representations of Lac zymograms of isolates of *Melanotus hepatochrous*. Band numbers start from the cathodic end. Rf values are given in parentheses.

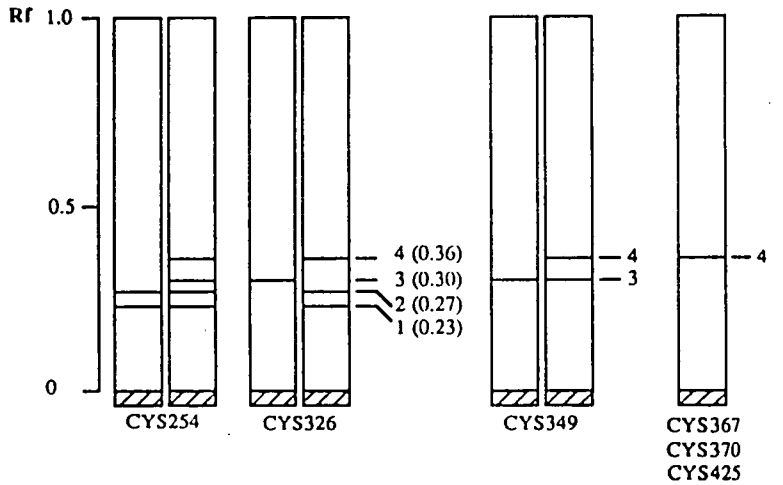


Fig. 6.7. Schematic representations of Per zymograms of isolates of *M. hepatochrous*. Band numbers start from the cathodic end and Rf values are given in parentheses.

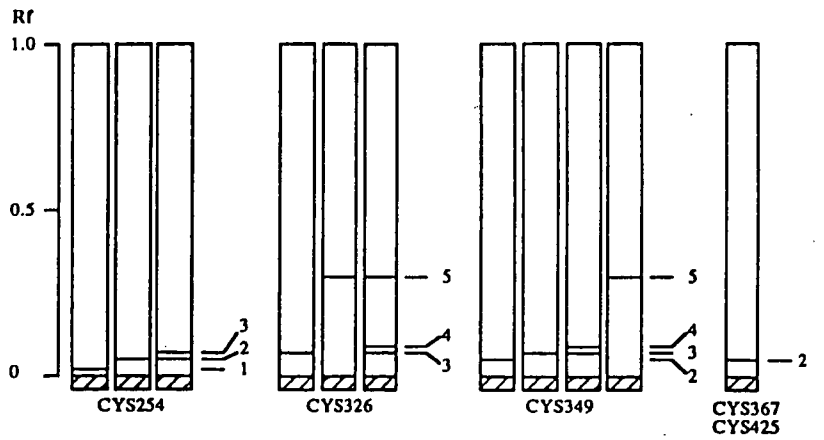


Fig. 6.8. Schematic representations of AcP zymograms of isolates of *M. hepatochrous*. Band numbers start from the cathodic end. Rf values: 1=0.02, 2=0.05, 3=0.07, 4=0.09 & 5=0.30.

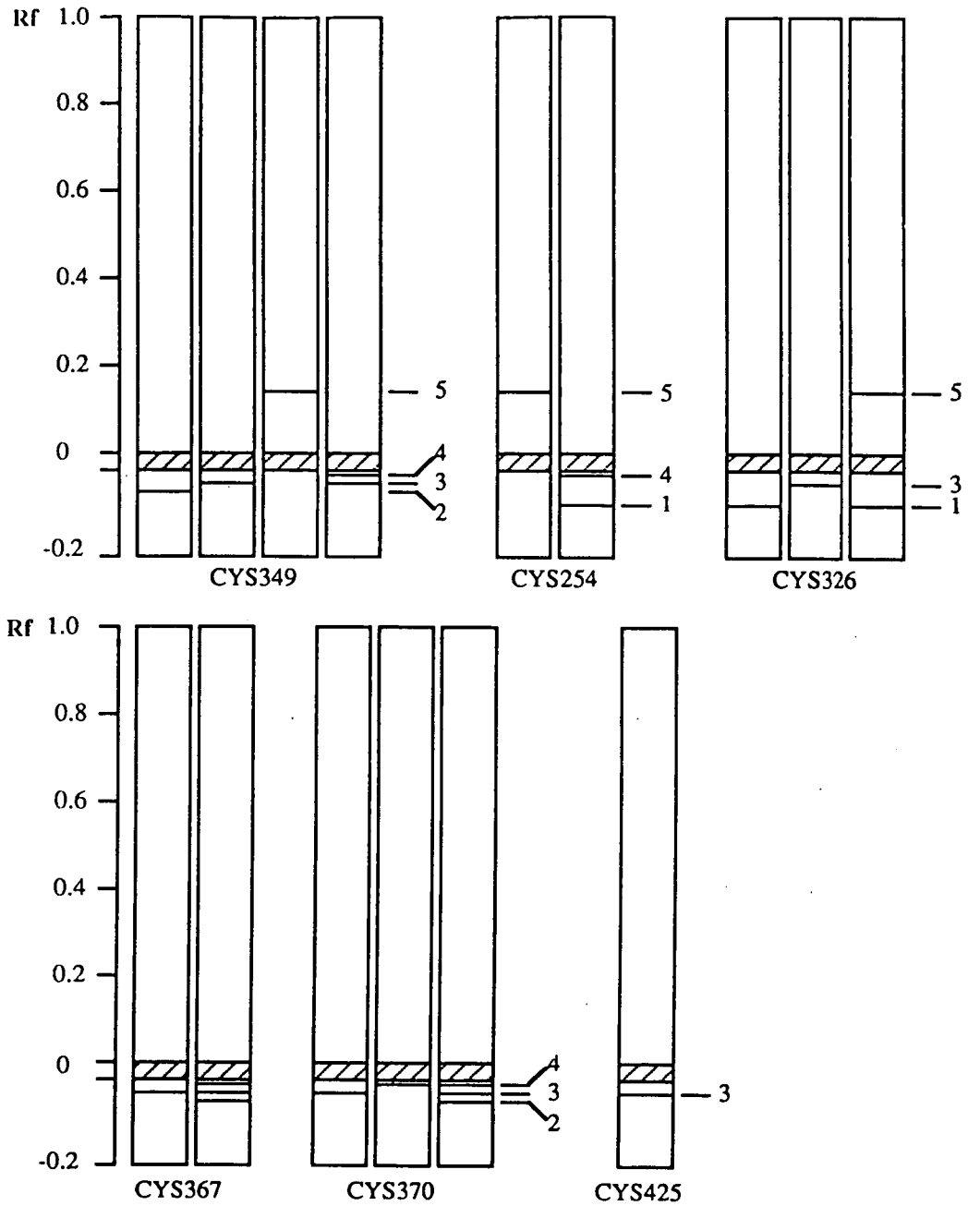


Fig. 6.9. Schematic representations of PE zymograms of isolates of *M. hepatochrous*. Band numbers start from the cathode end. R_f values: 1=-0.08, 2=0.05, 3=0.03, 4=0.01 & 5=0.14.

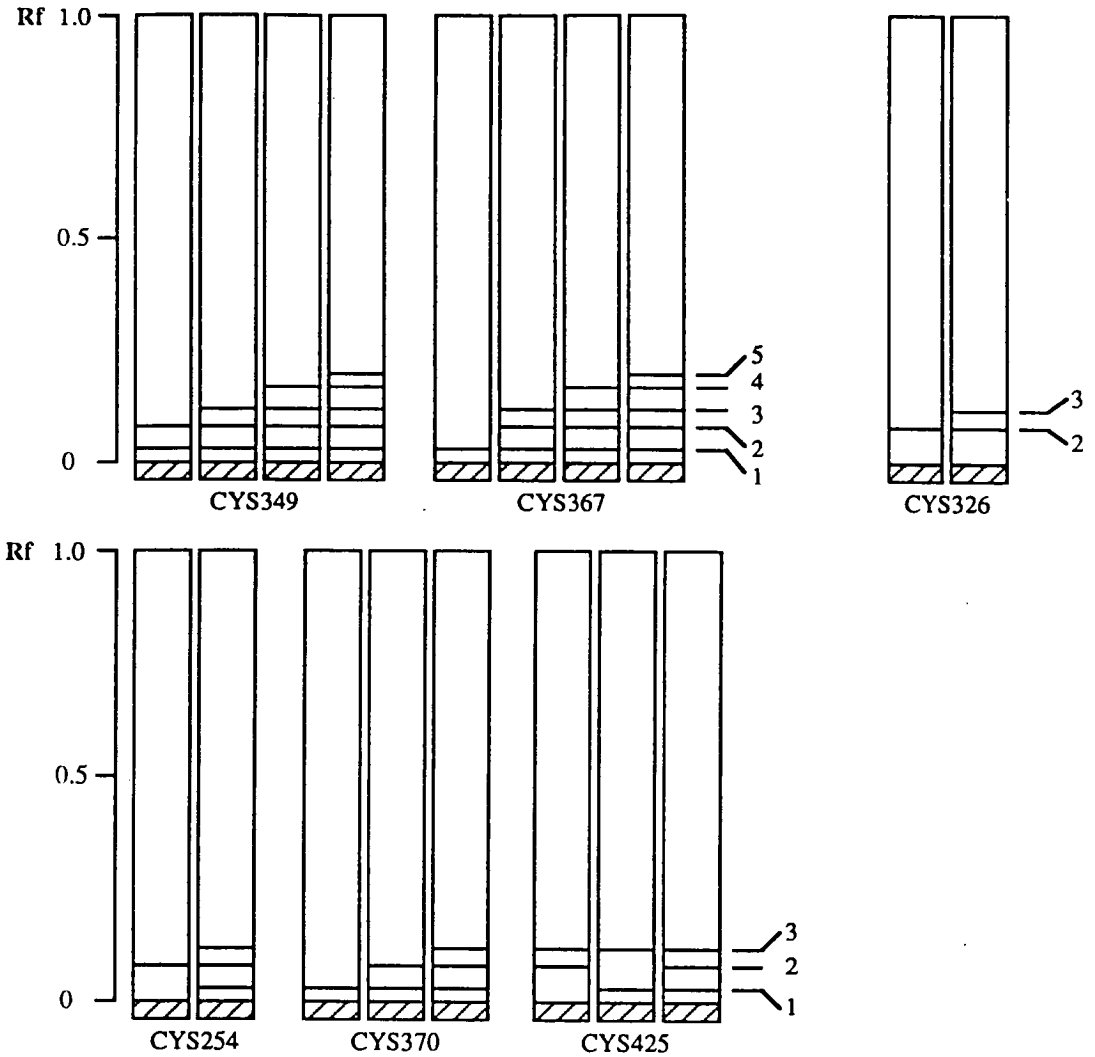


Fig. 6.10. Schematic representations of PG zymograms of isolates of *M. hepatochrous*. Band numbers start from the cathode end. R_f values: 1=0.03, 2=0.08, 3=0.12, 4=0.17 & 5=0.20.

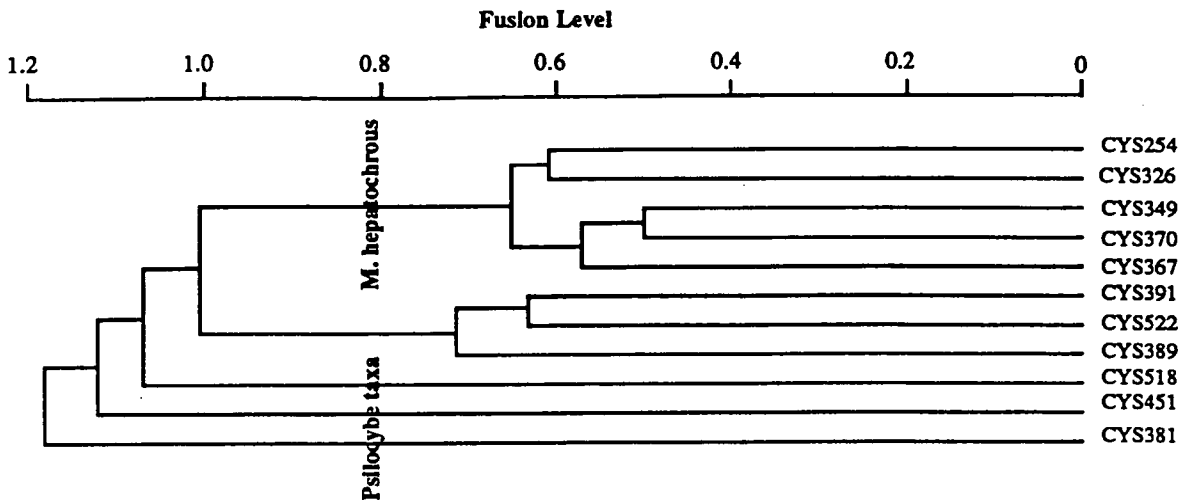
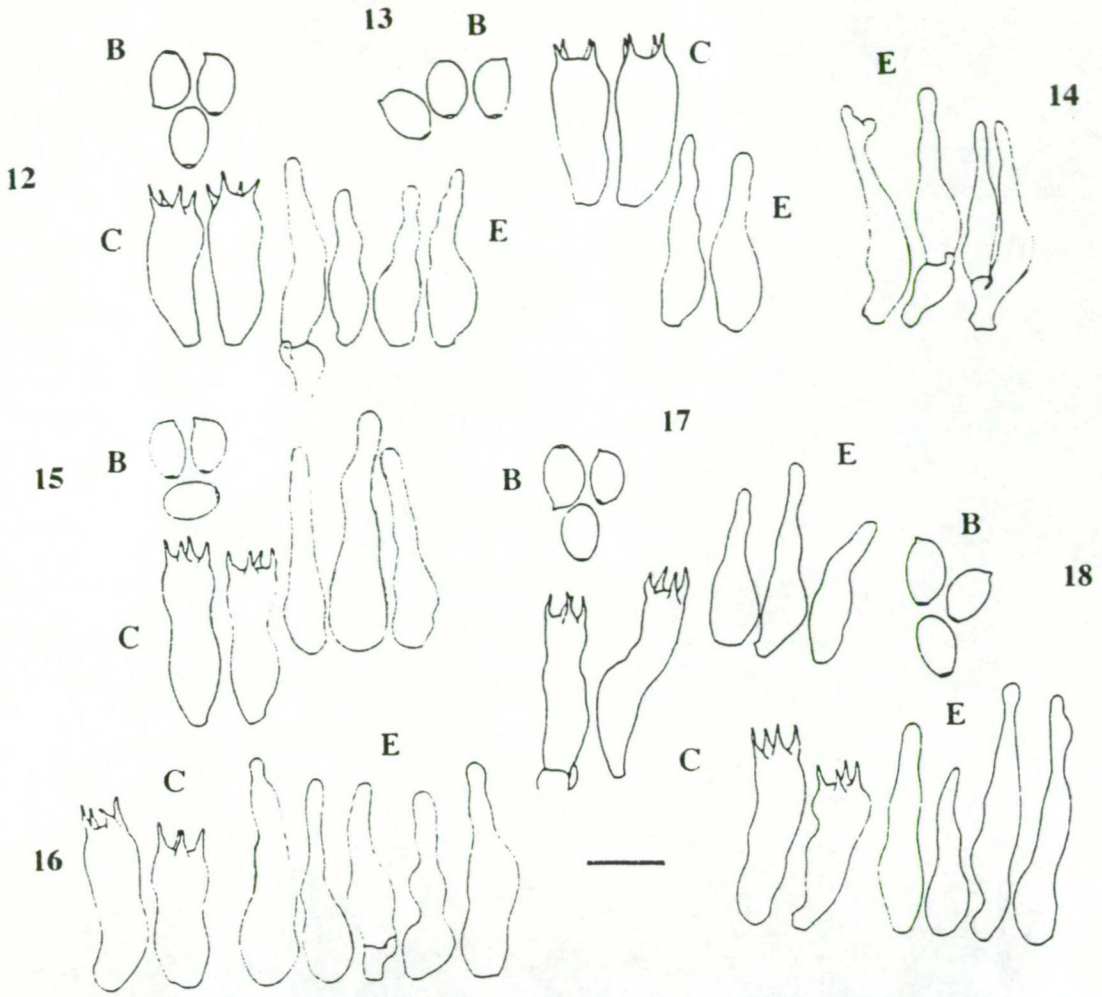


Fig. 6.11. Dendogram from UPGMA cluster analysis based on the band frequencies of all the enzymes examined for isolates of *Melanotus hepatochrous* with isolates of *Psilocybe* taxa included for comparison.



19

Figs. 6. 12 -19. *Melanotus hepatochrous*. B: spores, C: basidia, and E: cheilocystidia. 12. CYS254. 13. CYS326. 14. CYS370. 15. CYS349. 16. CYS425. 17. Rodway166. 18. Rodway670. 19. Habit of CYS461.

Chapter 7

Genus *Pholiota* (Fr.) Kummer

7.1. Introduction

Pholiota was originally established by Fries (1821) as a tribe of *Agaricus*. According to Fries, species with scaly pileus and stipe and a more or less persistent annulus were placed in this tribe. *Flammula* was another tribe erected by Fries to include taxa which were not obviously scaly and without a persistent annulus. Fries later acknowledged the close affinity between these tribes. Kummer was the first person to assign generic ranks to these two tribes (in Smith & Hesler 1968). The genus *Dryophila* was proposed by Quélet in 1886 to encompass both tribes but dividing them into the subgenera *Pholiota* and *Flammula*. Kühner and Romagnesi (1953) followed Quélet's concept in using the name *Dryophila*. Singer and Smith (1946) erected the genus *Kuehneromyces* to include species in the *Pholiota-Flammula* group that have broad germ pore and the spore appears more or less truncate. In the same paper, Singer & Smith combine the two genera *Pholiota* and *Flammula* into a single genus *Pholiota*. These authors expressed the opinion that the generic differences between *Pholiota* and *Flammula* were too insignificant to maintain each as a separate genus. This modern concept of *Pholiota* is accepted by mycologists such as Moser, Horak, Orton, Dennis and Pegler (as well as Australian mycologists in general).

The size of the pileus varies in *Pholiota* from species to species, can be from <1 cm. (e.g. *Pholiota pseudosiparia*) to >15 cm. (e.g. *P. aurivella*, *P. malicola*), the majority are in the 1-5 cm. range. Various colours predominantly in the yellow and brown series are noted in the pileus, in some there are also tints of red or grey. The shapes of the pileus vary from conical to convex, rarely hemispherical, to plano-convex at times umbonate to broadly umbonate, or even slightly depressed. The surface may be dry, moist, greasy, viscid or slimy viscid with a visible thin glutinous layer. It may be scaly,

either aggregates of fibrils or pronounced development of scales such as those in the subgenus *Pholiota*, fibrillose or glabrous. Attachment of lamellae generally adnate or adnate with a tooth, adnexed but seldom decurrent. Colour of the lamellae when young varies from whitish, pallid, pale yellow or brownish and becoming browner with spores. Lamellae vary from narrow to broad, close to subdistant. Stipe is central, often paler coloured above and darker near the base, surface may be glabrous, fibrillose or scaly, dry and rarely viscid, stuffed or hollow, with or without rhizomorphs attached at base, these may be whitish, yellowish or brownish. Context usually pallid or pale yellow or more often concolorous with the surface, thick or thin. The inner veil is generally cortinate or arachnoid and fugacious, in some more or less membranous forming a persistent superior or median annulus and at times evanescent. Remnants of the inner veil at times become appendiculate along the margin of the pileus. Evidence of the outer veil is in the form of its remnants as fibrils or scales on the surface of the pileus or stipe and it is virtually undeveloped in some species.

Spore deposits are predominantly of some shades of brown, basically dark brown. The spores are smooth, melleous brown both in water and in 5%KOH, ellipsoid to subovate in face view, inequilateral to bean-shaped in profile. Germ pore minute and obscure, indiscernible or just visible under oil immersion, evident to prominent and appearing slightly truncate. Basidia are generally 4-spored, more rarely 2-spored forms also occur, generally clavate or obovate. Pleurocystidia are present in most species either as projecting leptocystidia (as in subgenus *Flammuloides*) or as chrysocystidia (as in subgenus *Pholiota*) or both types may occur together (e.g. *P. schraderi*) or completely absent (e.g. subgenus *Flammula*). Cheilocystidia are almost always present forming a more or less sterile band in some while intermixing with basidia or basidioles in others. They may be similar in shape to pleurocystidia (if present) or different and generally smaller, very rarely with or as chrysocystidia. Caulocystidia absent or when present are either scattered or in tufts.

Subhymenium may be indistinct or a defined region of non- or gelatinised filamentous or subcellular hyphae with thick or thin walls, pigmented or not, incrusted or smooth. Gill trama is generally regular to somewhat interwoven, consisting of non- or gelatinised hyphae, thin- or thick-walled, pigmented or not. The cuticle often consists of repent hyphae or collapsed trichoderm, non-gelatinised or a thin surface layer of gelatinised hyphae, hyaline or pigmented, smooth or with incrusted walls, interwoven or radially arranged, usually resting on a distinct region of brownish hyphae known as the hypodermium or which is undifferentiated in some. Hyphae in these tissues often bear clamp connections.

Carpophores appearing solitary or scattered or gregarious or caespitose, terrestrial (on earth, humus, on ground among grass) or lignicolous (on dead or living trees), or on woody or leafy debris or among mosses (*Sphagnum* and *Polytrichum*), or on charcoal and burnt ground, from sheltered to exposed areas inside or outside woods or forests.

Smith & Hesler (1968) consider the colour and surface characters of pileus to be highly important. According to them, these characters are also useful in delineation at the infrageneric level. They also indicate that there are likely correlations between the pileus characters and the subhymenium. The colour of the lamellae in the early stages, especially before or when the veil breaks, is important as the true colour of the lamellae is obscured by the production of spores with time. The surface characters of the stipe are considered important at the infrageneric and even sectional level. They propose the use of the colour of spore deposit as a character for infrageneric differentiation pending on further studies using more standardized conditions for comparisons. The wall character of pleurocystidia has been used by Smith & Hesler (*loc. cit.*) as a sectional character in the subgenus *Flammuloides*. However, too much emphasis placed on small morphological differences has the danger of leading to unjustified splitting as

shown in the study of species in stirps *Adiposa* by Farr *et al.* (1977).

Smith & Hesler (1968) broaden the modern concept of *Pholiota* in their treatment of the North American species of *Pholiota.* However, their incorporation of *Kuehneromyces* (in subgenus *Hemipholiota*, Section Mutabiles) and some species (while excluding others) of *Phaeomarasmius* (in subgenus *Flavida*) into *Pholiota* appears unjustified according to Singer (1986). Singer (*loc. cit.*) argued that *Kuehneromyces* is separable from *Pholiota* and *Galerina* on EM derived characters such as the surface and wall features of spores. This view, Singer claims (1986), is supported by results from the work of Clemençon (1972) and Pegler & Young (1972) which include the most constant differences between *Kuehneromyces* and *Pholiota* such as the "Kuehneromyces-Kelch" and warty surface of the spores. Singer also maintains that the pileus and spore characters support a natural generic unit for the species in *Phaeomarasmius* and thus are separable from other genera in both Strophariaceae and Cortinariaceae. However, Smith & Hesler (1968) use the development of the pileus cuticle in an evolutionary context to query the status of *Phaeomarasmius* as a separate genus.

As a result of the differences between these two concepts of *Pholiota*, two sets of taxonomic treatment of the genus are in existence (Table 7.1). For the purpose of the present project, the treatment of Smith & Hesler is followed.

Of the 38 species included in tribus *Pholiota* and *Flammula* by Cooke (1898), only nine are retained in *Pholiota* based on the modern concept. From the early literature it is evident that not many taxa from Tasmania have been described or reported for this genus. In L. Rodway's fungal collections only *Pholiota mutabilis* (*Kuehneromyces mutabilis*) is included. Hongo & Mills (1988) reported *P. malicola* var. *malicola* after Hongo's collecting trip in Tasmania. Eygelsheim (1981) reported *P. spectabilis*, *P. squarrosa*, *P. recedens* and *P. praecox* from Tasmania. Of these, *P. spectabilis* is

now more suitably known as *Gymnopilus spectabilis* and *P. praecox* is transferred to *Agrocybe praecox* (Smith & Hesler 1968). For this part of the project, attempts have been made to collect the previously reported taxa as well as discovering taxa not previously reported.

7.2. Results

A total of nine putative species belonging to four subgenera were delineated based on morphological characters. The four subgenera thus delineated were *Phaeonaematoloma*, *Flammula*, *Pholiota* and *Flammuloides*. Two putative species each were delineated for subgenera *Phaeonaematoloma* and *Pholiota*; one for subgenus *Flammula* and five for subgenus *Flammuloides*. For convenience, the results are discussed according to subgenera in each of the study.

As a result of some groups being morphologically more distinct than others, only morphological and electrophoretic studies were carried out such as in the case of *P. malicola*, while still others whose spores failed to germinate, wild isolates were obtained from various parts of the carpophores whenever possible to use in electrophoretic study in addition to morphological comparisons. In taxa which showed variations in morphology, all three approaches (morphology, electrophoresis and mating) were employed.

7.2.1. Morphological studies

7.2.1.1. Subgenus *Phaeonaematoloma*

Two putative species were identified within subgenus *Phaeonaematoloma* as defined by both Singer (1986) and Smith & Hesler (1968). Neither fitted any described species of this subgenus and were referred to as *Pholiota* sp. A and sp. B. Both taxa were exannulate forms. There were obvious distinctions between the two taxa in both macro- and micro-morphological characters.

Table 7.2 gives a summary of the major macrocharacters of the two taxa. Specimens of *Pholiota* sp. A (CYS284 & CYS509) possessed both slimy viscid pileus and stipe when moist. *Pholiota* sp. B had slimy viscid pileus but the stipe was dry and flocculose to sometimes appearing more or less glabrous. The two taxa differed in the colour of pileus and the general habit of the carpophore. *Pholiota* sp. A was generally delicate in appearance. *Pholiota* sp. B on the other hand varied from slight to robust. Distinct differences were noted in the surface features of the stipe. The stipe of *Pholiota* sp. A was glabrous, slimy viscid with an obvious layer of gluten covering the lower two thirds. The typical form of *Pholiota* sp. B was observed to have a dry, white flocculose stipe. However, the floccose surface of stipe was easily abraded thus appearing more or less glabrous.

Table 7.3 gives a summary of the microcharacters of *Pholiota* sp. A and sp. B. The main difference between the two taxa at this level was the presence of leptocystidia in addition to chrysocystidia in *Pholiota* sp. A. Another notable difference was in the larger mean size of cheilocystidia in *Pholiota* sp. B. Other characters, such as the mean measurements of spores suggested slightly smaller and narrower spores in *Pholiota* sp. A otherwise microcharacters of both taxa showed similar size range.

7.2.1.2. Subgenus *Flammula*

Only a single taxon corresponding to *P. malicola* was identified from the Tasmanian collections.

Table 7.4 gives a summary of the observed macroscopic characters of specimens in collections of *P. malicola* from Tasmania. It shows variations in the colour of pileus. The colour of the pileus ranged from pale yellow, waxy yellow to orange brown. The pileus varied from dry to greasy and hygrophanous. The stipe was stuffed at first then

becoming hollow with age. There was no properly formed annulus and only a velar zone was visible.

Table 7.5 gives a summary of the major microcharacters of specimens from Tasmania. It appeared that the spore characters were relatively consistent across the collections. The variations noted in spore size were within the circumscribed size range of *P. malicola* sensu Smith & Hesler (1968) and *P. alnicola* sensu Jacobsson (1986). Occasionally 2-spored basidia were noted in the present study with a single collection (CYS357) which had mainly 2-spored basidia. Cheilocystidia were abundant and generally forming a more or less sterile band. The size range and shape of cheilocystidia were also consistent within the circumscription of this taxon.

7.2.1.3. Subgenus *Pholiota*

Two taxa in this subgenus were delineated based on morphological criteria. As a result of the strongly viscid to glutinous pileus and scaly stipe, both taxa were placed in section Adiposae with one corresponding to *P. aurivella*(Fr.) Kummer and the other to *P. squarrosipes*Clel..

7.2.1.3.1. *P. aurivella* (Fr.) Kummer

Table 7.6 gives a summary of the major macrocharacters of the collections of *P. aurivella* from SE Tasmania. Variations were noted in the colour and surface features of the pileus. The pileus of all specimens were glutinous, the layer of gluten was particularly noticeable when collected wet. The glutinous surface was apparent in young carpophores. The colour of the pileus ranged from bright lemon yellow to golden orange brown. Tawny brown scales of various arrangement were present in most specimens, they were easily abraded giving a few a more or less glabrous appearance.

Table 7.7 gives a summary of the microcharacters of the Tasmanian collections of *P.*

aurivella. It shows variations in the mean size of spores and cystidia. Table 7.8 shows comparisons of the Tasmanian material with herbarium and published data of *P. aurivella* and closely related species in the same stirps. It shows an apparent overlap in the size range of spores between the Tasmanian and North American specimens, with *P. limonella* and *P. albietis* having narrower spores. Slight variations in veil colours were noted, from pallid to yellow. An obvious difference between them was the host plants. The Tasmanian representatives of *P. aurivella* were found on sassafras (*Atherosperma moschatum*) and infrequently myrtle-beech (*Nothofagus cunninghamii*). The North American representatives were found on hardwood (e.g. alder, elm, etc.) and conifers.

7.2.1.3.2. *P. squarrosipes* Clel.

The Tasmanian specimens corresponded to *P. squarrosipes* Clel. with extensions to the described range of both macroscopic and microscopic characters. Syntype collections of this taxon had been included for comparison.

Variations were noted in the colour of pileus, surface features of pileus and stipe features in the Tasmanian specimens (Table 7.9). The colour of pileus of most specimens was ochre or cinnamon or deep cinnamon brown. However, one collection (CYS458) showed more yellow in the pileus than majority of the specimens . Comparison with syntype material showed that some variations noted in the Tasmanian material were also noted in the syntypes by Cleland (Table7.10).

Wide variations in microcharacters were noted from the Tasmanian collections, in particular the shape and size of chrysocystidia. Three forms had been observed in the Tasmanian collections and were referred to from hereon as normal (mucronate, ventricose form), long-snouted form (ventricose form with a prominent apical protuberance >10 µm. long) and long slender based form (Fig.7.1). All three forms

were observed in the Tasmanian collections with the majority having the normal form and only occasional occurrence of the latter two forms. However, specimens of one collection (CYS458) possessed consistently long-snouted forms with the apical protuberance >10 µm. long (Fig. 7.1). Another collection (CYS424) possessed consistently long slender based forms (Fig. 7.1). In the case of CYS424, cheilocystidia and basidia appeared similarly slender in shape (Fig. 7.1). In almost all the syntype material, chrysocystidia were of the normal form with a prominent apical protuberance, the long snouted forms were present but rare. Variations in other microcharacters are given in Appendix V.

The habitat range for this taxon is very wide, from general ground litter of woody or leafy debris, amongst grass or mosses in relatively sheltered areas to bare, clayey or rocky ground and fairly exposed. Such a habitat range seemed to coincide with that of the syntypes (Table 7.10). It fruited early in the season from April through to July and appeared to be wide-spread in Tasmania with specimens collected from the north west, north east, south west, central and south east. Growth habit ranged from caespitose to gregarious or scattered. Its occurrence appeared to be associated with slight disturbance.

Canonical discriminant analysis (CDA) of microcharacters

Pholiota sp. B was included in CDA with *P. squarrosipes* for comparison as an outgroup species. The results of CDA shows that 97.4% of the total variation in spores is due to SCV1 (first canonical variate generated from spore variables), i.e. the spore length. Only 2.4% of the total variation is attributed to SCV2 (second canonical variate generated from spore variables), i.e. facial width of spores. Fig.7.2 shows the scatter plot based on the first two mean canonical variates generated from the spore variables (MSCV1 & 2). Two clusters are evident, one corresponds to *Pholiota* sp. B and the other to *P. squarrosipes* comprising both the syntypes and Tasmanian specimens.

They are clearly resolved along the axis of MSCV1. This part of the analysis shows that the two taxa are clearly separable on spore characters in particular the spore length and that the Tasmanian collections and the South Australian syntypes form a single group.

The second part of CDA is based on cystidia variables. The results show that 54.6% of the total variation in cystidia variables is due to CV1 (first canonical variate generated from the cystidia variables) and 24.5% to CV2 (second canonical variate generated from the cystidia variables). Fig. 7.3 shows the scatter plot based on the first two mean canonical variates generated from the cystidia variables (MCV1 &2). Two evident clusters are resolved along the axis of MCV1, i.e. length of cheilocystidia. The variation along the axis of MCV2 is resulted mainly from the contrast between the length and width of chrysocystidia. These two taxa are again shown to be separable on cystidia characters. Variation in cystidia characters of specimens of *Pholiota* sp. B was noted to be due more to the variation in length of cheilocystidia than to the contrast between length and width of chrysocystidia. However, variation in cystidia characters in specimens of *P. squarrosipes* (both Tasmanian and syntype material) was noted to be more as a result of the contrast between length and width of chrysocystidia than to the length of cheilocystidia.

Fig. 7.4 shows a dendogram resulted from UPGMA cluster analysis using all the mean canonical variates from CDA. Again two clusters are evident corresponding to *Pholiota* sp. B (B) and *P. squarrosipes* (A & T). This dendogram further exemplifies the close affinity between the syntypes and Tasmanian specimens.

7.2.1.4. Subgenus *Flammuloides*

Morphological examination resulted in the separation of five putative taxa in subgenus *Flammuloides*. Tables 7.11 summarizes the macro-characters of these five taxa

respectively. Species in section *Flammuloides* were characterized by the thick-walled pleurocystidia and only CYS257 met this criterion in the sense of Smith & Hesler (1968). This collection was referred to as *Pholiota* sp. C. However, specimens of 16 other collections which were very similar to *Pholiota* sp. C in most respects of macrocharacters but possessed both thin- and thick-walled pleurocystidia were grouped together and were referred to as *Pholiota* sp. D. As a result of the inconsistency in wall thickness of the pleurocystidia, sp. D was tentatively placed in section *Flammuloides*. *Pholiota* sp. D did not fit any published description of *Pholiota*. It was distinct from *P. multicingulata* in the more delicate stature as well as the paler colour of the pileus. Another outstanding feature of this taxon was that the stipe was always pallidly coloured initially and at the base of which were the conspicuous yellow rhizomorphs (Table 7.11). These rhizomorphs were also evident in the field when the fungus was still attached to the woody substrate (e.g. rotten log).

P. highlandensis was separated from the rest by its carbon-rich (burnt) habitat and to a certain extent the coloured veil. This specialised habitat placed it in section Carbonicola. All the specimens of collections identified as *P. highlandensis* were collected in two fruiting seasons from a single locality where the ground was burnt recently (within the last 12 months of the first visit). Various attempts to collect this taxon from similar habitats had failed. The colour of the pileus varied from cinnamon brown to dark brown in most specimens. Variations were noted in the veil characters. Certain populations appeared to have better developed veil features than others. Basically in such populations, the remnants of the russet-coloured veil was evident in the form of a russet or ferruginous brown velar zone on the stipe and/or with scattered fuzzy patches along the margin of the pileus. In others there was no visible trace of the veil at all. It suggested inconsistency in the veil features. The colour of the veil appeared to be influenced by environmental factors as well. The russet or ferruginous brown colour was not evident in young carpophores in the field and appeared to be achieved with maturity.

The remaining collections belonging to this subgenus appeared to be most suitably placed in section Spumosae. Two taxa were delineated in section Spumosae. One corresponded to *P. multicingulata* Horak, with CYS524 as a collection positively identified by Dr. Horak during his visit in Tasmania. It was noted that *P. multicingulata* was a variable species both in macro- and micro-morphology. The multizonate stipe was described as the diagnostic character for *P. multicingulata.* However, the multizonate stipe was not always evident. In many cases, this feature was only observed in young carpophores. Variations were also noted in the pileus. Firstly, the colour exhibited various shades of brown and secondly, the surface features varied from virgate (with brown steaks), concentric rings of broad appressed scales to more or less glabrous. The other taxon consisted of a single collection morphologically similar to *P. multicingulata* but with glutinous veil. This was the main character that separated it from *P. multicingulata.* It occupied similar habitat to *P. multicingulata* and was referred to as *Pholiota* sp. E (CYS266).

Table 7.12 gives a summary of the micro-characters of the five taxa in subgenus *Flammuloides. P. highlandensis* appeared to have smaller and narrower spores whereas *Pholiota* sp. E had slightly wider spores. Spores of *Pholiota* sp. C besides being longer also appeared to be more elongate in profile than the other taxa. Wall thickness of pleurocystidia appeared a variable character in specimens of both *P. multicingulata* and *Pholiota* sp. D. Horak (1983) did not emphasised the wall thickness of pleurocystidia of *P. multicingulata.* In the Tasmanian specimens, the majority had thin-walled pleurocystidia but in some both thin- and thick-walled pleurocystidia were observed on the same basidiome. The wall thickness in such cases seldom exceeded 0.8 µm. Table 7.12 also shows variations in the contents of pleurocystidia across the five taxa. Similar variations were also noted in the comparison of *P. multicingulata* with *P. austrospumosa* and *P. spumosa* (Table 7.13).

The three Tasmanian taxa of *P. highlandensis*, *P. multicingulata* and *Pholiota* sp. D in the subgenus *Flammuloides* were included in CDA as well as herbarium specimens belonging to this subgenus using spore and cystidia variables. The analysis indicated that 93.7% of the total variation was due to the first canonical variate (SCV1), i.e. the facial width of spores. Only 5.9% of the total variation was attributed to the second canonical variate (SCV2), i.e. spore length. Fig. 7.5 shows the scatter plot using the first two mean SCV as axes. It shows two loose clusters where each contains approximately two subclusters. These clusters were resolved along the first MSCV axis. There is a slight overlap in the spore characters between *Pholiota* sp. D (A) and *P. multicingulata* (B). These two taxa together form a loose cluster with three herbarium specimens, *P. austrospumosa*(D), *P. iterata* (G) and *P. stratosa* (H), on the outer fringes of this group. The second loose cluster consists mainly of the two taxa of *P. highlandensis* (K) and *P. spumosa* (C) (herbarium material only) as well as two other herbarium specimens, *P. piceina* (F) and *P. bakerensis* (E). All taxa showed very similar spore length but were separated on facial width of spores.

In the second part of the analysis using cystidia variables, 65.7% of the total variation was attributed to the width of cheilocystidia and only 19.8% was due to length of pleurocystidia. Fig. 7.6 shows the scatter plot based on mean CV generated from the CDA of cystidia variables. Approximately three clusters were resolved along the axis of MCV1 with varying degrees of overlap. They corresponded approximately to *P. highlandensis*, *P. multicingulata* and *Pholiota* sp. D. The remaining taxa are scattered among these three clusters.

Fig. 7.7 shows the dendogram derived from the cluster analysis using all the mean CVs generated from the CDA. At the two cluster level, the two clusters more or less corresponded to the two loose clusters (Cluster 1 & II) resolved using the spore

variables. At the two subcluster level, Cluster Ia included all the collections of *P. highlandensis* including the type collection (T) and Cluster Ib consisted of the three collections of *P. spumosa* and two smaller taxa (Smith73446, *P. piceina* & Smith16727, holotype of *P. bakerensis*) showing affinity to *P. spumosa* in terms of spore and cystidia characters. The relationships between the components of Cluster II appeared more complicated. At the similar subcluster level to Cluster 1, three subclusters were noted. They roughly corresponded to IIa (mainly of collections of *P. multicingulata*, plus Smith9318, holotype of *P. iterata* and T228300, holotype of *P. austrospumosa*), IIb (mainly of collections of *Pholiota* sp. D & several collections of *P. multicingulata*) and IIc (a single collection of Smith64684, holotype of *P. stratosa*). As a result of the overlap observed in the cystidia variables, collections of *P. multicingulata* and *Pholiota* sp. D were intermixed in the dendogram. The Tasmanian collections of *P. highlandensis* appeared as a relatively homogeneous taxon in terms of both spore and cystidia characters when compared with the other taxa in the same subgenus.

7.2.2. Electrophoretic studies

7.2.2.1. Subgenus *Flammula*

Lac (Laccase)

Four bands were scored across all the isolates. The various combinations of these four bands indicated the intercollection variability (Fig. 7.8). Band 2 at R_f 0.34 was the dominant band occurring in 100% of the isolates. The remaining three bands occurred only occasionally with a low occurrence of <8%.

Per (Peroxidase)

As in the case of Lac isozymes, only four bands of Per activities were scored across all the isolates. Table 7.14 shows that Bands 2 and 3 at R_f 0.37 and 0.39 respectively occur in 33.3 and 51.8% of the isolates respectively. Band 3 appeared to be the common band noted in various combinations with the remaining bands across the

isolates (Fig. 7.9). The combination of Bands 1, 2 and 3 suggested greater affinity between the four collections of CYS334, 335, 355 and 383 than with CYS357.

AcP (Acid Phosphatase)

Activity of acid phosphatase was not detected in any of the isolates of *P. malicola* nor those of other taxa of *Pholiota* in general.

PE (Pectinesterase) & PG (Polygalacturonase)

For PE activities, four bands were scored across all the isolates. Bands 1 and 3 at R_f 0.04 and 0.27 respectively were noted to occur in 51.8 and 66.7% of the isolates (Table 7.14). Band 1 was conspicuously absent from the isolates of CYS334 (Fig. 7.10) and Band 2 was not detected in the available isolates of CYS335, 357 and 383.

A total of six bands were scored across the isolates for PG activities (Fig 7.11). Of these, Band 2 at R_f 0.14 occurred in 63% of the isolates (Table 7.14). Comparison of the occurrence of this band between collections showed that it had a lower occurrence in isolates of CYS334. Two other less dominant bands at R_f 0.04 and 0.47 respectively recorded 40.7 and 51.8% (Table 7.14). Band 1 at R_f 0.04 was detected in all the isolates of CYS334 and its occurrence was very low in isolates of other collections. Similar observation was noted for Band 6 at R_f 0.27.

UPGMA cluster analysis using band frequency was performed by including an outgroup species, *P. aurivella*. The results are presented as a dendogram in Fig. 7.12. It shows two distinct clusters corresponding to *P. malicola* and *P. aurivella* respectively.

7.2.2.2. Subgenus *Pholiota*

7.2.2.2.1. *P. aurivella*

Lac

A total of 11 bands were detected across the isolates of *P. aurivella*. The dominant bands were Bands 10, 2, 5 and 4 occurring in 75, 62.5, 45.8 and 41.7 % of isolates in that order (Table 7.15). The remaining bands probably represented both intra- and inter-collection variation. Fig. 7.13 shows a schematic representation of the laccase zymogram pattern noted in all the isolates of *P. aurivella*. Bands 1, 6 and 8 were only observed in two separate isolates with Bands 6 and 8 noted in the same isolate. As a result, these bands were considered to be inconsistent and were excluded from the cluster analysis.

Per

Activities of Per isozymes were noted to be less vigorous than Lac isozymes. Only two bands, Bands 1 & 2, at R_f 0.51 and 0.56 respectively were detected in all the isolates of this group with low percentage occurrence of 25 and 29.2% respectively. Fig 7.14 shows the schematic diagram of Per activities scored in the isolates of this group.

PE & PG

Intercollection variability was noted in PE activities. Though a total of seven bands were scored consistently across the isolates, the % occurrence of individual band activities was relative low compared to the previous two enzyme systems. Only four bands, Bands 2 (R_f 0.17), 5 (R_f 0.45), 6 (R_f 0.47) and 7 (R_f o.48) occurred in >25% of the isolates. Variations in banding patterns were largely expressed as different combinations of these four bands (i.e. Bands 2, 5, 6, & 7), plus the occasional addition of other bands (Fig.7.15). It was noted that the isolates of CYS116 and 128 produced fewer detectable zones of PE activities than the other isolates.

A lower degree of variability was noted in the PG activities with a total of four bands scored across the isolates (Fig. 7.16). Of these, Bands 3, 1 and 2 were noted to occur in 100, 66.7 and 50% of the isolates respectively (Table 7.15). Variations in banding patterns mainly involved the various combinations of these three bands in addition to Band 4.

7.2.2.2.2 *P. squarrosipes*

Lac

For Lac activities, a total of 12 bands were scored consistently across all the isolates of collections of *P. squarrosipes*. Two dominant bands were noted in majority of the isolates. Band10 (R_f=0.59) scored in isolates of 11 out of 12 collections (Fig. 7.17) included in this study was the most dominant band occurring in 74.7% of the isolates (Table 7.16). The second dominant band was Band 2 (R_f=0.23) scored in isolates of 10 out of 12 collections (Fig 7.17) totalling 51.7% of the isolates (Table 7.16). Four other bands of lesser dominance were Bands 11 (44.8%), 9 (35.6%), 7 (33.3%) and 4 (29.9%) in descending order. Intercollection variations were noted in the combination of the 12 bands and combination of bands appeared to generally involve the above mentioned six bands in addition to the less dominant bands.

Per

A total of 15 bands corresponding to the various regions of Per activities were scored consistently across all the isolates of *P. squarrosipes*. Due to the great number of bands detected, no allelic designation was assigned. As noted in previous electrophoretic studies involving isolates of other genera of the family, Per and Lac bands, showing similar mobility, were detected again in the presence of hydrogen peroxide. In this case, Bands 6 (R_f=0.32), 8 (R_f=0.36)and 15 (R_f=0.63) were previously detected in the absence of hydrogen peroxide when staining for Lac activities (Fig. 7.18). These bands except Band 8 were excluded from UPGMA average linkage cluster analysis. The inclusion of Band 8 of Per isozymes and exclusion of Band 5 (of

the same R_f) of Lac isozymes was the detection of this band in more isolates (36.8%) in the presence of hydrogen peroxide compared to 4.3% in its absence.

PE & PG

For PE activities, nine bands were consistently scored for all the isolates of *P. squarrosipes*. Two dominant bands, Bands 3 (R_f=0.25) and 7 (R_f=0.39), were noted across the isolates occurring in 92.2 and 41.1% of isolates respectively (Table 7.16). Band 6 (R_f=0.37) appeared to alternate with Band 7 (Fig.7.19) and they were probably alleles at the same locus. This band was noted in 26.7% of the isolates. Band 9 (R_f=0.44) was noted exclusively in the isolates of CYS14 whilst showing similarity in Band 3 with isolates of other collections (Fig. 7.19).

Of the 12 bands scored for the activities of PG (Fig 7.20), only four bands occurred consistently in >20% of the isolates. Bands 2 (R_f=0.11) and 5 (R_f=0.46) were the two dominant bands occurring in 64.4 and 46.7% of the isolates respectively (Table 7.16). Two backrunners (R_f=-0.04 & -0.02 respectively) were noted to occur exclusively in isolates of CYS14 (Fig 7.20). The absence of these two bands from the other isolates in the group may suggest that this genetic variation could be of some importance.

UPGMA cluster analysis using band frequencies was performed by including a morphologically distinct species, *P. aurivella*. The result is presented as dendogram in Fig. 7.21. It shows a distinct hiatus between collections of *P. squarrosipes* and *P. aurivella* with CYS14 being comparatively distant from the two former groups.

7.2.2.3. Subgenus *Flammuloides*

7.2.2.3.1. *P. highlandensis*

Lac

A total of six bands of laccases were consistently scored across the isolates of *P.*

highlandensis. Bands 4 (at R_f 0.37) was the most dominant band occurring in 98% of the isolates, followed by Bands 5 (at R_f 0.47) and 6 (at R_f 0.49) of 44 and 49% respectively (Table 7.17).

While isolates of five collections showed consistent band patterns, variability not observed in the others was noted in two collections, CYS529 and CYS538. On the whole, isolates of CYS529 showed similar activities in the three dominant bands mentioned above when compared with other collections in the group (Fig. 7.22). While all collections showed activity at Bands 4, 5 and 6, only isolates of collections CYS529 and 538 exhibited activities recorded as Bands 1, 2 and 3. Isolates of both CYS529 and 538 showed activity at Band 1 (R_f 0.28). Only some isolates of CYS538 exhibited activity at Band 2 whilst Band 3 was detected only in some isolates of CYS529.

Per

A total of 12 bands were scored across the isolates, of these, four (Bands 3, 4, 5 and 11) were previously detected in Lac staining in the absence of peroxide ions (Fig. 7.23). No Per activity was detected in the isolates of CYS453. The activity of peroxidase isozymes of *P. highlandensis* was considered to be more variable than the laccase activity previously noted. The relative frequency of band occurrence was low (Table 7.17) and varied from Band 1 which only occurred in some isolates of CYS538 to Band 7 which occurred in at least some isolates of four out of the seven collections tested (Fig. 7.23). Each of the collection showed a different set of isozyme activities however only in the case of Band 1 was the activity unique to a single collection.

PE & PG

Eight prominent bands of PE activity were scored across all the isolates. Of these, Band 7 occurred in 91% of the isolates with Bands 5 and 6 in 73 and 45% respectively (Table 7.17). Only Band 1 occurred in <10% of the isolates while the remaining bands in >20% of the isolates. The band pattern of each collection included various

combinations of the three major bands in addition to the lesser bands (Fig. 7.24).

A total of nine bands were scored for PG activities. Two dominant bands, Bands 3 (at R_f 0.24) and 4 (at R_f 0.28) occurred in 67 and 45% of the isolates.respectively (Table 7.17). The remaining bands varied considerably between the different collections. For example, Band 1 was detected only in some isolates of CYS530, similarly Band 2 was only noted in CYS529. Isolates of CYS528 and 529 showed greater affinity than with isolates of other collections in the occurrence of Band 5 which was virtually absent in the isolates of other collections (Fig 7.25).

7.2.2.3.2. *P. multicingulata*

Lac

Nine bands were scored across the isolates for Lac activities with three dominant bands, Bands 5 (at R_f 0.46), 6 (at R_f 0.48) and 7 (at R_f 0.51) occurring at 88.3, 80.8 and 65.9% respectively of the isolates (Table 7.18). The most frequently observed combinations of bands included at least one of these three bands in addition to the other bands (Fig.7.26). The only exception was CYS537 which lacked these three bands but showed similar activities in Bands 2, 3 and 4 with other collections(Fig. 7.26).

Per

A total of 11 bands were recorded for Per activities across the isolates of *P. multicingulata*. Of these, a few bands were noted to be detected previously in the absence of peroxide. They included Band 5 at R_f 0.38, Band 7 at R_f 0.46, Band 8 at R_f 0.51 and Band 9 at R_f 0.54 (Fig. 7.27). There was a lower percentage occurrence for these bands in the presence of peroxide except Bands 6 and 8 (Table 7.18). The two dominant bands were 7 and 8 occurring in 55.8 and 66.3% of the isolates respectively.

PE & PG

For PE activities, a total of eight bands were scored across all the isolates. Table 7.18 gives the percentage occurrence of the four dominant bands across the isolates. Their percentage occurrence was moderately low with Band 7 scoring only 40%. However, the banding patterns appeared to involve various combinations of the four major bands. Fig. 7.28 shows the schematic representations of the banding patterns produced by the isolates of collections in *P. multicingulata.* It also indicates the degree of variability within this group.

For PG activities, six bands were scored across the isolates. Of these, Bands1 and 5 at R_f 0.31 and 0.43 respectively were the two dominant bands. Band 1 occurred in >95% of the isolates followed by Band 5 at 62.3% (Table 7.18). Two other less dominant bands (Bands 3 & 6) were noted to occur in isolates of the various collections. These four bands formed the major band composition of the various banding patterns (Fig. 7.29).

7.3.2.3.3. *Pholiota* sp. D

Lac

A total of eight bands were scored across all the isolates of *Pholiota* sp. D. Of these, Bands 2 at R_f 0.32 and 4 at R_f 0.40 were dominant and occurred in 79 and 56.4% of the isolates respectively (Table 7.19). At least one or all of these three more dominant bands were noted to occur in various combinations with the less dominant bands (Fig. 7.30). Isolates of CYS271 showed similarity in its solitary band at R_f 0.54 with isolates of other collections in *Pholiota* sp. D (Fig. 7.30). A band of the same mobility was also detected in some isolates of *P. multicingulata* (See Fig. 7.26).

Per

A total of nine bands were scored across all the isolates. Two bands, 5 (at R_f 0.33) and

6 (at R_f 0.37), occurred in 85 and 60% of the isolates respectively (Table 7.19) followed by Band 8 at R_f 0.55 scoring 46.7%. These three bands formed the basis of the banding patterns of the isolates of the various collections in this group except CYS271 (Fig. 7.31). Two bands at R_f 0.16 and 0.22 respectively were detected in all the isolates of CYS271 exclusively (Fig. 7.31).

PE & PG

For PE activities, a total of 11 bands were scored across the isolates. Of these, Bands 9 (R_f 0.57) and 2 (R_f 0.37) were the dominant bands occurring in 82.7 and 80.8% of the isolates respectively (Table 7.19). Two other less dominant bands at R_f 0.12 and 0.41 respectively occurred in >50% of the isolates (Table 7.19). Various combinations of bands appeared to include at least one of these three bands except isolates of CYS271 where no PE activities were detected (Fig. 7.32).

For PG activities, a total of five bands were scored consistently across all the isolates. Bands 2 and 5 at R_f o.22 and 0.44 respectively occurred in 57.7 and 44.2% of the isolates respectively (Table 7.19) except isolates of CYS271. A solitary band of PG activity at R_f 0.12 was detected exclusively in isolates of CYS271 (Fig. 7.33).

Fig. 7.34 shows representations of laccase zymograms of *P. squarrosipes* and the main taxa (*P. highlandensis*, *P. multicingulata* and *Pholiota* sp. D) in subgenus Flammuloides indicating species distinctiveness, and similarly for peroxidase and pectic zymograms (Figs. 7.35 & 7.36).

UPGMA cluster analysis

Two sets of UPGMA cluster analyses were performed for the four taxa in subgenus Flammuloides, one using the band frequencies of Lac, PE and PG isozymes and the other that of Per isozymes only. Results of the two analyses are shown in Fig 7.37 and Fig. 7.38. The dendogram constructed is based on the squared average distance

between clusters. At the four cluster level, Fig. 7.37 shows four groups corresponding to *P. highlandensis*(K), *P. multicingulata*(B), *Pholiota* sp. D (A) and a single collection of CYS529 (*P. highlandensis*). The separation of CYS529 from the '*highlandensis*' proper was as a result of the variations in pectic isozymes.

Due to the variations noted earlier in the Per isozymes, it was expected that the UPGMA cluster analysis would not produce any obvious clusters corresponding to the three taxa. Fig. 7.38 shows the dendogram produced from this second analysis. At the same truncation level as in Fig. 7.37, five single collections (CYS528, CYS501, CYS532, CYS271 and CYS530) were separated from a loose cluster (C) consisting of mixed collections of the three taxa. Within the loose cluster, three putative subclusters could be discerned roughly coresponding to *Pholiota* sp. D (C1), *P. multicingulata* (C2) and *P. highlandensis* (C3) with some dispersal of collections of each group.

7.2.3. Mating Compatibility studies

7.2.3.1. Subgenus *Pholiota*

7.2.3.1.1. *P. aurivella*

The results indicated a bipolar incompatibility mating system for CYS159 and two mating types (A: 01, 04, 08 & 13 and a: 02, 05, 06 07, 10 11 & 12) were recovered from the polarity matrix. Monokaryotic isolates of CYS116, 128, 154 and 157 were intercompatible with isolates of the two mating types of CYS159 (Table 7.20).

7.2.3.1.2. *P. squarrosipes*

Isolates of two collections (CYS16 &11) were used in the polarity matrices. The results indicated a bipolar incompatibility mating system in both cases and two mating types (CYS16: A - 01, 04, 17, 19 & a - 03, 07, 08, 12, 13, 15 16 & 18 and CYS11: A - 01, 08, 09 & a - 03, 04, 05, 06, 07, 10, 13 & 14) were recovered. CYS16 and CYS11 were shown to be intercompatible between themselves as well with the monokaryotic

isolates of other collections within this group (Table 7. 21). The mating types of both collections were found to be interincompatible with the isolates of CYS14 (Table 7.21).

7.2.3.2. Subgenus *Flammuloides*

7.2.3.2.1. *P. highlandensis*

Ten monokaryotic isolates of CYS532 were used in a polarity matrix to determine the mating system. The results indicated a bipolar incompatibility mating system with two mating types recovered (A: 01, 04, 07, 08 & 11 and a: 02, 03, 05, 06 & 09). Monokaryotic isolates of six other collections placed in the same taxon as CYS532 were intercompatible with the mating types of CYS532 (Table 7.22).

7.2.3.2.2. *P. multicingulata*

Isolates of CYS501 were used in the polarity matrix to determine the mating system of this species. The results indicated a bipolar incompatibility system and two mating types (A: 01, 02, 05, 06, 07, 08, 11 & 12 and a: 03, 04, 09 & 10) were recovered. Monokaryotic isolates of 13 other collections placed in this taxon as CYS501 based on morphological characters were intercompatible with the mating types of CYS501 (Table 7.23).

7.2.3.2.3. *Pholiota* sp. D

Isolates of CYS482 were used in the polarity matrix to determine the mating system of this taxon. The results indicated a bipolar incompatibility mating system and two mating types (A: 01, 02, 03, 05, 07 & 10 and a: 04, 06, 08, 09, 11 & 12) were recovered. Isolates of 12 other collections in the same taxon as CYS482 based on morphological criteria were intercompatible with the mating types of CYS482 (Table 7.24) except CYS271. Isolates of CYS271 were also interincompatible with isolates of three other collections in taxon C (Table 7.24).

Isolates of the two mating types of CYS482 were shown to be interincompatible with isolates of collections of *P. multicingulata* (Table 7.24), another taxon in the same subgenus. In these incompatible crosses, there were visible antagonistic reactions between the inocula. Such reactions included production of yellow brown pigments and a growth inhibitory effect.

7.2.4. Observations on some cultures of *Pholiota*

One interesting observation was noted in the cultures of CYS271, i.e., acanthocytes (in the sense of Farr) resembling those observed in *Stropharia* were produced in culture (Fig. 7.39). Cultures of other taxa of *Pholiota* did not produce acanthocytes but clusters of slender fusiform cells were noted on the surface of older cultures (Fig. 7.39). Table 7.25 gives a summary of the taxa that produced these cell types in culture.

7.3. Discussion

A total of 12 taxa belonging to four subgenera were delineated from the results of morphological comparisons, electrophoresis, and mating compatibility. Of these, three (*Pholiota* sp. A, *Pholiota* sp. B & *Pholiota* sp.D) are previously undescribed species which will be formally described in the chapter on New Species. Two other taxa (*Pholiota* taxon 1, CYS14 & taxon 2, CYS271) were delineated from their initial grouping based on results of both electrophoretic and mating compatibility studies. Delineation of these two taxa using means other than morphology introduces problems into a taxonomic system in which species are essentially defined by morphological criteria. As the material available is only a relatively small number of basidiomes from single collections, no concept of the morphological limits could be established for either of these two taxa. Therefore it is probably premature to grant them true species status and a conservative approach has been adopted by accepting them as distinct, separate taxa (*Pholiota* taxon 1 from *P. squarrosipes* and *Pholiota* taxon 2 from *Pholiota* sp. D).

The cases illustrated above accentuate some limitations of traditional morphological based taxonomic systems. Two further taxa (*Pholiota* taxon 3 & taxon 4) are delineated based on single morphological character which is distinct and consistent enough to separate them from the rest of the taxa. Again specific epithet will not be given them for the simple reason that more distinctive characters are required to establish their species status. The remaining five species (*P. malicola, P. aurivella, P. squarrosipes, P. highlandensis & P. multicingulata*) have been previously described and are shown to be separable through morphological comparisons, isozyme analysis or mating compatibility.

The occurrence of acanthocytes (in the sense of Farr) has not been previously reported in genera other than *Stropharia.* This cell type has been considered in the present study to be effective in separating *Stropharia* from the other genera of Strophariaceae. For the first time, this cell type is observed in cultures of *Pholiota* in this study. Acanthocytes were noted in some cultures of one collection (CYS271) of *Pholiota* but not in the basal mycelium of carpophore as observed in *Stropharia.* There is really insufficient information to suggest the general occurrence of acanthocytes in *Pholiota* and further studies are required. As for the clusters of fusiform cells noted in some other cultures of Pholiota, it is not clear of what taxonomic significance they have. Further investigations into the development and consistency of these structures may provide a clearer understanding of this character and therefore elucidate its usefulness as a taxonomic character.

Subgenus Phaeonaematoloma

The two taxa delineated here are morphologically distinct from each other as well as from other taxa in the genus. Both are exannulate forms with a subcellular hypodermium and thus are best placed in Section *Phaeonaematoloma* (Singer) Singer s. Smith & Hesler (1968) and Singer (1986).

P. fieldiana sp. nov. (*Pholiota* sp. A)

P. fieldiana sp. nov. is characterised by both pileus and stipe being strongly viscid when moist. The gluten layer is clearly visible clinging to the lower two-thirds of the stipe and this is a feature which Singer (1986) emphasised in the separation of taxa of subgenus *Phaeonaematoloma* from the brown-spored taxa of *Naematoloma* (=*Hypholoma*). This feature is reminiscent of *Myxacium* in the Cortinariaceae. *P fielidana* is a very unusual and rare *Pholiota* not only in its restricted occurrence, only in the wetter regions of temperate rainforest of *Nothofagus-Atherosperma* dominance, but also in the presence of both leptocystidia and chrysocystidia. Smith & Hesler (1968) did not consider the presence of two kinds of cystidia unique. However, this is one character that renders *P. fieldiana* unusual amongst the other Tasmanian species of *Pholiota* which possess only one kind of pleurocystidia. Taking all these into consideration, it is clear that this fungus is a new and distinctive species. It appears to be almost as rare as *P. aberrans* Smith & Hesler which, for a long period, was known from the type collection only, it has since been reported from one other locality as of *Hypholoma anomalum* (synonym of *P. aberrans*) (Dennis 1961). Only two collections are made for *P. fieldiana* and both are from the same locality but in different years.

P. visco-fumosa sp. nov.(*Pholiota* sp. B)

P. visco-fumosa sp. nov. is also found in similar habitats to *P. fieldiana* but is often associated with woody litter on the forest floor. The specimens of one collection (CYS256) from the north-west coast were found growing on moss-covered rotten wood and tend to have smaller carpophores than the southern specimens. This fungus shows a broader range of distribution than *P. fieldiana.* There are obvious macromorphological distinctions, such as the colour of pileus and stipe features (viscid in *P. fieldiana* and dry in *P. visco-fumosa*) to display an evident hiatus between them. The larger spores of *P. visco-fumosa* separate it from *P. squarrosipes* as shown in the

CDA analysis. These distinctive differences ensure this fungus a separate species status.

Subgenus Flammula

Species in this subgenus are known to form a natural and distinct group within the genus *Pholiota* (Jacobsson 1986). There is such uniformity in many respects of morphological characters that species delineation within this subgenus is not easily achieved. Jacobsson (*loc. cit.*) recognizes this difficulty and suggests the use of cultural characters including mating compatibility data to supplement morphological characters in species delineation.

Recalcitrant spore germination is encountered in this subgenus consequently the number of isolates available for cultural experiments has been severely truncated in this study. The results indicated the presence of a single species based on both morphological and electrophoretic studies. The morphological results indicate a relatively uniform taxon with variability falling within the circumscription of *P. malicola* (Kauff.) A. H. Smith. This name is adopted here instead of *P. alnicola* s. Jacobsson mainly because much work, especially cultural studies, is still needed to understand the relationships between the *malicola/alnicola*-like taxa. Two observations suggest some deviation of the Tasmanian specimens from both the European and North American representatives of this taxon. Firstly, the occasional presence of 2-spored basidia is reported for the first time for species in the subgenus of *Flammula.* The predominant presence of 2-spored basidia in specimens of one collection suggests the existence of 2-spored form in nature albeit only rarely. Secondly, there is no obvious association with deciduous wood as in the case of the North American representatives. Though the fungus is often found in close proximity to temperate rainforest with *Nothofagus cunninghamii* dominance, it appears to be associated more with disturbance and frequently fruit beside roads or tracks, on apparently bare and rocky ground. Occurrence on the forest floor with

Nothofagus leaf litter is infrequent. Unlike some of the European specimens, association with conifers is also not obvious.

The electrophoretic results show variability in the isozymes used in the study (Figs. 7.8 - 11) yet they also indicate an obvious affinity between isolates of the different collections (Fig. 7.12) grouped as *P. malicola*. The evident hiatus between collections grouped as *P. malicola* and *P. aurivella* in Fig. 7.12 shows that there are distinct differences between the two taxa and suggests there is only one taxon represented within the collections grouped as *P. malicola*.

Subgenus Pholiota

Two species corresponding to *P. aurivella* (Fr.) Kummer and *P. squarrosipes* Clel. are delineated based on morphological and electrophoretic results. *Pholiota* taxon 1 was delineated from collections of *P. squarrosipes* through the pectic isozyme data and mating compatibility.

P. aurivella (Fr.) Kummer

All the Tasmanian material included in this composite study correspond well to the concept of *P. aurivella* in the sense of Smith & Hesler (1968) and Farr *et al.* (1977) particularly the morphological aspects. The results of mating compatibility studies indicated the existence of a single biological species exhibiting a bipolar mating incompatibility system. Though the bipolar incompatibility system concluded from the mating results contradicts the published results of Farr *et al.* (*loc. cit.*) and Jacobsson (1989), the finding is consistent with earlier reports of Mounce, Martens & Vandendries and Vandendries (in Farr *et al.* 1977 and Jacobsson 1989).

The species status of *P. aurivella* is questioned recently by Kuyper & Tjallingii-Beukers (1986) and Jacobsson (1987). According to Kuyper & Tjallingii-Beukers

(1986), the modern circumscription of *P. aurivella* is in clear conflict with the description of *Agaricus aurivellus* by Batsch. They propose *P. cerifera* (P. Karst.) P. Karst. to replace *P. aurivella* (Batsch ex Fr.) Kummer which they considered to be incorrect. Jacobsson (1987) proposes the inclusion of *P. aurivella* in*P. adiposa* which he considers a more appropriate name for this taxon. However, he (Jacobsson 1990) later put forward a more convincing argument to retain *P. aurivella*, and *P. adiposa* is reduced to a synonym.

P. aurivella in the sense of Smith & Hesler (1968) differs from *P. adiposa* by possessing dry, scaly stipe and smaller spores. Evidence of the dry stipe is obvious in the basidiomes of the F_1 generation obtained from the fruiting trial (Figs. a & b in Appendix II). The stipe remains dry from the button stage through to the time when pileus begins to open and expand. On the other hand, the stipe of *P. adiposa* is viscid or with slimy scales. However, the viscid scales on the stipe may not be a reliable character since the gluten is very thick on the surface of the pileus, there is no guarantee that some of it may not have reached the stipe and be misinterpreted as part of the stipe. Smith & Hesler (1968) acknowledge the close affinity between these two taxa and that confusion can easily arise but fail to elucidate any other distinctions between them. Jacobsson's concept of this species seems to suggest one resolution of this problem and *P. aurivella* is thus considered synonymous with *P. adiposa* sensu Jacobsson.

There is no past record of either *P. adiposa* or *P. aurivella* in Tasmania. This study provides the first thorough record of this species in SE Tasmania. It is unlikely that the species is introduced since its occurrence has always been on native trees (*Nothofagus cunninghamii* and *Atherosperma moschatum*) and exhibiting a different mating incompatibility system from the North American representatives. This interpretation of *P. aurivella* as a native Australasian species is at odds with Horak (1971).

P. squarrosipes Clel.

P. squarrosipes Clel. has not been reported in Tasmania, again this study gives the first thorough report for Tasmania as well as the first analysis of the inherent variability of this species in Australia. The results of the study show that *P. squarrosipes* is quite a variable species occurring in a broad habitat range. This species appears to be endemic to Australia. (or perhaps at least the Australasian region). Variations in macro-morphological characters include colour of the pileus, surface features of pileus and the general stature of basidiome. The broad habitat range indicates that this fungus is a good survivor in the field. It is in fact a common fungus and fruits from April to July.

Two unusual forms, one with long snouted chrysocystidia and the other with slender based chrysocystidia, were observed in this study. They exhibit distinctive differences in microcharacters not seen consistently in the syntypes and other Tasmanian collections, and this indicates some interesting development within *P. squarrosipes*. Though only one collection was made for each (CYS458 & CYS424), it nevertheless suggests the possibility of divergence which may eventually lead to new varieties provided that the morphological differences noted in each of these two forms are not environmentally induced and are only some kind of temporary phenotypic expression. It seems unlikely that the differences are environmentally induced, since the two cystidial forms are also observed in majority of the specimens of *P. squarrosipes* though with a relatively infrequent occurrence. Another possible explanation of these two unusual morphs in the collections grouped as *P. squarrosipes* is that the rare morphs represent a relative infrequent combination of genes within the *P. squarrosipes* population. Thus they may represent a rare but normal form of *P. squarrosipes*.

Mating results indicated two intersterility groups within collections grouped as *P. squarrosipes*, one corresponding to *P. squarrosipes* at large and the other a single collection (CYS14). There is a possibility that CYS14 is a sibling species to *P.*

squarrosipes and not one of the forms mentioned above. The phenomenon of sibling species has not been reported in this family in any previous reports for Australia. The implication of this is the likelihood of speciation in process here. Since CYS14 is morphologically indistinguishable from other collections of *P. squarrosipes* it is possible that a single mutation step may have resulted in the interincompatibility observed and that this genetic differentiation and that illustrated in the pectic zymograms could have occurred before phenotypic differentiation. If this is the case, morphological comparison would not separate CYS14 from other specimens of *P. squarrosipes*, and as shown in this study delineation can only be achieved through isozyme analysis and mating compatibility data. This further illustrates the effectiveness of these two approaches in species delineation at the species level.

Pholiota taxon 1 (CYS14)

As mentioned above, morphological similarity between *P. squarrosipes* and CYS14 has masked the genetic variations which were partly revealed in some isozyme data and more apparently in mating incompatibility. Thus the mating and electrophoretic studies have uncovered a complex situation where two biological taxa share a common morphology and the only practical step is to refer to the *P. squarossipes* complex and await further details which would allow complete separation of Taxon 1. For this reason, Taxon 1 will not be given species status at this stage.

Subgenus Flammuloides

Results of morphological comparisons show that there is much overlap in micromorphology in the species in this subgenus. For example, the spore length of the six taxa delineated in the study fell in a range of 6.7 -8.75 µm. This observation coincides with similar observations of Smith & Hesler (1968) in the North American species of this subgenus. However, these results show that width of spores, in particular facial width, is much more useful in the separation of taxa within this subgenus than spore length. Further, Smith & Hesler (*loc. cit.*) recognise the

inconsistency in the wall thickness of pleurocystidia in some species included in section Flammuloides. They set the dividing line between 'thin' and 'thick' at 0.5 µm. (*loc. cit.*). In this study, inconsistency in wall thickness is particularly noticeable in specimens of *P. multicingulata.*

Another variation is noted in the contents of pleurocystidia. The contents of pleurocystidia in specimens of all taxa included in this subgenus in this study show variations from hyaline homogenous, pale yellow homogenous, granular yellow brown to golden brown. Smith & Hesler (1968) have outlined similar variations in the projecting type of pleurocystidia in subgenus Flammuloides. For this subgenus, this study has accepted the description 'chrysocystidioid' by some authors to refer to those pleurocystidia with yellow content. True chrysocystidia are usually pleurocystidia with amorphous content, i.e. they have the refractive inclusions as revived in KOH The use of Melzer's iodine has shown chemical differences between the refractive inclusion in chrysocystidia and the ones with pale yellow content (*loc. cit.*). In fact, throughout *Pholiota*, pleurocystidia with a wide range of contents are observed. Smith & Hesler suggest that this can be attributed to the chrysocystidia originating in this genus.

On the whole, macromorphology is found to be more effective than micromorphology in species delineation in this subgenus.

Isozyme analysis showed promising results that indicate the potential of this parameter as a tool in species delineation. The three extracellular enzyme systems of laccase, pectinesterase and polygalacturonase are relatively distinct for the taxa tested. Laccase in particular is very useful in a preliminary screening since the incubation time required is short (3 da.) and on the whole the banding pattern is distinct for each taxon. Furthermore, pectic isozymes provide an additional level of support to the results of laccase. Thus these three enzyme systems supplement morphological comparisons in

species delineation. The analysis of peroxidase enzymes appears more complicated. These enzymes are known to be complex and *in vitro* interactions between bands could create complications (Gottlieb 1981 & 1984). Less defined distinctions are achieved for each taxon than in the previously mentioned enzymes. Thus, it is suggested that there is a need to search for other markers to add to the three effective ones mentioned earlier.

P. highlandensis (Peck) Smith & Hesler

Habitat remains the most consistent and reliable criterion for the delimitation of *P. highlandensis* from other species in this subgenus in Tasmania. Smith & Hesler (1968) use veil colour to separate *P. highlandensis* from *P. carbonaria* Smith, the latter is separated from the former in the distinctly red veil. In this study, the veil character is found to be rather inconsistent and therefore not a reliable taxonomic character. Chance fruiting of dikaryotic isolates from Tasmanian collections under laboratory conditions showed that the initial colour of the veil is pallid or pale cinnamon (Fig. 7.40a). The colour of the veil remnants changed to russet or reddish brown with age (Fig. 7.40b) thus ruling out the possibility that the colour was due to spore deposits. This colour change raises doubt as to the reliability of colour of veil as a taxonomic character. Jacobsson (1989) points out the unreliability or limitations of morphological characters of this and other closely related species and suggests the use of intercompatibility test for species confirmation. Results from the present study also indicate a similar finding that species delineation based on morphological characters is fraught with danger.

Within the collections designated as *P. highlandensis* a single biological species is identified from the mating results. Jacobsson (*loc. cit.*) reports tetrapolar incompatibility mating system for the European representatives of *P. highlandensis*. Contrary to that report, the present study indicates a bipolar incompatibility mating system with multiple alleles. It would be interesting in future studies to explore the

relationships of mating compatibility of isolates of representatives of this species from northern and southern hemisphere as well as closely related species such as *P. carbonaria* A.H. Smith.

Pholiota taxon 3 (*Pholiota* sp. C)

The single collection of CYS257 is separated from other taxa, in particular *P. multicingulata*, on the basis of the thick-walled pleurocystidia. As a result, it is placed in section Flammuloides. Because of the inconsistency in wall thickness of pleurocystidia in some species of this section, ignoring the wall thickness of pleurocystidia of *Pholiota* sp. C (CYS257) would place it in section Spumosae and probably considered a collection of *P. multicingulata*. While the wall character is consistent within specimens of CYS257 it is inconsistent in specimens of *P. multicingulata*. However, wall thickness of pleurocystidia in majority of the specimens of *P. multicingulata* seldom exceed 1.5 μm. which is the lower limit of the wall thickness (1.5-2.7 μm.) of CYS257. Therefore, the wall character of pleurocystidia of CYS257 is too consistent to be ignored and as a result it is more appropriate to consider CYS257 a separate taxon. There are similarities noted between CYS257 and *P. multicingulata* in many aspects of morphology, this is possibly in a parallel situation to that observed between *P. squarrosipes* and CYS14. As a result of the lack of information from other approaches, this fungus will not be given a specific epithet until more distinctions between them can be obtained.

Pholiota taxon 4 (*Pholiota* sp. E)

The specimens in the single collection of CYS266 resemble *P. multicingulata* in many respects morphologically except for the glutinous veil. Like *Pholiota* sp. C, the veil is the only morphological character separating it from *P. multicingulata*. The other known species in this subgenus with a glutinous veil is *P. velaglutinosa* Smith & Hesler. The veil character is distinct enough to set it apart and this fungus is considered

a separate taxon but specific epithet will not be given pending on further studies. Unfortunately the lack of isolates prevent any further investigation using the isozyme or mating approaches.

P. multicingulata Horak

The study shows that *P. multicingulata* is a species that shows a broad range of variations in morphological characters. This study found that the multizonate stipe that gives the species its name is not always evident and examination of young basidiomes is often required to confirm its presence. Other macromorphological variations were noted in the colour and surface features of the pileus.

As mentioned earlier, inconsistency in wall thickness of pleurocystidia was noted between specimens of different collections. Pleurocystidia in majority of specimens possessed walls <0.4 µm. (below the boundary set by Smith & Hesler) thick. However several collections (including CYS501 whose mating types were established in the mating study) possessed pleurocystidia with wall thickness ranging from 0.8 to 1.5 µm. but more often <0.8 µm.

In the protologue of this species, pleurocystidia are described as chrysocystidioid (Horak 1983). It is believed that such a description refers to pleurocystidia with yellow content as mentioned earlier and not chrysocystidia with amorphous content. The inclusion of several other species in this subgenus in morphological comparisons resulted in some interesting findings. Firstly, spore characters in particular spore facial width are more effective in the separation of taxa than cystidia characters which showed a greater degree of overlap across the taxa. Secondly, there is no obvious affinity with *P. highlandensis* indicating that they are distinct and separate species. Thirdly, morphometric analyses failed to separate *P. austrospumosa* Hongo from *P. multicingulata* implying close affinity between the two species. Finally, affinity with the northern hemisphere species is less forthcoming.

Variations are also noted in laccase, peroxidase and pectic enzymes. For example several collections, CYS501, 397, 376 and 537, showed some distance from the main bulk of collections in this group (Fig. 7.37). However, the overall result is that the variations noted in these collections are not great enough to justify erecting a separate taxon. Furthermore, the banding patterns of laccase and pectic isozymes in particular are characteristic for isolates of this species.

Results from mating incompatibility show the existence of a single biological species exhibiting bipolar incompatibility system. The wall character of pleurocystidia is proven to be of no taxonomic value in this species through the successful mating between isolates from specimens with thick-walled pleurocystidia (e.g. CYS501) and those with thin-walled pleurocystidia (e.g. CYS385).

In addition to its occurrence in New Zealand and Western Australia, *P. multicingulata* also occurs in Victoria and Tasmania. This coincides with Horak's (1983) prediction that it is a widely distributed species in the Australasian region.

P. austrospumosa Hongo, a species collected from the highlands of Papua New Guinea, together with *Pholiota* taxon 3 and taxon 4, show close affinity with *P. multicingulata* especially in morphology (Fig. 7.7, Table 7.13). In view of the Gondwanic association between New Guinea and Australia (Axelrod & Raven 1982), it is not surprising that these taxa are closely related. It is suggested here that together they form a group of closely related species probably forming the southern hemisphere equivalent to the '*spumosa*' complex in the northern hemisphere.

Pholiota pallidocaulis sp. nov. (*Pholiota* sp. D)

Pholiota pallidocaulis displays distinctive macromorphology that separates it from the other taxa in this subgenus. It is characterised by the pale-coloured pileus in mature

basidiomes, pale-coloured slender stipe and conspicuous yellow rhizomorph. Variations are noted in the colour of pileus and stipe. Young basidiomes tend to have a deeper shade than the mature ones. The change in colour may be related to different developmental stages and may also be partially affected by environmental factors. The pale-coloured stipe is very pronounced in young basidiomes and is also evident in mature ones. The yellow rhizomorph is noted to be a relatively reliable field character for this fungus.

There is notable degree of overlap in microcharacters between this taxon and other taxa in this subgenus. There is overlap in spore size with *P. multicingulata* to a certain degree but the greater spore width separates it from *P. multicingulata.* It is also separable from *P. multicingulata* in having broader cheilocystidia. As a result of the thick-walled pleurocystidia, *P. pallidocaulis* is placed in section Flammuloides. Unlike *P. multicingulata*, the wall character of pleurocystidia appears a relatively consistent character across the specimens between different collections though a small number of specimens exhibited some inconsistency even on the same basidiome.

Isolates of the various collections showed characteristic banding patterns for this taxon in the isozymes tested. Good separation is obtained using laccase and pectic isozyme data. Variations are noted in isolates of CYS271 in the possession of exclusive bands. This is particularly pronounced in the peroxidase isozymes where this collection is completely separated from the bulk of collections of *P. pallidocaulis.* Another collection, CYS533, also showed variation in peroxidase that resulted in its grouping with collections of *P. multicingulata.* But peroxidase is known to be complex and exhibit complication through *in vitro* interaction (Gottlieb 1984).

Two intersterility groups are delineated in *Pholiota pallidocaulis* corresponding to the majority of collections in this group and CYS271. However, neither morphological

comparisons nor other isozyme analysis has resulted in any conclusive separation despite its exclusion from *P. pallidocaulis* as shown in the dendogram based on peroxidase data. Differences in laccase and pectic isozymes (Figs. 7.34 & 7.36) of CYS271 are apparently not great enough to warrant a separation. However, the inability to breed with the mating groups of CYS482 indicates the presence of a genetic barrier. It is possible that incompatibility could also be caused by changes that occurred in culture under laboratory conditions since there are selective forces present. Or simply there may be a genuine genetic barrier that prevents mating to proceed. Whichever is the likely cause for the genetic barrier, CYS271 is regarded as a sibling species to *P. pallidocaulis*. The isozyme analysis shows that CYS271 is closer to *P. pallidocaulis* than to other taxa of subgenus Flammuloides included in the comparison. This may suggest a certain affinity or similarity in this portion of the total genome between the two taxa and thus an indication of recent divergence.

Pholiota taxon 2 (CYS271)

Pholiota taxon 2 as mentioned above is only separated from *P. pallidocaulis* through mating incompatibility. This phenomenon is again analogous to that between *P. squarrosipes* and *Pholiota* taxon 1. Once again, two biological taxa sharing a common morphology are uncovered through mating study. The inconclusive outcome of isozyme analysis may be attributed to genetic differentiations that have occurred relatively recently. Thus, pending on further studies to obtain more details this taxon will not be granted species status.

7.4. Taxonomy

The following includes key, observations and descriptions of the Tasmanian representatives of previously described species of *Pholiota* as well as new taxa delineated from the above studies. A synopsis of the taxa delineated from this study is presented in Table 7.26.

Key to species of *Pholiota* in SE Tasmania

1 Pileus dry or merely greasy, hygrophanous and usually glabrous 2

1' Pileus tacky or viscid when moist with or without a gluten layer, scaly, squamulose, virgate or appearing glabrous 3

2 Pleurocystidia present 3

2' Pleurocystidia absent .....**3. malicola**

3 Stipe viscid with lower two-thirds covered with a layer of gluten; pileus with an olivaceous tinge drying to ochraceous brown, glabrous, striate and hygrophanous; pleurocystidia of two kinds as chrysocystidia and hyaline, slightly projecting leptocystidia ..... **1. fieldiana sp. nov.**

3' Not as above 4

4 Stipe whitish flocculose to coarsely fibrillose, sordidly coloured with age; pileus slimy viscid, dark blond or clay coloured or smoky grey, concentric rings of whitish squamules at disc when young then more or less glabrous ..... **2. visco-fumosa sp. nov.**

4' Stipe scaly, fibrillose or almost smooth, whitish or pallid or dingy brown 5

5 Pleurocystidia as chrysocystidia and usually embedded 6

5' Pleurocystidia projecting 7

6 Both pileus and stipe scaly with recurved scales, lemon yellow or golden brown, strongly glutinous with a thick gluten layer in moist weather, usually >5 cm. across, on rotten or damaged myrtle (*Nothofagus*) or sassafras (*Atherosperma*) ..... **4. aurivella**

6' Pileus squamulose or almost glabrous, slimy viscid, fulvous, ochre, honey or dull brown, margin at first appendiculate with veil remnant, usually exannulate, annulus when present semi-permanent, gregarious or caespitose on bare and rocky ground, on leafy or woody ground litter or amongst grass and mosses ..... **5. squarrosipes (including 6. taxon 1)**

7 Pleurocystidia consistently thick-walled, 0.8 -2.5 µm., spores tending to elongate ellipsoid ..... **10. taxon 2**

7' Pleurocystidia thin-walled or intermixed with thin- and thick- walled 8

8. On burnt ground ..... **7. highlandensis**

8' On wood or general ground litter 9

9 Stipe always whitish or pallid at first becoming sordidly coloured with age or bruised brownish, yellow rhizomorph below; pileus varies from beige, brownish orange to raw sienna; spores elliptic both in face and side view, 6.7 - 8.75 (9.2) x (5) 5.4 - 6.7 x 5 - 6.25 µm. .....**9. pallidocaulis sp. nov. (including 11. taxon 3)**

9' Stipe brownish, fibrillose, pileus generally darker shade of brown 10

10 Stipe scabrous below velar zone, light to dark brown, veil glutinous firm cortina; pileus hazel brown darker at disc, virgate with dark brown streaks .. **12. taxon 4**

10' Stipe with multiple brown fibrillose zones near base; veil a firm cortina, non-viscid; pileus slimy viscid, light brown throughout darker at disc, with concentric rings of appressed scales at disc or merely virgate ..... **8. multicingulata**

1. *Pholiota fieldiana* sp. nov.

This is an unusual and rare *Pholiota* which is very typical of the subgenus *Phaeonaematoloma* possessing a viscid pileus and stipe as well as more or less subcellular hypodermium. It has been collected from the same locality in different fruiting seasons, despite its very restricted distribution, it is so distinctive that a name is warranted. A full description of this species is included in the chapter on New Species.

2. *Pholiota visco-fumosa* sp. nov.

This previously undescribed species lacks the typical scaly features found in most species of *Pholiota.* Its general appearance in the best conditions resembles a species of *Stropharia* with flocculose stipe. However, the colour of spore mass places it in the

of *Stropharia* with flocculose stipe. However, the colour of spore mass places it in the Pholiotoideae. The presence of subcellular hypodermium indicates some affinity with subgenus *Phaeonaematoloma* and is considered best included there at this stage. This species will be formally described in the chapter on New Species.

3. *Pholiota malicola* (Kauff.) A. H. Smith, *Ann. Myc.* 32: 480, 1934.

Flammula sulphurea Peck, *New York State Mus. Bull.* 157: 26, 1912.

(non *F. sulphurea* Massee, 1902)

Flammula alnicola var. *marginalis* Peck, *New York State Mus. Ann. Rept.* 54: 167, 1901. *Flammula malicola* Kauffman, *Amer. J. Bot.* 13: 24, 1926.

Illustrations: Figs. 7.41 - 46.

Material examined: see Appendix IIIE.

Observations

Morphological characters conform well to those observed by Hongo & Mills (1988). Some variations are noted in the colour of pileus, from waxy yellow to dull orange brown. Within the relatively wide range of morphologies no evidence could be found to suggest the existence of more than one species. There appears an association with disturbance in habitat which has not been previously reported, this disturbance may be caused by man, or may be a natural occurrence e.g. this fungus is found on the upturned root-ball of windblown *Eucalyptus* species.

4. *Pholiota aurivella* (Fr.) Kummer, *Der Fuhrer in die Pilzkunde*:.83, 1871.

Pholiota adiposa (Batsch ex Fr.) Kummer, *Der Führer in die Pilzkunde*, 1871, sensu Batsch p.p., Fr., Jacobsson in *Windahlia* 19: 26, 1990, non Ricken & J. Lange.

Illustrations: Figs. 7.47 - 49.

Material examined:

U.S.A., Washington, on alder, Smith13193 (MICH); Michigan, on dead *Quercus*

rubra stump, Mazzer6018 (MICH); Alaska, Wells-Kempton 8/24/64-3 (MICH); Minnesota, on wood in deciduous woods, Weaver1040 (MICH); Tenneessee, on elm, Hesler12508 (MICH).

AUSTRALIA, Tasmania, see Appendix IIIE.

Observations

This study presents the first report of its occurrence in Tasmania. The Tasmanian specimens corresponded well to the description of *P. aurivella* in Jacobsson (1990), and to a lesser extent in Smith & Hesler (1968) and Farr *et. al.* (1977). Its distribution seems to be restricted in the wetter regions of temperate rainforests of *Nothofagus - Atherosperma* dominance. It is almost always found growing on damaged or rotten trunks of *A. moschatum* (sassafras) and *N. cunninghamii* (myrtle beech).

5. *Pholiota squarrosipes* Clel., *Proc. Roy Soc. S. Aust.* 57, p190. 1933.

Type citation: Cleland did not specify the type in the protologue. Thus, there is a need for lectotypication.

Lectotype (selected here): South Australia, Encounter Bay, 9. v. 1931, AD12142!

Syntypes: South Australia, Encounter Bay, 25. v. 1928, AD11954! South Australia, Upper Tunkahlla (sic) Creek, 4.vi. 1930, AD12050! South Australia, Encounter Bay, 9. v. 1931, AD11958! South Australia, Back Valley, Encounter Bay, 24. v. 1933, AD12200!

Selected illustrations: Shepherd & Totterdell (1988), p. 89; and Cole *et al* (1984), plate 5 (as *Pholiota* sp).

Illustrations: Figs. 7. 50 - 57, 59 - 61.

The following is a description of the typical form of this species occurring in Tasmania:

Pileus 20 - 50 mm. broad, at first convex then subumbonate or plano-convex to almost plane in fully expanded basidiomes; viscid to slimy viscid, fine squamules scattered on

surface otherwise glabrous; velar remnants appendiculate along margin of pileus; margin slightly incurved; fulvous or honey brown (5D6) or pale golden brown (5D7) throughout or dull brown (6E5), darker at disc, deeper colour when young tending to dull brown (6E6) or dark brown(7F6). *Lamellae* adnate or adnate with a tooth, slightly decurrent when fully expanded, greyish yellow. (4C4) when veil breaks, becoming browner (5D6 to 6E5) with spores, moderately broad (3-5 mm.). *Stipe* 30 - 73 x 3 - 7 mm., scaly immediately below veil line, becoming coarsely fibrillose towards base, ± equal to slightly flexuose, white mycelium at base, hollow, creamy white (4A3) above velar zone, becoming brown (6E5-6) towards base. *Context* pale yellow (3A3), thin to thick and firm. *Veil* firm cortina or submembranous, pale cinnamon 5D5) to concolorous with pileus, forming a semi-persistent ring, evanescent.

Spores cocoa or snuff brown (6E5-7) in mass, 6.2 - 8.3 (-9.2) x 4.2 - 5 x 4.2 - 5µ, ovate to subellipsoid in face view, inequilateral to bean shape in profile, smooth, melleous brown in 5%KOH, germ pore minute but distinct. *Basidia* 4-spored, sterigmata up to 5µ in length, 20 -30.8 x 6.7 - 7.9µ, clavate or obovate, yellowish brown near edge of lamellae otherwise hyaline. *Pleurocystidia* as chrysocystidia, 26.7 - 51.7 x 8.3 - 13.3 (-15)µ, mucronate or ventricose with prominent apical protuberance or an obtuse apex, generally with amorphous body within or contents golden brown throughout in 5%KOH or highly granular. *Cheilocystidia* 24.2 - 33.3 x 4.5 - 7.5µ, forming a ±sterile band, hyaline or pale yellowish brown, intermixed with chrysocystidioid forms, elongate clavate or lageniform. Caulocystidia scattered, variable in shape and size, hyaline or with contents similar to chrysocystidia.

Subhymenium of gelatinised filamentous hyphae. *Trama* regular, turning yellowish brown in 5%KOH, refringent (or oleiferous) hyphae scattered amongst the hyaline ones, hyphae 4 - 24 µm. broad. *Epicutis* of repent hyphae, heavily incrusted, with brown pigments, lying over smooth-walled hyphae, 3 - 12 µm. broad, clamp connections present in all the above tissues.

Habit & Habitat gregarious to caespitose on ground amongst grass and mosses, on

bare ground amongst rocks or on general ground litter of leafy or woody debris, most frequently in disturbed sites such as parks and roadside.

Two different forms of this species are also noted. But since only single collection is obtained for each form, more material is necessary before assigning variety status to either of them. However, the differences noted between these two forms and the typical form warrant special mention and they are discussed separately below.

Form A (based on CYS458)

Illustrations: Figs. 7.53 & 60.

This form has a stronger yellow tone in the colour (golden to yellowish brown, 5D7-8) of the pileus and slightly stouter in stature. The main difference in microscopic morphology is in the shape of chrysocystidia where the apical protuberance is at least one third to half the length of the main body (a range of 5.8 - 22.9 µm.). Unlike other collections, all the basidiomes in this collection show consistency in this character. Other morphological characters are similar to the typical form.

Form B (based on CYS424)

Illustrations: Fig. 7.54.

The macromorphology of this form is indistinct from the typical form. However, it differs from the typical form in the shapes and sizes of chrysocystidia, cheilocystidia and basidia. Chrysocystidia generally have very elongated basal regions measuring up to one third of the total length (48.3 - 63.3 [69.2] x 8.3 - 11.7 µm. [mean of 10 = 52.58 x 9.88 µm.]). Similar variation is noted in cheilocystidia (25.8 - 44.2 x 5 -7.9 µm. [mean of 10 = 32.04 x 6.04 µm.]). Basidia are longer than those in the typical form reaching a length of 32.5 µm. These differences noted are again consistent within the collection.

Material examined: see Appendix IIIE.

Observations

AD12142 is selected as the lectotype because it is closest to the typical form found in fresh collections examined.

P. squarrosipes has not been previously reported from Tasmania. It has a wide distribution in Tasmania. This study also shows that *P. squarrosipes* is variable in its macromorphology as seen in the different colour tones of the pileus as well as the various surface features. Variations in micromorphology are represented by two other forms of this species and exemplified in the long-snouted form and slender elongate based form of chrysocystidia. The adaptability of this species to different habitats is demonstrated in its wide habitat range.

6. *Pholiota* taxon 1 (CYS14)

Illustration: Fig. 7.58.

Pileus 27 - 52mm broad, umbonate, viscid, glabrous, browner shade of buff (between 4B5 & 5B5). *Lamellae* sinuate or adnate with a tooth, pale buff (4A3), up to 5 mm. broad, close. *Stipe* 50 -80 x 2 - 5 mm., equal, scaly near base, concolorous with pileus. *Context* whitish, more or less firm. *Veil* cortinate, evanescent.
Spores snuff brown (6E5) in mass, 6.7 - 8.3 x 4.2 - 5 x 4.2 - 4.6 µm. (mean [25/1] = 7.22 x 4.52 x 4.33 µm.), smooth, melleous (5%KOH), ovate to subellipsoid in face view, slightly elongate or bean shape in profile, germ pore distinct. *Basidia* 20.8 - 25 x 7.5 - 8.3 µm., 4-spored, generally hyaline, tending to brown near gill edge. *Pleurocystidia* as chrysocystidia, 41.7 - 60 (66.7) x 11.7 - 17.5 µm., with yellow amorphous body (5%KOH) within, ventricose or mucronate,with a more or less prominent apical portion. *Cheilocystidia* 22.5 - 36.7 x 5.8 - 7.5 µm., hyaline or with yellowish brown contents, clavate or subutriform. *Caulocystidia* in clusters, hyaline or

brown, clavate.

Habit & Habitat Solitary on leafy debris.

Material examined: Tasmania, Arve Loop, off Arve Road, near Geeveston, 21. vi. 1988, CYS14.

Observations

This taxon is only separated from *P. squarrosipes* through isozyme and mating studies, otherwise it is morphologically indistinct from the Tasmanian material of *P. squarrosipes*.

7. *Pholiota highlandensis* (Peck) Smith & Hesler, *The North American species of* Pholiota, p.287, figs. 330, 332 - 335; pls. 67a, 70b, 72; 1968.

Agaricus carbonarius Fries, *Obs. Myc.* 2: 33, 1818.

Flammula carbonaria (Fr.) Kummer, *Der Fuhrer in die Pilzkunde*, p.82, 1871.

Flammula highlandensis (Peck) Peck, *New York State Mus. Ann. Rept.* 50: 138, 1897.

Gymnopilus carbonarius (Fr.) Murrill, *Mycologia* 4: 256, 1912.

Pholiota carbonaria (Fr.) Singer, *Agaricales*, p.517, 1951.

Dryophila carbonaria (Fr.) Quélet, *Enchir. Fung.*, p.70, 1886.

Illustrations: Figs. 7.62 - 66.

Material examined: see Appendix IIIE.

Observations

Tasmanian specimens correspond in most aspects to the description of *P. highlandensis* except for the variations noted in the veil. The pallid or pale cinnamon brown veil is one of the characters that separates this species from *P. carbonaria* A. H. Smith which has a

ferruginous red veil. This veil or remnants of it is not always obvious in the Tasmanian specimens, however, when present it is noted to be pale cinnamon or remnants of it appearing as russet brown fibrillose patches on the stipe and margin of pileus. Variations are noted in pleurocystidia in both shape and wall thickness. Variation is also noted in the apex of cystidia, from simple, obtuse to variously branched (usually bifurcate).

8. *Pholiota multicingulata* Horak, *Aust. J. Bot. suppl.* 10: 33 (1983)
Illustratiopns: Figs. 7.67 - 71, 74 & 75.

Pileus 14 - 34 mm. broad, more conical or convex when young, then subumbonate to plano-convex; viscid to slimy viscid when moist; virgate with dark brown streaks or with concentric rings of broad appressed scales otherwise glabrous; dark brown (7F6) at disc, paler brown (7E6 to 6E5) towards periphery, margin slightly incurled. *Lamellae* broadly adnate, ivory coloured (4B3) then becoming browner (5D4 to 6D4-5) with spores, broad (up to 6 mm. wide), crowded, 3-5 tiers between, 33 reaching stipe. *Stipe* 26 - 38 x 4 - 5 (11!) mm., ±cylindric, rings of dark brown scales below veil line, more pronounced when young and fresh, becoming less obvious with age, stuffed at first then hollow, white mycelia at base, dingy brown (close to dingy 6E6 to 7E6) near base, paling towards apex, brown rhizomorphs at base. *Context* yellowish white (3A2), thick, firm. *Veil* cortinate, pale yellow to whitish.
Spores deep snuff brown (6E6-8) to dark brown (7F8) in mass, 7.5 -9.2 (-10) x (4.6-0 5 - 5.8 (6.2) x 5 - 5.8µ, smooth, ellipsoid in face view, slightly inequilateral in profile, melleous brown in 5%KOH, germ pore minute and inconspicuous. *Basidia* 4-spored, 20 -27.5 (30.8) x 7.1 -8.3µ, clavate or cylindric clavate, sterigmata up to 6µ in length, hyaline or pale yellow brown (5%KOH) near edge of lamellae. *Pleurocystidia* projecting, 57.5 - 72.2 x 10 -20.8µ, fusoid ventricose with obtuse or bifurcate apex, wall thickness variable (<0.5 - 2µ), hyaline or with yellow brown colloidal content or

with yellow brown plug in neck region, amorphous collar on the exterior around base of neck, occasional amorphous cap over apex, pedicel long or short, clamp connections at base. *Cheilocystidia* (37.5-) 40 - 63.3 x 13.3 - 18.3 (23.3) µm., similar in shape and contents to pleurocystidia, forming a loose sterile band, thin- or thick-walled, amorphous collar or cap present in some. *Caulocystidia* in clusters, clavate, majority thin-walled.

Subhymenium of gelatinised filamentous hyphae. *Trama* ±regular, hyphae 4 -24 (-26) µm. broad, yellow brown in 5%KOH. *Epicutis* a layer of repent, incrusted hyphae, brown pigments in walls, 4 -8µ broad, clamp connections present.

Habit & Habitat solitary or scattered to gregarious or subcaespitose on ground, leafy or woody litter, rotten wood, eucalypt wood chips or pine bark mulch.

Material examined: see Appendix IIIE.

Observations

This is the first report of *P. multicingulata* in Tasmania. It is very widespread, growing on ground litter or rotten wood in both wetter and dryer regions of temperate rainforests as well as the wetter regions of wet sclerophyll. It is also found on eucalypt wood chips and pine bark mulch in cultivated patches in garden. It is first suggested from this study that this is a variable species in the same sense as *P. spumosa* in the northern hemisphere. Its affinity with *P. austrospumosa* is demonstrated in the morphometric studies.

9. *Pholiota pallidocaulis* sp. nov.

This is a relatively common fungus growing on rotten wood or woody debris on the forest floor in temperate rainforest or wet sclerophyll. This hitherto undescribed species is rather inconspicuous amongst the fungal flora in similar habitats. Its most distinctive features are the pallid stipe and conspicuous yellow rhizomorph. The colour of pileus varies from pale cinnamon brown to pale orange brown. It will be formally described in

the chapter on New Species.

10. *Pholiota* taxon 2

Illustration: Fig.7.72.

Pileus 21 - 31 mm. broad, broadly umbonate to plano-convex, viscid when moist, glabrous, topaz or brownish orange (close to 5C5),browner (6E6) at disc. *Lamellae* adnexed, greyish yellow (4B4) becoming browner with spores, close. *Stipe* 26 - 32 x 2 - 3 mm., more or less equal, slightly broadening at base, fibrillose, yellowish white (close to 4A2) darkening when bruised, white mycelium at base, with yellow rhizomorph. *Context* whitish, olivaceous in $FeSO_4$, slow reaction in Melzer's. *Veil* evanescent.

Spores mustard brown (5E6) in mass, 7.5 - 8.3 (-9.2) x 5 - 5.8 x 5 - 5.8 (-6.2) µm. (mean [25/1] = 7.83 x 5.75 x 5.59 µm.), ellipsoid in both face and side view, smooth, melleous brown, thick-walled, germ pore minute but visible under oil immersion.

Basidia 17.5 - 23.3 (-24.2) x 7.5 - 10 (-10.8) µm., 4-spored, sterigmata up to 5.8 µm. long. *Pleurocystidia* 53.3 - 78 (-80.8) x 10 - 20.8 µm., projecting, wall in ventricose region up to 1.2 µm. thick, hyaline or with yellowish brown colloidal contents, fusoid ventricose, apex obtuse or branched. *Cheilocystidia* 24.2 - 54.2 x 9.2 - 19.2 µm., hyaline or with yellow brown colloidal contents, thin-walled, fusoid ventricose. *Caulocystidia* broadly fusoid or irregularly shaped, thin- or thick-walled, hyaline or with yellow colloidal contents.

Subhymenium slightly gelatinised, defined layer up to 20 µm. thick. *Trama* regular, hyphae 12 - 22 µm. broad. *Epicutis* of repent hyphae incrusted with yellow brown pigments, clamped. *Hypodermium* of broader hyphae.

Habit & Habitat Solitary of subgregarious on ground.

Material examined Tasmania, Snug Falls Track, 26. vi. 1989, CYS271.

Observations

Like *Pholiota* taxon 1, this taxon was previously grouped together with *Pholiota pallidocaulis* and is separable only through isozyme and mating studies. It bears similarities to specimens in *Pholiota pallidocaulis* and is morphologically indistinguishable from them.

11. *Pholiota* taxon 3

Illustration: Fig. 7.73.

Pileus 13 -21 mm. broad, convex to plano-convex or broadly umbonate, viscid, three concentric rings of brown squamules near disc, light brown (close to 6D5), darker when young. *Lamellae* adnate, buff (close to 4A3), becoming browner with spores, close, edges even. *Stipe* 27 - 45 x 1.5 - 2 mm., equal, hollow, superior evanescent fibrillose zone, pale yellow (3A2) just above the fibrillose zone becoming browner (6D6) near base. *Context* moderately thin.

Spores chestnut brown (6F7 - 8) in mass, 7.5 -8.3 (9.2) x 5 - 5.8 (6.2) x 5 - 5.8 µm., smooth, melleous brown, elliptic in face view, slightly inequilateral in profile, germ pore distinct but apex not truncate. *Basidia* 18.3 - 23.3 x 7.9 - 10.8 µm.., 4-spored, clavate to obovate. *Pleurocystidia* 55.8 - 70 x 17.5 - 29.2 µm., projecting, with golden brown contents in 5%KOH or hyaline,fusoid ventricose, wall thicken in ventricose region, up to 2.5 µm., rarely <0.8 µm.. *Cheilocystidia* 20 - 44.2 x 10.8 - 21.7 µm., majority with thin wall, some with wall up to 1.2 µm., hyaline or with pale yellow homogenous contents, similar in shape to pleurocystidia but broader in the ventricose region and either sessile or short pedicellate. *Caulocystidia* scattered, 44.2 - 76.7 x 15 - 25.4 µm.., similar in shape to pleurocystidia, with thick or thin wall, generally with golden brown contents or golden brown amorphous body.

Subhymenium filamentous, gelatinised. *Trama* regular. *Epicutis* repent hyphae,

gelatinised,clamp connections present.

Habit & Habitat scattered on fallen manfern trunk.

Material studied: Tasmania, NW coast, Milkshakes Hill Reserve, picnic area, 16. vi. 1989, CYS257.

Observations

As a result of the thick-walled pleurocystidia, this taxon is placed in section Flammuloides of subgenus *Flammuloides*. It appears close to *P. subminor* Smith & Hesler in the thick-walled pleurocystidia. Since only a single collection was made, it will not be formally described until additional collections are available to establish the consistency of the wall character of pleurocystidia and other likely variations and its distribution.

12. *Pholiota* taxon 4

Illustration: Figs. 7.76-77.

Pileus (28-)32 - 86 mm. broad, conical to convex when young, then broadly umbonate to almost plane, virgate, slimy viscid with a thin gluten layer, dark brown (7F6) at disc, hazel or rust brown (6E8) throughout. *Lamellae* broadly adnate, greyish yellow (4B5) at first, then becoming oak brown (5D6) with spores. *Stipe* 30 -39 x 9 -11 mm., ±cylindric, light brown (6D5) becoming darker (7F6) towards base, stuffed at first then becoming hollow with age, white mycelia at base. *Context* watery beige (4C3), thick, firm, olivaceous in $FeSO_4$, yellow in 5%KOH, tawny in Melzer's. *Veil* cortinate, glutinous, pale yellow.

Spores snuff brown (6E6) in mass, 7.5 - 8.3 (8.7) x 5 - 5.4 x 4.6 - 5.4 µm., smooth , melleous brown in 5%KOH, ellipsoid in both face and side view, germ pore minute and

inconspicuous. *Basidia* 4-spored, 24.2 - 40.8 x 7.5 - 10.8 µm.,clavate to cylindric clavate. *Pleurocystidia* projecting, 48.5 - 67.5 x 15 - 19.2 µm., fusoid ventricose, apex obtuse, wall thicken in some in ventricose region, majority thin-walled, hyaline or with yellow brown colloidal content (5%KOH). *Cheilocystidia* 28.3 - 37.5 x 8.3 - 14.2 µm., forming a loose sterile band, thin-walled, hyaline, clavate. *Subhymenium* a region of slightly gelatinised, filamentous hyphae . *Trama* regular, hyphae 4 - 18µ broad, appearing pale yellow in 5%KOH (Check!). *Epicutis* a layer of gelatinised, repent hyphae over undifferentiated broader hyphae. Clamp connections present.

Material examined: Tasmania, Hobart, campus of Uni. of Tasmania, on eucalypt wood chips, A. K. Mills, 20 vi. 1989, CYS266.

Observations

This taxon differs from *P. multicingulata* in the glutinous veil and the darker shade of brown colour of the pileus. The microscopic characters are very similar to *P. multicingulata* differing only minimally in the slightly smaller and narrower spores.

Table 7.1. Systematic treatments of the genus *Pholiota*.

Singer (1986)	
Genus	Pholiota
Subgenus	1. Hemipholiota Singer ex Singer
Sections	Destruentes Konr. & Maubl., Sordidae Singer, Myxannulatae Hongo, Albocrenulatae Singer
Subgenus	2. Pholiota
Sections	Flavidula Smith & Hesler, Pholiota, Adiposae Konr. & Maubl.
Subgenus	3. Flammula (Fr.) Singer
Sections	Udae (Fr.) Singer, Albivelatae Smith & Hesler, Subsiccae (Lange) Singer, Delubricatae Singer, Lubricae (Fr.) Singer
Subgenus	4. Phaeonaematoloma (Singer) Singer
Sections	Glutinigerae Singer, Novembres Singer
Subgenus	5. Plocoloma Singer
Smith & Hesler (1968)	
Genus	Pholiota
Subgenus	1. Flavidula Smith & Hesler
Section	Flavidula
Subgenus	2. Hygrotrama Smith & Hesler
Sections	Confragosae (Singer) Smith & Hesler, Hygrotrama.
Subgenus	3. Hemipholiota
Sections	Sordidae, Mutabiles, Hemipholiota, Variabilisporae Smith & Hesler.
Subgenus	4. Phaeonaematoloma
Sections	Albivelatae Smith & Hesler, Phaeonaematoloma (Singer) Singer.
Subgenus	5. Flammula
Section	Flammula.
Subgenus	6 Pholiota
Sections	Pholiota, Adiposae.
Subgenus	7. Flammuloides Smith & Hesler
Sections	Flammuloides, Carbonicola Smith & Hesler, Spumosae Smith & Hesler, Lubricae.

Table 7.2. Summary of macrocharacters of the two taxa of *Pholiota* in subgenus *Paeonaematoloma*. Colour of pileus is from fresh specimens and that of lamellae is taken from young basidiome whenever possible in particular when the veil has just broken.

Character	*Pholiota* sp A	*Pholiota* sp B
Pileus		
viscidity	glutinous with a layer of gluten	glutinous, viscid or tacky, sometimes with a layer of gluten
colour	dark brown (6E7) at disc, golden with an olivaceous tinge (4C6) throughout when moist drying to ochraceous buff (close to 4A4)	dark blond (4C3-4) to clay (5D4-5) or smoky grey (3C2) or greyish yellow (4B4) light brown (6D4) when young
surface	glabrous, striate	with concentric rings of whitish squamules at disc easily abraded then appearing glabrous
diameter	25 - 32 mm.	(12) 22 - 60 mm.
Lamellae		
attachment	depressed adnate to adnexed	broadly adnate
colour	yellowish grey (4C5) becoming browner with spores	dull to greyish yellow (3B3-3C4)
Stipe	slimy viscid with lower two-thirds covered with a layer of gluten, glabrous	dry, whitish flocculose to coarsely fibrillose and appearing sordidly coloured with age
l x w	3 - 4.5 x 38 - 42 mm.	2 - 6 x (23) 38 - 87 mm.
Spore print	cigar brown (6E6)	dark brown (6F6-7)
Habitat & Growth habit	solitary to scattered on moss-covered ground in shady areas of temperate rainforests	subcaespitose to subgregarious on ground litter or rotten wood or amongst mosses in sheltered areas of temperate rainforests

Table 7.3. Summary of microcharacters of the two taxa of *Pholiota* in subgenus *Phaeonaematoloma*. Mean and standard deviation are given for measurements of length (l), width (w), facial width of spores (f) and profile width (p)in µm. where n equals the number of collections used to calculate the mean.

Character	*Pholiota* sp A (n=2)	*Pholiota* sp B (n=7)
Spores	truncate, thick-walled (->0.8 µm.) elongate ellipsoid	truncate, wall up to o.4 µm. thick elongate ellipsoid in face view to slightly inequilateral in profile
l x f x p (µm)	10.02 ±0.61 x 6.10 ±0.29 x 5.89 ± 0.34	10.25 ±0.59 x 6.23 ±0.42 x 6.17 x ±0.42
Basidia	4-spored	4-spored, sterigmata slightly swollen at base
l x w (µm.)	25.02 ± 2.42 x 8.60 ±0.56	26.88 ±2.13 x 8.46 ±0.70
Pleurocystidia	2 types:	as chrysocystidia only
a) leptocystidia		
l x w (µm.)	56.04 ±7.86 x 15.75 ± 3.59	
b) chrysocystidia		
l x w (µm.)	45.85 ± 3.78 x 12.23 ±1.52	43.39 ± 5.98 x 14.14±1.97
Cheilocystidia	hyaline, forming a ±sterile band	hyaline, forming a ±sterile band
l x w	27.86 ±4.40 x 8.15 ± 0.89	40.55 ±7.56 x 7.74 ± 1.28
Caulocystidia	in clusters above veil line, hyaline or with pale yellow wrinkled content	in clusters, similar to cheilocystidia in shape but more variable, hyaline

Table 7.4. Summary of macrocharacters of selected collections of *Pholiota malicola* from Tasmania.

	CYS20	CYS171	CYS180	CYS226	CYS334	CYS383
Pileus						
colour	waxy straw yellow (close to 3B5)	amber yellow (4B5-6), pale orange brown at diac	orange brown (between 5B5 & 6B6)	dull honey colour (dull shade of 4C6)	light yellow (4A5) cinnamon brown (6D6) at disc	dull orange brown (between 5B5 & 6B6)
surface	greasy & hygrophanous	greasy to tacky, hygrophanous	greasy hygrophanous	±dry hygrophanous	hygrophanous	
diameter	-> 95 mm. across	->120 mm. across	->78 mm. across	->107 mm. across	->80 mm. across	->165 mm. across
Veil	white cortina, evanescent	-	white cortina, evanescent	white cortina evanescent	whitish cortina evanescent	whitish cortina evanescent
Lamellae						
attachment	adnate	adnate	adnate	adnate	adnate	adnate
colour when young	straw coloured (between 3A4 & 3B4)	pale yellow (3A3)	pale buff (close to 4B4)	pallid (2A2)	light yellow (3A3)	-
Stipe						
colour	pale yellow (2A2 -3) becoming dingy brown near base	whitish when young becoming dingy brown with age near base	pale straw (3A3) becoming dingy brown near base	dull yellow (3B3) becoming dingy brown near base	pale yellow (3A3) above velar zone becoming dingy brown near base	dingy brown
l x w (mm.)	86-160 x 10-12	8-15 mm. thick & ->31.5 mm. long	15-27 x 5-11	58-150 x 5-12	-	76-145 x 18-52
Growth habit & Habitat	caespitose on ground by roadside	gregarious on ground by side of track	caespitose on ground, sheltered	caespitose on ground amongst grass	caespitose on ground under *Eucalyptus delegatensis*, by roadside, ±exposed	caespitose on stony ground, ±exposed

Table 7.5. Summary of major microcharacters of collections of *Pholiota malicola* from Tasmania.

Collections	Spores	Basidia	Cheilocystidia
CYS20	smooth, wall thin, germ pore minute but distinct elliptic in face view, inequilateral in profile tawny in Melzer's	majority 4-spored, more rarely 2-spored	hyaline, clavate with rounded apex or mucronate
	7.80 x 4.40 x 4.31	24.83 x 7.88	33.25 x 10.65
	±0.41 ±0.22 ±0.17	±1.79 ±0.82	±3.37 ±1.51
CYS171	as in CYS20	majority 4-spored	hyaline, clavate with rounded apex
	7.82 x 4.76 x 4.56	26.00 x 7.33	29.04 x 7.17
	±0.47 ±0.20 ±0.20	±1.76 ±0.53	±1.86 ±0.61
CYS177	as in CYS20 epiculus evident, tawny in Melzer's	majority 4-spored	hyaline, elongate clavate
	7.28 x 4.40 x 4.30	26.50 x 6.77	33.00 x 6.46
	±0.20 ±0.22 ±0.19	±1.56 ±0.52	±3.33 ±1.03
CYS180	as in CYS177	majority 4-spored	hyaline, elongate clavate or variable in shape
	7.94 x 4.77 x 4.45	26.17 x 8.25	33.83 x 8.96
	±0.31 ±0.23 ±0.24	±2.26 ±0.87	±3.75 ±1.83
CYS226	as in CYS177	majority 4-spored	hyaline, clavate or variable in shape
	7.37 x 4.51 x 4.34	26.79 x 7.25	33.12 x 7.25
	±0.33 ±0.25 ±0.21	±1.80 ±0.63	±4.89 ±1.33
CYS334	as in CYS177	majority 4-spored	hyaline, thin-walled, clavate or varying in shape
	7.89 x 4.80 x 4.73	26.83 x 7.94	33.63 x 7.50
	±0.34 ±0.20 ±0.14	±2.14 ±0.44	±4.26 ±1.79
CYS357	as in CYS177	majority 2-spored more infrequently 4-spored or 1-spored	hyaline, elongate clavate or clavate mucronate or varying in shape
	7.38 x 4.50 x 4.27	26.67 x 7.77	33.83 x 9.31
	±0.46 ±0.21 ±0.14	±4.41 ±0.57	±4.11 ±2.06
CYS383	as in CYS177	majority 4-spored also 2-spored	hyaline, elongate clavate or obovate
	7.07 x 4.60 x 4.41	26.87 x 8.67	36.81 x 12.02
	±0.37 ±0.26 ±0.22	±2.32 ±0.37	±2.18 ±1.93

Table 7.6. A summary of the major macrocharacters and habitats of the Tasmanian specimens of *P. aurivella* included in morphological studies. Colour given for lamellae is from young carpophores unless specified otherwise.

Collection	Pileus	Lamellae	Stipe	Habitat
CYS21	Bright orange brown, glutinous viscid dark brown scales scattered in in gluten 95-145 mm.	Adnate with a tooth, slightly emarginate yellow	Scaly especially below velar zone, recurved scales dry	small clusters on sassafras
CYS116	Lemon yellow glutinous viscid dark brown scales in gluten layer 62 mm.	adnate with a tooth pallid or pale buff	more scaly near base dry	solitary on fallen sassafras
CYS128	lemon yellow, orange brown when young glutinous viscid tawny brown scales 100-105 mm.	adnate & slightly emarginate greyish yellow	scaly immediately below velar zone, scales whitish dry	small clusters on fallen sassafras log
CYS154	Bight lemon yellow glutinous viscid rusty brown recurved scales 48-62 mm.	adnate & slightly emarginate pallid then becoming cocoa brown with spores	scaly immediately below velar zone, pallid to pale straw recurved scales	solitary or in small on dead sassafras
CYS157	dull lemon yellow shiny gluten layer 6-8 concentric rings of tawny brown scales 90-97 mm.	adnate with a tooth, slightly emarginate pallid	coarsely fibrillose when mature more scaly when young dry	small clusters on dead myrtle stump
CYS159	bright ochraceous yellow, deep orange brown when young glutinous viscid dark tawny brown scales 52-70 mm.	adnexed pallid	recurved scales immediately below velar zone dry	small clusters on dead myrtle stump
CYS181	Ochre yellow glutinous viscid tawny brown scales in gluten layer 80-98 mm.	emarginate pallid then becoming ferruginous brown with spores	sclay immediately below velar zone tawny brown near base dry	solitary on fallen myrtle trunk
CYS324	ochre yellow to orange brown glutinous viscid tawny brown scales in gluten layer 75-115 mm.	emarginate ferruginous brown with spores	whitish sclaes near base ±fibrillose above dry	in clusters on damaged, living sassafras, high up on trunk
CYS325	Light yellow glutinous viscid tawny brown scales in gluten layer, very compact at disc, spreading towards the periphery 75-120 mm.	adnate pale yellow then becoming light brownish with spores	±fibrillose ferruginous brown dry	in clusters on damaged sassafras, same host as CYSS21

Table 7.7. A summary of microcharacters of Tasmanian specimens of *P. aurivella* including range, mean and standard deviation. Legend: l=length; w=width at the broadest part; f=width of spore in face view; and p=width of spore in profile. Colour given for individual spore is observed in 5%KOH, colour code for spore mass is according to Methuen (K & W 1978).

Collection	Spore (l x f x p)	Basidia (l x w)	Chrysocystidia (l.x w)	Cheilocystidia (l x w)
CYS21	dark brown (6F7-8) in mass melleous brwon germ pore minute. but distinct	4-spored	with amorphous body or with yellow brown necropigments throughout	hyaline or with yellow brown nercopigments throughout intermixed with chrysocystidioid forms
	7.82 x 5.39 x 5.11	22.02 x 7.50	41.15 x 12.75	34.55 x 10.14
	±0.36 ±0.25 ±0.19	±1.58 ±0.85	±3.84 ±1.12	±3.97 ±0.87
CYS116	rust brown (6E8) in mass melleous brown germ pore minute but distinct	4-spored	as above	as above
	7.60 x 5.06 x 5.02	20.67 x 7.17	37.71 x 11.67	33.25 x 10.62
	±0.39 ±0.19 ±0.23	±0.88 ±0.47	±4.67 ±1.03	±4.75 ±2.23
CYS128	in mass melleous brown germ pore minute but distinct	4-spored	as above	as above
	7.72 x 5.42 x 5.40	22.50 x 7.12	40.23 x 11.14	32.54 x 9.04
	±0.29 ±0.23 ±0.26	±1.36 ±0.42	±3.44 ±0.91	±3.36 ±1.16
CYS154	melleous brown germ pore minute but distinct	4-spored	as above	as above
	8.27 x 5.64 x 5.45	22.29 x 6.96	40.54 x 10.23	40.00 x 10.04
	±0.27 ±0.22 ±0.29	±1.16 ±0.34	±2.37 ±0.99	±5.68 ±1.29
CYS157	melleous brown germ pore minute butdistinct	4-spored	as above	as above
	7.83 x 5.24 x 5.11	22.29 x 6.71	35.56 x 9.33	30.17 x 8.50
	±0.32 ±0.29 ±0.18	±1.02 ±0.46	±2.73 ±1.39	±2.32 ±1.36
CYS159	melleous brown germ pore minute but distinct	4-spored	as above	as above
	7.69 x 5.15 x 5.11	22.29 x 7.52	40.64 x 11.02	32.71 x 10.75
	±0.43 ±0.25 ±0.22	±1.59 ±0.77	±4.21 ±1.01	±3.16 ±2.11
CYS181	melleous brown germ pore minute but distinct	4-spored	as above	as above
	7.45 x 5.03 x 4.93	19.92 x 6.65	41.50 x 13.31	35.75 x 11.37
	±0.24 ±0.21 ±0.20	±1.07 ±0.48	±2.57 ±1.00	±4.74 ±1.71
CYS324	dark brown (6F8) in mass melleous brown germ pore minute but distinct	4-spored	as above	as above
	7.84 x 5.38 x 5.17	24.52 x 7.06	46.13 x 11.81	36.37 x 9.91
	±0.44 ±0.27 ±0.27	±1.67 ±0.57	±6.72 ±1.73	±5.01 ±1.87
CYS325	dark brown (6F8) in mass melleous brown germ pore minute but distinct	4-spored	as above	as above
	8.30 x 5.47 x 5.28	26.33 x 8.79	46.33 x 12.12	37.58 x 9.27
	±0.22 ±0.26 ±0.30	±0.83 ±0.42	±4.18 ±2.02	±6.20 ±1.28
CYS361	melleous brown germ pore minute but distinct	4-spored	as above	as above
	7.35 x 5.01 x 4.88	21.67 x 6.48	40.46 x 12.23	35.69 x 9.25
	±0.41 ±0.21 ±0.23	±1.76 ±0.39	±3.88 ±0.59	±3.94 ±1.37
CYS372	melleous brown germ pore minute but distinct	4-spored	as above	as above
	7.15 x 4.91 x 4.75	22.83 x 7.88	42.12 x 10.16	37.12 x 9.60
	±0.45 ±0.20 ±0.19	±2.12 ±1.08	±3.60 ±0.88	±5.17 ±1.98

Table 7.8. Selected morphological characters of *Pholiota aurivella* and closely related species from literature or examination of fresh or herbarium material.

Specimen	Spore range (µm.) l x w	Colour of young gills	Chrysocystidia	Host	Source
P. aurivella					
OKM3028	9 - 11.5 x 5.5 -6	yellow	+	hardwood	Farr *et al.* 1978
Smith13193	8.3 - 10 x 5 - 6.7	-	+	hardwood on alder	N America (MICH!)
Hesler12508	6.7 - 9.2 x 4.6 - 5.8	-	+	hardwood on elm	N. America (MICH!)
CYS21	7.3 - 8.7 x 4.8 - 5.8	pale sulphur yellow	+	hardwood on sassafras	Tasmania
CYS116	6.7 - 8.3 x 4.6 - 5.6	pale buff	+	hardwood on sassafras	Tasmania
CYS128	7.1 - 8.3 x 4.8 - 5.8	pallid	+	hardwood on sassafras	Tasmania
CYS154	7.9 - 8.7 x 5 - 5.8	pallid	+	hardwood on sassafras	Tasmania
CYS157	7.5 - 8.3 x 4.8 - 5.8	pallid	+	hardwood on myrtle	Tasmania
CYS159	7.1 - 9.0 x 4.8 - 5.6	pallid	+	hardwood on myrtle	Tasmania
CYS181	7.1 - 8.1 x 4.6 - 5.4	pallid	+	hardwood on myrtle	Tasmania
CYS324	7.5 - 9.0 x 5 - 5.8	?	+	hardwood on sassafras	Tasmania
CYS325	7.9 - 8.7 x 5 - 5.8	pale yellow	+	hardwood on sassafras	Tasmania
CYS361	6.7 - 8.1 x 4.6 - 5.4	?	+	hardwood ?	Tasmania
CYS372	6.7 - 8.3 x 4.4 - 5.4	?	+	hardwood ?	Tasmania
P. limonella	6.5 - 7.5 x 3.7 - 4.7	pallid	+	hardwood	Farr *et al.* 1978.
P. albietis	6.7 - 7.5 x 3.7 - 4.7	pallid	-*	conifer	Farr *et al.* 1978.
P. connata	7 - 9.3 x 4 - 5.5	yellow	+**	hardwood	Farr *et al.* 1978.

* Not observed by Farr and co-workers, but reported by Smith and Hesler.

** Not reported by Smith and Hesler, but observed by Farr and co-workers.

Table 7.9 Macroscopic characters and habitat of selected Tasmanian collections of *Pholiota squarrosipes*.

	CYS16	CYS14	CYS106	CYS424	CYS458	CYS496
Pileus						
colour	fulvous	buff	ochre brown	cinnamon brown	yellowish brown	cinnamon brown
surface	tacky, glabrous	viscid, glabrous	greasy when moist	viscid, ±glabrous	viscid, squamulose	slimy viscid, squamulose, then ±glabrous
margin	-	-	appendiculate with veil remnants	-	appendiculate with veil remnants	appendiculate with veil remnants
diam. (mm.)	31-34	27-52	15-33	18-32	16-40	20-30
Lamellae						
attachement	adnate	adnate with a tooth	adnate	adnate	adnate or with a tooth	adnexed
colour when young	pallid	pale buff	greyish buff	greyish yellow	greyish yellow (4B3)	greyish orange (5C3)
Stipe						
colour	pale fulvous	concolorous with pileus	pale snuff brown	very pale cinnamon brown	pale cinnamon brown	dingy corn colour
annulus	+	-	present at first, then evanescent	-	present at first, then evanescent	present, then evanescent
surface	scaly, recurved scales below ring	coarsely scaly towards base	scaly, recurved scales below ring	coarsely fibrillose towards base	scaly immediately below ring	scaly immediately below ring
l x w (mm.)	45-58 x 5	50-75 x 2-5	14-28 x 2-4	28-48 x 3-4	38-46 x 3.5-6	47-73 x 4-5
Habitat	on leafy debris amongst *Polytrichum*	on leafy debris	on clayey soil, fairly exposed	on ground amongst grass	on mossy ground in recently burnt area	on mossy ground amongst grass

Table 7.10. Comparison of morphological characters between syntype and Tasmanian material of *Pholiota squarrosipes*. * taken from Cleland's (1933) protologue.

	Syntypes	Tasmanian material
Pileus		
colour	waxy yellow brown, Raw Sienna III to Empire Yellow IV*, darker at disc and paling towards the periphery	golden or light brown (5E7) or cinnamon brown (5D5-6) at disc and yellowish brown (5C5-7) or honey brown towards the periphery
surface	nearly smooth, sometimes fibrillose	with pale-coloured squamules at first otherwise glabrous
margin	slightly wavy	at first appendiculate with veil remnants
Lamellae		
attachment	adnate or with decurrent tooth	adnate, with a tooth or adnexed
colour when young	near Saccardo's umber* or yellow ochre	pallid or greyish yellow
Stipe		
surface	shaggy with fibrillose scales more coarsely fibrillose near base	recurved scales below ring tending to coarsely fibrillose towards base
Habitat	on ground near eucalypts or wood	on woody or leafy litter, amongst grass or mosses in relatively sheltered areas to exposed clayey or rocky ground
Spore print	umber	coaco or snuff brown
Spore		
l x f x p (µm.), mean	6.99 x 4.53 x 4.46 ±0.37 ±0.23 ±0.25	6.73 x 4.51 x 4.40 ±0.44 ±0.27 ±0.26
Basidia		
l x w (µm.), mean	19.21 x 6.40 ±2.54 ±0.81	21.70 x 7.18 ±2.75 ±0.93
Chrysocystidia		
l x w (µm.), mean	38.96 x 11.95 ±5.80 ±1.86	39.55 x 12.20 ±6.72 ±2.08
Cheilocystidia		
l x w (µm.), mean	26.38 x 5.99 ±2.38 ±0.79	27.50 x 6.32 ±4.01 ±0.95

Table 7.11. Summary of major macrocharacters between taxa in the subgenus *Flammuloides* in SE Tasmania.

	P. highlandensis	P. multicingulata	Pholiota sp C	Phliota sp D	Pholiota sp E
Pileus					
colour	cinnamon to dark brown (6D6-7, 6E6-7 or 7E5-6) darker at disc	dark brown at disc (7F6) paling to light brown (5C5 or5D7) towards the periphery	buff (close to 4A3) throughout	varies from light yellow (4A4), beige (4C3), brownish orange (5C5-6) to raw sienna (6D5-7)	dark brown (7F6) at disc hazel brown (6E8) towards the periphery
shape	convex to plano-convex or broadly umbonate	conical when young, becoming convex, plano-convex or sub-umbonate or with a slight central depression	convex to broadly umbonate	convex, plano-convex or subumbonate	conical when young, convex to plano-convex or subumbonate
surface	virgate or glabrous	dark brown appressed scales in concentric rings or virgate or glabrous	three concentric rings of brown squamules at disc	compact rings of whitish squamules at disc otherwise glabrous	virgate with dark brown streaks
	tacky to viscid when moist	slimy viscid	viscid when moist	viscid when moist	slimy viscid with gluten layer
diameter	18-40 mm.	18 - 67 mm.	13-21 mm.	14 - 45 mm.	28-86 mm.
Veil	cortina, pale cinnamon or russet brown evanescent	firm cortina, pallid to pale yellow (4A3) evanescent	probably cortinate evanescent	cortina, pallid or cream coloured (4A3), evanescent	cortina, glutinous, pale yellow (close to 4A3)
Lamellae					
attachment	broadly adnate to adnexed	broadly adnate or adnate with a tooth	adnate	broadly adnate or adnexed	broadly adnate
colour	greyish yellow (4C4) becoming browner (5D4-5, or 6E4-5) with spores	greyish yellow (4B3 to 4C5) becoming brownish (5D5, 5E5)with spores	pallid (close to 4A2) becoming brown with spores	pale yellow (4B3) becoming brown with spores	greyish yellow (4B5) then becoming browner (5D5-6) with spores
Stipe					
colour	dull clay to cinnamon brown (5D5 to 6D5)	pale straw near apex becoming brownish towards base	pale straw near apex becoming light brown near base	pallid becoming sordidly coloured with age or bruised brownish	light brown (6D5) near apex darker brown (7F6) towrads base
shape	±equal or slightly flexuose, abrupt	equal or flexuose, base abrupt or sub-bulbous	±equal, abrupt	equal, slightly flexuose, base abrupt or sub-bulbous	slighly enlarged near base
surface	coarsely fibrillose immediately below veil line or reddish brown scales otherwise ±glabrous	fibrillose or with multiple brown squamulose zones near base, very pronounced when young	coarsely fibrillose otherwise glabrous	±glabrous	scabrous below velar zone
rhizomorph	+, white	+, brown	none	+, yellow	
l x w(mm.)	18-46 x 2.5-5	20-55 x 3-6 (11)	27-45 x 1.5-2	25-54 x 2-4	30-39 x 9-11
Spore print	snuff to hazel brown (6E5-7)	deep snuff brown (6E6-8) to dark brown (7F8)	snuff brown (6E5)	snuff brown (6E5)	snuff brown (6E6)

Table 7.12. Summary of major microcharacters between taxa of subgenus *Flammmuloides* from SE Tasmania. Mean measurements (in µm.) were given for each taxon where n = number of collections used to calculate the mean, l = length, f=spore facial width, p= spore profile width, w= width in the widest part, Q_f = ratio of mean spore length to mean spore facial width and Q_p = ratio of mean spore length to mean spore profile width. Only size range (in µm.) is given for caulocystidia due to the greater degree of variation.

	P. highlandensis (n = 8)	**P. multicingulata** (n = 25)	**Pholiota sp C** (n = 1)	**Pholiota sp D** (n = 16)	**Pholiota sp E** (n = 1)
Spores					
germ pore	minute & inconspicuous	minute & inconspicuous	distinct but not truncate	minute & inconspicuous	minute & inconspicuous
l x f x p	7.14 x 4.60 x 4.52	8.11 x 5.43 x 5.33	8.66 x 5.49 x 5.42	8.07 x 5.71 x 5.59	7.79 x 5.08 x 5.02
Q_f	1.55	1.49	1.58	1.41	1.53
Q_p	1.58	1.52	1.60	1.44	1.55
Basidia					
no. of sterigmata	4	4	4	4, more rarely 2	4
l x w	19.86 x 6.94	23.79 x 8.08	20.96 x 9.17	21.77 x 8.15	27.92 x 9.17
Pleurocystidia					
wall thickness	thin-walled	thin- or thick-walled	consistently thick-walled, up to 2.5µm	thin- or thick-walled	thin-walled
contents	hyaline and yellow brown	hyaline or with yellow brown plug in neck region or yellow brown throughout	hyaline or with homogenous yellow brown contents throughout	hyaline or yellow brown	hyaline or pale yellow brown throughout
shape	elongate lageniform	lageniform	lageniform	lageniform	lageniform
apex	obtuse, subacute single or bi- or tri-furcate	obtuse or rounded single or bifurcate	obtuse or slightly flattened or tapered	obtuse or rounded or subacute, single or bifurcate	obtuse or rounded single
l x w	55.85 x 12.10	66.50 x 17.24	68.50 x 21.46	64.23 x 17.23	59.53 x 17.00
Cheilocystidia					
wall thickness	thin-walled	thin- or thick-walled	thin- or thick-walled	thin- & thick-walled	thin-walled
contents	hyaline	hyaline	hyaline or yellow brown	hyaline or yellow brown	hyaline
	forming a ±sterile band	loose band inter-mixed with basidia	forming a ±sterile band	forming a ±sterile band	loose band inter-mixed with basidia
l x w	32.95 x 10.59	51.01 x 16.25	38.58 x 16.17	38.59 x 13.19	37.42 x 13.04
Caulocystidia	in clustrers	scarce to none	scarce & scattered	in clusters or rows	none
contents	hyaline or with yellow brown content	hyaline	with yellow brown refractive content	hyaline or with pale or yellow brown content	
shape	variable in shape & size	generally club-shaped	similar in shape to cheilocystidia but larger	variable in shape & size	
l x w	27.5-81.64 x 8.33-13.33	25.83-66.67(88) x 7.5-22.42	44.17-76.67 x 15-25.42	33.33-82.5 x 7.5-16.67(31.67)	

Table 7.13 Comparison of morphological characters between *P. multicingulata*, *P. austrospumosa* and *P. spumosa*.

	P. multicingulata	*P. austrospumosa*[1]	*P. spumosa*[2] (Scandinavian)	*P. spumosa*[3] (North America)
Pileus				
viscid	+ or ++	+	+ to ++	++ or +
colour	ochraceous or umber* dark brown at disc light or ochraceous brown towards margin	rust brown to light on disc, ochraceous along margin	orange to light brown at disc, pale or lemon yellow towards margin	brownish to tawny at disc olive ochre towards margin
	fibrillose or scaly*	with rust brown appressed scales	at scaly then glabrous or fibrillose	glabrous or fibrillose-streaked (virgate)
Lamellae				
attachment	emarginate* broadly adnate or with a tooth	adnate or adnexed	adnate to slightly emarginate with tooth	adnate to adnexed
colour	clay-coloured* greyish yellow	pale yellowish	pale to lemon yellow with an olive flush	"sulphur yellow" to "citron yellow" or pale greenish yellow
Veil	cortina, pallid to pale yellow	cortina, pallid yellow	-	yellow
	evanescent	evanescent	evanescent	evanescent
Stipe	multiple brownish fibrillose zones or coarsely fibrillose	fibrillose to squamulose	floccose with veil remnants when young ±glabrous with age	thin coating of yellow fibrils from veil
spores	deep snuff brown (6E6-8) in mass	colour in mass n/a	rusty brown in mass (6E7-8)	colour in mass n/a
	smooth, ovate with germ pore*	smooth, elliptic to ovate, somewhat inequilateral germ pore minute	smooth, ovoid-ellipsoid, only slightly inequilateral in profile, with germ pore	smooth, oblong to elliptic bean shaped to somewhat inequilateral in profile germ pore distinct
	6.5-8 x 4.5-5 µm.*	6.5-8 x 4.5-5.5 µm.	6-8(-9) x 3.5-4.5 µm.	7-9 x 4-4.5 µm.
Pleurocystidia				
projecting	+	+	+	+
shape	fusoid to lageniform with yellow brown covering*	fusoid ventricose neck broad, apex obtuse to rounded	lageniform fusiform neck often with thin coating of crystals	fusoid ventricose subacute to obtuse apex rarely with mucilaginous coating
wall	thin or thick-walled	thin-walled	n/a	thin-walled
content	hyaline or with yellow plug in neck region or yellowish brown throughout	homogenous and yellowish	hyaline, sometimes with yellow contents	hyaline to yellowish, colloidal in the neck
	40-70 x 10-15 µm.*	39-61 x 11-15.5 µm.	50-8- x 10-16 µm.	40-60 (68) x 7-14 (16) µm.
Habitat	on rotten wood of Mrytaceae* on ground litter or rotten wood in wet rainforest or on eucalypt wood chips	on decaying wood	on ground of coniferous or mixed forests, also on decaying stumps or logs of coniferous trees	on soil, in coniferous woods or on logs or stumps

[1] From protologue of *P. austrospumosa* (Hongo 1989).

[2] From description of Nordic specimens of *P. spumosa* (Jacobsson 1990).

[3] From description of North American specimens of *P. spumosa* (Smith & Hesler 1968).

* From protologue of *P. multicingulata* (Horak 1983).

Table 7.14. Percentage occurrence of major band activities of Lac, Per, PE and PG in isolates of *Pholiota malicola*.

Enzyme	Band	Rf	% occurrence
Lac	2	0.34	100
Per	2	0.37	33.3
	3	0.39	51.8
PE	1	0.04	51.8
	3	0.27	66.7
PG	1	0.04	40.7
	2	0.14	63
	6	0.47	51.8

Table 7.15. Percentage occurrence of major band activities of Lac, Per, PE and PG in isolates of *Pholiota aurivella*.

Enzyme	Band	Rf	% occurrence
Lac	2	0.37	62.5
	4	0.43	41.7
	5	0.47	45.8
	10	0.66	75
Per	1	0.51	25
	2	0.56	29.2
PG	1	0.08	66.7
	2	0.28	50
	3	0.31	100

Table 7.16. Percentage occurrence of major band activities of Lac, Per, PE and PG in isolates of *Pholiota squarrosipes.*

Enzyme	Band	Rf	% occurrence
Lac	2	0.23	51.7
	10	0.59	74.7
	11	0.61	44.8
PE	3	0.25	92.2
	7	0.39	41.1
PG	2	0.11	64.4
	5	0.46	46.7

Table 7.17. Percentage occurrence of dominant band activities in Lac and Per in isolates of collections of *Pholiota highlandensis.*

Enzyme	Band	R_f	% occurrence
Lac	4	0.37	98
	5	0.47	45
	6	0.49	51
PE	5	0.35	73
	6	0.40	45
	7	0.43	91
Per	7	0.36	29.1
	8	0.38	32.7
	11	0.49	32.7
PG	3	0.24	67
	4	0.28	45

Table 7.18. Percentage occurrence of dominant band activities in Lac, Per, PE and PG in isolates of collections in *P. multicingulata*.

Enzyme	Band	R_f	% occurrence	Enzyme	Band	R_f	% occurrence
Lac	4	0.42	19.1	**Per**	4	0.34	41.9
	5	0.46	88.3		6*	0.42	40.7
	6	0.48	80.8		7*	0.46	55.8
	7	0.51	65.9		8*	0.51	66.3
	8	0.54	21.3		9*	0.54	4.65
PE	1	0.18	36.5	**PG**	1	0.31	96.5
	3	0.35	28.2		3	0.38	23.5
	6	0.48	36.5		5	0.43	62.3
	7	0.51	40		6	0.47	28.2

Table 7.19. Percentage occurrence of dominant band activities in PE and PG in isolates of collections in *Pholiota* sp D.

Enzyme	Band	R_f	% occurrence	Enzyme	Band	R_f	% occurrence
Lac	2	0.32	79	**PE**	1	0.12	59.6
	4	0.40	56		2	0.37	80.8
	8	0.54	33.9		5	0.41	51.9
					9	0.57	82.7
Per	3	0.25	18.3	**PG**	2	0.22	57.7
	5	0.33	85		3	0.31	25
	6	0.37	60		5	0.44	44.2
	8	0.55	46.7				

Table 7.20. Results of mating compatibility tests between the known mating types of CYS159 and isolates of other collections of *Pholiota aurivella.*

Collection & isolate no.	No. of mono-karyons	Collection & isolate no.	No. of mono-karyons	No. of pairings	Total no. of positive pairings	Total no. of negative pairings
CYS159 (01, 02, 08 & 10)	4	X CYS116 (01, 02, 03 & 04)	4	16	16	0
		X CYS128 (01, 02, 03 & 04)	4	16	16	0
		X CYS154 (05 & 08)	2	8	8	0
		X CYS157 (01, 02, 03 & 04)	4	16	16	0

Table 7.21. Results of crosses between the mating types of CYS16 and the isolates of other collections grouped together as *Pholiota squarrosipes.*

Collection & isolate no.	No. of mono-karyons	Collection & isolate no.	No. of mono-karyons	Total no. of pairing	No. of positive pairing	No. of negative pairing
CYS16 (07, 17, 18 & 19)	4	x CYS8 (01, 02, 03 &04)	4	16	16	0
		x CYS11 (01, 07, 10 & 14)	4	16	16	0
		x CYS14 (01, 02, 05 & 10)	4	16	0	16
		x CYS18 (01, 02, 03 & 04)	4	16	16	0
		x CYS366 (01, 02, 03 & 04)	4	16	16	0
		x CYS424 (01, 02, 03 & 04)	4	16	16	0
		x CYS458 (01, 02, 05 & 06)	4	16	16	0
		x CYS496 (01, 02, 05 & 06)	4	16	16	0

Table 7.22. Results of confrontations between isolates of the mating types of CYS532 and isolates of other collections of *P. highlandensis*.

Collection & isolate no.	No.of mono-karyon		Collection of isolate no.	No. of mono-karyon	Total no. of pairing	No. of positive pairing	No. of negative pairing
CYS532 (05 & 11)	2	x	CYS453 (03, 04, 06 & 09)	4	8	8	0
		x	CYS497 (01, 02, 08 & 12)	4	8	8	0
		x	CYS528 (01 - 04)	4	8	8	0
		x	CYS529 (01 - 04)	4	8	8	0
		x	CYS530 (01 - 04)	4	8	8	0
		x	CYS538 (02 - 05)	4	8	8	0

Table 7.23. Results of crosses between mating types of CYS501, *Pholiota multicingulata*, and isolates of other collections of the same taxon.

Collection & isolate no.	No. of mono-karyons		Collection & isolate no.	No. of mono-karyons	Total no. of pairings	No. of positive pairings	No. of negative pairings
CYS501 (01, 03, 06 & 09)	4	X	CYS331 (01, 02 & 03)	3	12	12	0
		X	CYS376 (01, 02, 03 & 05)	4	16	16	0
		X	CYS385 (01 - 04)	4	16	16	0
		X	CYS397 (01, 03, 04 & 06)	4	16	16	0
		X	CYS413 (01, 02, 04 & 06)	4	16	16	0
		X	CYS426 (01 - 04)	4	16	16	0
		X	CYS440 (01 - 04)	4	16	16	0
		X	CYS466 (01, 02, 04 & 05)	4	16	16	0
		X	CYS475 (01, 02 & 04)	3	12	12	0
		X	CYS485 (01 - 04)	4	16	12	4
		X	CYS490 (01 - 04)	4	16	16	0
		X	CYS493 (01 - 04)	4	16	16	0
		X	CYS512 (01 - 04)	4	16	16	0

Table 7.24. Results of crosses between the mating types of CYS482 (*Pholiota* sp. D) and isolates of other collections of sp. D and isolates of collections of *P. multicingulata*as well as between the isolates of CYS271 and three other collections of sp. D.

Species Collection & isolate no.	No. of mono-karyon		Species Collection & isolate no.	No. of mono-karyon	Total no. of pairing	No. of positive pairing	No. of negative pairing
Pholiota sp. D			*Pholiota* sp. D				
CYS482	4	x	CYS4	1	4	4	0
(02, 08, 10 & 12)			(01)				
		x	CYS30	3	12	12	0
			(01, 02 & 03)				
		x	CYS43	1	4	2	0*
			(03)				
		x	CYS54	2	8	8	0
			(01 & 03)				
		x	CYS86	1	4	2	2
			(01)				
		x	CYS271	3	12	0	12
			(01, 02 & 03)				
		x	CYS289	3	12	12	0
			(01, 02 & 03)				
		x	CYS329	3	12	12	0
			(01, 02 & 03)				
		x	CYS411	4	16	16	0
			(01, 02, 03 & 04)				
		x	CYS488	4	16	16	0
			(01, 02, 03 & 05)				
		x	CYS502	4	16	16	0
			(01, 02 03 & 04)				
		x	CYS510	4	16	16	0
			(01, 02, 03 & 04)				
CYS271	3	x	CYS43	1	3	0	3
(01, 02 & 03)			(03)				
		x	CYS86	1	3	0	3
			(01)				
		x	CYS411	4	12	0	12
			(01, 02, 03 & 04)				
Pholiota sp. D			*P. multicingulata*				
CYS482	4	x	CYS331	2	8	0	8
(02, 08, 10 & 12)			(01 & 03)				
		x	CYS376	2	8	0	8
			(01 & 03)				
		x	CYS385	2	8	0	8
			(01 & 03)				
		x	CYS397	2	8	0	8
			(01 & 04)				
		x	CYS426	2	8	0	8
			(01 & 02)				
		x	CYS466	2	8	0	8
			(01 &02)				
		x	CYS501	2	8	0	8
			(01 & 03)				
		x	CYS512	2	8	0	8
			(03 & 04)				

Table 7.25. Cultures of some taxa of *Pholiota* producing unusual cell types.

Taxa & collection	Acanthocytes	Clusters of fusiform cells
Pholiota sp D		
CYS411		+
CYS482		+
CYS488		+
CYS502		+
CYS271	+	
P. highlandensis		
CYS497		+
CYS529		+
CYS530		+
CYS532		+
CYS538		+
P. multicingulata		
CYS426		+
CYS490		+

Table 7.26. Synopsis of taxa of *Pholiota* delineated in the study.

Subgenus Phaeonaematoloma
- *species*: 1. fieldiana sp. nov.
- 2. visco-fumosa sp. nov.

Subgenus Flammula
- *species*: 1. malicola

Subgenus Pholiota
- *Section* Adiposae
- *species*: 1. aurivella
- 2. squarrosipes
- 3. taxon 1

Subgenus Flammuloides
- *Section* Carbonicola
- *species*: 1. highlandensis
- *Section* Flammuloides
- *species*: 1. pallidocaulis sp. nov.
- 2. taxon 2
- 3. taxon 3
- *Section* Spumosae
- *species* 1. multicingulata Horak
- 2. taxon 4

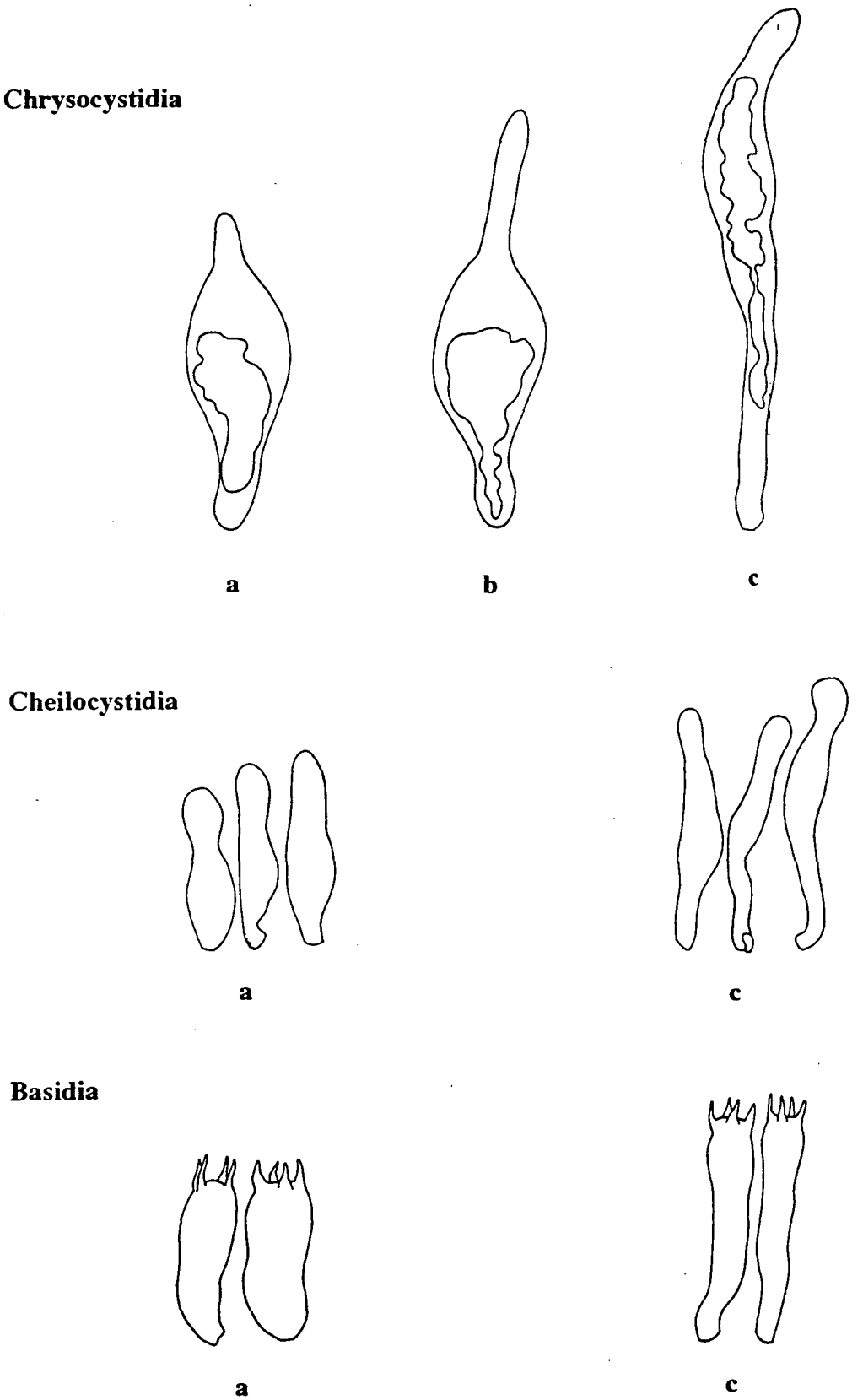


Fig. 7.1. Variations in cystidia and basidia characters in Tasmanian specimens of *Pholiota squarrosipes*. a) normal form, b) long snouted form, and c) long slender based form.

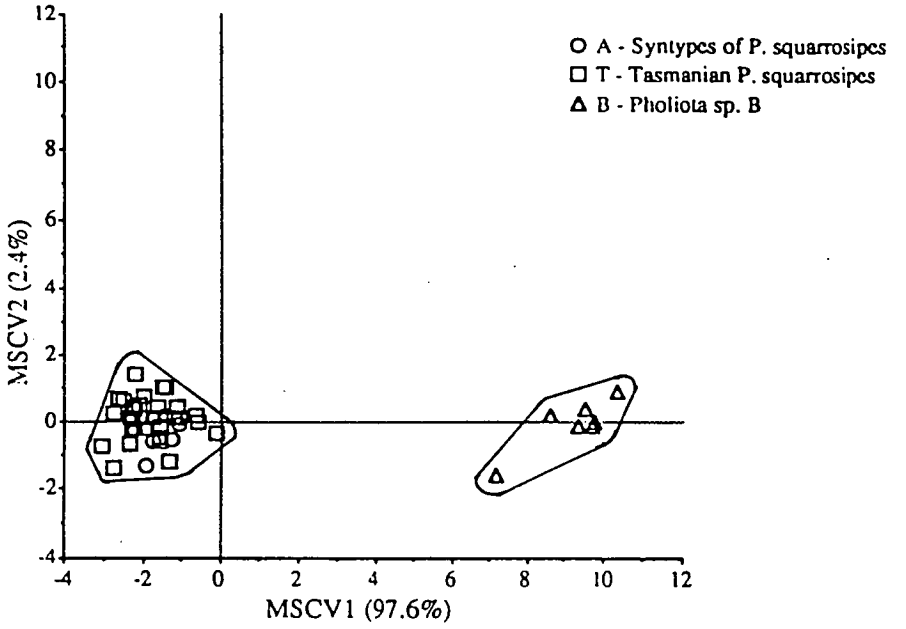


Fig. 7.2. Scattergram of mean canonical variates (MSCV1 & MSCV2) generated from CDA of spore variables for collections of *Pholiota squarrosipes* (both syntype & Tasmanian material) and *Pholiota* sp. B.

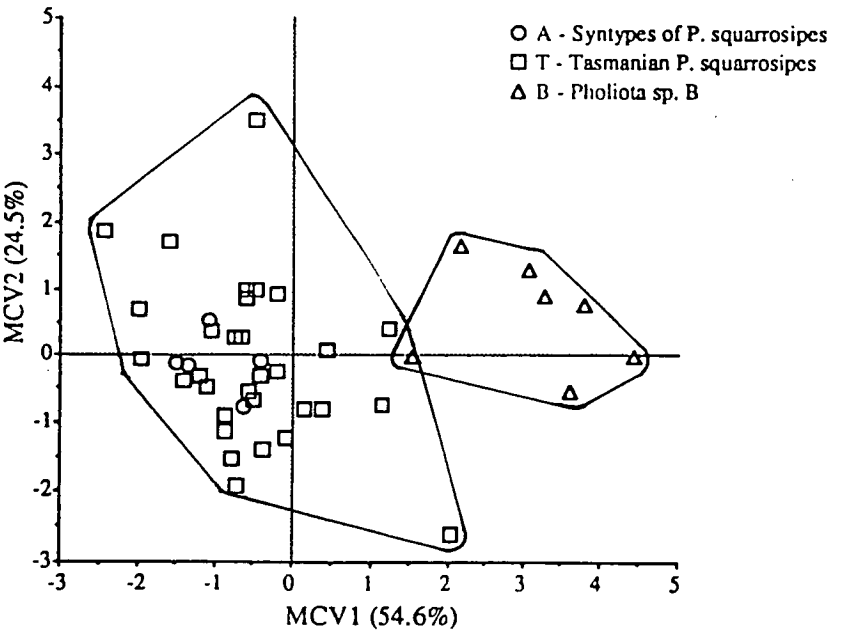


Fig. 7.3. Scattergram of mean canonical variates (MCV1 & MCV2) generated from CDA of cystidia variables for collections of *Pholiota squarrosipes* (both syntype & Tasmanian material) and *Pholiota* sp. B.

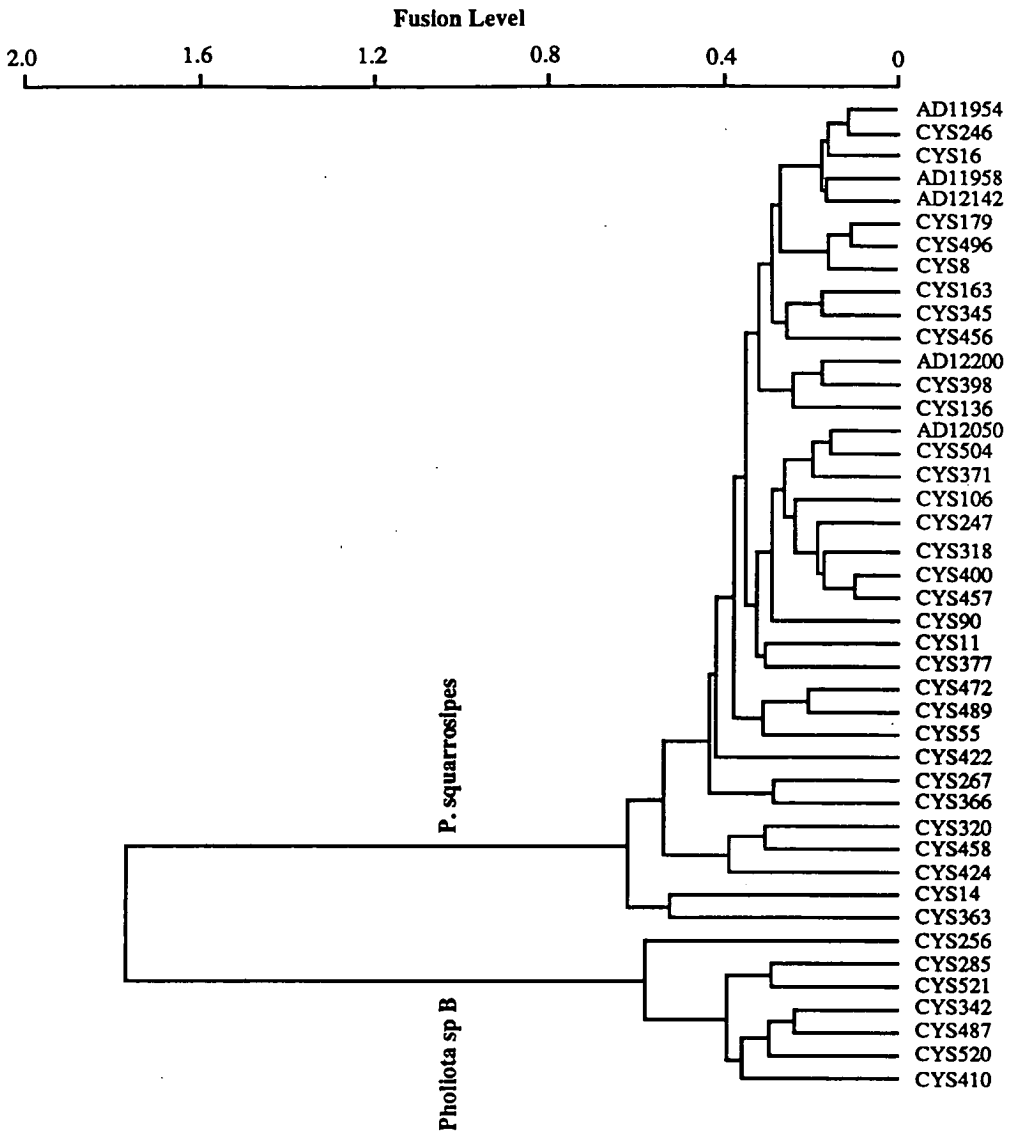


Fig. 7.4. Dendogram from UPGMA cluster analysis based on all the mean canonical values for Tasmanian and syntype collections of *Pholiota squarrosipes* and *Pholiota* sp B.

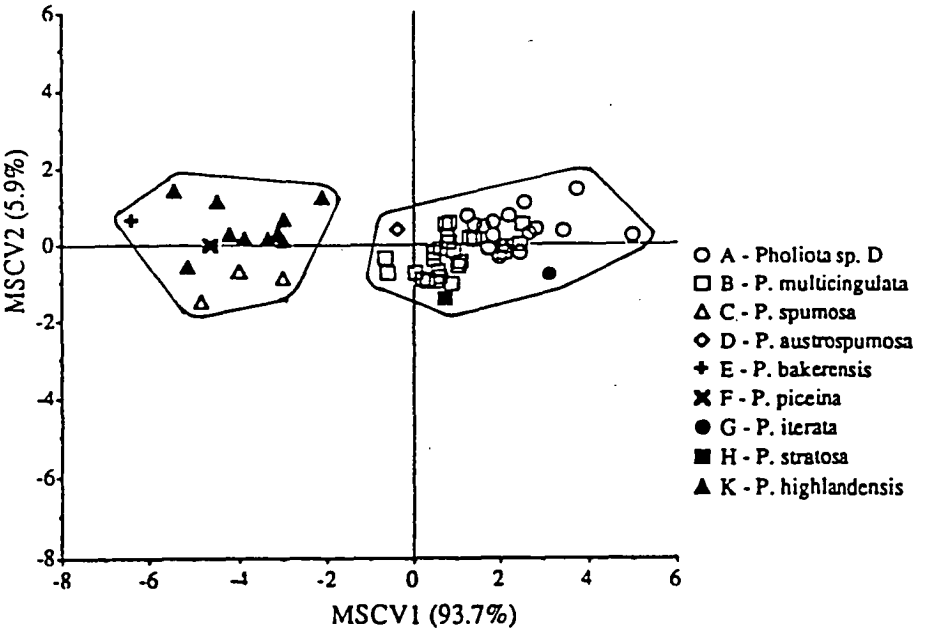


Fig. 7.5. Scattergram of mean canonical variates (MSCV1 & MSCV2) generated from CDA of spore variables for collections of *Pholiota* sp. D, *P. multicingulata*, *P. highlandensis* and herbarium material of *Pholiota* species in subgenus *Flammuloides*.

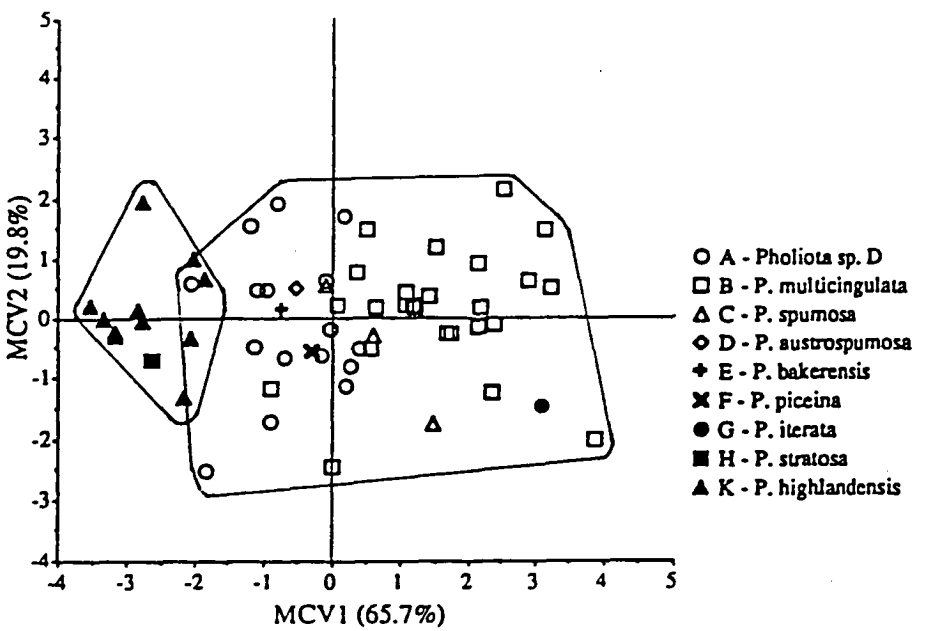


Fig. 7.6. Scattergram of mean canonical variates (MCV1 & MCV2) generated from CDA of cystidia variables for collections of *Pholiota* sp. D, *P. multicingulata*, *P. highlandensis* and herbarium material of *Pholiota* species in subgenus *Flammuloides*.

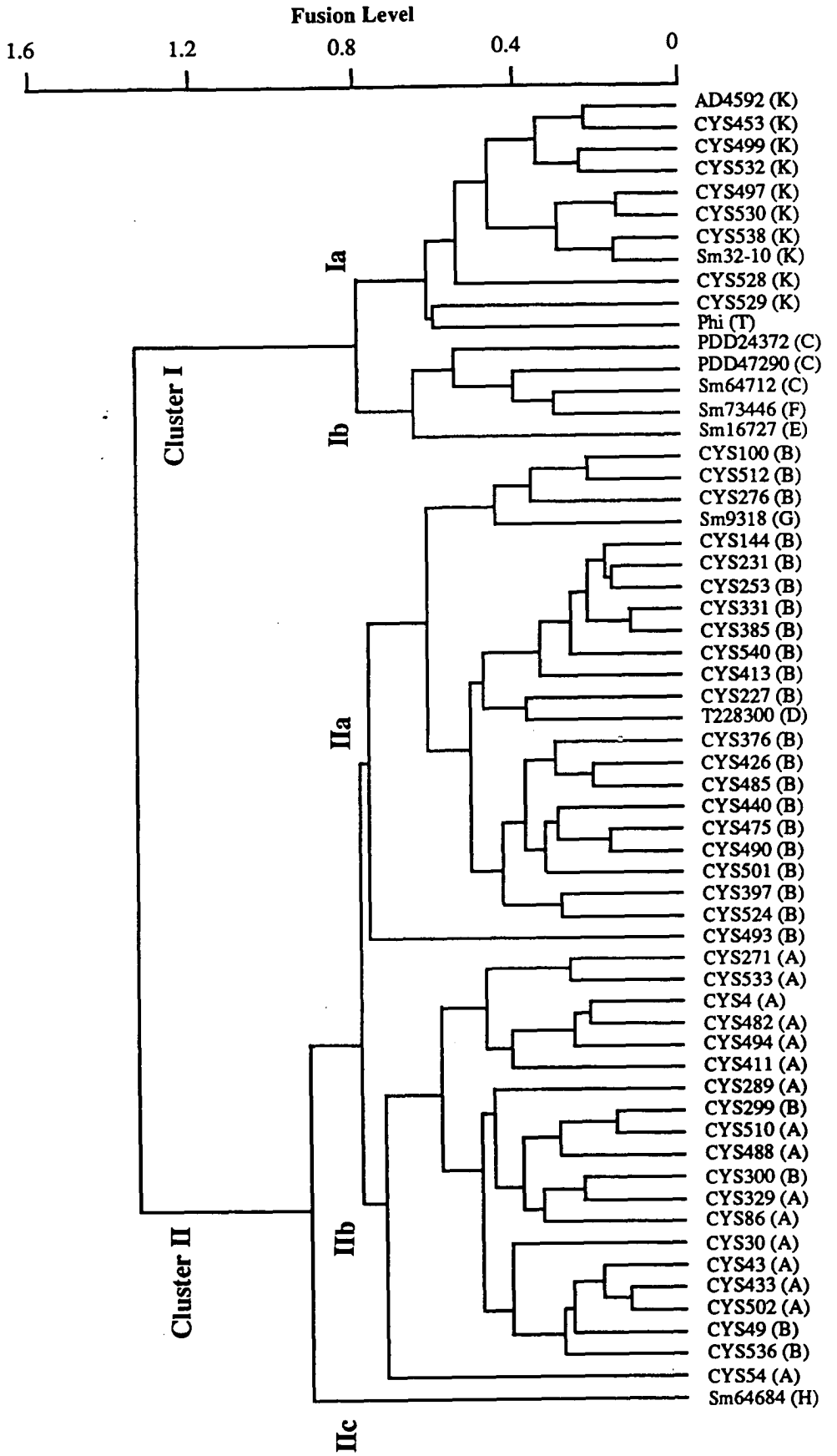


Fig. 7.7. Dendogram from UPGMA cluster analysis based on all the mean canonical variates for collections in subgenus Flammuloides. See text for symbols of clusters.

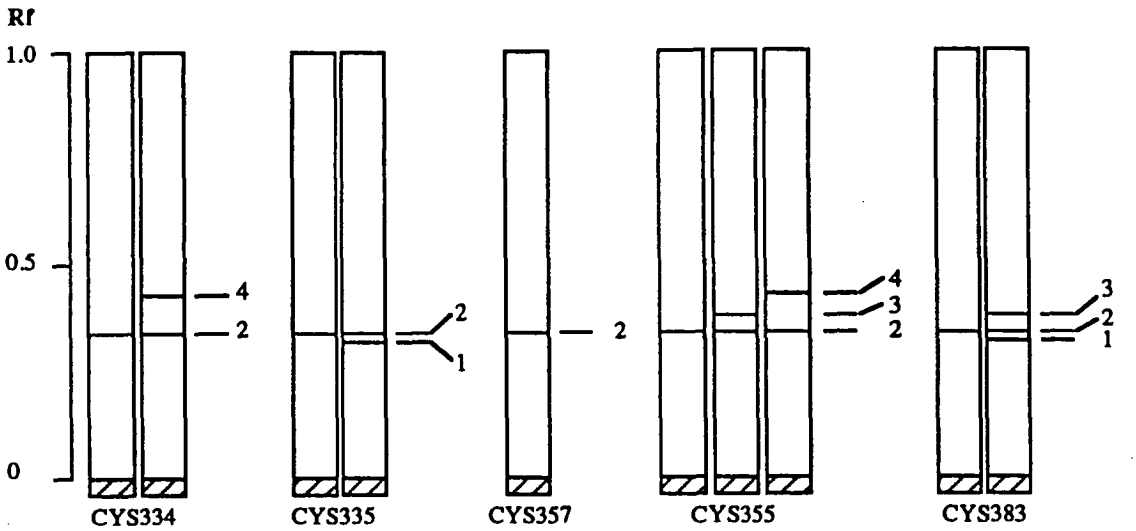


Fig. 7.8. Schematic representations of laccase zymograms of isolates of *Pholiota malicola*. Band numbers start from the cathodic end. Rf values: 1=0.32, 2=0.34, 3=0.38 & 4=0.43..

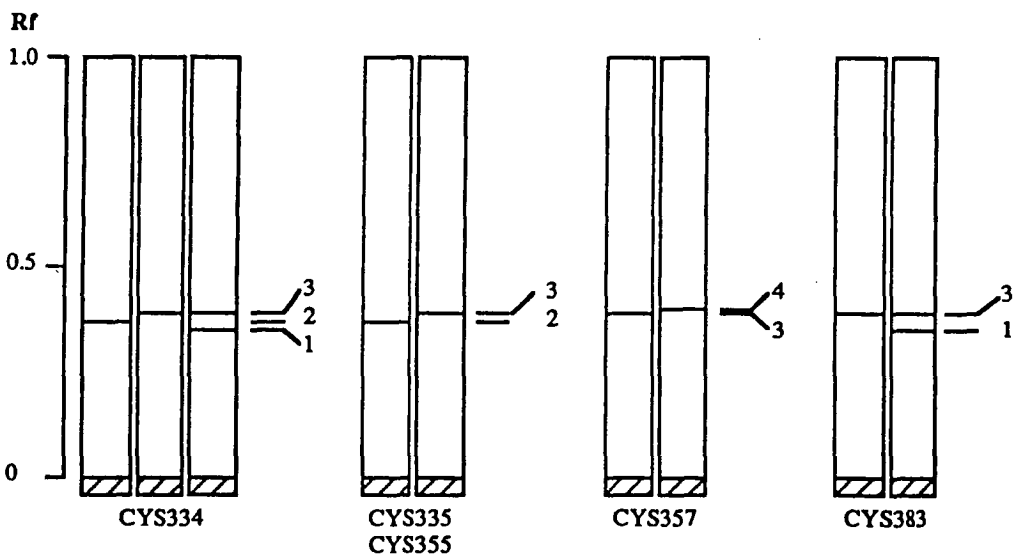


Fig. 7.9. Schematic representations of Per zymograms of isolates of *Pholiota malicola*. Band numbers start from the cathodic end. Rf values: 1=0.35, 2=0.37, 3=0.39 & 4=0.40.

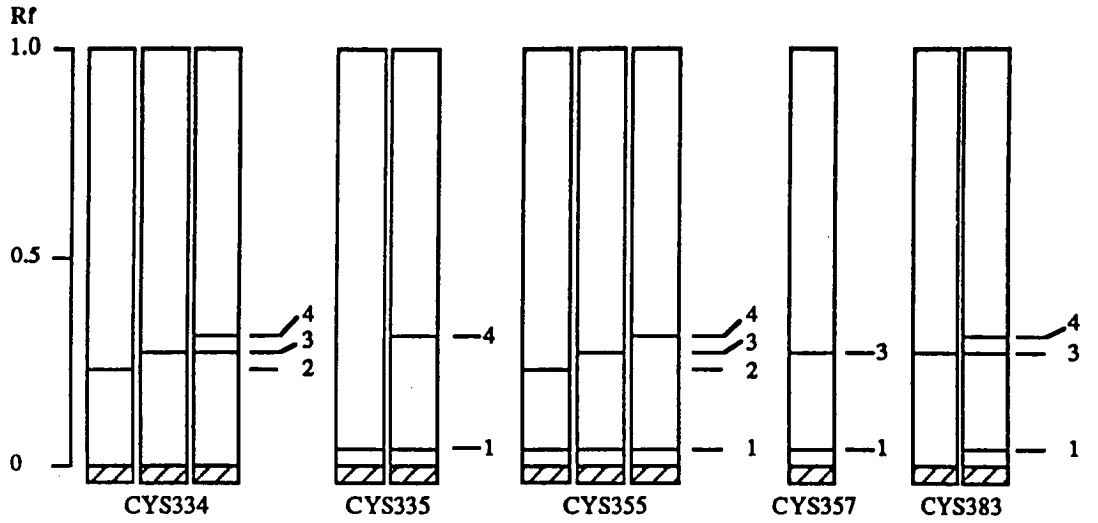


Fig. 7.10. Schematic representations of PE zymograms of isolates of *Pholiota malicola*. Band numbers start from the cathodic end. Rf values: 1=0.04, 2=0.23, 3=0.27 & 4=0.31.

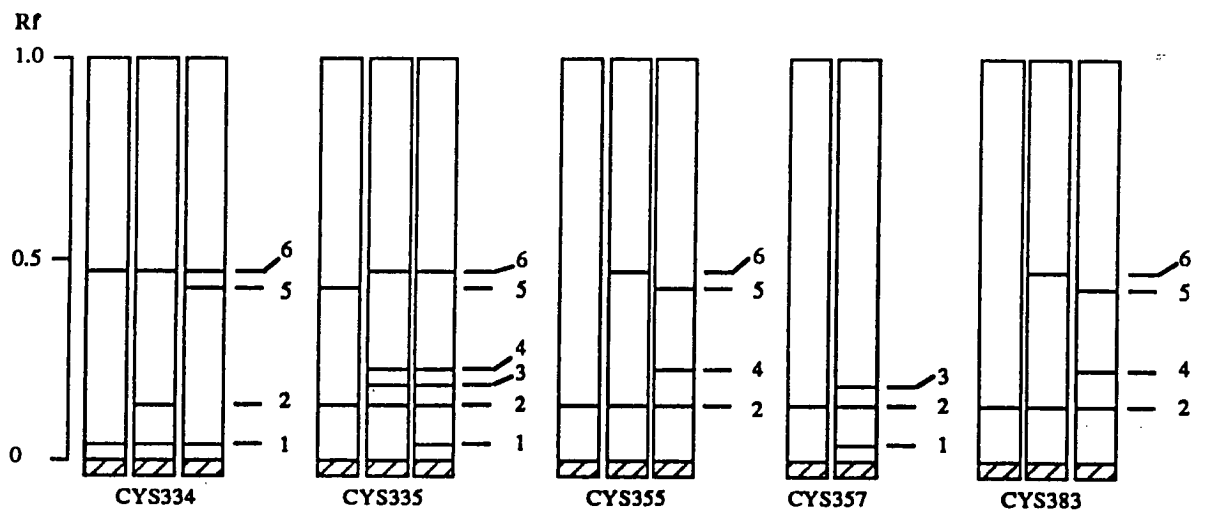


Fig. 7.11. Schematic representations of PG zymograms of isolates of *Pholiota malicola*. Band numbers start from the cathodic end. Rf values: 1=0.04, 2=0.14, 3=0.19, 4=0.23, 5=0.43 & 6=0.47.

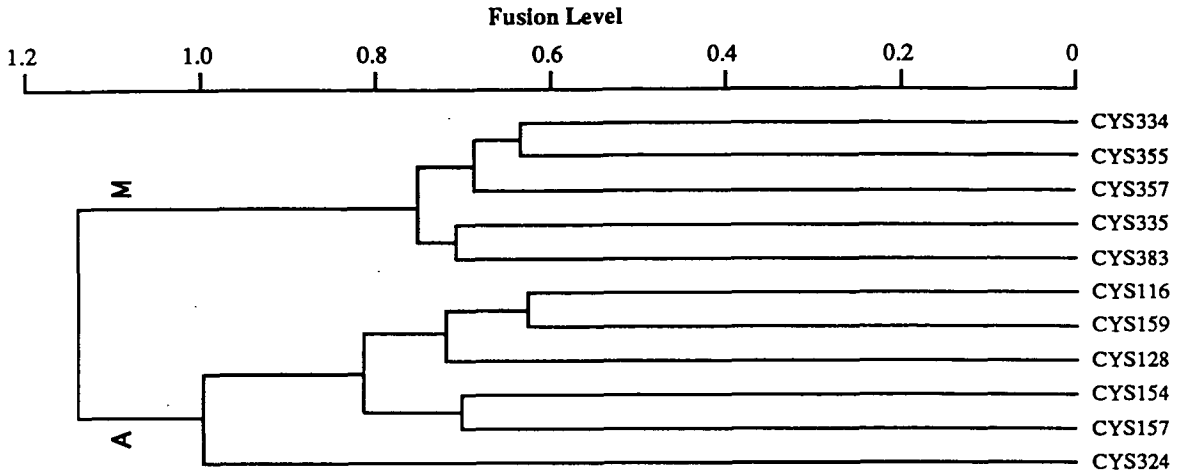


Fig. 7.12. Dendogram from UPGMA cluster analysis based on band frequencies of the tested enzymes showing relationships of collections of *Pholiota malicola* (M) and *P. aurivella* (A).

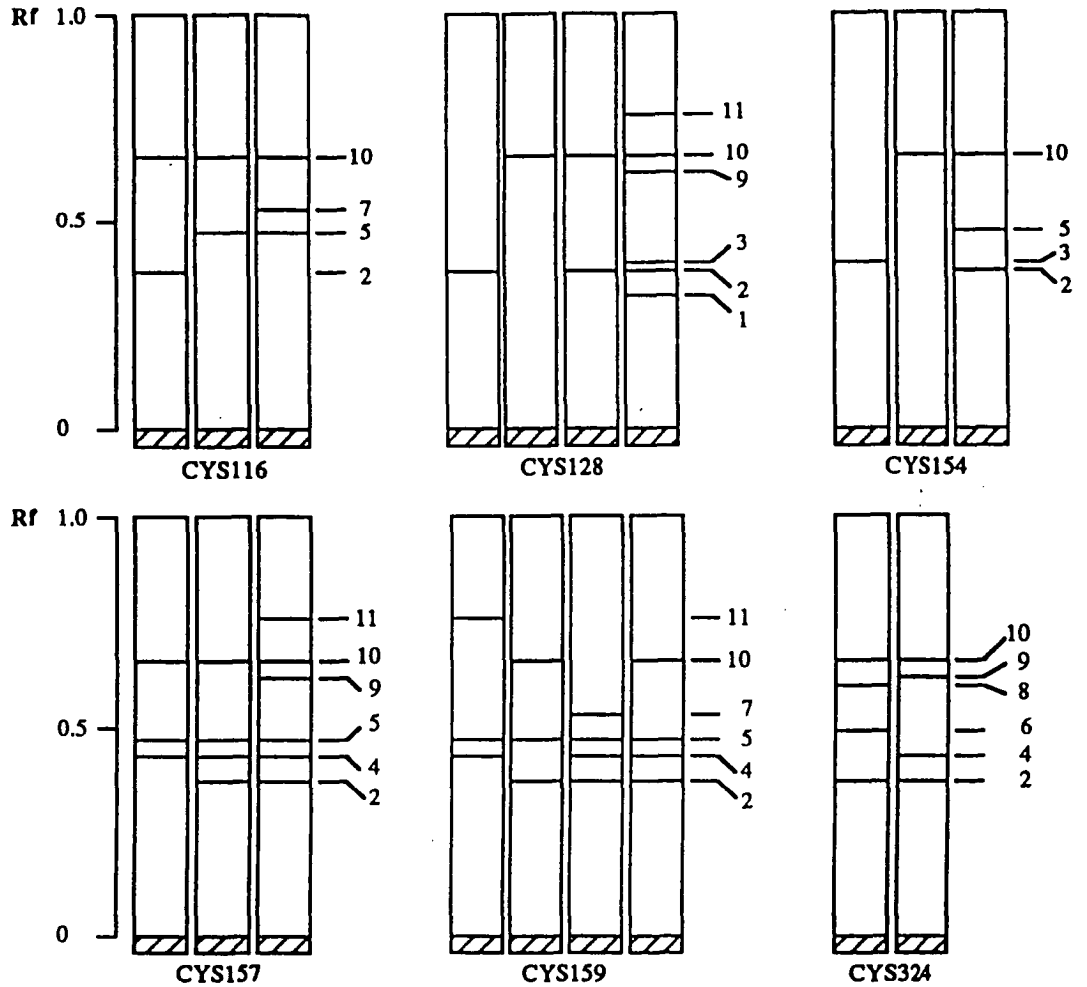


Fig. 7.13. Schematic representations of Lac zymograms of isolates of *Pholiota aurivella*. Band numbers start from the cathodic end. Rf values: 1=0.31, 2=0.37, 3=0.39, 4=0.43, 5=0.47, 6=0.49,7=0.53, 8=0.60, 9=0.62 & 10=0.66.

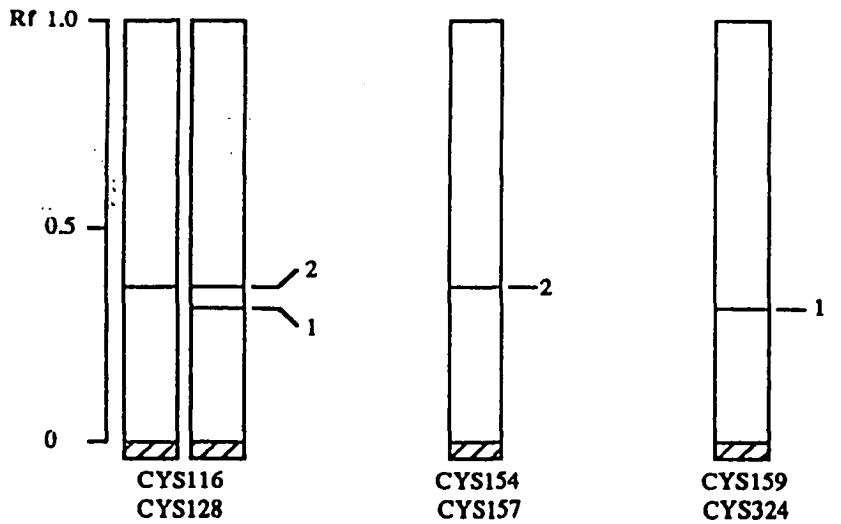


Fig. 7.14. Schematic representations of Per zymograms of isolates of *P. aurivella*. Band numbers start from the cathodic end. Rf values: 1=0.51 & 2=0.56.

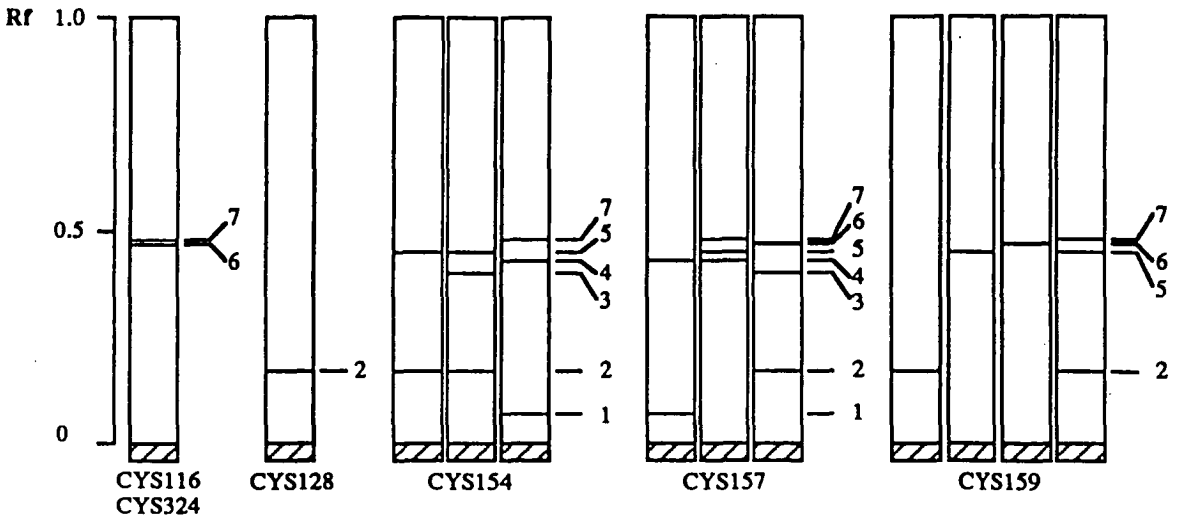


Fig. 7.15. Schematic representations of PE zymograms of isolates of *P. aurivella* included in the study. Band numbers start from the cathodic end. Rf values: 1=0.07, 2=0.17, 3=0.40, 4=0.43, 5=0.45, 6=0.47 & 7=0.48.

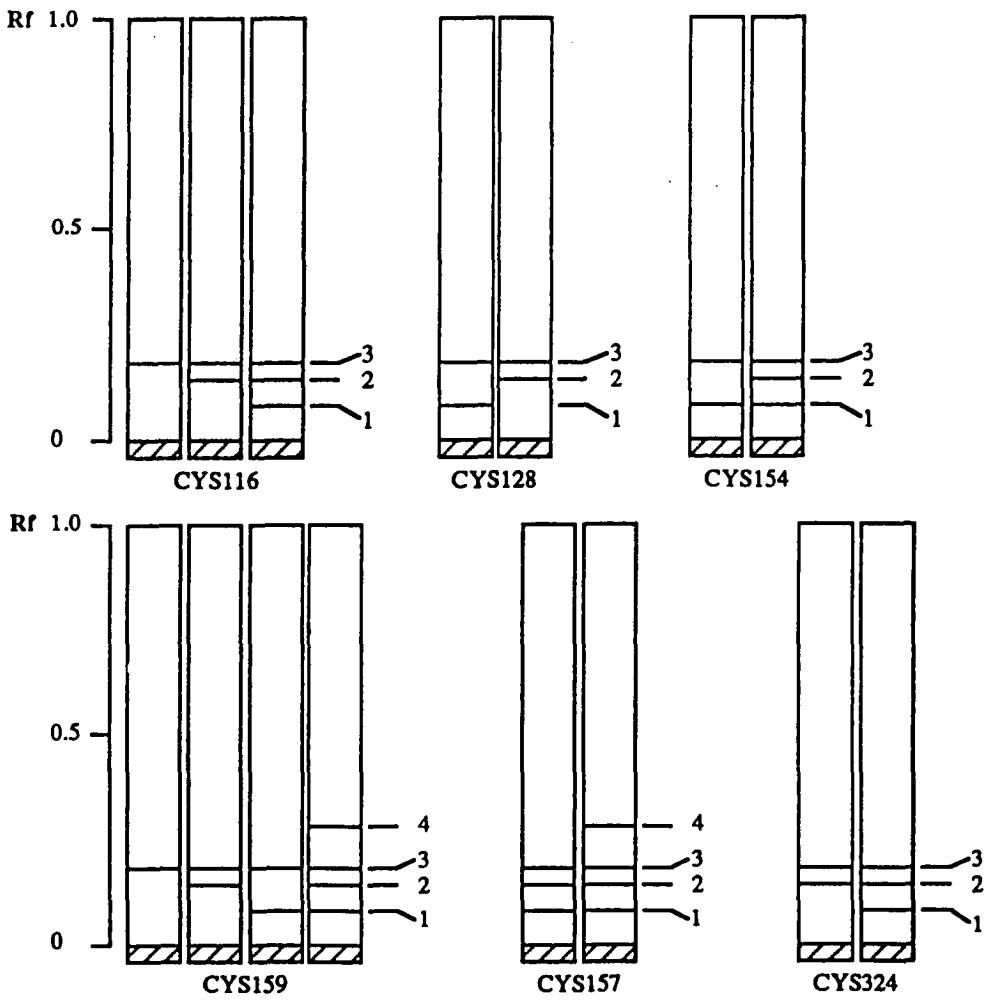


Fig. 7.16. Schematic representations of PG zymograms of isolates of *P.aurivella* included in the study. Band numbers start from the cathodic end. Rf values: 1=0.08, 2=0.28, 3=0.31 & 4=0.36.

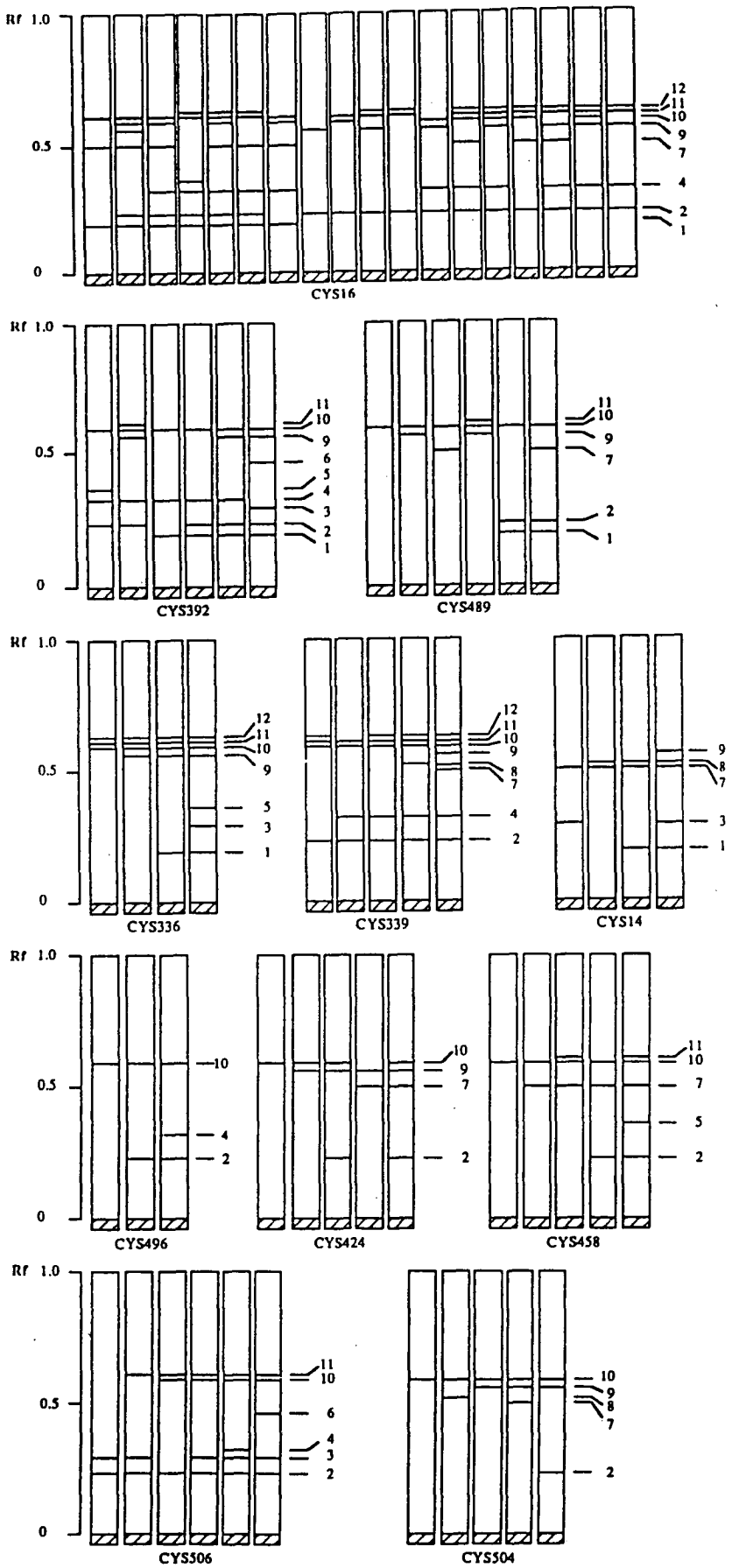


Fig. 7.17. Schematic representations of Lac zymograms of isolates of collections of *Pholiota squarrosipes*. Band numbers start from the cathodic end. R_f values: 1=0.19, 2=0.23, 3=0.29, 4=0.32, 5=0.36, 6=0.46, 7=0.50, 8=0.52. 9=0.56, 10=0.59, 11=0.61 & 12=0.63.

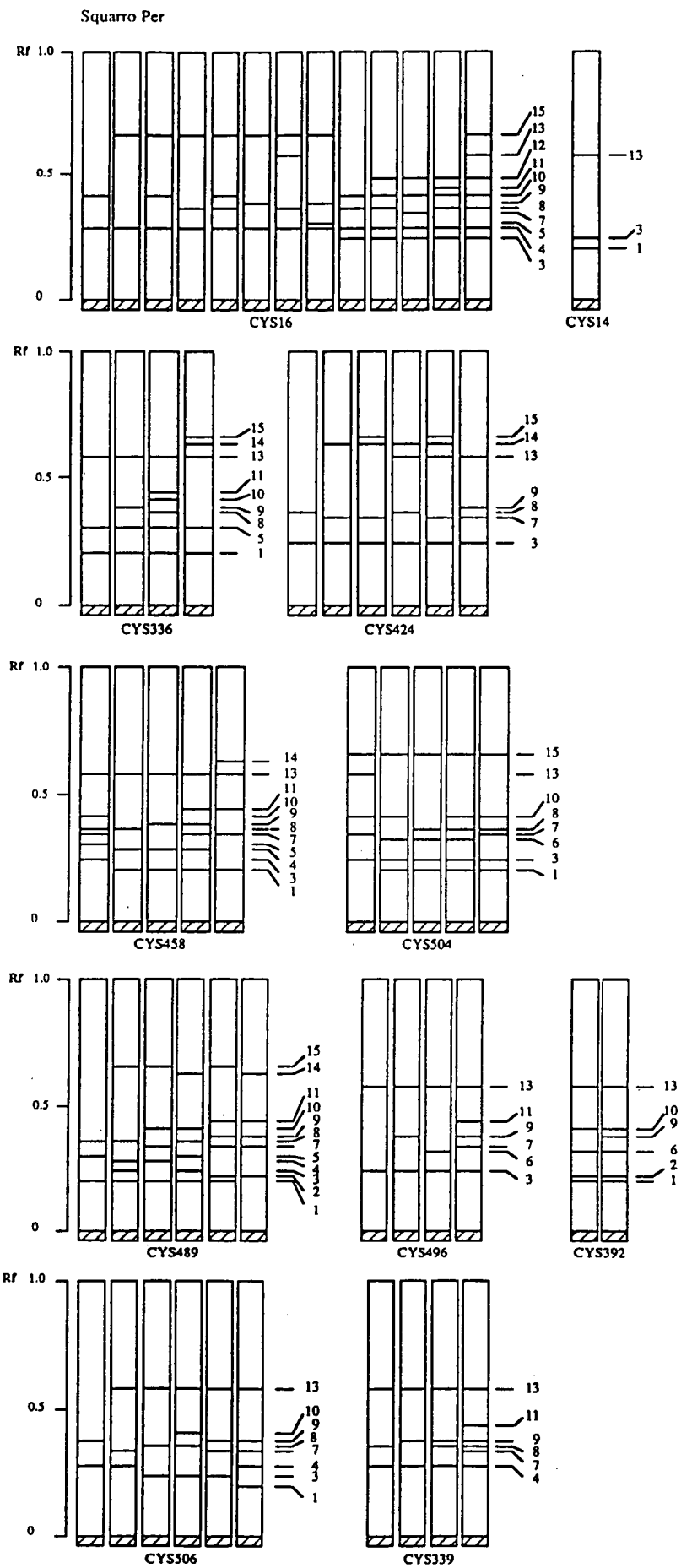


Fig. 7.18. Schematic representations of Per zymograms of isolates of collections of *Pholiota squarrosipes*. Band numbers start from the cathodic end. R_f values: 1=0.20, 2=0.22, 3=0.24, 4=0.28, 5=0.30, 6=0.32, 7=0.34, 8=0.36, 9=0.38, 10=0.41, 11=0.44, 12=0.48, 13=0.58, 14=0.63 & 15=0.66.

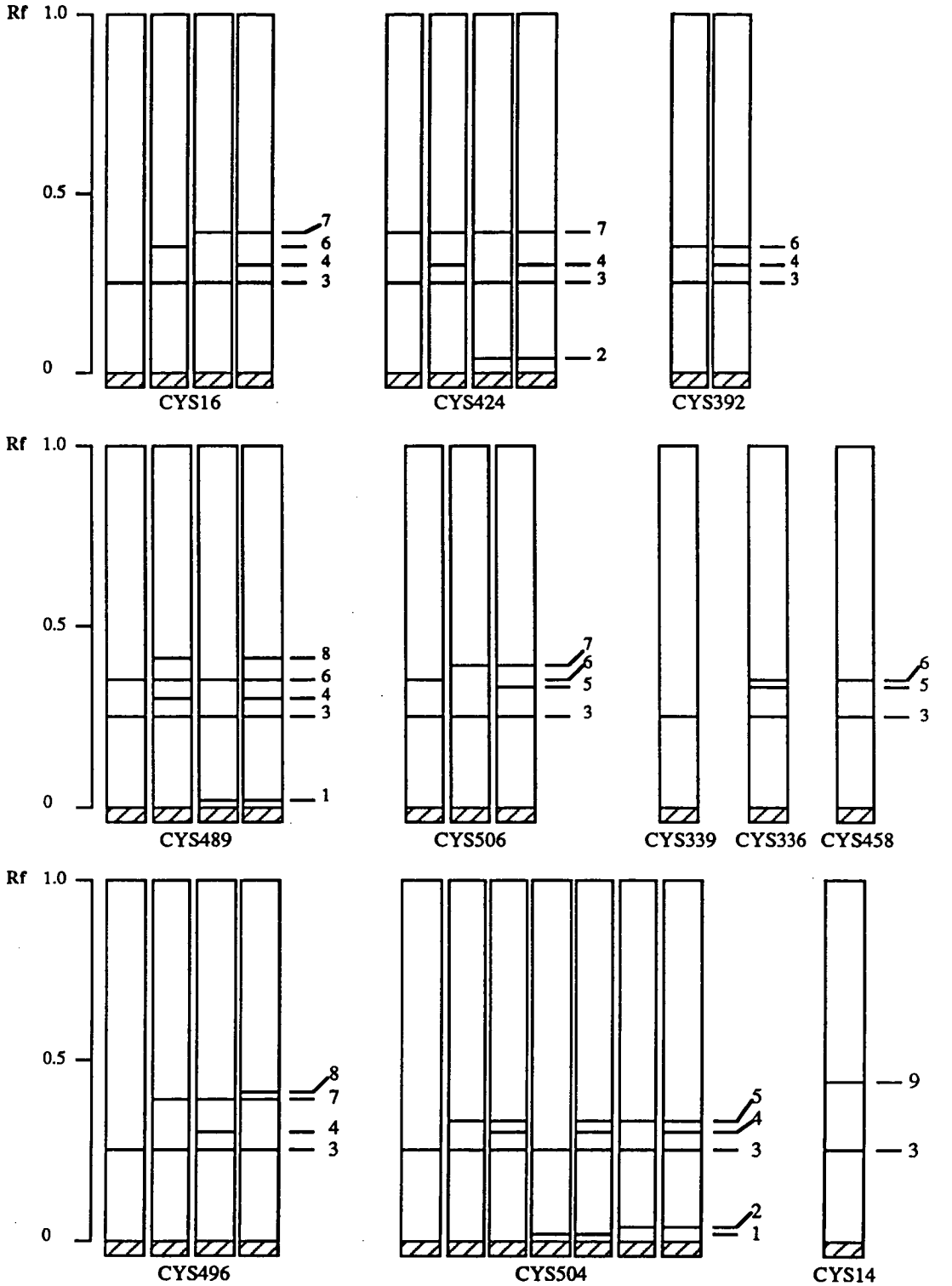


Fig. 7.19. Schematic representations of PE zymograms of isolates of *Pholiota squarrosipes*. Band numbers start from the cathodic end. Rf values: 1=0.02, 2=0.05, 3=0.25, 4=0.30, 5=0.33, 6=0.37, 7=0.39, 8=0.41 & 9=0.44.

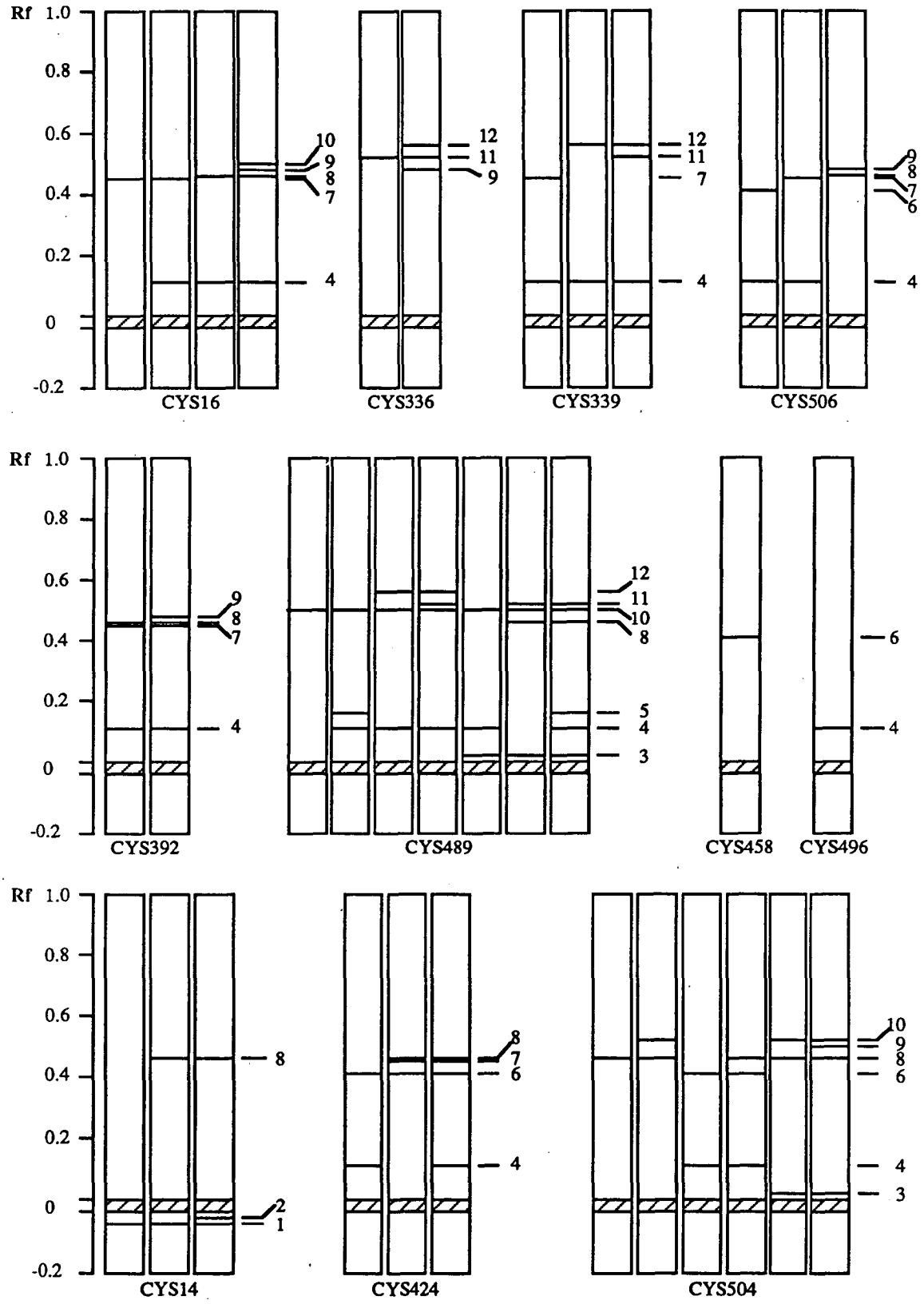


Fig. 7.20. Schematic representations of PG zymograms of isolates of *Pholiota squarrosipes*. Band numbers start from the cathodic end. Rf values: 1=-0.04, 2=-0.02, 3=0.02, 4=0.11, 5=0.16, 6=0.41, 7=0.45, 8=0.46, 9=0.48, 10=0.50, 11=0.52 & 12=0.56.

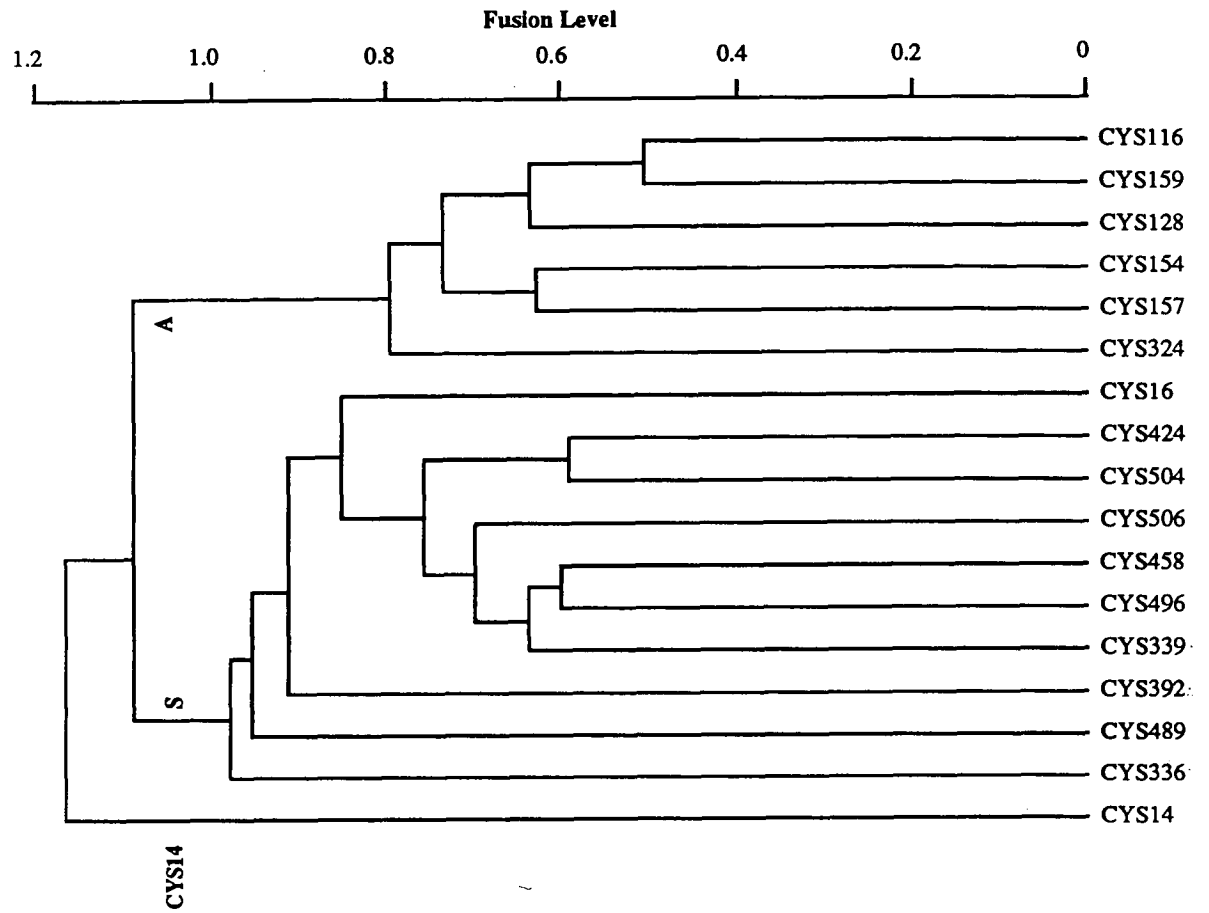


Fig. 7.21. Dendogram derived from UPGMA cluster analysis based on band frequencies of Lac, Per, PE and PG of isolates of collections of *Pholiota aurivella* (A) and *P. squarrosipes* (S).

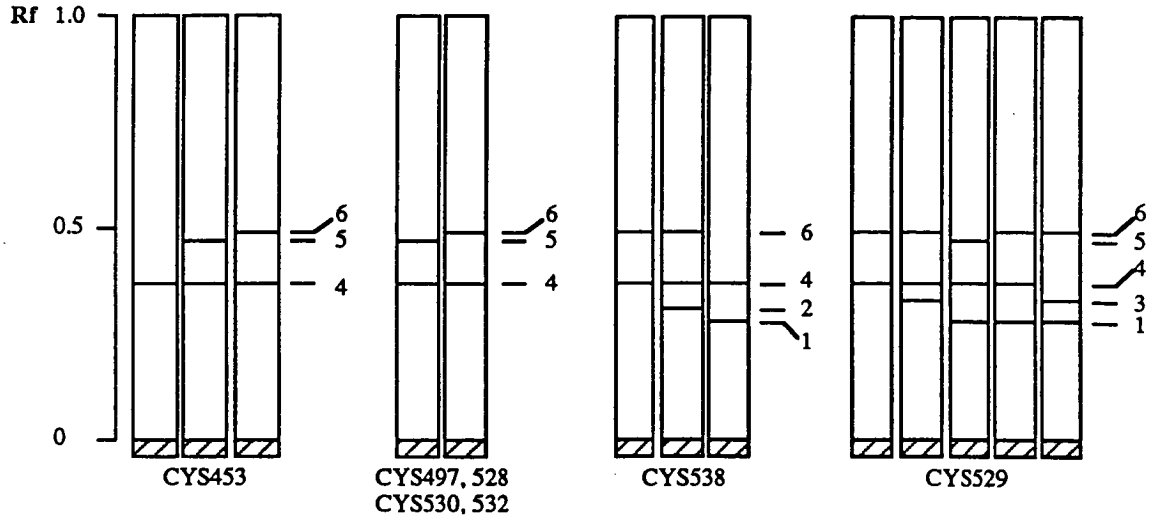


Fig. 7.22. Schematic representations of Lac zymograms of isolates of *P. highlandensis*. Band numbers start from the cathodic end. Rf values: 1=0.28, 2=0.31, 3=0.33, 4=0.37, 5=0.47 and 6=0.49

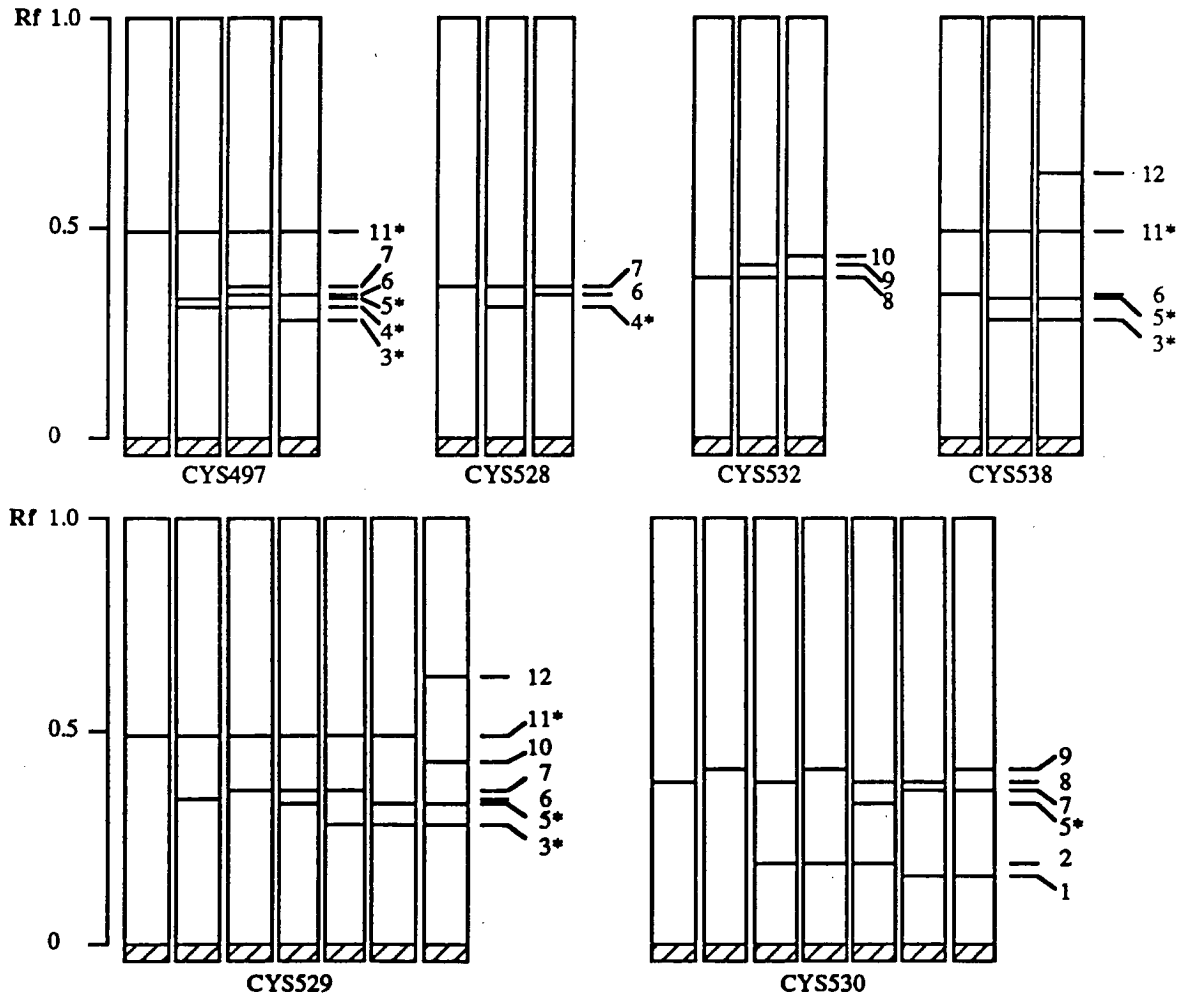


Fig. 7.23. Schematic representations of Per zymograms of isolates of *P. highlandensis*. Band numbers start from the cathodic end. Bands marked with an asterisk were previously detected in the absence of hydrogen peroxide. Rf values: 1=0.16, 2=0.19, 3=0.28, 4=0.31, 5=0.33, 6=0.34, 7=0.36, 8=0.38, 9=0.41, 10=0.43, 11=0.49 and 12=0.63.

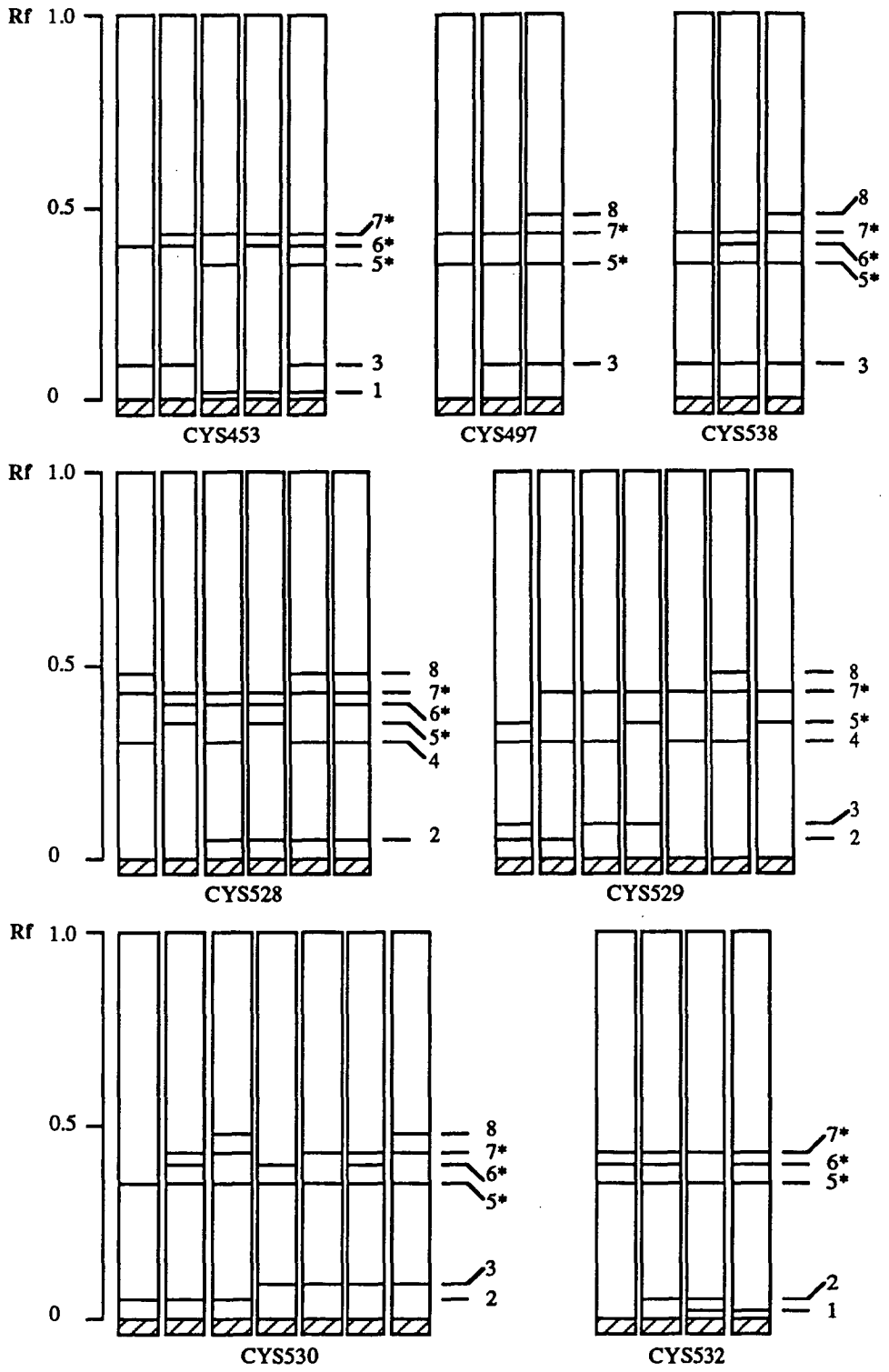


Fig. 7.24. Schematic representations of PE zymograms of isolates of *P. highlandensis*. band numbers start from the cathodic end and dominant bands are marked with an asterisk. Rf values: 1=0.02, 2=0.05, 3=0.09, 4=0.30, 5=0.35, 6=0.40, 7=0.43 and 8=0.48.

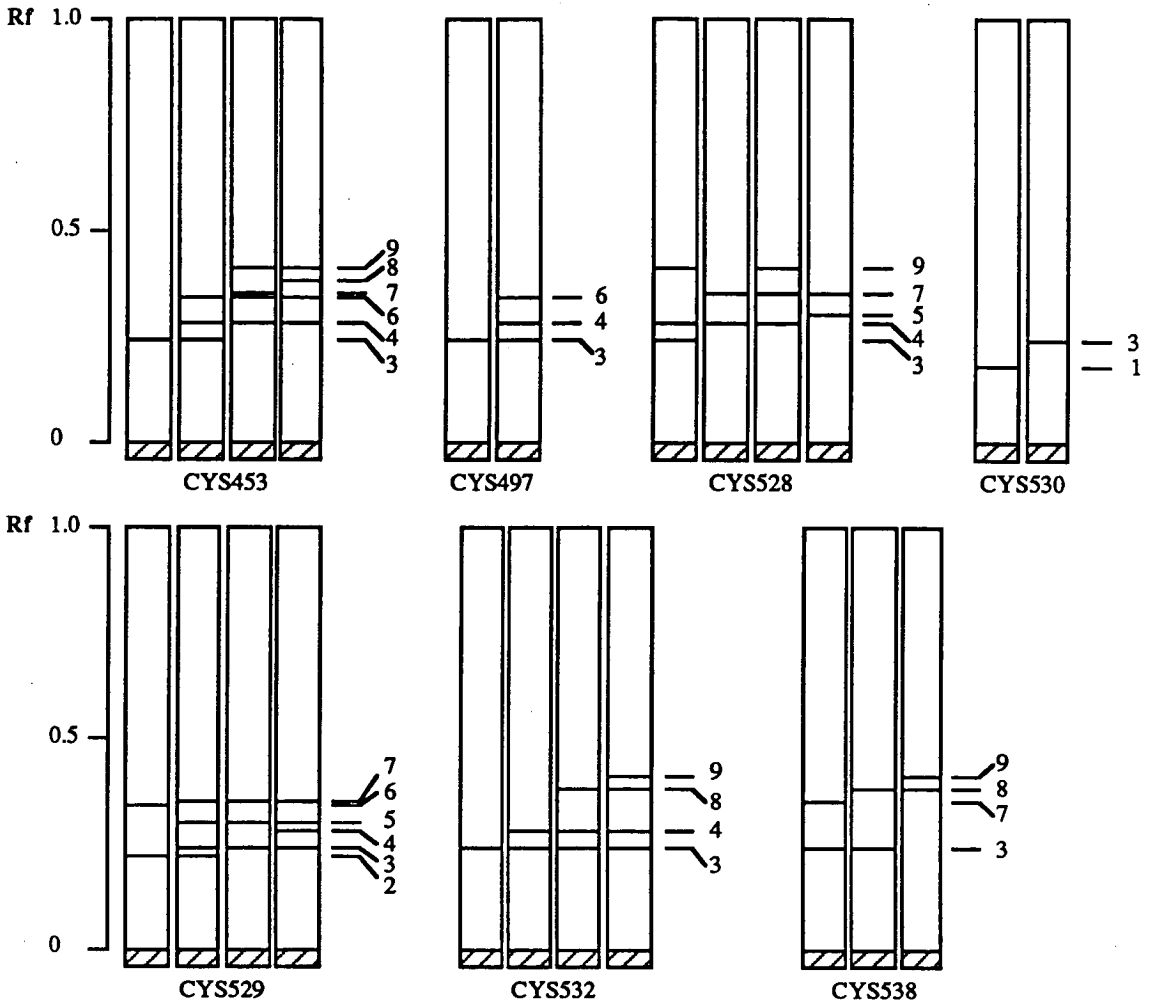


Fig. 7.25. Schematic representations of PG zymograms of isolates of *P. highlandensis*. Band numbers start at the cathodic end. Rf values: 1=0.18, 2=0.22, 3=0.24, 4=0.28, 5=0.30, 6=0.34, 7=0.35, 8=0.38 and 9=0.41.

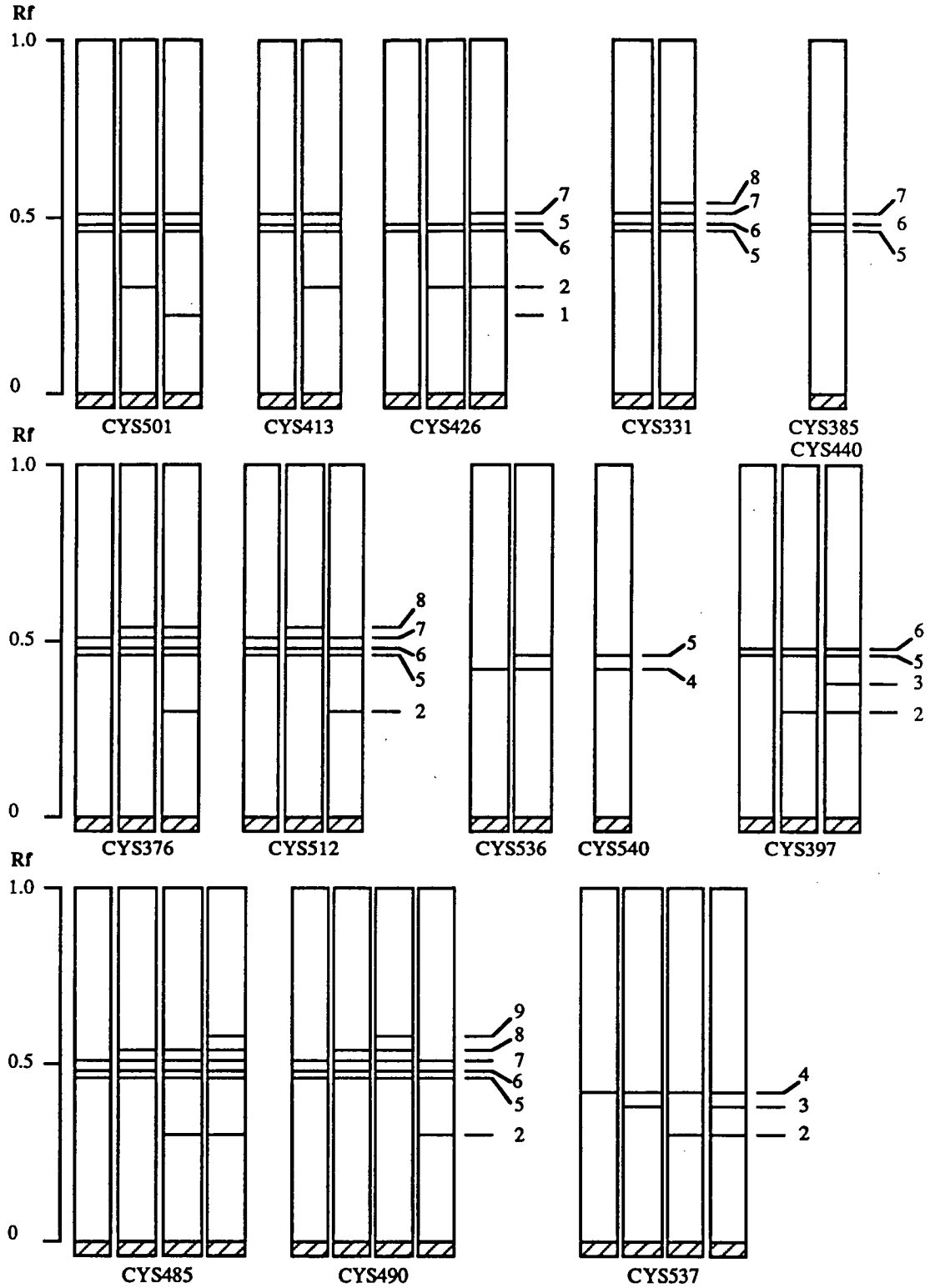


Fig. 7.26. Schematic representations of Lac zymograms of isolates of *P. multicingulata* included in the study. Band numbers start from the cathodic end. Rf vlaues: 1=0.22, 2=0.30, 3=0.38, 4=0.42, 5=0.46, 6=0.48, 7=0.51, 8=0.54 & 9=0.58.

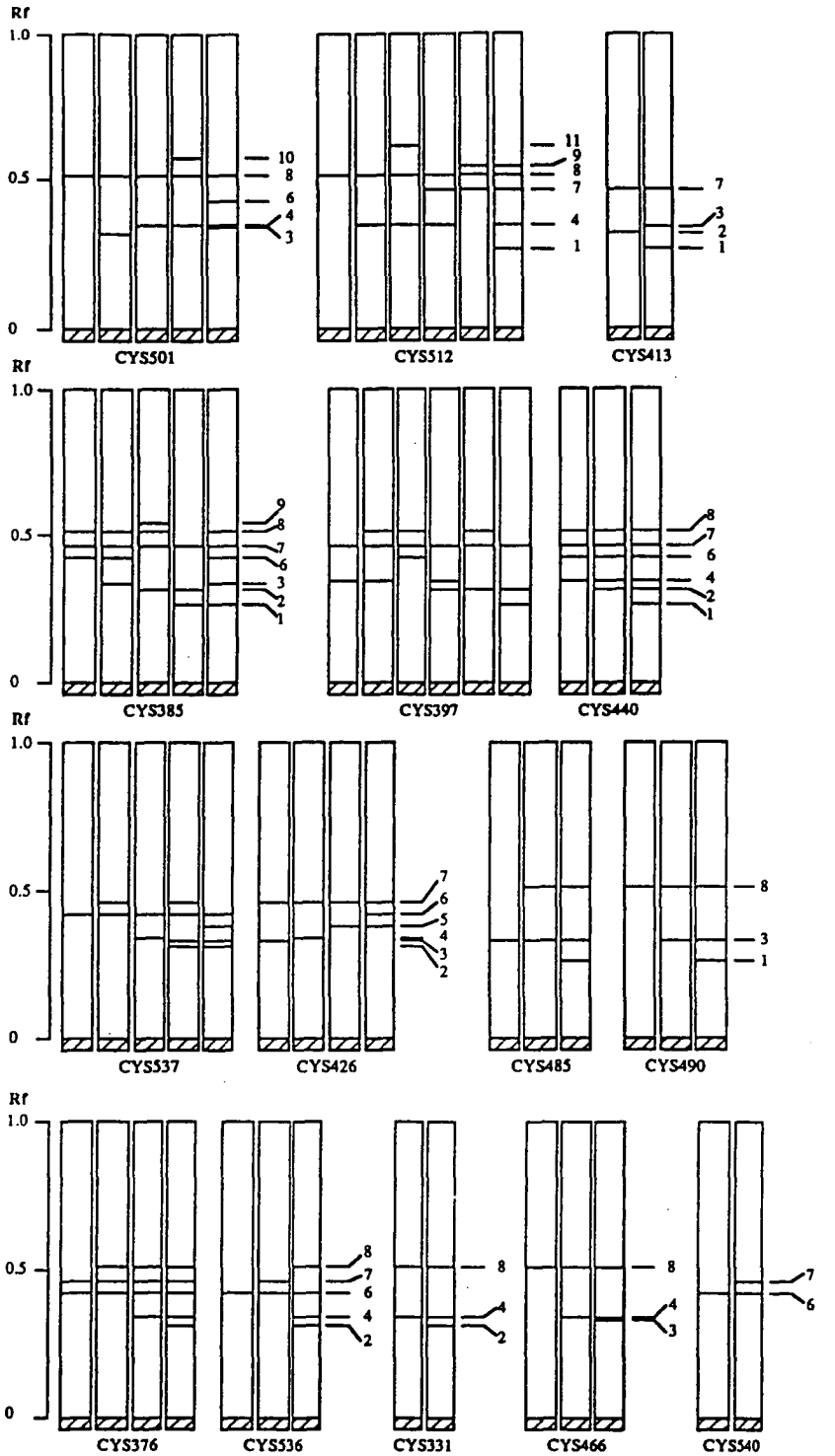


Fig. 7.27. Schematic representations of Per zymograms of isolates of *Pholiota multicingulata* included in the study. Band numbers start from the cathodic end. R_f values: 1=0.26, 2=0.31, 3=0.33, 4=0.34, 5=0.38, 6=0.42, 7=0.46, 8=0.51, 9=0.54, 10=0.57 & 11=0.61.

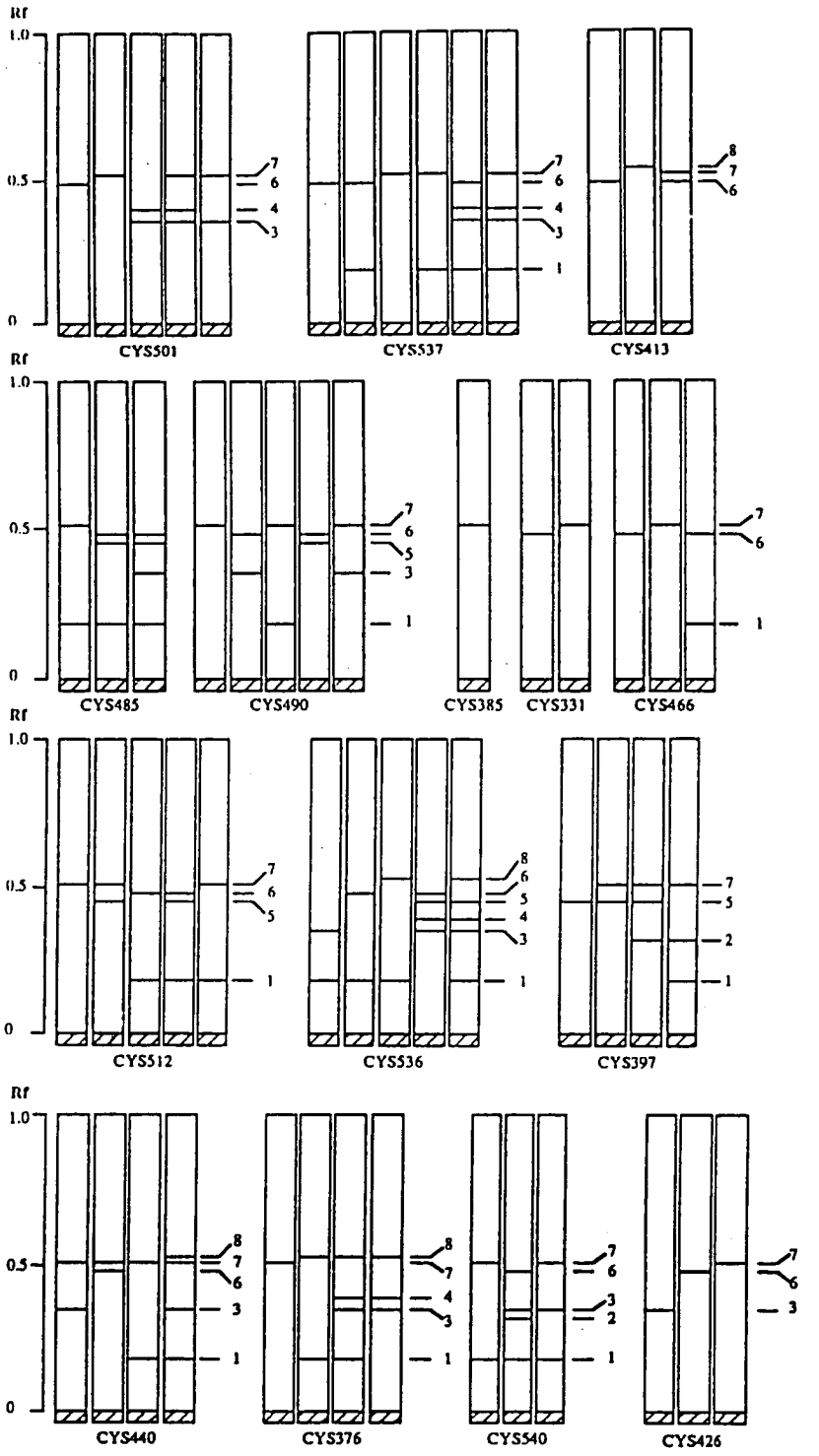


Fig. 7.28. Schematic representations of PE zymograms of isolates of *Pholiota multicingulata* included in the study. Band numbers start from the cathodic end. R_f values: 1=0.18, 2=0.32, 3=0.35, 4=0.39, 5=0.45, 6=0.48, 7=0.51 & 8=0.53.

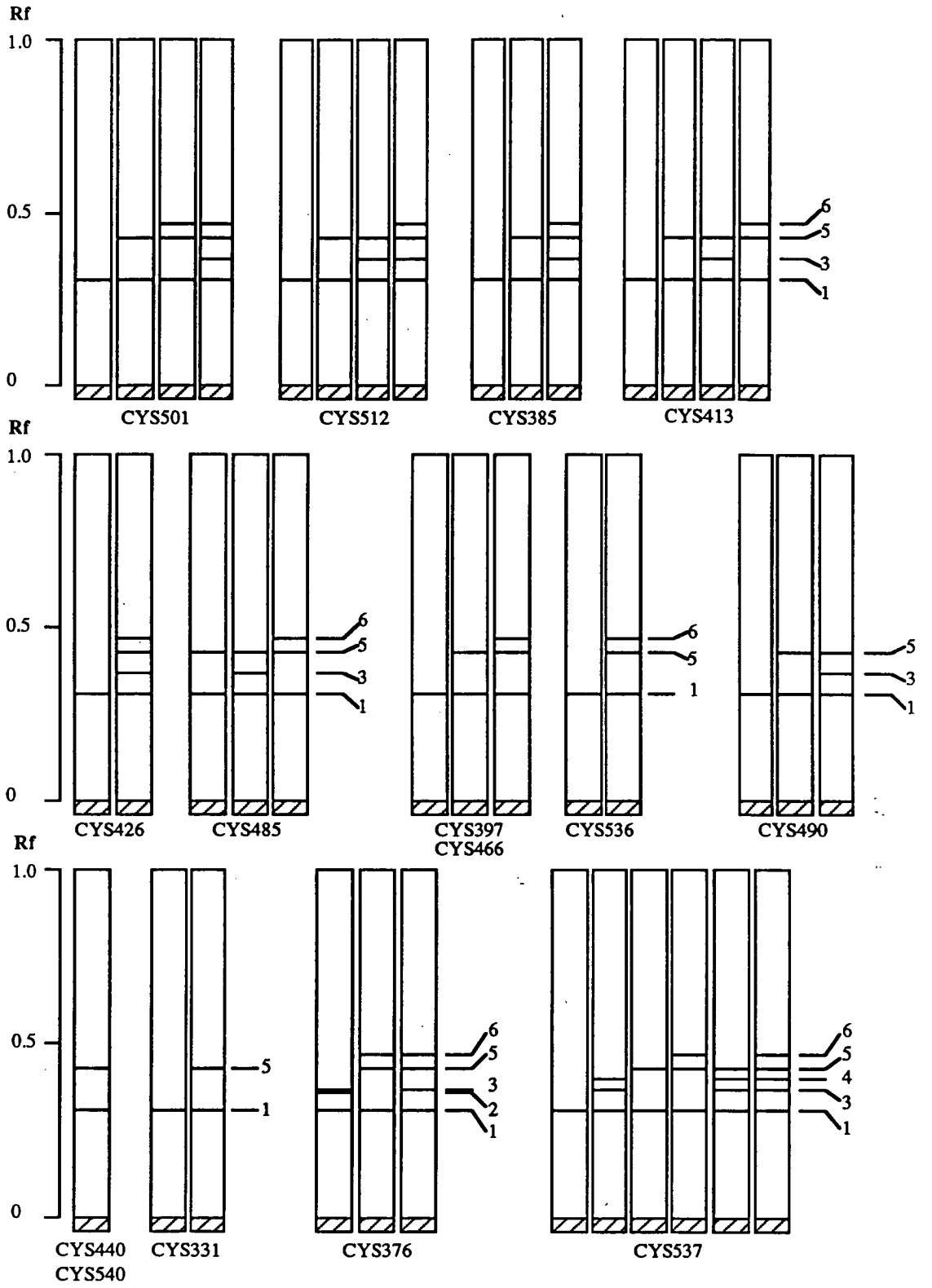


Fig. 7.29. Schematic representations of PG zymograms of isolates of *P. multicingulata* included in the study. Band numbers start from the cathocid end. Rf values: 1=0.31, 2=0.36, 3=0.37, 4=0.40, 5=0.43 & 6=0.47.

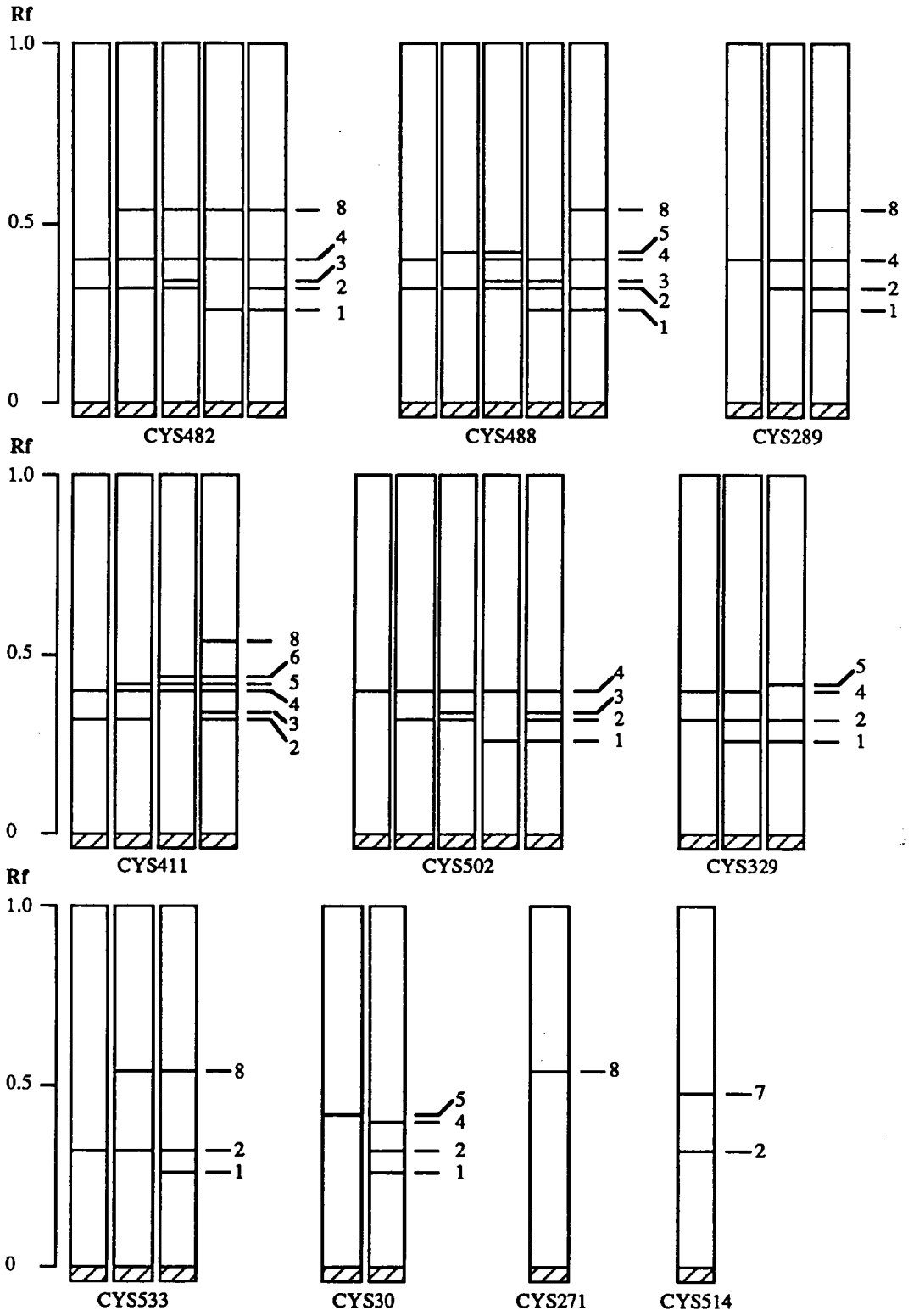


Fig. 7.30. Schematic representations of Lac zymograms of isolates of *Pholiota* sp D. Band numbers start from the cathodic end. Rf values: 1=0.26, 2=0.32, 3=0.34, 4=0.40, 5=0.42, 6=0.44, 7=0.48 & 8=0.54.

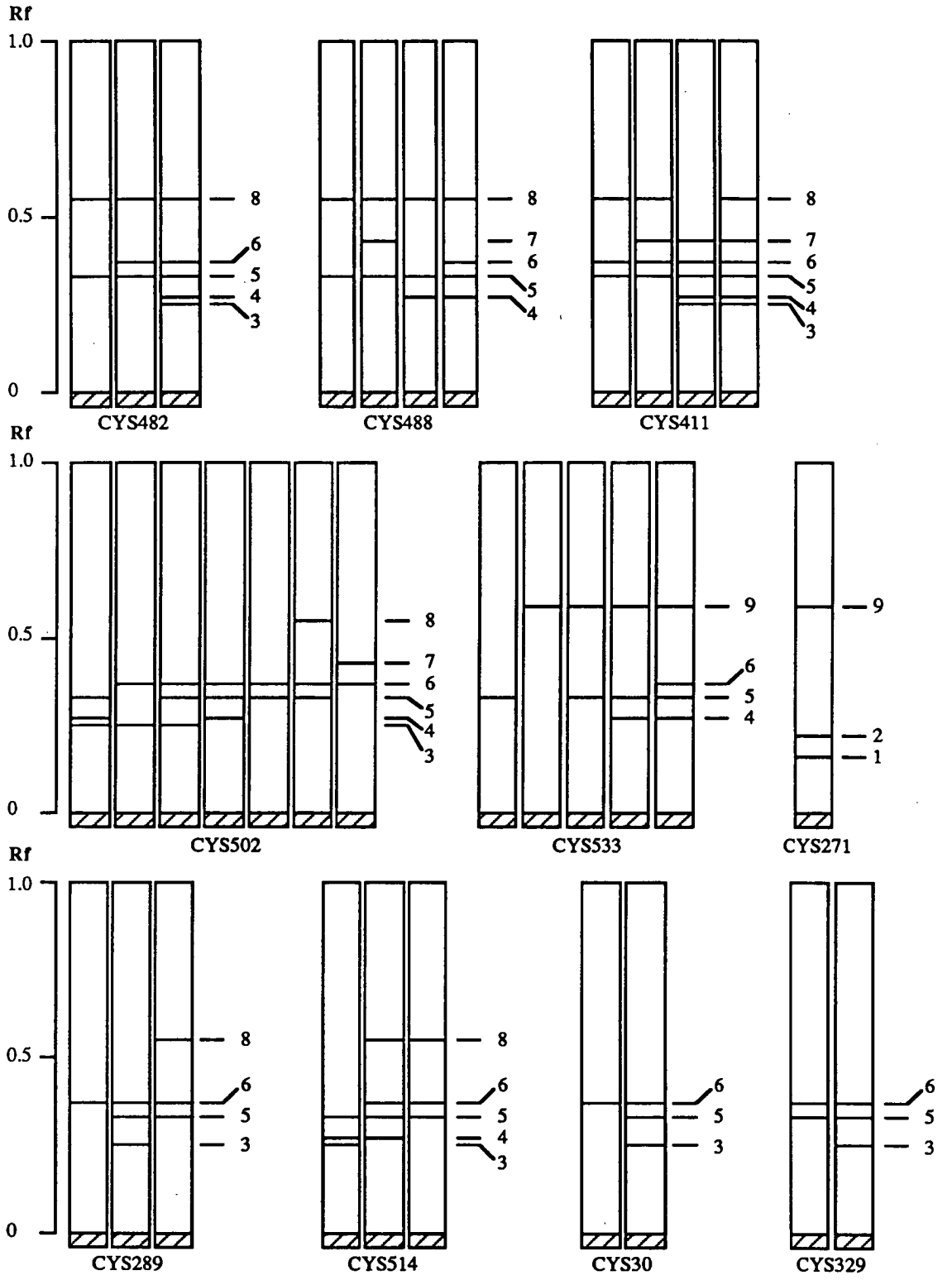


Fig. 7.31. Schematic representations of Per zymograms of isolates of *Pholiota* sp D. Band numbers start from the cathodic end. Rf values: 1=0.16, 2=0.22, 3=0.25, 4=0.27, 5=0.33, 6=0.37,7=0.43, 8=0.55 & 9=0.59.

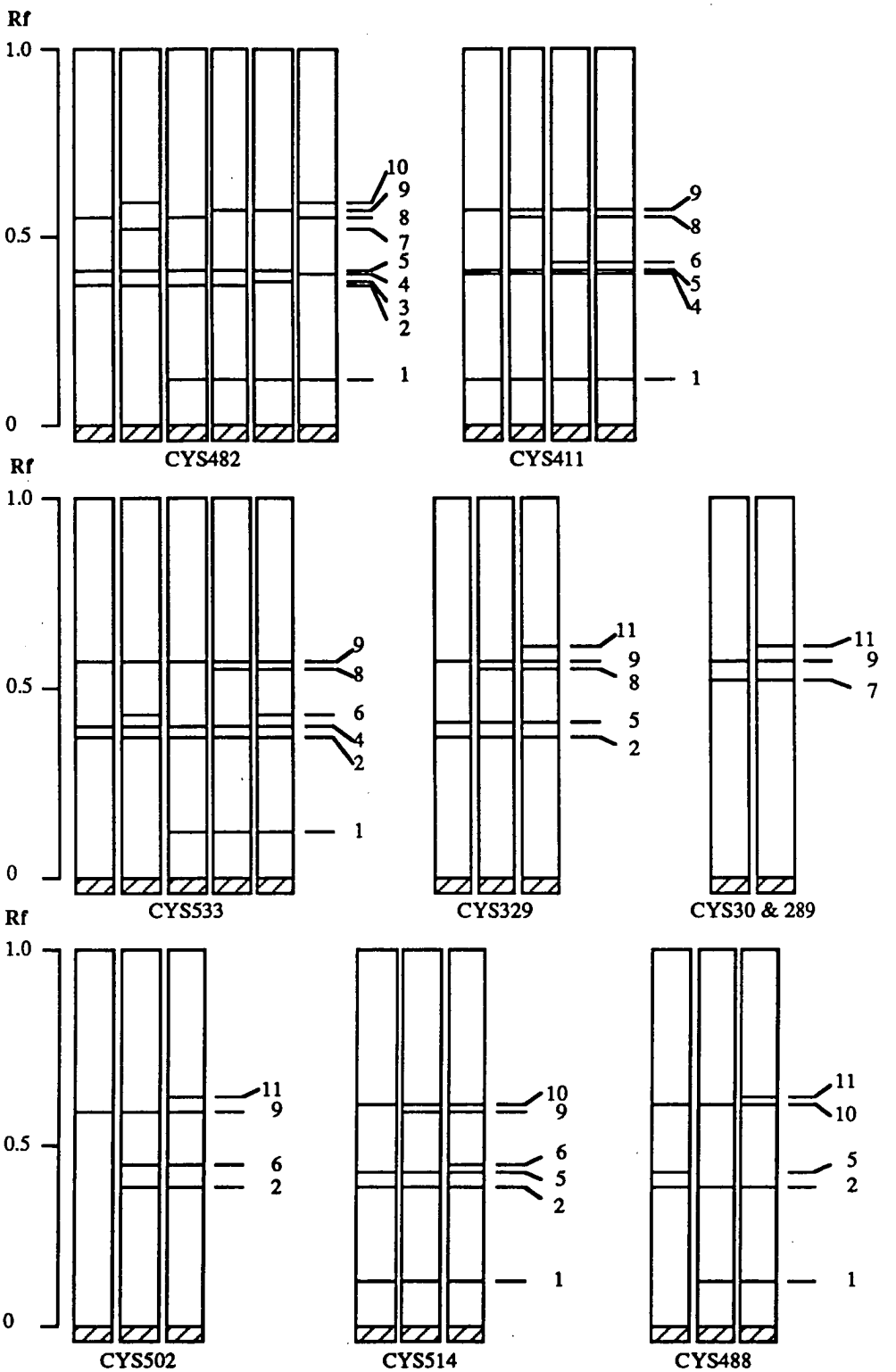


Fig. 7.32. Schematic representations of PE zymograms of isolates of *Pholiota* sp D. Band numbers start from the cathodic end. Rf values: 1=0.12, 2=0.37, 3=0.38, 4=0.40, 5=0.41, 6=0.43,7=0.52,8=0.55,9=0.57, 10=0.59 & 11=0.61.

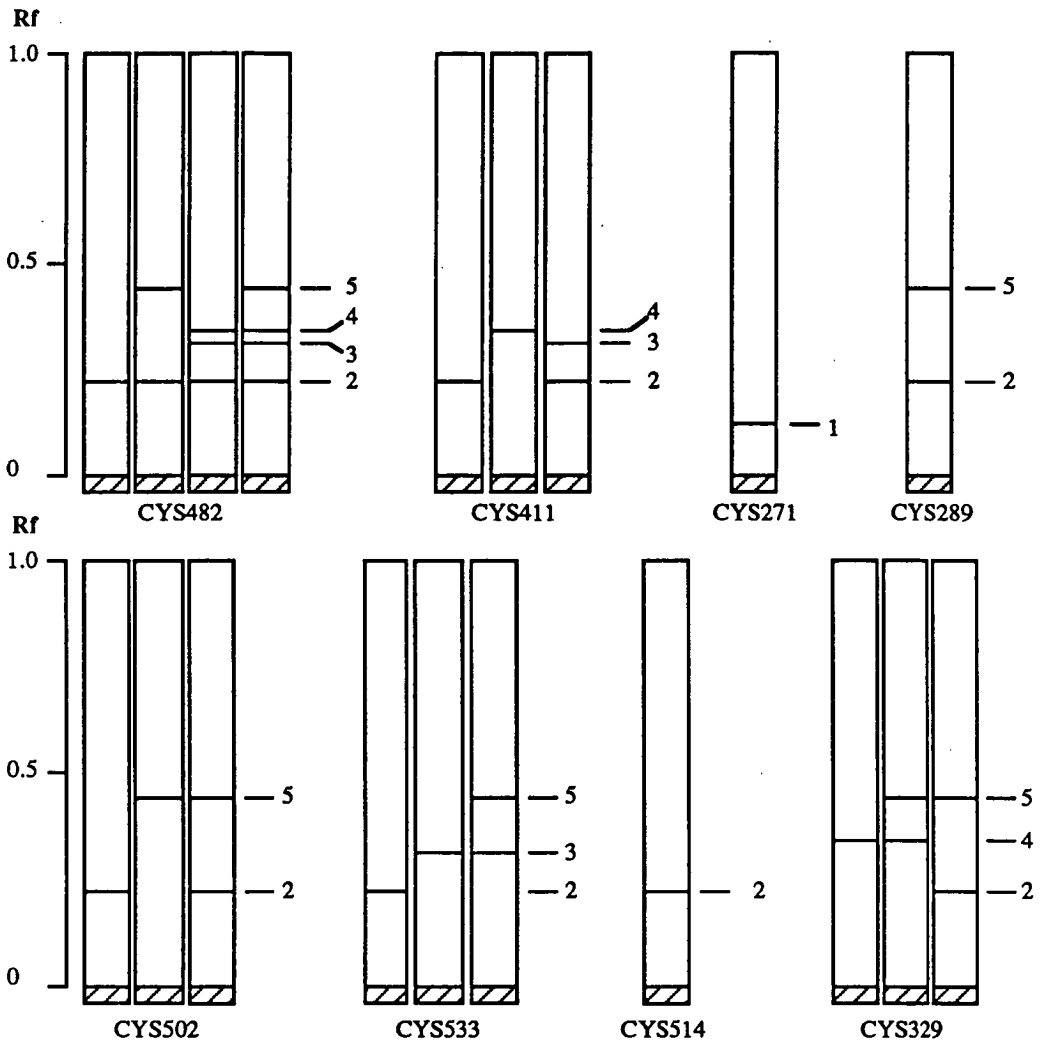


Fig. 7.33. Schematic representations of PG zymograms of isolates of *Pholiota* sp D. Band numbers start from the cathodic end. Rf values: 1=0.12, 2=0.22, 3=0.31, 4=0.34 & 5=0.44.

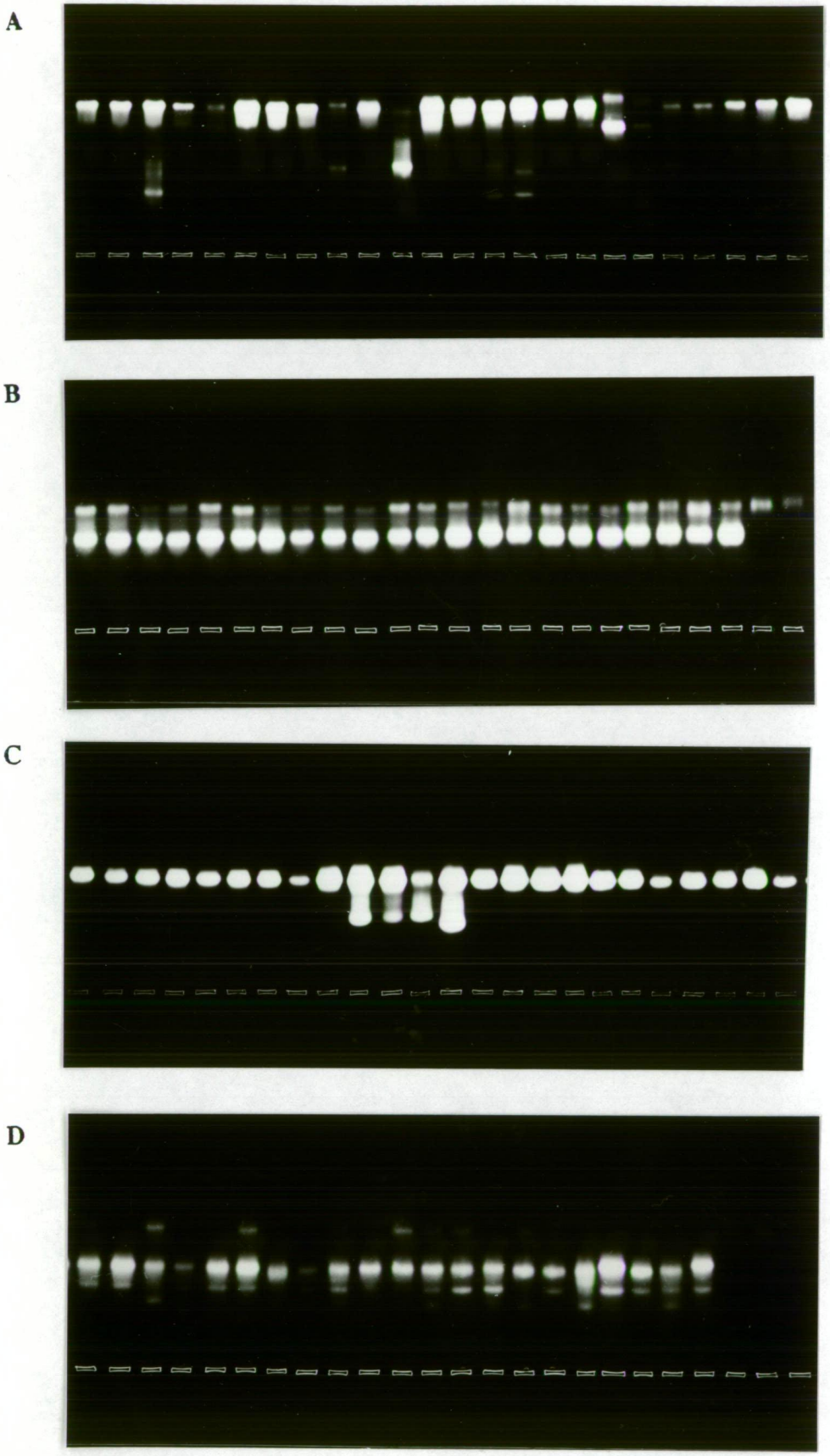


Fig. 7.34. Representative photograms of laccase zymograms of isolates of *Pholiota squarrosipes* (A) *P. highlandensis* (B), *P. multicingulata* (C) and *Pholiota* sp. D (D) showing species distinctiveness (x 0.8).

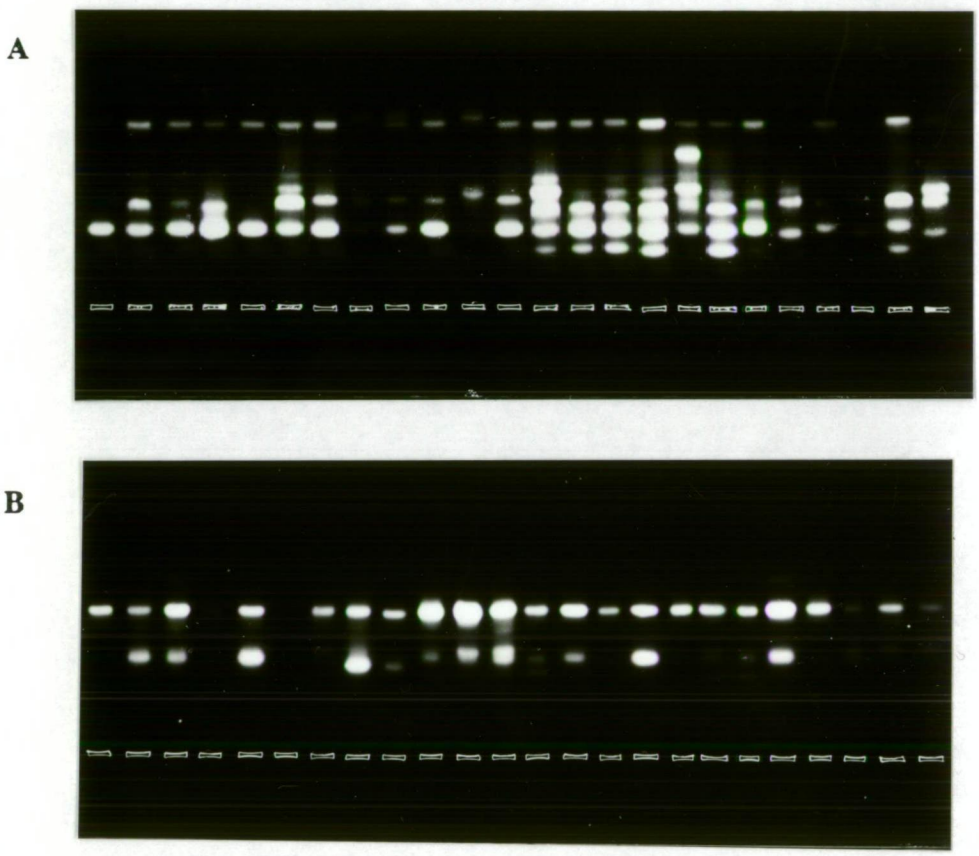


Fig. 7.35. Representative photograms of peroxidase zymograms of isolates of *Pholiota squarrosipes* (A) and *P. multicingulata* (B) showing species distinctiveness (x 0.8).

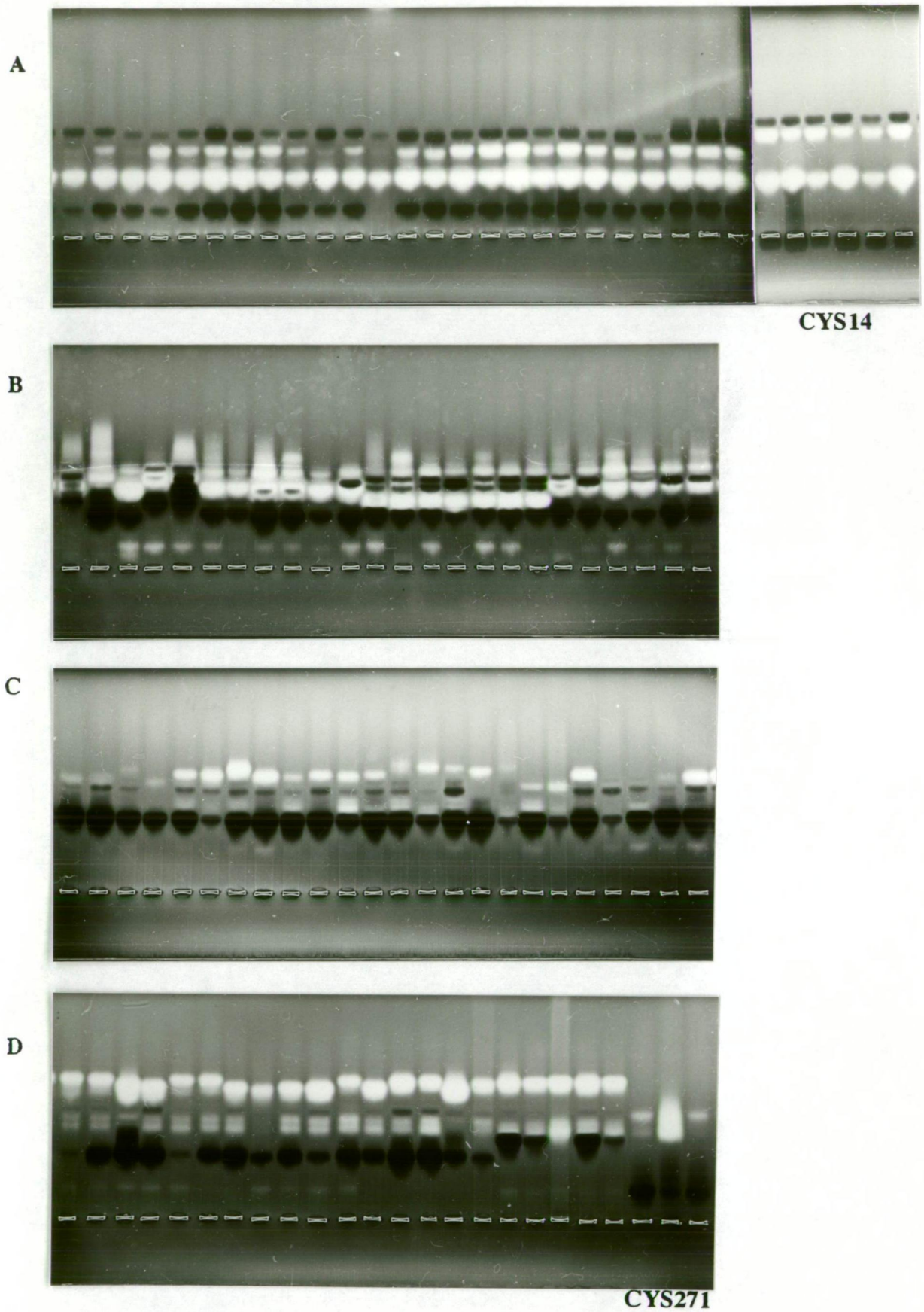


Fig. 7.36. Representative photograms of pectic zymograms of isolates of *Pholiota squarrosipes* (A) *P. highlandensis* (B), *P. multicingulata* (C) and *Pholiota* sp. D (D) showing species distinctiveness. White bands represent PE (pectinesterase) and dark bands PG (polygalacturonase) activities (x 0.8).

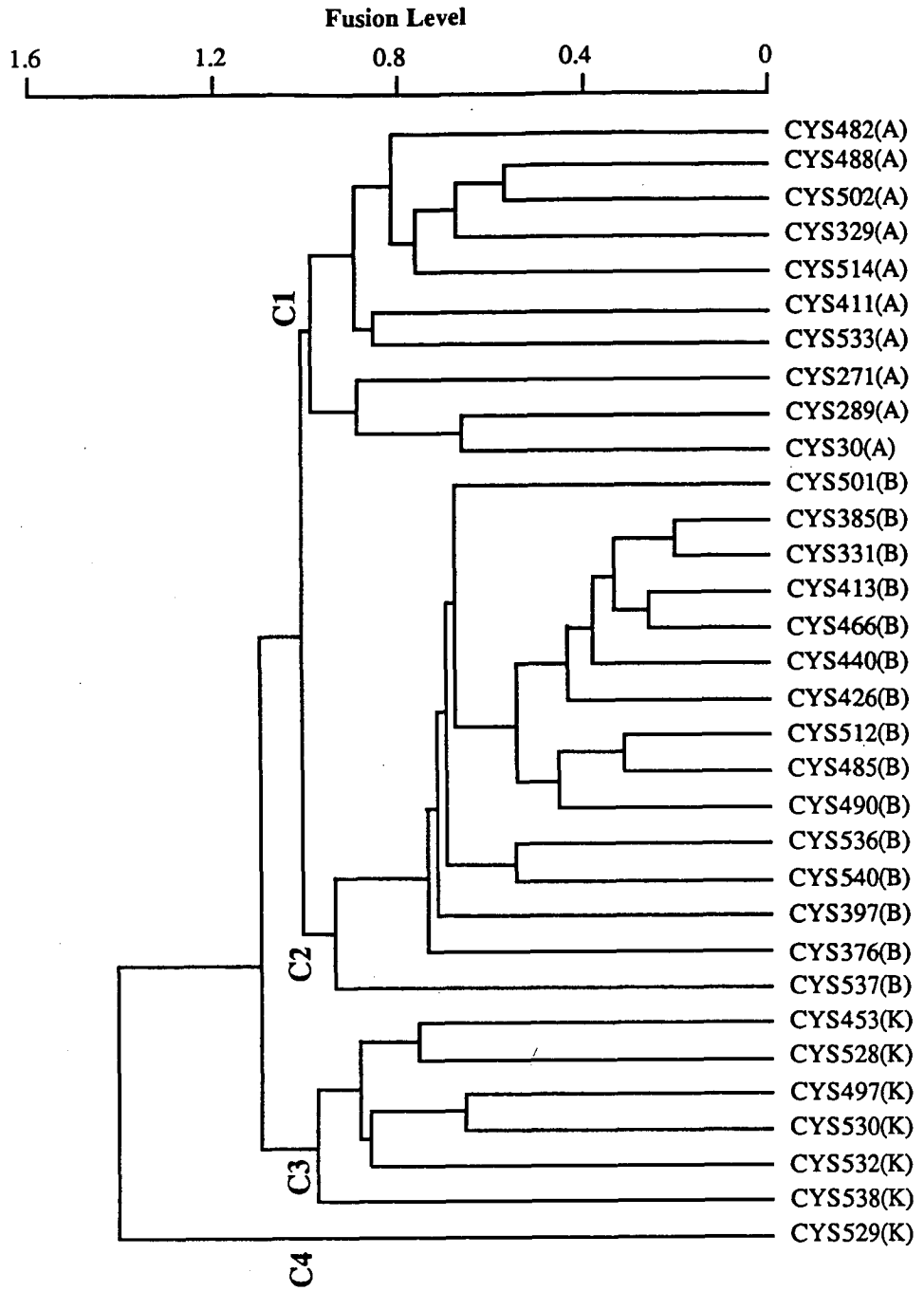


Fig. 7.37. Dendogram from UPGMA cluster analysis based on band frequencies of laccase and pectic isozymes from collections of subgenus *Flammuloides*.

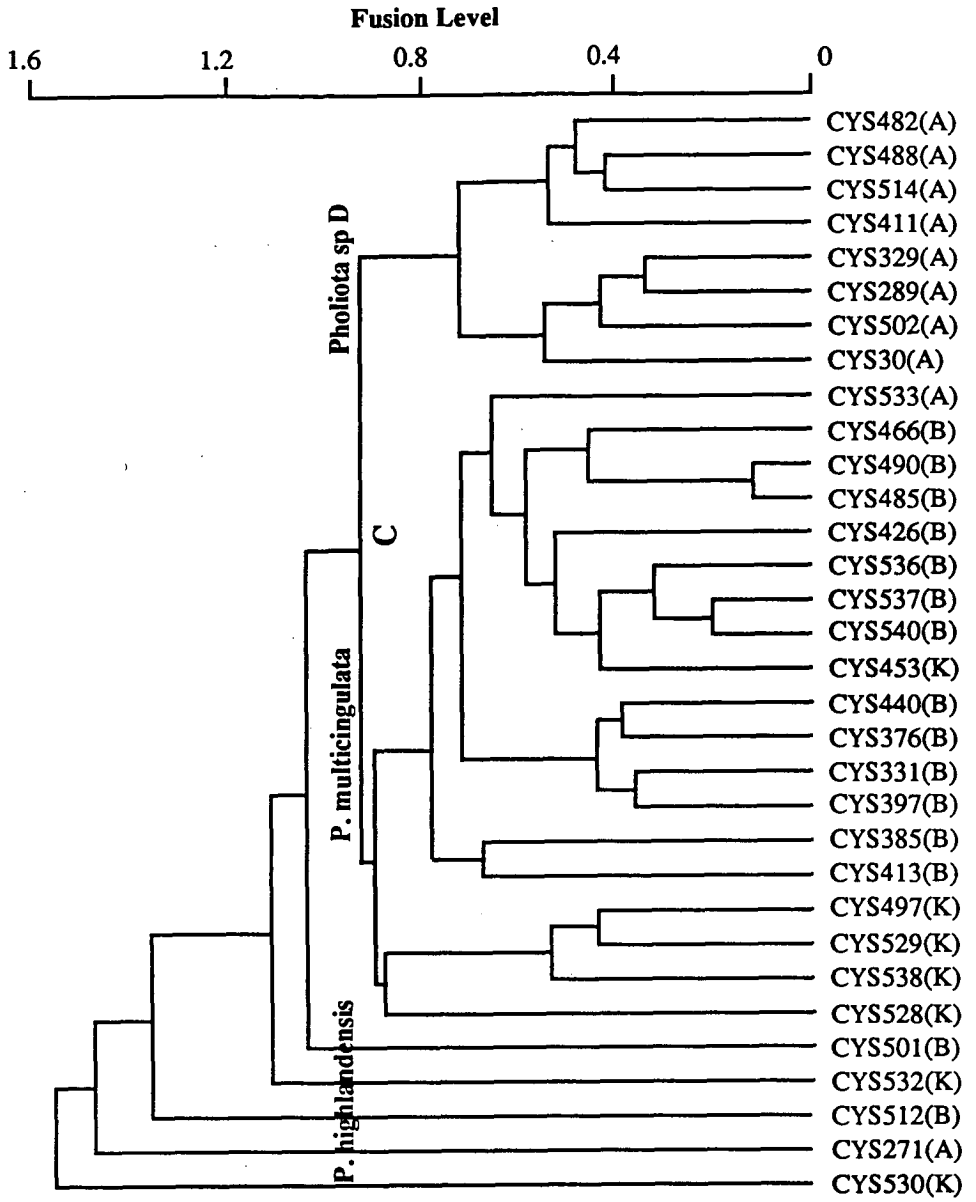
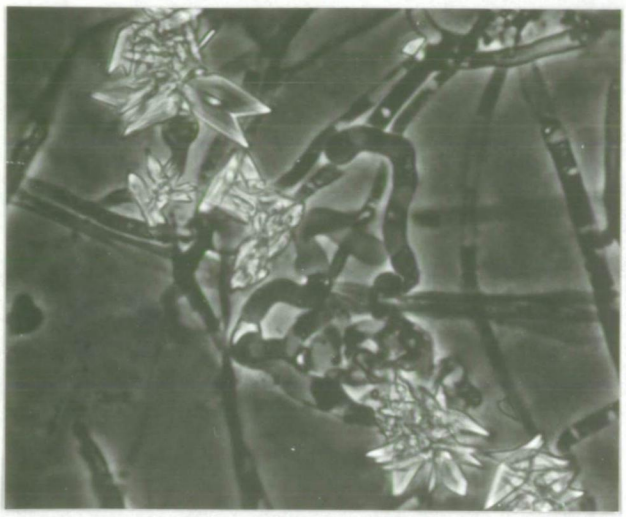


Fig. 7.38. Dendogram from UPGMA cluster ananlysis based on band frequencies of peroxidase isozymes only for collections of subgenus Flammuloides.

A



B

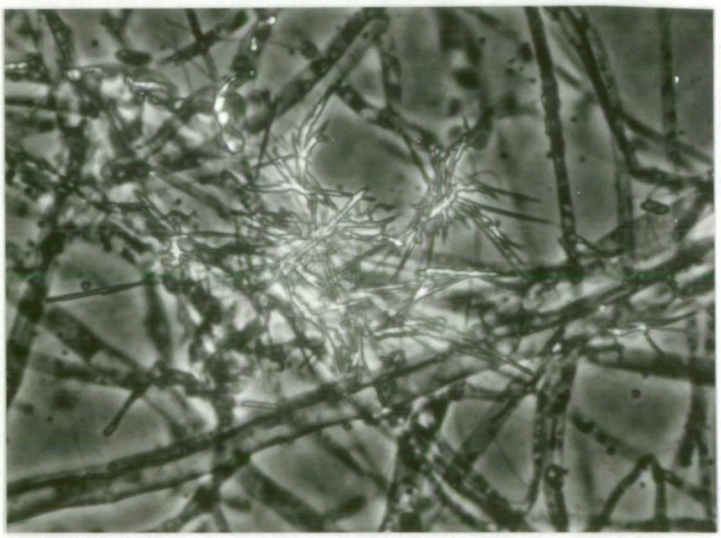
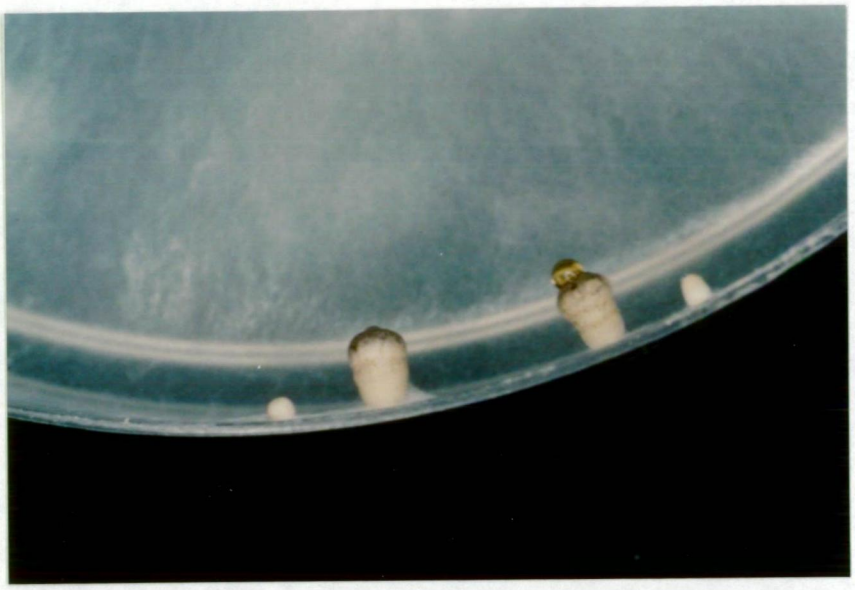


Fig. 7.39. Unusual cell types in *Pholiota* cultures. a) Acanthocyte-like cells in cultures of CYS271, *Pholiota* sp. D, and b) clusters of fusiform cells in cultures of CYS493, *P. multicingulata.*

a



b

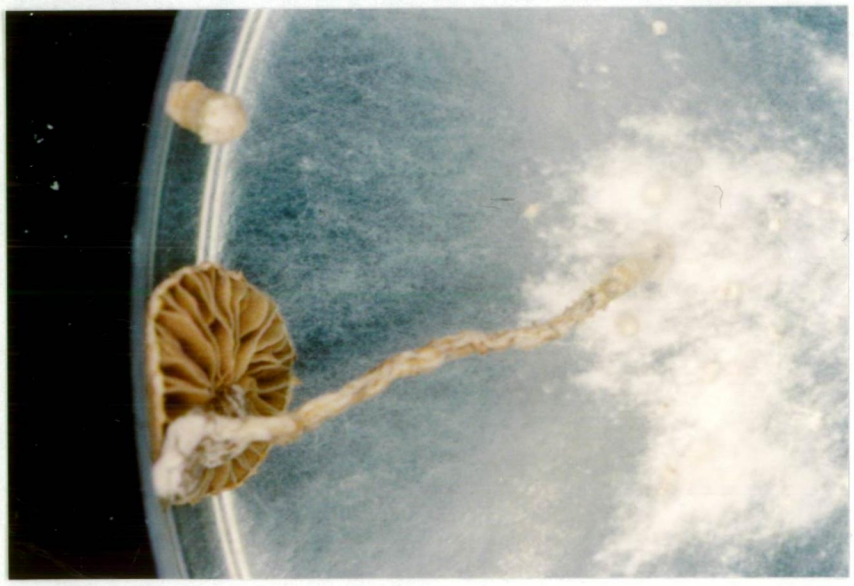
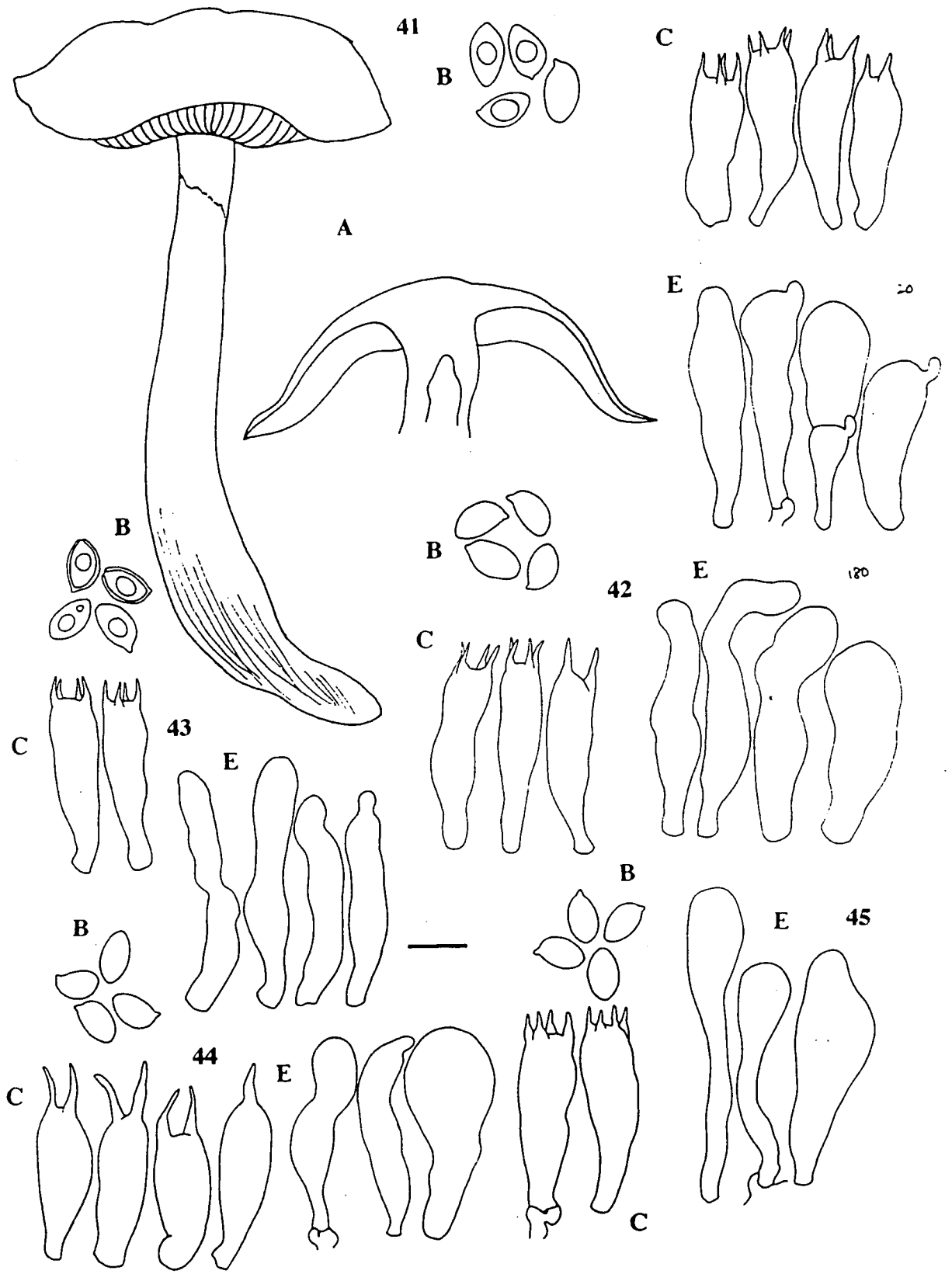
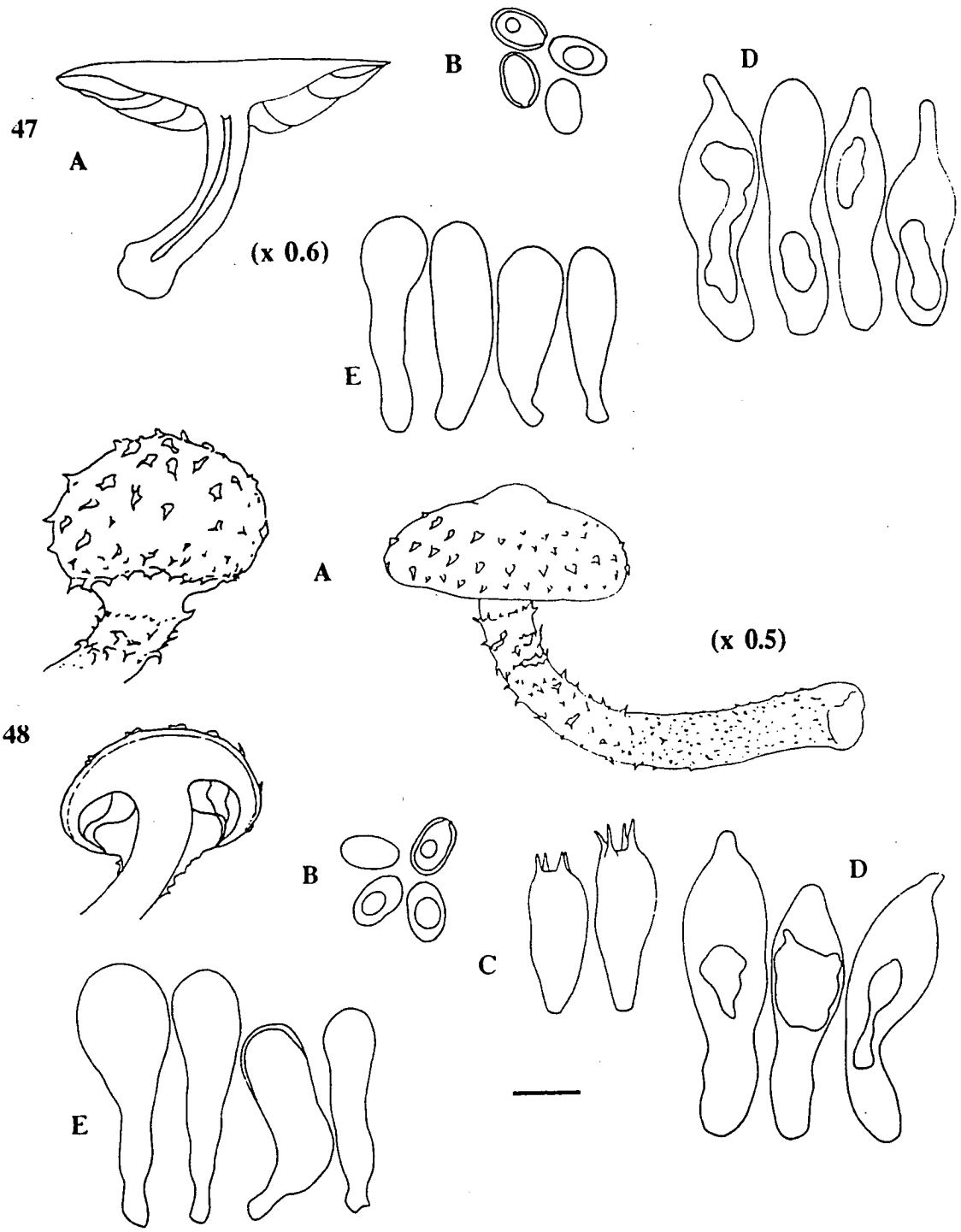


Fig. 7.40. Occasional fruiting in diakaryotic cultures of *Pholiota highlandensis*. a) Button stage with pallid veil, and b) more mature carpophore with slightly coloured veil remnants on stipe.



Figs. 7.41 - 45. *Pholiota malicola.* A: habit, B: spores, C: basidia, and E: cheilocystidia. 41. CYS20. 42. CYS180. 43. CYS226. 44. CYS357. 45. CYS383.

Fig. 7.46. Growth habit of *Pholiota malicola*.

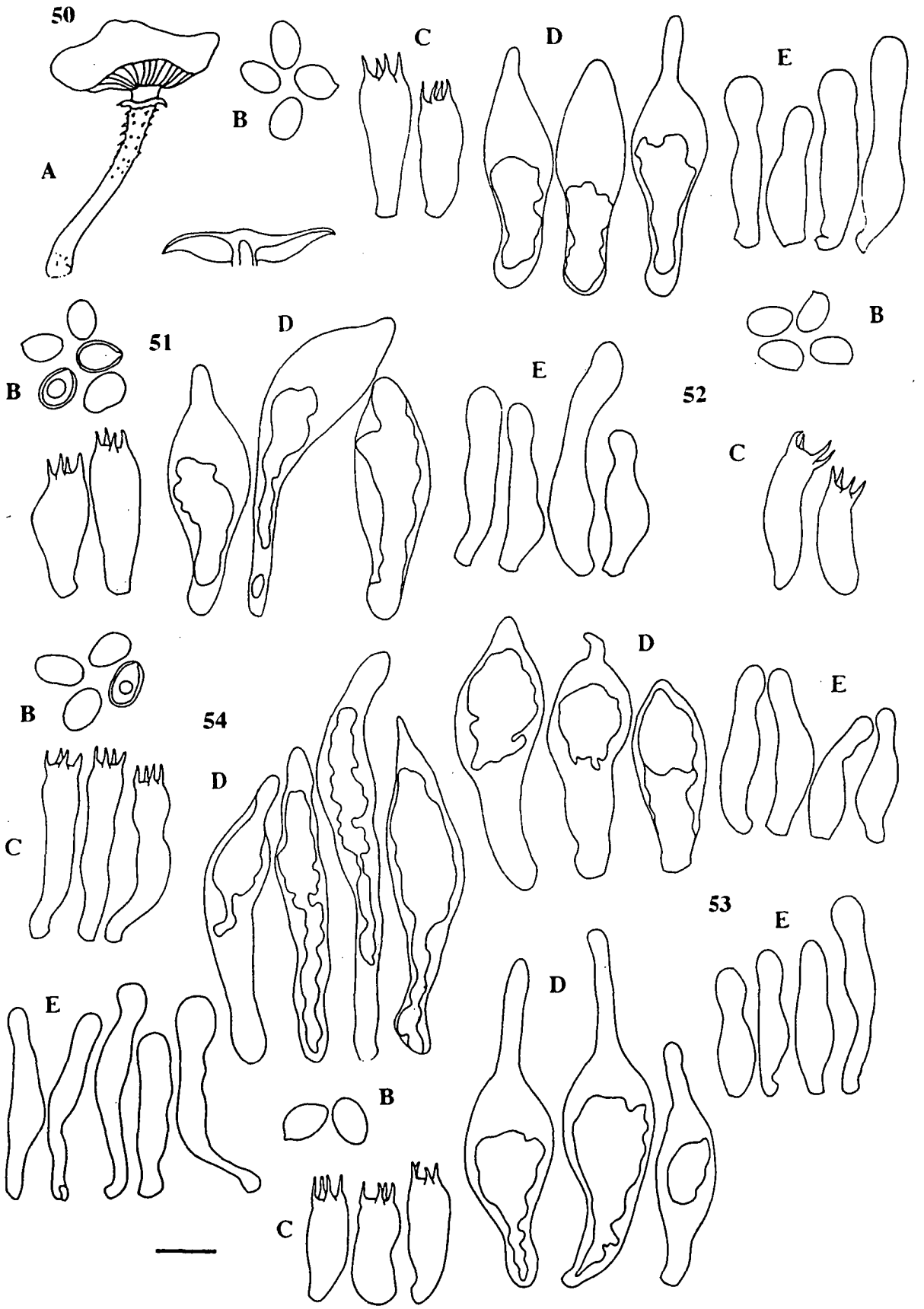


Figs. 7.47 - 48. *Pholiota aurivella.* A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 47. CYS157. 48. CYS159.

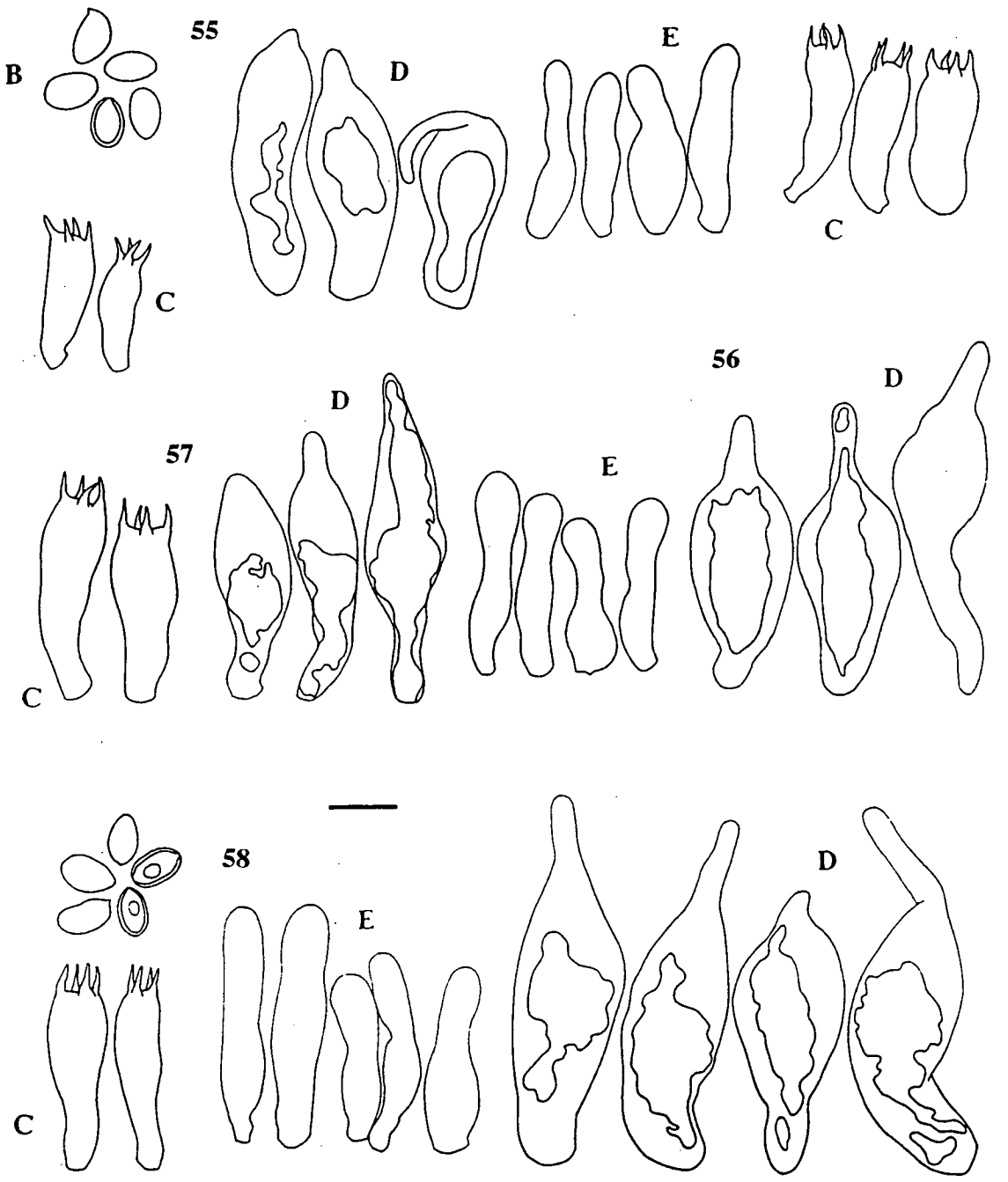
a

b

Fig. 7.49. Growth habit of *Pholiota aurivella*. a) CYS157 and b) CYS159.



Figs. 7.50 - 54. *Pholiota squarrosipes*. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 50. CYS16. 51. CYS496. 52. AD12142 (lectotype). 53. CYS458. 54. CYS424.



Figs. 7.55 - 58. *Pholiota squarrosipes*. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 55. AD11958 (syntype). 56. CYS489. 57. CYS2647. 58. *Pholiota* taxon 1, CYS14.

Fig. 7.59. Habit of *Pholiota squarrosipes*, the common form.

a

b

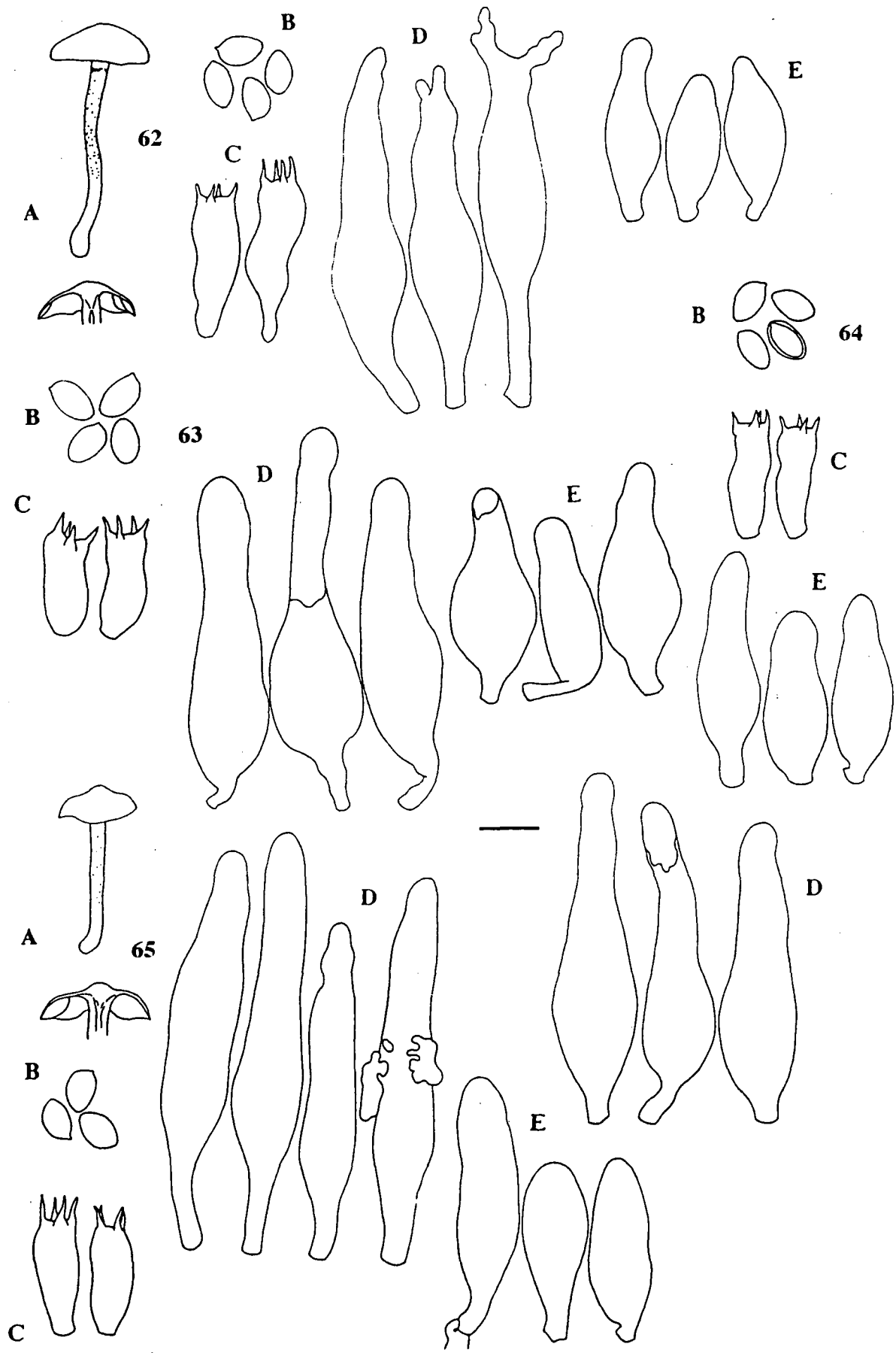
c

Fig. 7.60. Variability in habit of *Pholiota squarrosipes.* a) CYS496, b) CYS267 and c) CYS458.

a

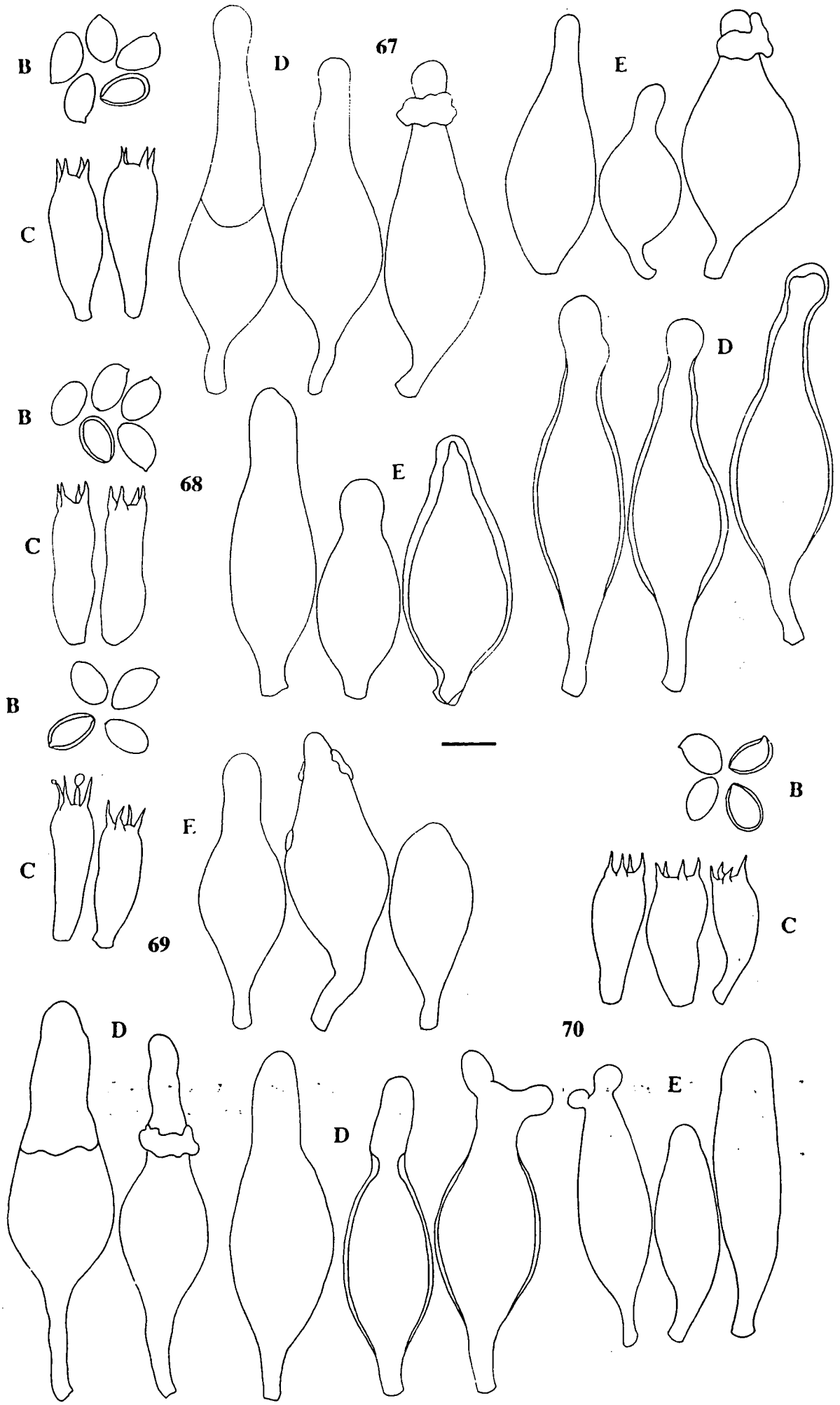
b

Fig. 7.61. *Pholiota squarrosipes* in various habitats. a) in relatively sheltered areas, amongst grass and mosses, and b) in apparently exposed area, on ground.



Figs. 7.62 - 65. *Pholiota highlandensis*. A: habit, B: spores, C: basidia, D: pleurocystidia, and E: cheilocystidia. 62. CYS532. 63. Type. 64. Smith32-10. 65. CYS329.

Fig. 7.66. Habit of *Pholiota highlandensis*, CYS538.


B
C
D
67
E
B
C
68
D
E
B
C
69
F
B
C
70
D
D
E

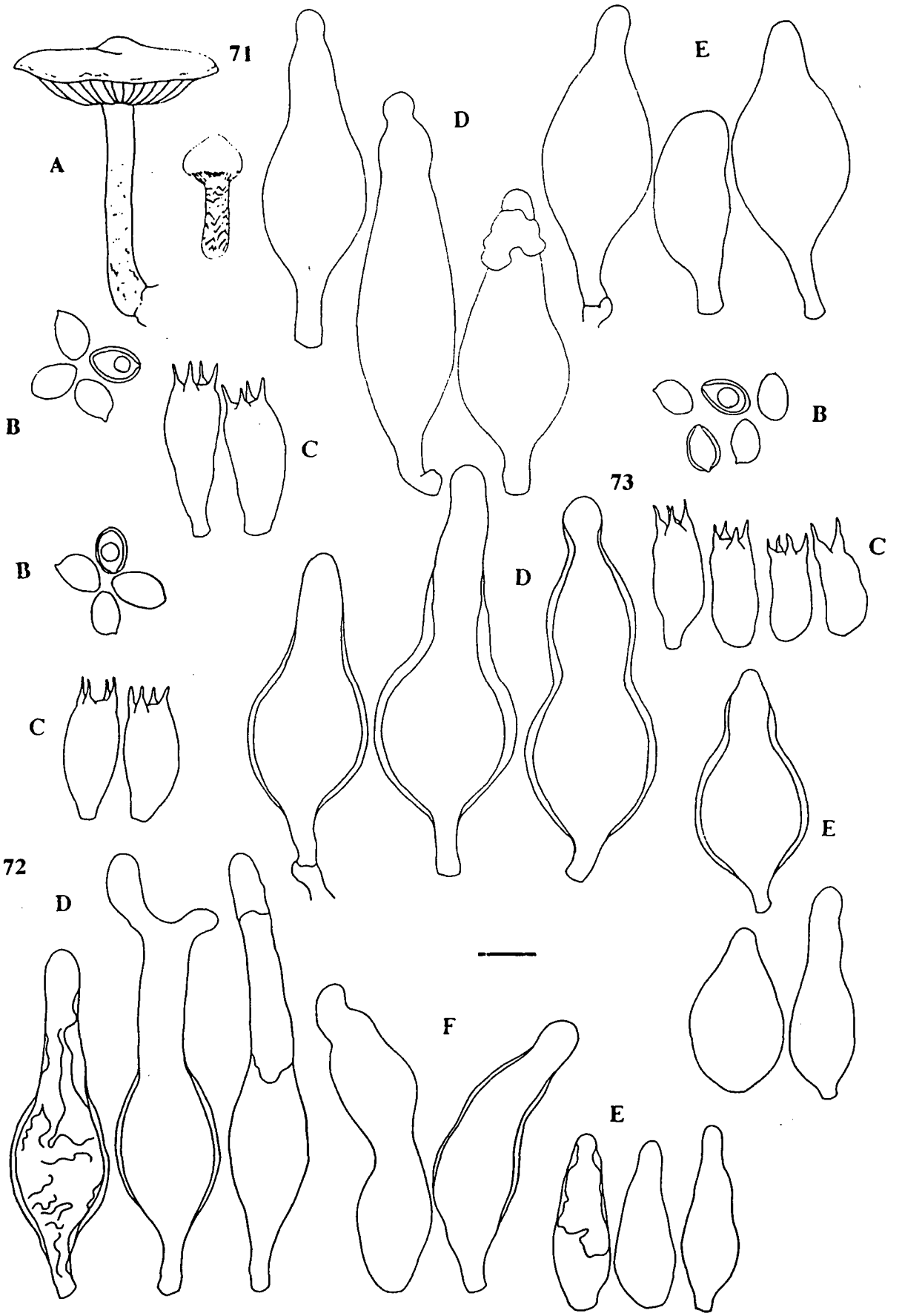


Fig. 7.71 - 73. A: habit, B: spores, C: basidia, D: pleurocystidia, E: cheilocystidia, and F: caulocystidia. 71. *Pholiota multicingulata*, CYS231. 72. *Pholiota* taxon 2, CYS271. 73. *Pholiota* taxon 3, CYS257.

Fig. 7.74. Habit of *Pholiota multicingulata*, CYS492.

a

b

Fig. 7.75. Variations in habit of *Pholiota multicingulata*, a) CYS299 and b) CYS475.

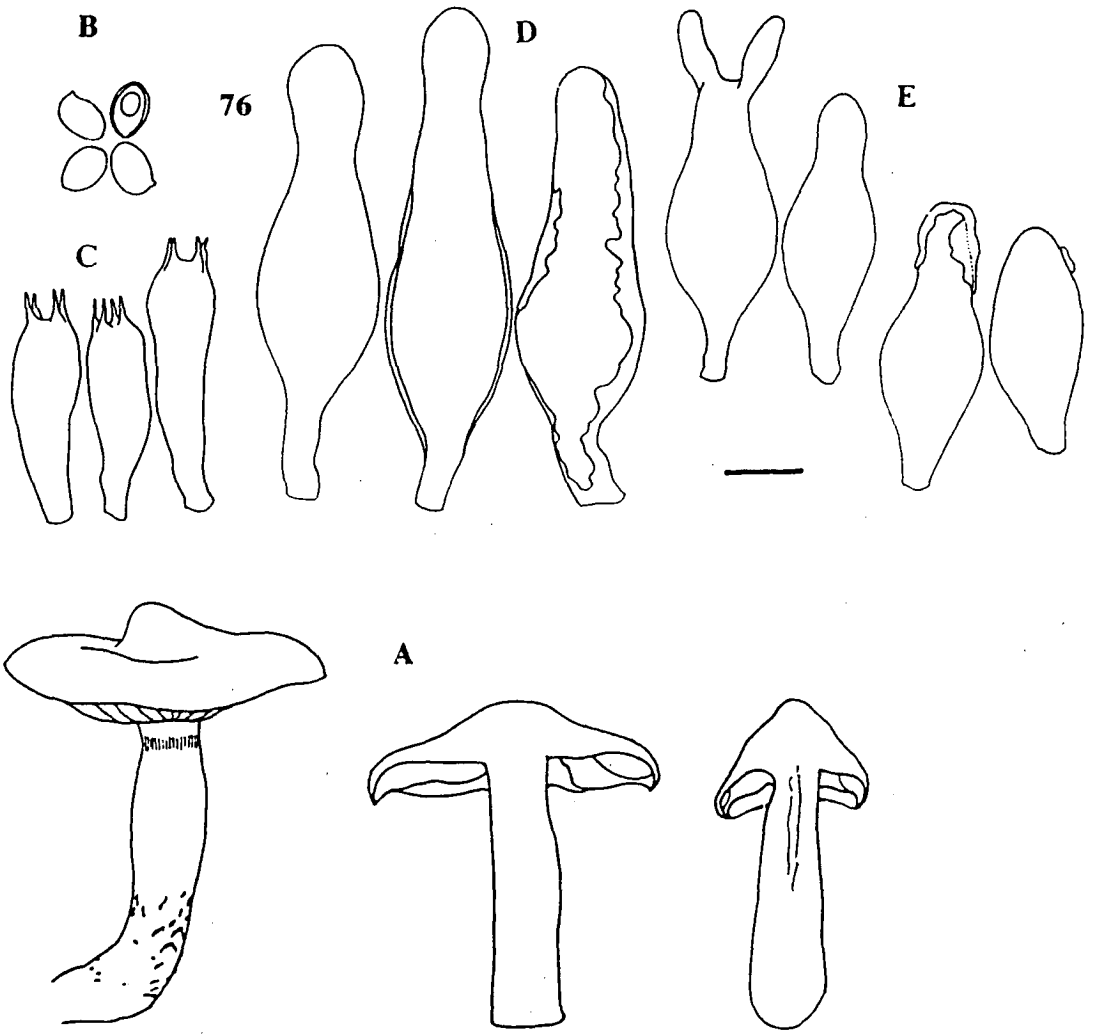


Fig. 7.76. Pholiota taxon 4, CYS266. A: habit, B: spores, C: basidia, D: pleurocystidia, and E: cheilocystidia.

Fig. 7.77. Habit of *Pholiota* taxon 4, CYS266.

Chapter 8

General Conclusions

This study has shown that the family Strophariaceae is represented by at least 31 taxa in Tasmania (Table 8.1). *Pholiota* is the genus with the greatest number of taxa (a total of 12 were identified). Genera in the subfamily Stropharioideae are relatively small in terms of species number. Table 8.2 gives an analysis of the representatives of this family identified and reported for Australia. It appears that many of the earlier species of *Psilocybe* and *Pholiota* are misidentified and the number reported for *Stropharia* and *Hypholoma* is probably underestimated. It would be safe to say that further study of these genera will yield additional numbers of species. This relative brief study which has for the most part been confined to the cool temperate SE corner of Australia has not attempted any analysis of the sub-tropical and tropical elements which can be expected within this family.

The study has shown the presence in Australia of a number of cosmopolitan species such as *Hypholoma fasciculare* and *Pholiota highlandensis*, widely dispersed species which may have had relatively recent introduction, e.g. *Stropharia semiglobata/S. stercoraria*, and species hitherto undescribed e.g. *Stropharia formosa* and *Pholiota fieldiana.*

This study has identified six taxa for *Stropharia* including two new species; six for *Hypholoma* including one new species and a variety; five for *Psilocybe* including two new species and resolving the relationships between the closely allied species in the '*P. subaeruginosa* complex'; one for *Melanotus* and 12 for *Pholiota* including three new species.

The use of morphological cirteria, both macro- and micro-characters for species

delineation has been found to be effective in most cases. The supplementary use of multivariate analysis appears useful when dealing with subtle differences between taxa particularly when the differences noted in the micro-characters of morphologically similar taxa are apparently part of a continuum such as in the case of *Stropharia semiglobata-S.stercoraria*. However, for the common species of *Hypholoma* and species of *Pholiota* in subgenus *Flammuloides* the considerable degree of overlap in the main micro-characters renders this approach less effective. Thus, the use of a multifaceted approach which includes complementary criteria other than morphology would certainly assist in elucidating the relationships between species.

Zymograms can provide tangible evidence of discrete parts of the total genome (Cruickshank 1989) because they can be examined for coincident similarity in overall pattern to provide evidence of close relationship or indicators of synonymy (Ferguson 1980). This study has found that laccase and pectic zymograms are species distinctive for most species in the family Strophariaceae. The genus *Hypholoma* appears to be the only exception which seems to exhibit a higher degree of conservativeness in the enzymes examined. Due to the complexity of the peroxidase enzymes (Gottlieb 1977), the peroxidase zymograms are less species distinctive.

The multifaceted approach employed in the study has successfully resolved the relationships between *Psilocybe subaeruginosa* and *P. australiana*, *P. eucalypta* and *P. tasmaniana*, identified sibling species in both *Stropharia* and *Pholiota*, and established *Pholiota. squarrosipes* Clel. and *P. multicingulata* Horak as species showing a wide range of morphological variations. In addition, such an approach also lends support to the applicability of the common species concept proposed for Hymenomycetes (Clémençon 1977) in the family Strophariaceae.

Psilocybe subaeruginosa Clel. is a taxon which as a result of various interpretations by

different mycologists, has been represented by four different names. This study has been able to show that certain morphological characters, such as the colour, size and shape of pleurocystidia, need to be interpreted with caution. The electrophoretic study show that laccase, peroxidase, acid phosphatase and pectic zymograms are species distinctive for this species. This conclusion is further supported by the complete intercompatibility results. Consequently, there is much doubt to the phylogenetic tree proposed by Guzmán (1983, p. 75) relating the various sections of *Psilocybe*. *P. cyanescens* Wakefield appears a close ally to *P. subaeruginosa* and future work similar to that conducted for *P. subaeruginosa* involving both species would no doubt be helpful in clarifying their relationships.

In the *Stropharia semiglobata/stercoraria* group, differences in interpretations have resulted in the grouping together of two very closely related taxa. Using the multifaceted approach, this study has shown that genetic barrier exists between the Tasmanian specimens of medium-spored form (*Stropharia semiglobata*) and large-spored form (*S. stercoraria*). Isolates from the two forms produced different zymograms for the enzymes tested, again indicating that the zymograms are species specific.

The combination of electrophoretic and compatibility approaches with morphological comparisons also show that fungi exhibiting wide variations in moprhology can be a single biological entity sharing a common gene pool and this is illustrated in the Tasmanian specimens of *Pholiota squarrosipes* and *P. multicingulata.*

There is scope for future work. Firstly, collecting has been concentrated in south-east Tasmania in this study, future collecting trips can be extended to other parts of Tasmania including a greater range of habitats then more taxa will certainly be added to Strophariaceae. Secondly, the nomenclatural confusion between *S. semiglobata* and

S. stercoraria needs to be clarified. However, *S. semiglobata* s. l. is probably an introduced species, only through the examination of a wide range of material particularly from Europe and by using a similar approach to that of this study would both parts of this problem be resolved. The first of which is to confirm the presence of both *S. semiglobata* and *S. stercoraria* in Europe; then to clarify their relationship to the Tasmanian representatives. Thirdly, there is the likelihood that *P. highlandensis* Peck and *P. carbonaria* A. H. Smith may not be separate species (Jacobsson 1990). This study has indicated the unreliability in the colour of partial veil as a diagnostic character. Future studies of the multifaceted approach employed here would help to verify the species status of these two taxa. Fourthly, it has been suggested in this study that as a result of the Gondwanic association between Australia and Papua New Guinea, *P. multicingulata* and *P. austrospumosa* be considered as closely related species in a complex parallel to the "*spumosa* complex" in the northern hemisphere. Future study can investigate the affinity of these two taxa to substantiate the above suggestion. Finally, the approach used in this study can certainly be extended to other species in the Agaricales.

The technique of electrophoresis of extracellular enzymes used in the present study is shown to be simple, quick and cost effective. The zymograms thus obtained are shown to provide strong evidence for separation to putative specific groups. Subjecting these groups to further stringent morphological and mating analysis would help to determine a coherent systematic arrangement within a genus (May & Royse 1988).

Table 8.1 Synopsis of all the taxa delineated in the study for each genera in the family Strophariaceae.

Subfamily **Strophalioideae** Singer

Genus ***Stropharia*** Kummer

Section Mundae (Fr.) Konr. & Maubl.

Species delineated: *S. coronilla* (Bull. ex Fr.) Quél. & *S. aurantiaca* Orton

Section Stropharia Singer

Species delineated: *S. formosa* sp. nov.

Section Stercophila (Romagnesi) Singer

Species delineated: *S. stercoraria* (Fr.) s. Saccardo,

S. semiglobata (Batsch ex Fr.) Quél. & *S. parvula* sp. nov.

Genus ***Hypholoma*** Fr.

Section Fascicularia Smith

Species delineated: *H. sublaterium* Fr., *H. brunnea* (Massee) Reid.,

H. fasciculare Huds. ex Quél., *H. fasciculare* var. *armeniacum* var. nov.

& *Hypholoma* taxon 1.

Section Tenacia Smith

Species delineated: *H. paludicolum* sp. nov. & *Hypholoma* taxon 2.

Genus ***Psiloeybe*** Kummer

Section Cyanescens Guzmán

Species delineated: *P. subaeruginosa* Clel. (syn. *P. australiana* Guzmán & Watling,

P. eucalypta Guzmán & Watling and *P. tasmaniana* Guzmán & Watling)

Section Semilanceatae Guzmán

Species delineated: *P. semilanceata* (Fr. ex Sacc.) Kummer & *P. alutacea* sp. nov.

Section Coprophilae Guzmán

Species delineated: *P. coprophila* (Bull. ex Fr.) Kummer

Section Aztecorum Guzmán

Species delineated: *P. brunneo-albescens* sp. nov.

Genus ***Melanotus*** Pat.

Species delineated: *M. heptochrous* (Berk.) Singer

Subfamily **Pholiotoideae** Singer

Genus ***Pholiota*** Kummer

Subgenus *Phaeonaematoloma*

Section Phaeonaematoloma

Species delineated: *P. fieldiana* sp. nov. &

P. visco-fumosa sp. nov.

Subgenus *Flammula*

Section Flammula

Species delineated: *P. malicola* (Kauff.) A. H. Smith

Subgenus *Pholiota*

Section Adiposae

Species delineated: *P. aurivella* (Fr.) Kummer,

P. squarrosipes Clel. & *Pholiota* taxon 1

Subgenus *Flammuloides*

Section Flammuloides

Species delineated: *P. pallidocaulis* sp. nov., *Pholiota* taxon 2

& *Pholiota* taxon 3

Section *Carbonicola*

Species delineated: *P. highlandensis* (Peck) Smith & Hesler

Section Spumosae

Species delineated: *P. multicingulata* Horak & *Pholiota* taxon 4

Table 8.2. Past records of the number of species in the family Strophariaceae (sensu Singer) in Australia.

Genus	No. of species reported	No. of species excluded	No. of doubtful species	Source
Stropharia	4	2	2	Cooke 1892
	4	2	2	Cleland 1934
	6			Shepherd & Totterdell 1988
	6			Present study (for Tasmania)
Hypholoma	4	1		Cooke 1892
	3	1		Cleland 1934
	7			Shepherd & Totterdell 1988
	6			Present study (for Tasmania)
Psilocybe	8	3	4	Cooke 1892 (including *Deconica*)
	11	6	2	Cleland 1934
	27	12	7	Guzmán & Watling 1978
	15			Shepherd & Totterdell 1988
	5			Present study (for Tasmania)
Melanotus	2	1	1	Cooke 1892 (as *Crepidotus*)
	1			Cleland 1934 (as *Crepidotus*)
	1			Present study (for Tasmania)
Pholiota	38	26	2	Cooke 1892
	23	15		Cleland 1934
	7	1		Pegler 1965
	30			Shepherd & Totterdell 1988
	12			Present study (for Tasmania)

Chapter 9

New Species

The new species recognized here do not conform to any known published descriptions and their affinity to existing species will be noted for each species.

There are altogether 31 taxa delineated in the project (see Table 8.1). Of these, 8 are given specific epithet and one a variety status. They will be formally described below, the Latin vocabulary is from Stearn (1973). Of the remaining taxa, 16 are previously described species and five are referred to as taxon 1- 5 due to the lack of information of their variation and distribution. Many of these latter taxa consisted of single collections and have been included in the section Taxonomy in the appropriate genera. Two taxa, *Pholiota* taxon 1 and *Pholiota* taxon 2, were delineated through mating compatibility studies and to a certain extent isozyme profiles. Whilst three other taxa, *Hypholoma* taxon 1, *Pholiota* taxon 3 and *Pholiota* taxon 4, were delineated based on morphological characters.

All the drawings of microcharacters are reproduced using an Olympus drawing tube, and the scale bar (10 μm.) is the same for each species and habit drawings are of natural size unless specified otherwise.

Genus *Stropharia*

1. *Stropharia formosa* sp. nov.

(*formosa*, handsome)

Selected illustration: Fuhrer & Robinson (1992), p. 59.

Illustrations: Figs. 9.1 - 5

Pileus 28 - 55 mm. latus, convexus vel subumbonatus, badius/spadiceus tinctus

violaceus vel brunneo-vinosus; pagina mucosa, strato tenui glutinoso et squamis latis appressis, facile exutis, tandem glabra; margo vestigiis appendiculatis. *Lamellae* adnatae, latae, luteo-griseae tandem griseo-brunneae, margine alburo. *Stipes* 56-80 (-99) mm. longus, (3-) 4.5 - 8 mm. crassus, cavus, albo-flocculosus, basi albo mycelio. *Contextus* albidus, firmus. *Velum* pallido luteum, submembranaceum, evanescens. *Sporae* in massa violaceo-fuscae, 9.6-11.7 x 5.8-7.5 (-7.9) x 5.8-7.5 µm., ellipsoideae vel subellipsoideae; poro germinali lato. *Basidia* 21.7 - 30.8 x 6.7 - 11.7 µm., plerumque tetra-spora, raro bi- vel tri-spora, clavata vel constricta ad medium. *Pleurocystidia* ut chrysocystidia, 33.7-54.2 x 10.8-18.3 µm., fusoide ventricosa vel elongato-subclavata, pro parte majore prominentibus protuberatibus. *Cheilocystidia* 23.3-34.2 x 6.7-16.2 µm., hyalina, tenuitunicata, clavata.
Subhymenium subcellulosum. *Trama* regularis, aetate interta. *Epicutis* stratum ex hypharum gelatinosarum repentibarum., brunneolarum, 2 - 4 µm. latarum, fibuligerium. Solitara vel dispersa in terra in sylva mixto.

Holotypus: Tasmania, Tahune Forest Reserve, 1. v. 1990, CYS341 (HO); isotypus in DAR conservatum.

Pileus 28 - 55 mm. broad, convex or subumbonate, a rich date brown with a vinaceous tint (close to 9F7 - 8) or vinaceous brown (7E4 -5); surface slimy viscid with a thin glutinous layer, broad appressed easily abraded scales then appearing glabrous; veil remnants appendiculate along margin. *Lamellae* adnate, broad, yellowish grey (4C2) then greyish brown (5D3); margin white. *Stipe* 56-80 (-99) x (3-)4.5 - 8 mm., hollow, white flocculose below veil line, white mycelium at base. *Context* whitish (close to 2A2), firm. *Veil* pale yellow (3A2), somewhat membranous, evanescent.
Spores violaceous black in mass, 9.6-11.7 x 5.8-7.5 (-7.9) x 5.8-7.5 µm., ellipsoid or subellipsoid, germ pore broad. *Basidia* 21.7 - 30.8 x 6.7 - 11.7 µm., majority 4-spored, more rarely 2- or 3-spored, clavate or constricted at waist. *Pleurocystidia* as

chrysocystidia, 33.7-54.2 x 10.8-18.3 µm., fusoid ventricose or elongate clavate, majority with prominent protuberance. *Cheilocystidia* 23.3-34.2 x 6.7-16.2 µm., hyaline, thin-walled, clavate.

Subhymenium subcellular. *Trama* regular, tending to interwoven with age. *Epicutis* a layer of gelatinised, repent, pale brown hyphae, 2 - 4 µm. broad, bearing clamp connections.

Solitary or scattered on general ground litter, of either leafy or woody debris.

Type: Tasmania, Tahune Forest Reserve, on ground litter beside track in mixed forest, 1. v. 1990 (HO), *Y. S. Chang*, CYS341; isotype in DAR.

Specimens examined: Tasmania, Mt Field National Park, on ground litter in mixed forest, 2. v. 1989, *Y. S. Chang*, CYS137; Mt Field National Park, Track to Lady Barron Falls, on ground litter on creek bank, 2. v. 1989, *Y. S. Chang*, CYS160; Tasman Peninsula, Fortesque Bay, behind sand dune, *A. K. Mills*, 25. ix. 1989, CYS327; Tahune Forest Reserve, on ground beside track, *B. Fuhrer*, 7. v. 1990, CYS358.

Observations

This fungus is a typical exannulate species of *Stropharia*. The most distinctive features in the field are the vinaceous brown colour of the pileus, the viscid to glutinous surface, the appendiculate veil remnants along the margin and the flocculose stipe. These characters place it in Section *Stropharia*. It is close to *S. hornemannii* and *S. ambigua* in stature but differs from them in the colour of pileus and the evanescent veil.

2. *Stropharia parvula* sp. nov.

(*parvulus*, very small)

Illustrations: Figs. 9.6 - 10.

Pileus 5-10 mm. latus, conico-convexus vel subcampanulatus, viscidus vel mucosus, dilute hygrophanus, glaber, aurantio-brunneus omino, magis brunneus ad discum, pallido bubalinus decolorans. *Lamellae* subdecurrentes, luteo-griseae tandem griseo-brunneae. *Stipes* 18-52 x 1 - 1.5 mm., aequalis, viscidus, strato tenui glutinoso infra velum, luteo albidus, sub-bulbosus basi. *Contextus* tenuis, pileo concolorus. *Velum* arachnoideum, evanescens.

Sporae 12.9-15.4 x 7.5-9.2 x 7.9-9.6 µm., elongato-ellipsoideae.

Basidia (18.3-) 21.7 - 35 x 9.2 - 19.2 µm., tetra-spora, clavata vel pyriformia.

Pleurocystidia ut chrysocystidia, 32.1-47.5 x 11.7-20 µm., fusoido-ventricosa.

Cheilocystidia 25.8-38.3 (-41.7) x (4.2-) 5-9.2 µm., hyalina, lageniformia vel elongato-clavata.

Subhymenium subcellulosum. *Trama* regularis, intertextus prope marginem lamellarum, hyphis 2 -16 µm. latis, pallido luteo-brunneolis (5%KOH). *Epicutis* stratum tenuis hypharum gelatinosarum repentibarum, 2 -4 µm. latarum, fibuligerium numerosarum. *Hypodermium* stratum hypharum latarum, incrustarum, luteo-brunnearum, 4 - 16 µm. latarum, fibuligerium. Mycelii basalis acanthocystae. Solitaria ad fimum *Macrorufum*.

Typus: Tasmania, Mt Field National Park, 17. vi. 1990, *Y. S. Chang*, CYS486 (DAR).

Pileus 5-10 mm. broad, conical-convex or subcampanulate, viscid to slimy viscid, slightly hygrophanous, glabrous, brownish orange (5C4-5) throughout, browner (6D5) at disc, fading to pale buff. *Lamellae* subdecurrent, yellowish grey then greyish

brown. *Stipe* 18-52 x 1 - 1.5 mm., equal, viscid with a thin glutinous layer below veil line, yellowish white (4A2), sub-bulbous at base. *Context* thin, concolorous with pileus. *Veil* arachnoid, evanescent.

Spores 12.9-15.4 x 7.5-9.2 x 7.9-9.6 µm., elongate ellipsoid. *Basidia* (18.3-) 21.7 - 35 x 9.2 - 19.2 µm., 4-spored, clavate or pyriformis. *Pleurocystidia* as chrysocystidia, 32.1-47.5 x 11.7-20 µm., fusoid ventricose. *Cheilocystidia* 25.8-38.3 (-41.7) x (4.2-) 5-9.2 µm., hyaline, lageniform or elongate clavate.

Subhymenium subcellular. *Trama* regular, tending to interwoven near edge of lamellae, 2 -16 µm. broad, pale yellowish brown (5%KOH). *Epicutis* a thin layer of gelatinised repent hyphae, 2 -4 µm. broad, bearing numerous clamp connections. *Hypodermium* a broad layer of encrusted, yellowish brown hyphae, 4 - 16 µm. broad, clamp connections present. Basal mycelium with acanthocytes.

Solitary on wallaby (*Macrorufus*) dung.

Type: Tasmania, Mt Field National Park, near car park, solitary on wallaby dung, 27. vi. 1990, *Y. S. Chang*, CYS486 (DAR).

Specimens examined: Tasmania, near Nugent, in slightly sheltered area, on wallaby dung, 15. v. 1989, *Y. S. Chang*, CYS364; Sandspit River Forest Reserve, past Robertson Bridge, *A. K. Mills*, 23. iv. 1991, CYS526.

Observations

This species was initially mistaken for an undernourished form of the *semiglobata*-like species. However, later collections indicated otherwise. *Stropharia parvula* is characterised by the very slender and delicate habit, the faint pinkish hue to the pileus the diameter of which seldom exceeds 10 mm. These field characters separate it from the other coprophilous species. It shares similar spore range (13-16 µm.) with *S. semiglobata*. It is apparently rare and is found on dung of native animals only. Its

association with the dung of native animals suggests that this may be an endemic species.

Genus *Hypholoma*

3. *Hypholoma fasciculare var armeniacum* Chang & Mills var. nov.

(*armeniacus*, apricot-coloured)

Selected illustration: Fuhrer & Robinson (1992), p. 37 (as *H. fasciculare*).

Illustrations: Figs. 9.11 - 15.

Similis *Hypholomati fasciculari* praeter colorem armeniacum pilei et lamellarum. Caespitosum ad lignum putridum.

Typus varietatis: Hobart, Fern Glades, slopes of Mt. Wellington, caespitose on very rotten wood, 19. iv. 1990, *Y. S. Chang*, CYS333 (HO).

Similar to *Hypholoma fasciculare* except for the apricot orange (5A7 to 6B7) colour of pileus and lamellae.

Caespitose on rotten wood.

Type: Hobart, Fern Glades, slopes of Mt. Wellington, caespitose on very rotten wood, 19. iv. 1990, *Y. S. Chang*, CYS333 (HO).

Material examined: See Appendix IIIB.

Observations

Hypholoma fasciculare var. *armeniacum* is similar to *H. fasciculare*, the normal sulphur tuft, in every respect except for the colour of pileus and lamellae. Both forms occur in similar habitats and even on the same piece of rotten wood. When they co-occur, a gradation of colour from sulphur yellow to apricot orange can be observed with the extremes at either end of the piece of wood. There are no discernable differences in

spores or other microscopic characters.

4. *Hypholoma paludicolum* sp. nov.

(*palus*, bog; *cola*, I inhabit)

Illustrations: Figs. 9.16 - 19.

Pileus 10 - 15 mm. latus, 8 - 10 mm. altus, convexus, hygrophanus, sordido luteo brunneus, fuscior ad discum, griseo luteus decolorans. *Lamellae* adnatae ad subdecurrentes, subdistantes, griseo brunneae (6E4). *Stipes* 48 -60 mm. longus, 1 -2 mm. crassus, cavus, flexuosus, decrescens basin versus, translucens aurantio brunneus fascior basi. *Contextus* tenuis, pileo concolorus.

Sporae 9.6 - 11.7 x 5.8 - 7.1 x 5.6 - 7.1 µm., subellipsoideae aspectu frontali, inaequilaterae aspectu laterali, poro germinali minuto sed evidenti. *Basidia* 23.7 - 32.5 (-35.8) x 6.7 -10.8 µm., plerumque tetra-spora, raro bi-spora. *Pleurocystidia* ut chrysocystidia, 45 - 77.5 x 10.8 - 17.5 µm., ventricosa, prominentibus apicali protuberatibus vel elongato subclavata. *Cheilocystidia* 26.7 - 44.8 x 6.1 - 9.2 µm., tenuitunicata, hyalina, clavata, sessilia vel pedicellato lageniformia, intermixtus chryspcystidiis.

Subhymenium subcellulosum. *Trama* regularis. *Epicutis* stratum hypharum repentibarum, incrustarum, brunnearum, fibuligerium. *Hypodermium* subcellulosum, hyphis latis, brevibus, fibuligeris constanti.

Solitarium inter Sphagnum et Polytrichum in areis udo et uliginoso.

Typus: Tasmania, Arve Road, prope Geeveston, 15. v. 1991, *Y. S. Chang*, CYS543 (HO).

Pileus 10 - 15 mm. broad, 8 -10 mm .high, convex, hygrophanous, dingy or sordid yellowsh brown (5E5), darker (6E5) at disc, fading to greyish yellow (close to 4B4). *Lamellae* adnate to subdecurrent, subdistant, greysih brown (6E4). *Stipe* 48 -60 mm.

long, 1 -2 mm. thick, hollow, flexuose, tapering towards base, translucent orange brown becoming browner at base. *Context* thin, concolorous with pileus. *Spores* 9.6 - 11.7 x 5.8 - 7.1 x 5.6 - 7.1 µm., subellipsoid in face view, slightly inequilateral in profile, germ pore minute but distinct. *Basidia* 23.7 - 32.5 (-35.8) x 6.7 -10.8 µm., majority 4-spored, more rarely 2-spored. *Pleurocystidia* as chrysocystidia, 45 - 77.5 x 10.8 - 17.5 µm., ventricose, with either or both long apical and basal portions. *Cheilocystidia* 26.7 - 44.8 x 6.1 - 9.2 µm., thin-walled, hyaline, clavate, sessile or pedicellate lageniform, intermixed with chrysocystidia. *Subhymenium* subcellular. *Trama* regular. *Epicutis* a layer of repent, incrusted, brown hyphae, clamp connections present. *Hypodermium* subcellular, with broad, short, clamped hyphae.

Solitary amongst *Sphagnum* and *Polytrichum* in areas wet and boggy.

Type: Tasmania, Arve Road, near Geeveston, 15. v. 1991, *Y. S. Chang*, CYS543 (HO).

Specimens examined: Arve Road, near Geeveston, solitary amongst Sphagnum, 6. v. 1991, *A. K. Mills*, CYS531; Mt Read, on muddy or boggy embankment, scattered, iv. 1991, *A. K. Mills*, AKM1001.

Observations

Hypholoma paludicolum is not typically gregarious and carpophores are either solitary or scattered in their natural habitats. The habitat, boggy areas, places it closer to species in section *Tenacia* Smith or *Psilocyboides* Singer. It appears close to *H. polytrichi* but differs in the larger and broader spores. The subdistant gills separate it from *H. udum*.

Genus *Psilocybe*

5. *Psilocybe brunneo-albescens* sp. nov.

(*brunneus*, brown; *albescens*, becoming white)

Illustrations: Figs. 9.20 - 23.

Pileus 7-12 mm. latus, acute umbonatus, glaber, striatus, unctuosus, valde hygrophanus, brunneo-albescens desiccatione. *Lamellae* adnatae, pallido-brunneae (6D5-E5). *Stipes* (18-) 20-31 x 1-2 mm., aequalis, farctus tandem cavus, basi fibrillosus, fibrillae albae. *Contextus* albidus, tenuis. *Velum* evanascens.
Sporae 6.7-7.5 x 4.2-4.6 x 4.2-5 µm., subellipsoideae, poro germinali evidenti. *Basidia* 20 -25.8 x 5.4 - 5.8 µm., tetra-spora, raro bi-spora, hyalina, obovata vel clavata; sterigmata usque ad 8.3 µm. longa. *Pleurocystidia* 31.7-54.2 x 5-9.2 µm., hyalina, lageniformia, apicibus 3-4 lobatis vel late obtusorum. *Cheilocystidia* 23.3-44.2 x 10.8-26.2 µm., obtusis inflata, late obovata vel polymorpha.
Subhymenium subcellulosum. *Trama* regularis, brunneola, hyphis incrustatis, 2.5 - 4.2 µm. latis. *Epicutis* stratum hypharum filamentosarum incrustarum brunnearum, 3.3 - 7.5 µm. latarum, fibuligerium.
Gregaria ad lignum putridum in sylva matura mixta.

Typus: Tasmania, Collinsvale, Myrtle Forest, 3. iv. 1991, CYS518 (HO).

Pileus 7-12 mm. broad, acutely umbonate, glabrous, striate, greasy, strongly hygrophanous, chestnut brown (6F7-8) turning whitish on drying. *Lamellae* adnate, pale brown (6D5-E5). Stipe (18-) 20-31 x 1-2 mm., equal, stuffed then hollow, basal part covered with white fibrils. *Context* whitish, thin. *Veil* evanescent.
Spores 6.7-7.5 x 4.2-4.6 x 4.2-5 µm., subellipsoid, germ pore distinct. *Basidia* 20 - 25.8 x 5.4 - 5.8 µm., 4-spored, rarely 2-spored, hyaline, obovate or clavate; sterigmata up to 8.3 µm. long. *Pleurocystidia* 31.7-54.2 x 5-9.2 µm., hyaline, apex lobed (3 or 4

lobes) or obtusely rounded, lageniform. *Cheilocystidia* 23.3-44.2 x 10.8-26.2 µm., inflated or broadly obovate or variable in shape.

Subhymenium subcellular. *Trama* regular, pale brown, with hyphae incrusted, 2.5 - 4.2 µm. broad. *Epicutis* a layer of filamentous, incrusted, brown hyphae, 3.3 - 7.5 µm. broad, clamp connections present.

Gregarious on rotten wood in mature mixed forest.

Type: Tasmania, Collinsvale, Myrtle Forest, 3. iv. 1991, CYS518 (HO).

Specimens examined: Tahune Forest Reserve, gregarious on woody debris on bank of creek, 5. vi. 1991, *A. K. Mills*, CYS552; Julius River Reserve, gregarious on very rotten myrtle beech (*Nothofagus*) log, 9. iv. 1991, *A. K. Mills*, AKM968.

Observations

The colour change (from brown to whitish) of the pileus is a useful field character in *P. brunneo-albescens* and there are not many species of *Psilocybe* that possess this character. In this respect, it is closest to *P. aztecorum* Heim *emend* Guzmán which also turns whitish on drying. However, it differs from *P. aztecorum* in not blueing at all though the latter is not a strongly blueing species. These two species also differ from each other in microscopic characters such as shape of spores and the pleurocystidia structure. There are not many lignicolous species of *Psilocybe* in Tasmania, the obvious lignicolous habitat and gregarious growth habit are two other useful field characters that distinguish *P. brunneo-albescens*.

The most distinctive microscopic character of this species is the inflated and variable shape of the cheilocystidia. Here it shows some affinity with *P. insconspicua* Guzmán & Horak, from Papua New Guinea. However, these two species differ from each other in both macro- and micro-morphology and are not likely to be in the same section. The pileus of *P. brunneo-albescens* is more acutely umbonate than *P. insconspicua* and it is

also differently coloured. It is more strongly hygrosphanous and more obviously striate at the margin. It differs from *P. insconspicua* in the gill colour and gill edge not being whitish and fimbriate. This fungus is apparently lignicolous while *P. insconspicua* is more or less terrestrial.

The pleurocystidia are less conspicuous in comparison to the cheilocystidia. This fungus shows some unusual diversity in the form of the multi-lobed apices of the pleurocystidia which perhaps suggests a tendency to diverge from the normal form of pleurocystidia.

6. *Psilocybe alutacea* sp. nov.

(*alutaceus*, leather coloured)

Illustrations: Figs. 9.24 - 28.

Pileus 10-13 mm. latus, conicus vel convexus, subviscidus, hygrophanus, glaber, striatus, alutaceo brunneus vel ochraceo brunneus. *Lamellae* adnatae, subdistantes, griseo brunneae (5D3), interdum subnebulosae, acie albis. *Stipes* 25-46 mm. longus, 1-2.5 mm. crassus, cylindricus, farctus, pallide brunneus.
Sporae 11.7-15.8 (-16.7) x 7.9-9.2 x 7.5-9.2 µm., ellipsoideae. *Basidia* 25.8 - 34.2 x 9.2 - 12.1 µm., tetra-spora, hyalina, obovata vel clavata. *Pleurocystidia* rara, 17.5-30.4 x 4.6-10 µm., lageniformia, longicollia. *Cheilocystidia* 22.5-35.9 (-44.2) x 5-10 µm., hyalina, longicollia, 6.7-15 µm., simplicia, bi- vel tri-furcata.
Subhymenium subcellulosum. *Trama* regularis, brunneola (5%KOH), hyphis 3.3 - 15 µm. latis. *Epicutis* stratum hypharum subgelatinosarum, incrustarum, brunneolarum, 2.5 - 5 µm. latarum, fibuligerium.
Solitaria vel subgregaria ad fimum vaccino.

Typus: Tasmania, Snug Falls Track, 30. v. 1990, *Y. S. Chang*, CYS391, (HO).

Pileus 10-13 mm. broad, conical, subviscid, hygrophanous, glabrous, striate, leathery brown to ochre brown (6E5-6 to 5F5-6). *Lamellae* adnate, subdistant, greyish brown (5D3) at time somewhat clouded, with white edge. *Stipe* 25-46 x 1-2.5 mm., cylindrical, stuffed, pale brown.

Spores 11.7-15.8 (-16.7) x 7.9-9.2 x 7.5-9.2 µm., ellipsoid. *Basidia* 25.8 - 34.2 x 9.2 - 12.1 µm., 4-spored, hyaline, obovate or clavate. *Pleurocystidia* rare, 17.5-30.4 x 4.6-10 µm., lageniform, long-necked. *Cheilocystidia* 22.5-359(-44.2) x 5-10 µm., hyaline, long-necked, 6.7-15 µm., simple, bi- or tri furcate.

Subhymenium subcellular. *Trama* regular, pale brown (5%KOH), with hyphae 3.3 - 15 µm. broad. *Epicutis* a layer of subgelatinised, incrusted hyphae with brown pigments, 2.5 - 5 µm. broad, with clamp connections.

Solitary to subgregarious on cow dung.

Type: Tasmania, Snug Falls Track, 30. v. 1990, *Y. S. Chang*, CYS391 (HO).

Specimens examined: Tasmania, Snug Falls Track, 30. v. 1990, on horse dung, *Y. S. Chang*, CYS389 and on cow dung, *Y. S. Chang*, CYS405; Snug Falls Track, on horse dung, 13. vi. 1990, *Y. S. Chang*, CYS448; Mt Field National Park, Pandanus Walk, 1050m, on wallaby dung, 16. iv. 1991, *Y. S. Chang*, CYS522.

Observations

This fungus was initially confused with the non-papillate form of *P. semilanceata*. Being coprophilous, it is also close to *P. fimetaria* but differs from the latter species in the absence of velar remnants and broader spores. The blueing reaction is not obvious and is only discernable on the gill edge, even the pale coloured stipe did not blue when bruised. Despite the slow blueing reaction, it is considered to be closer to the blueing temperate coprophilous species.

Genus *Pholiota*

7. *Pholiota fieldiana* sp. nov.

(*Fieldiana*, of Mt Field National Park)

Illustrations: Figs. 9.29 - 31.

Pileus 25-32 mm. latus, conicus vel subumbonatus, viscidus, glaber, hygrophanus, striatus, olivaceo-luteus omino, brunneus umbone, aurantio-brunneus desiccatione. *Lamellae* depresso-adnatae vel adnexae, luteo-griseae tandem luteo-brunneae. *Stipes* 38-43 mm. longus, 3-4.5 mm. crassus, glutinosus infra velum linea extensus basin versus, apice pallide luteus, brunnescens prope basin. *Contextus* pallido aurantio-brunneus vel pallido cinnamomeo-brunneus, tenuis.

Sporae 9.2-11.7 x 5.4-6.7 x 5-6.7 µm., ellipsoideae vel inaequilaterales, poro germinali conspicuo. *Basidia* 20 - 29.2 x 7.5 - 10 µm., tetra-spora, hyalina, anguste obovata vel subpyriformia. *Pleurocystidia* biformia, ut chrysocystidia et abundans, pedicello longo in subhymenio, 34.2-53.7 x 8.7-14.6 µm., et leptocystidia, hyalina, rara, 48.3-65 x 17.5-22.5 µm., saccata vel lecythiformia. *Cheilocystidia* 20-36.7 x 6.7-10 µm., hyalina, pedicellata, clavata vel lecythiformia.

Subhymenium subcellulosum. *Trama* regularis, hyphis tunicis tenuibus, hyalinis ad pallido luteo-brunneolis (5%KOH), 6 -20 µm. latis. *Epicutis* stratum hypharum gelatinosarum, repentibarum, infirme incrustatarum, 2 -4 µm. latarum, fibuligerium. *Hypodermium* subcellulosum.

Solitaria vel dispersa in terra inter muscos in sylva temperata.

Typus: Tasmania, Mt Field National Park, Nature Trail, 660 m., 2. viii. 1990, *Y. S. Chang*, CYS509 (HO).

Pileus 25-32 mm. broad, conical to subumbonate, slimy viscid, glabrous, hygrophanous, striate, olivaceous yellow (4C6) throughout, brown (6E7) at disc,

fading or drying to brownish orange. *Lamellae* depressed adnate to adnexed, yellowish grey (4C5) then yellowish brown. *Stipe* 38-43 x 3-4.5 mm., slimy viscid below veil line extending to the base, pale yellow above, browner near base. *Context* pale orange brown to pale cinnamon brown, thin.

Spores 9.2-11.7 x 5.4-6.7 x 5-6.7 µm., ellipsoid in face view, inequilateral in profile, germ pore conspicuous. *Basidia* 20 - 29.2 x 7.5 - 10 µm., 4-spored, hyaline, narrowly obovate or subpyriform. *Pleurocystidia* of two types, as chrysocystidia and abundant, with long pedicel embedded in the subhymenium, 34.2-53.7 x 8.7-14.6 µm., and as leptocystidia, hyaline, rare, 48.3-65 x 17.5-22.5 µm., saccate or lecythiform. *Cheilocystidia* 20-36.7 x 6.7-10 µm., hyaline, pedicellate, clavate or lecythiform.

Subhymenium subcellular. *Trama* regular, hyphae with thin wall, hyaline to pale yellow brown (5%KOH), 6 -20 µm. broad. *Epicutis* of thin layer of gelatinised, repent hyphae,slightly incrusted, 2 - 4 µm. broad, with clamp connections. *Hypodermium* subcellular.

Solitary to scattered on ground amongst mosses (*Rhizogonium*) in temperate rainforest.

Type: Tasmania, Mt Field National Park, Nature Trail, 660 m., 2. viii. 1990, *Y. S. Chang*, CYS509 (HO).

Specimens examined: Tasmania, Mt Field National Park, Nature Trail, 660 m., 3. vii. 1989, *Y. S. Chang*, CYS284.

Observations

The macroscopic and microscopic characters of *Pholiota fieldiana* conform well to those of the genus *Pholiota*. The viscid pileus and stipe placed it in subgenus *Phaeonaematoloma*. Singer (1986) placed all *Naematoloma*-like species with glutinous stipe in subgenus *Phaeonaematoloma*. It is close to *P. myosotis* (the type species of

the subgenus) in having both the pileus and stipe viscid but differs from it in habitat. They are also different in the general stature of the carpophores, where *P. myosotis* is slender , *P. fieldiana* is stout. Other differences noted between these two taxa are in the colour of the pileus and size range of spores.

An unusual character noted in the specimens of *P. fieldiana* is the presence of both chrysocystidia and leptocystidia on the sides of lamellae. The occurrence of two kinds of pleurocystidia has been noted in other *Pholiota* species in this subgenus. Such an occurrence is uncommon across the genus. Not much significance has been placed on this character and Smith & Hesler (1968) do not consider this as a unique character. Among the species recognized by Smith & Hesler (1968), *P. schraderi* (Peck) Overholts has the combination of chrysocystidia and leptocystidia. However, the Tasmanian material differs from *P. schraderi* in having both viscid pileus and stipe as well as the more prominent germ pore of the spores. In two of Singer's (1969) species, i.e., *P. myxacioides* and *P. majalis*, the combination is chrysocystidia and metuloids. The Tasmanian specimens differ from these two species in the presence of leptocystidia instead of metuloids and the more abundant chrysocystidia.

P. fieldiana is considered closest to *P. aberrans* Smith & Hesler. Independent examination of the Tasmanian specimens by Dr. R. Shaeffer of University of Michigan Herbarium (per. comm.) who compared the Tasmanian material with the holotype of *P. aberrans*, showed that these two species are not the same and differ from each other in both macro- and micro-scopic characters.

8. *Pholiota visco-fumosa* sp. nov.

(*viscosus*, viscous; *fumosus*, smoky)

Illustrations: Figs. 9.32 - 37.

Pileus 1.5 - 4.0 cm. latus, plano convexus vel subumbonatus, glutinosus, striatus, annulis concentricis squamellularum albidarum ad discum, squamellulae facile exutae et glabrescens; argillaceus tandem fumosus. *Lamellae* late adnatae, griseo luteae tandem brunnescens sporaris. *Stipes* 23-87 mm. longus, 2-6 mm. crassus, aequalis, flexuosus, siccus, cavus, albidus, flocculosus, glabrescens et sordido brunnescens. *Contextus* pallide luteus. *Velum* arachnoideum, evanescens.

Sporae 9.2-11.7 x 5.8-7.1 (-7.5) x (5.4-) 5.8-6.7 (-7.1) µm., elongato ellipsoideae; poro germinali lato. Basidia (20.8-) 23.3 - 30 (-30.8) x 7.1 - 9.6 (-10.4) µm., tetraspora, clavata. *Pleurocystidia* ut chrysocystidia, 32.5-57.5 x (8.7-) 10-17.5 (-19.2) µm., mucronata, contento amorpho. *Cheilocystidia* 20-36.7 x 6.7-10 µm., hyalina, pedicellata, clavata vel lecythiformia.

Subhymenium subcellulosum. *Trama* regularis, hyphis 6 -20 µm. latis. *Epicutis* stratum hypharum gelatinosarum repentibarum fibuligerium. *Hypodermium* subcellulosum.

Subcaespitosa vel subgregaria ad terram vel ligno carioso vel inter muscos, in areis perfugio sylvae temperatae.

Typus: Tasmania, Mt Field National Park, Nature Trail, 660 m., 16. iv. 1991, *Y. S. Chang*, CYS520, (HO).

Pileus 1.5 - 4.0 cm. broad, plano-convex to subumbonate, surface slimy viscid, striate, with concentric rings of whitish squamules at disc, easily abraded and becoming glabrous, dark blond to clay (4C4 to 5D5) then smoky grey (close to 4B4). *Lamellae* broadly adnate, dull to greyish yellow (3B3-3C4) then browner with spores. *Stipe*

23-87 x 2-6 mm., equal, flexuose, dry, hollow, whitish, flocculose, becoming glabrous and sordid brown. *Context* pale yellow. *Veil* arachnoid, evanescent. *Spores* 9.2 - 11.7 x 5.8 - 7.1 (-7.5) x (5.4-) 5.8-6.7 (-7.1) µm., elongate ellipsoid, broad germ pore. *Basidia* (20.8-) 23.3 - 30 (-30.8) x 7.1 - 9.6 (-10.4) µm., 4-spored, clavate. *Pleurocystidia* as chrysocystidia, 32.5-57.5 x (8.7-)10-17.5(-19.2) µm., mucronate, with amorphous content. *Cheilocystidia* 20-36.7 x 6.7-10 µm., hyaline, pedicellate, clavate or lecythiform.

Subhymenium subcellular. *Trama* regular, with hyphae 6 - 20 µm. broad. *Epicutis* a gelatinised layer of repent hyphae, with clamp connections.

Subcaespitose to subgregarious on ground litter or rotten wood or amongst mosses in sheltered areas of temperate rainforest.

Type: Tasmania, Mt Field National Park, Nature Trail, 660 m., 16. iv. 1991, *Y. S. Chang*, CYS520 (HO).

Specimens examined: Tasmania, Little Florentine Valley, off Five Road, on ground litter, 17. v. 1989, *Y. S. Chang*, CYS184; NW Coast, Pine Track, off Tayatea Road, gregarious on fallen manfern trunk, 16. vi. 1989, *Y. S. Chang*, CYS256; Mt Field National Park, off Nature Trail, gregarious on ground litter, 3. vii. 1989, *Y. S. Chang*, CYS285, 2. v. 1990, *Y. S. Chang*, CYS342, & 27. vi. 1990, *Y. S. Chang*, CYS487; Tasman Peninsula, Balt Spur, gregarious on mossy ground, 6. vi. 1990, *Y. S. Chang*, CYS410; Mt Field National Park, Pandanus Walk, 1050 m., 16. iv. 1991, *Y. S. Chang*, CYS521.

Observations

The white flocculose to scaly stipe of *Pholiota visco-fumosa* is reminiscent of species of *Stropharia*. But the colour of the individual spores (melleous brown) and spore print brings it closer to *Pholiota*.

This species appears to be moderately variable particularly in the marcoscopic characters. The two most obvious variations noted are the stature of the carpophores varying from slender to very robust and the colour of the pileus which varies from buff to smokey grey. Much variation is also noted in the surface feature of the stipe which in the best conditions appears whitish flocculose whilst more often just glabrous.

9. *Pholiota pallidocaulis* sp. nov.

(pallidus. pale; *caulis*, stem)

Illustrations: Figs. 9.38 - 45.

Pileus 14 - 45 mm. latus, late convexus vel subumbonatus, mucosus viscidus, annulis concentricis squamellularum albidarum ad discum vel facie glabro, luteolus tandem brunneo-aurantius tandem brunnescens omnino. *Lamellae* late adnatae vel adnexae, usque ad 5mm latae, luteolae tandem brunnescens sporis. *Stipes* 25 - 54 mm. longus, 2 - 4 mm. crassus, plus minusve aequalis; pallidus, aetate color sordidescens, basis sub-bulbosa vel abrupta, myceli albido et rhizomorpha luteo basi. *Contextus* albidus, firmus. *Velum* arachnoideum, cremicolor, evanescens.

Sporae 7.5 - 9.2 (10) x 5 - 5.8 x 5 - 6.2 µm., subellipsoideae vel leviter inaequilaterales, poro germinali minuto et inconspicuo. *Basidia* 18.7 - 32.5 x 7.5 - 10.8 µm., tetra-spora, obovata vel clavata, luteo brunneum prope lamellae acie. *Pleurocystidia* 53.3 - 69.2 x 16.7 - 21.7 µm., projicientia, tunicies 0.8 - 1.7 µm. crassa, hyalina, fusoido ventricosa, apicibus obtusis vel furcatis. *Cheilocystidia* 26.7 - 43.3 x 11.7 - 22.5 µm., facientia plus minusve taenia steriles, hyalina vel contentis luteo brunneolis, tunicis crassi vel tenui.

Dispersa vel gregaria ad lignum putridum.

Typus: Tasmania, Hobart, campus universitis, 26. vi. 1990, *Y. S. Chang*, CYS482 (HO).

Pileus 14 - 45 mm. broad, broadly convex or subumbonate, slimy viscid, with concentric rings of whitish squamules at disc or with a glabrous appearance, light yellow (4A4) then brownish orange (5C5-6) becoming light brown (6D7) throughout. *Lamellae* broadly adnate or adnexed, up to 5 mm. broad, pale yellow (4B3) then becoming brown with spores. *Stipe* 25 - 54 mm. long, 2 -4 mm. thick, more or less equal, pallid, becoming sordidly coloured with age, base sub-bulbous or abrupt, white mycelium and yellow rhizomorph at base. *Context* white, firm. *Veil* arachnoid, cream coloured, evanescent.

Spores 7.5 - 9.2 (10) x 5 - 5.8 x 5 - 6.2 µm., subellipsoid or slightly inequilateral, germ pore minute and inconspicuous. *Basidia* 18.7 - 32.5 x 7.5 - 10.8 µm., 4-spored, obovate or clavate, yellowish brown near gill edge. *Pleurocystidia* 53.3 - 69.2 x 16.7 - 21.7 µm., projecting, with thick wall, 0.8 - 1.7 µm. thick, hyaline, fusoid ventricose, with apex obtuse or branched. *Cheilocystidia* 26.7 - 43.3 x 11.7 - 22.5 µm., forming a more or less sterile band, hyaline or with yellowish brown content, with thick or thin wall.

Subhymenium filamentous, gelatinised. *Trama* regular. *Epicutis* filamentous, hyphae incrusted, with brown pigments, bearing clamp connections. Refringent hyphae present in stipe trama, gill trama and epicutis.

Scattered or gregarious on woody debris.

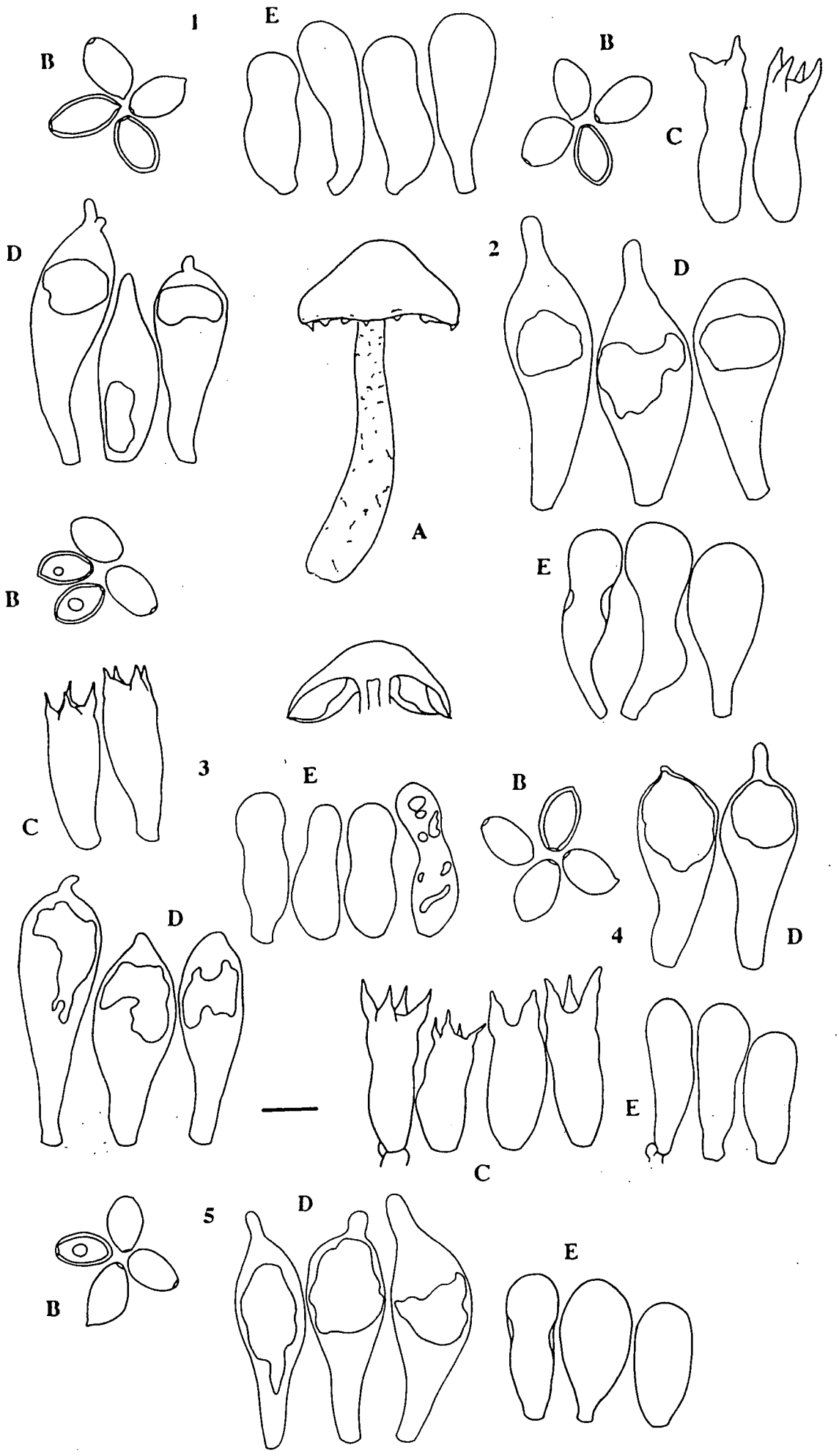
Type: Tasmania, Hobart, university campus, 26. vi. 1990, *Y. S. Chang*, CYS482 (HO).

Specimens examined: See Appendix IIIE for other collections of this species.

Observations

This is the third most common *Pholiota* species (after *P. squarrosipes* and *P.*

multicingulata) encountered in the woodland habitats (both cool temperate rainforests and wet sclerophyll). *P. pallidocaulis* is recognized by the apparent pale coloured stipe and conspicuous yellow rhizomorphs. It appears close to those species in stirps Condensa which show variations in wall thickness of pleurocystidia (Smith & Hesler 1968). However, this species differs from them in not having obvious association with conifers.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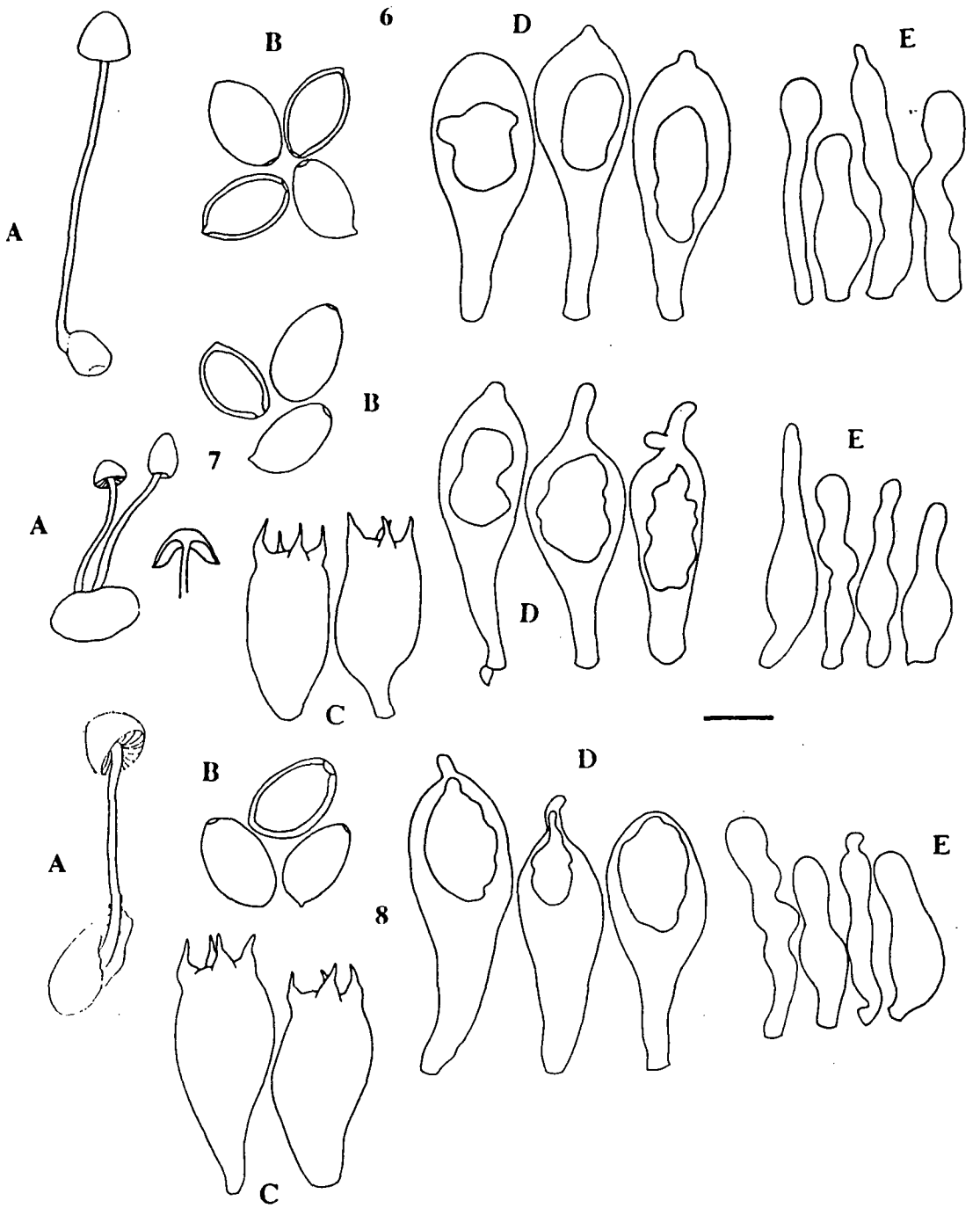

Figs. 9.6 - 8. *Stropharia parvula* sp. nov. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 6. CYS486 (type). 7. CYS364. 8. CYS526.

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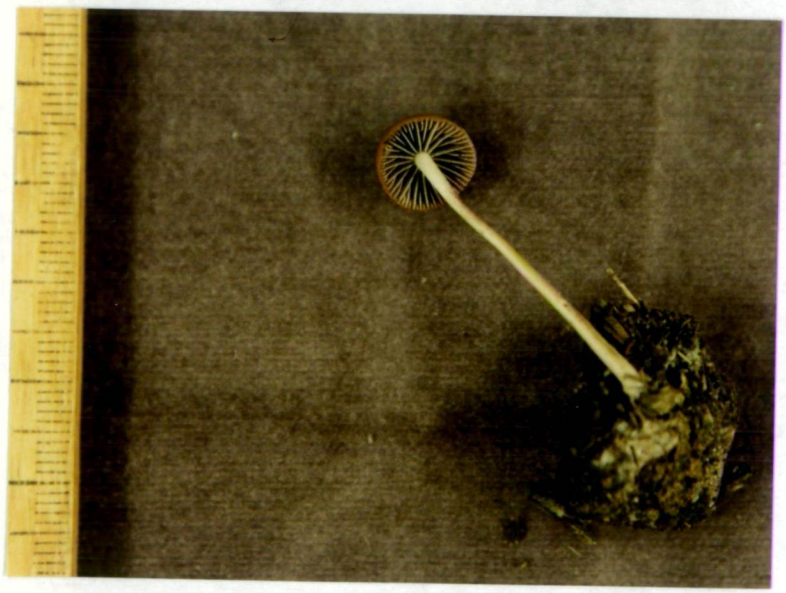
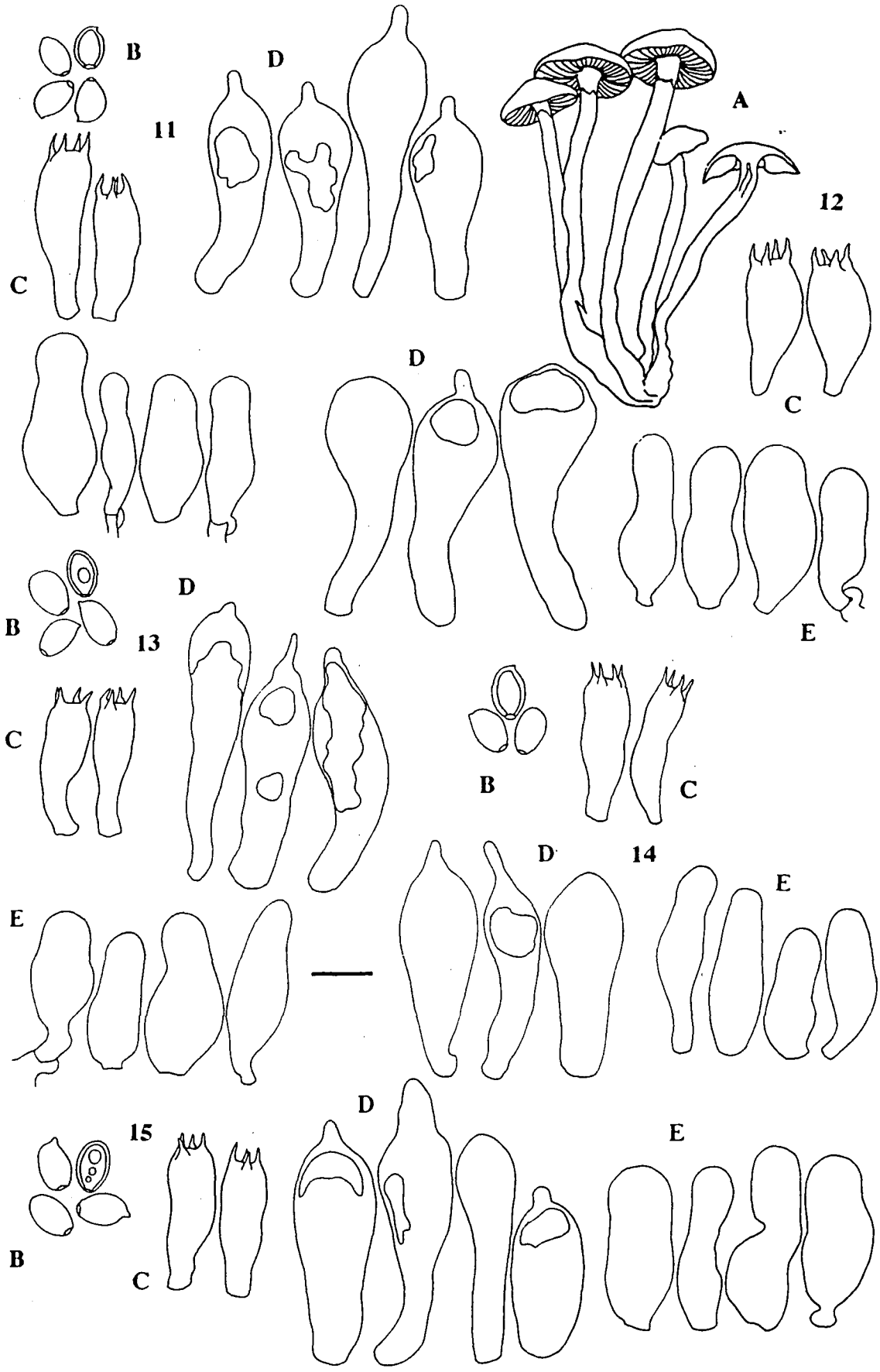
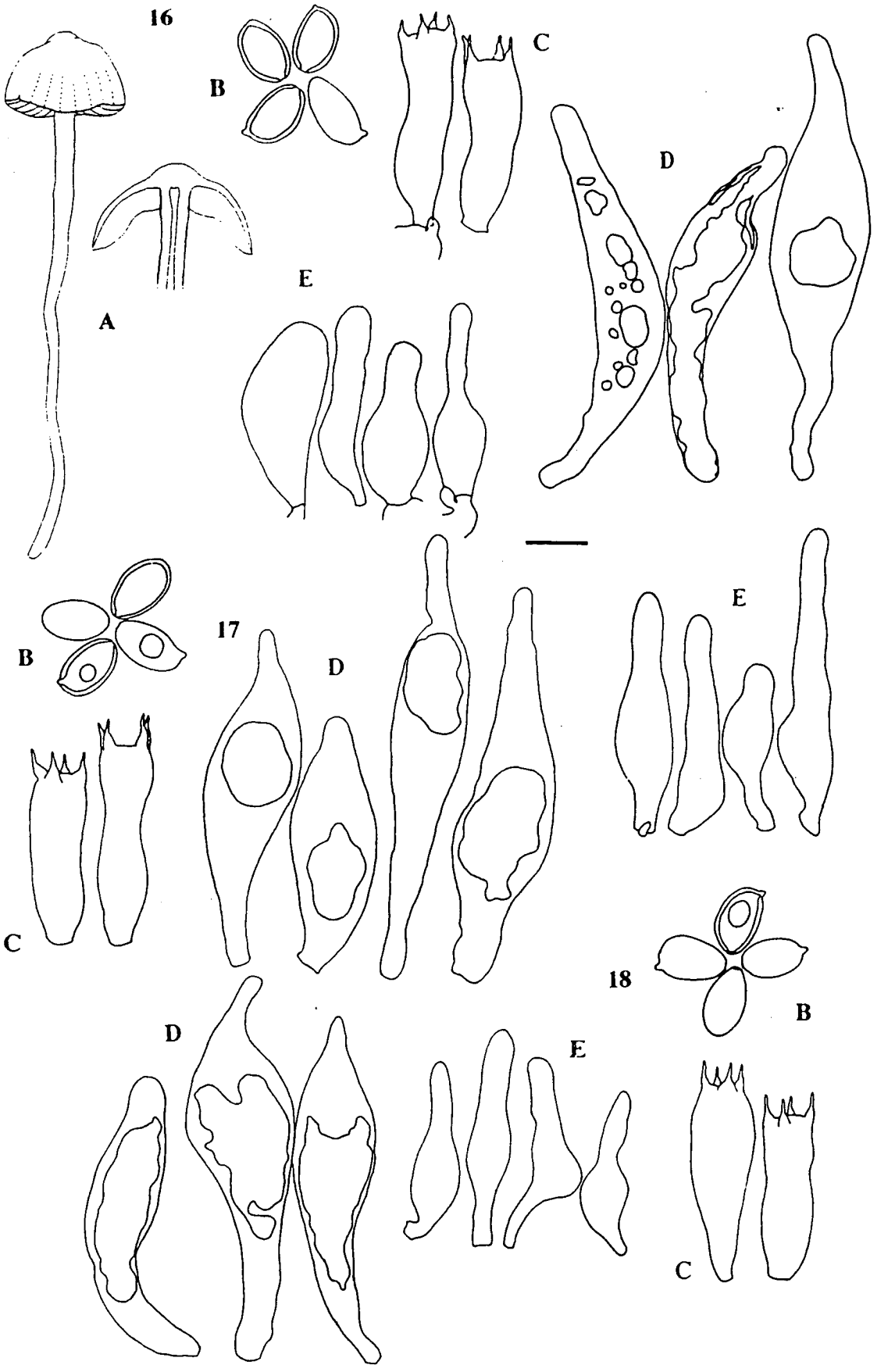


Figs. 9.9 - 10. Habit of *Stropharia parvula* sp. nov. 9. CYS364. 10. CYS526.

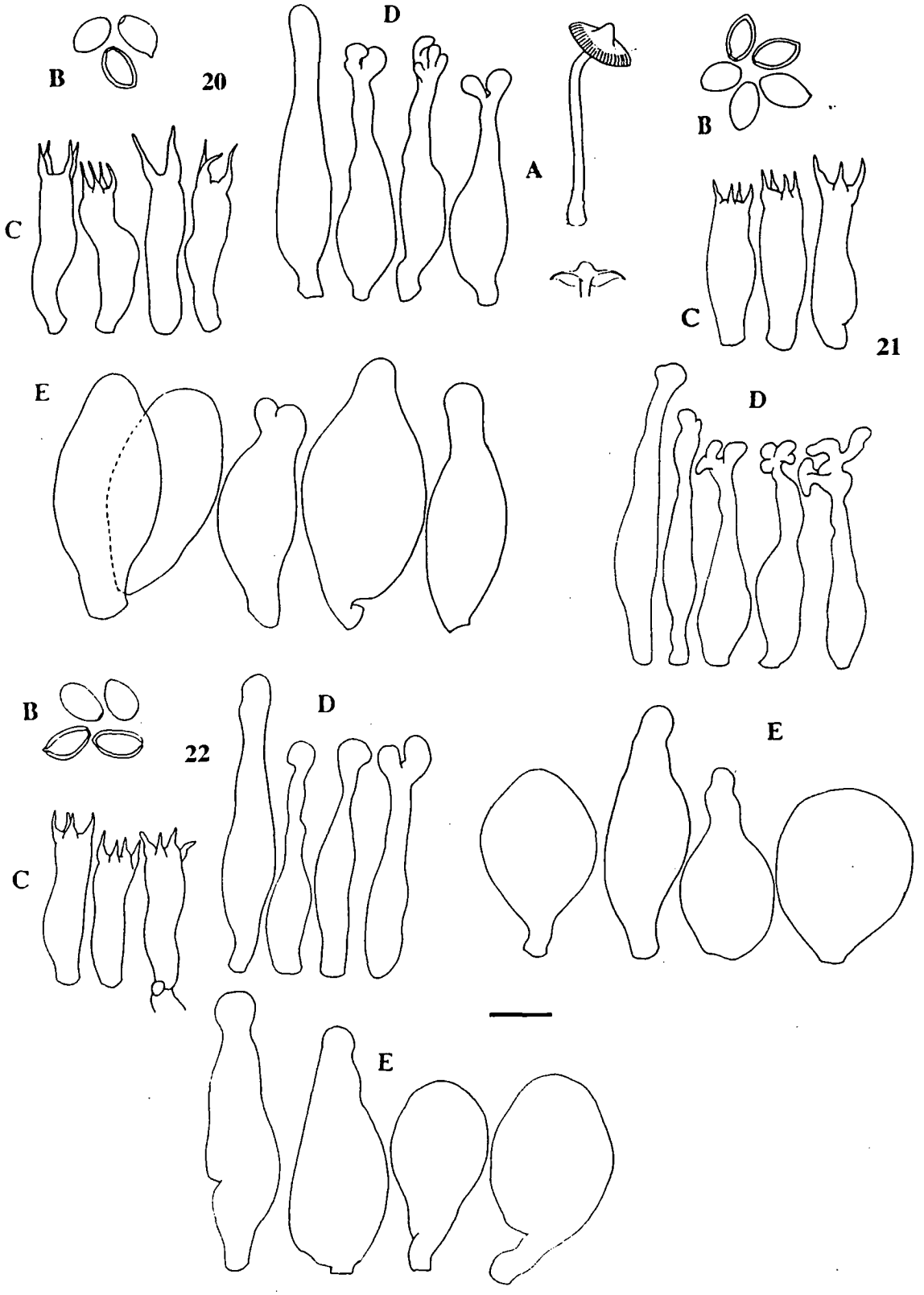


Figs. 9.11 - 15. *Hypholoma fasciculare* var *armeniacum* var. nov. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 11. CYS333 (type). 12. CYS108. 13. CYS81. 14. CYS133. 15. CYS78.



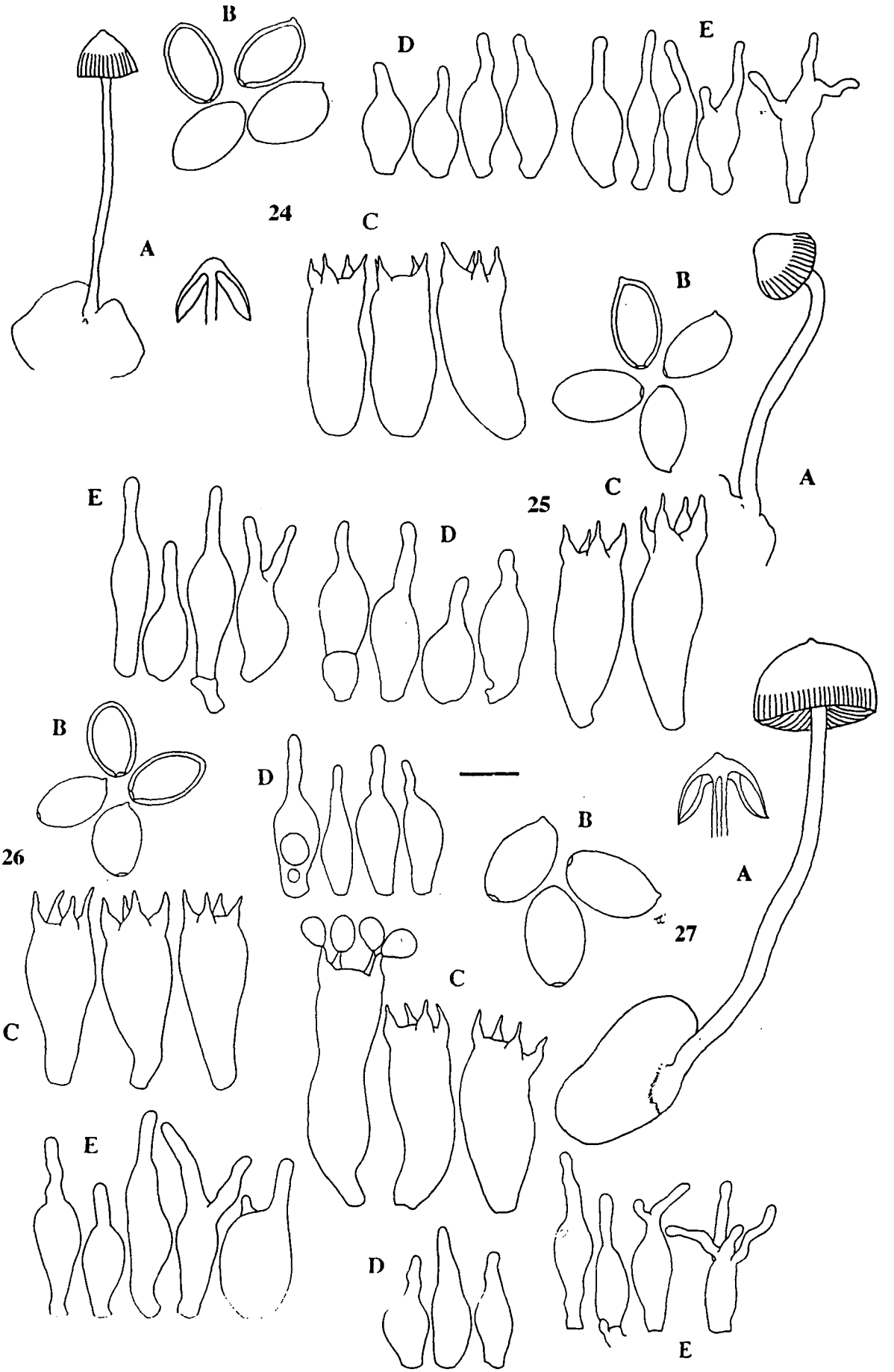
Figs. 9.16 - 18. *Hypholoma paludicolum* sp. nov. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 16. CYS531. 17. CYS543 (type). 19. AKM1001.

Fig. 9. 19. Habit of *Hypholoma paludicolum*, CYS531.



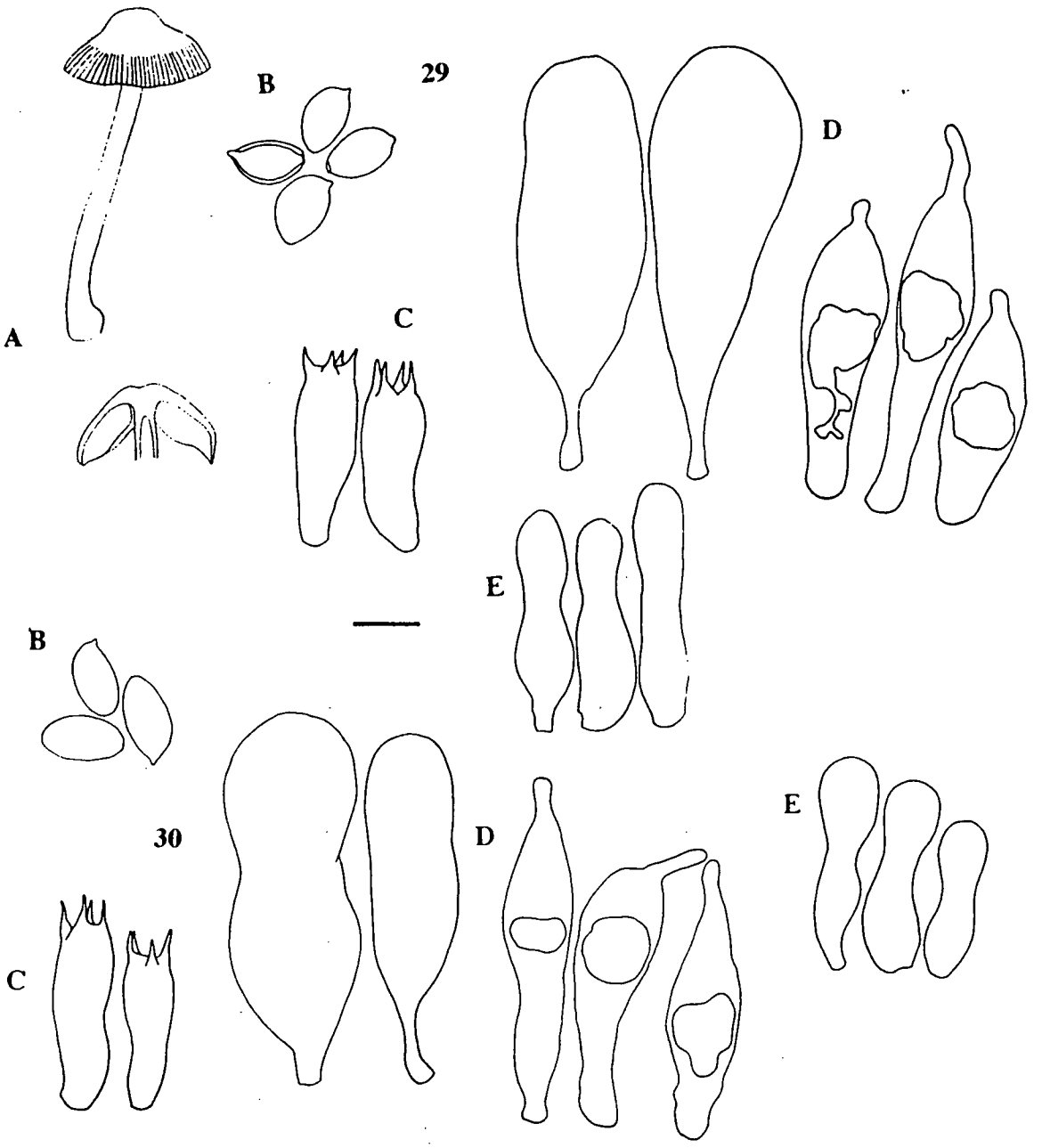
Figs. 9.20 - 22. *Psilocybe brunneo-albescens* sp. nov. A: habit. B: spores. C: basidia, D: pleurocystidia, and E: cheilocystidia. 20. CYS518 (type). 21. CYS552. 22. AKM968.

Fig. 9.23. Habit of *Psilocybe brunneo-albescens*, CYS518 (type).



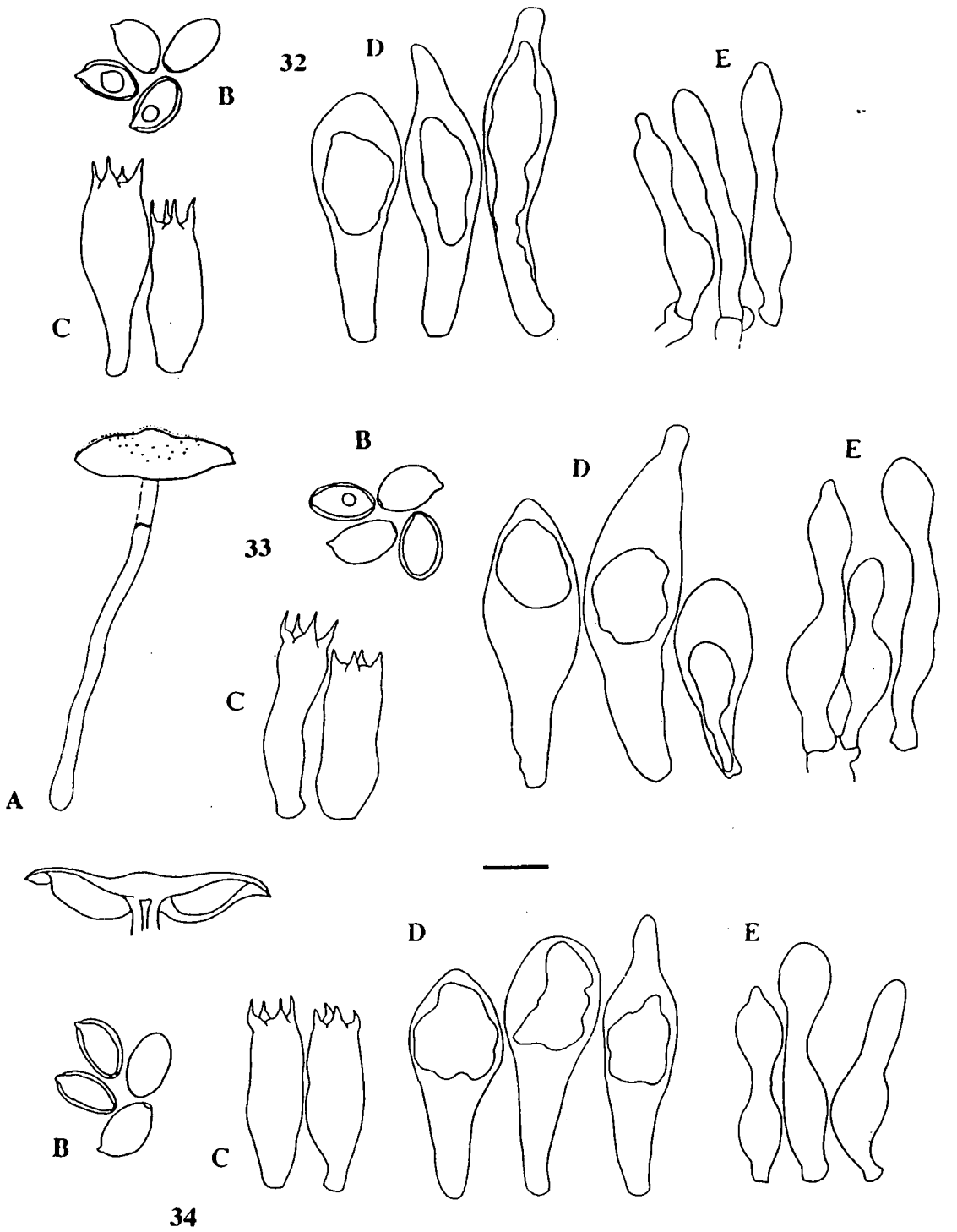
Figs. 9.24 - 27. *Psilocybe alutacea* sp. nov. A: habit, B: spores, C: basidia, D: pleurocystidia, and E: cheilocystidia. 24. CYS391 (type). 25. CYS389. 26. CYS405. 27. CYS522.

Fig. 9.28. Habit of *Psilocybe alutacea*, CYS391 (type).



Figs. 9.29 - 30. *Pholiota fieldiana* sp. nov. A: habit, B: spores, C: basidia, D: pleurocystidia as leptocystidia and chrysocystidia, and E: cheilocystidia. 29. CYS509 (type). 30. CYS284.

Fig. 9.31. Habit of *Pholiota fieldiana*. CYS509 (type).



Figs. 9.32 - 34. *Pholiota visco-fumosa* sp. nov. A: habit, B: spores, C: basidia, D: chrysocystidia, and E: cheilocystidia. 32. CYS520 (type). 33. CYS410. 34. CYS256.

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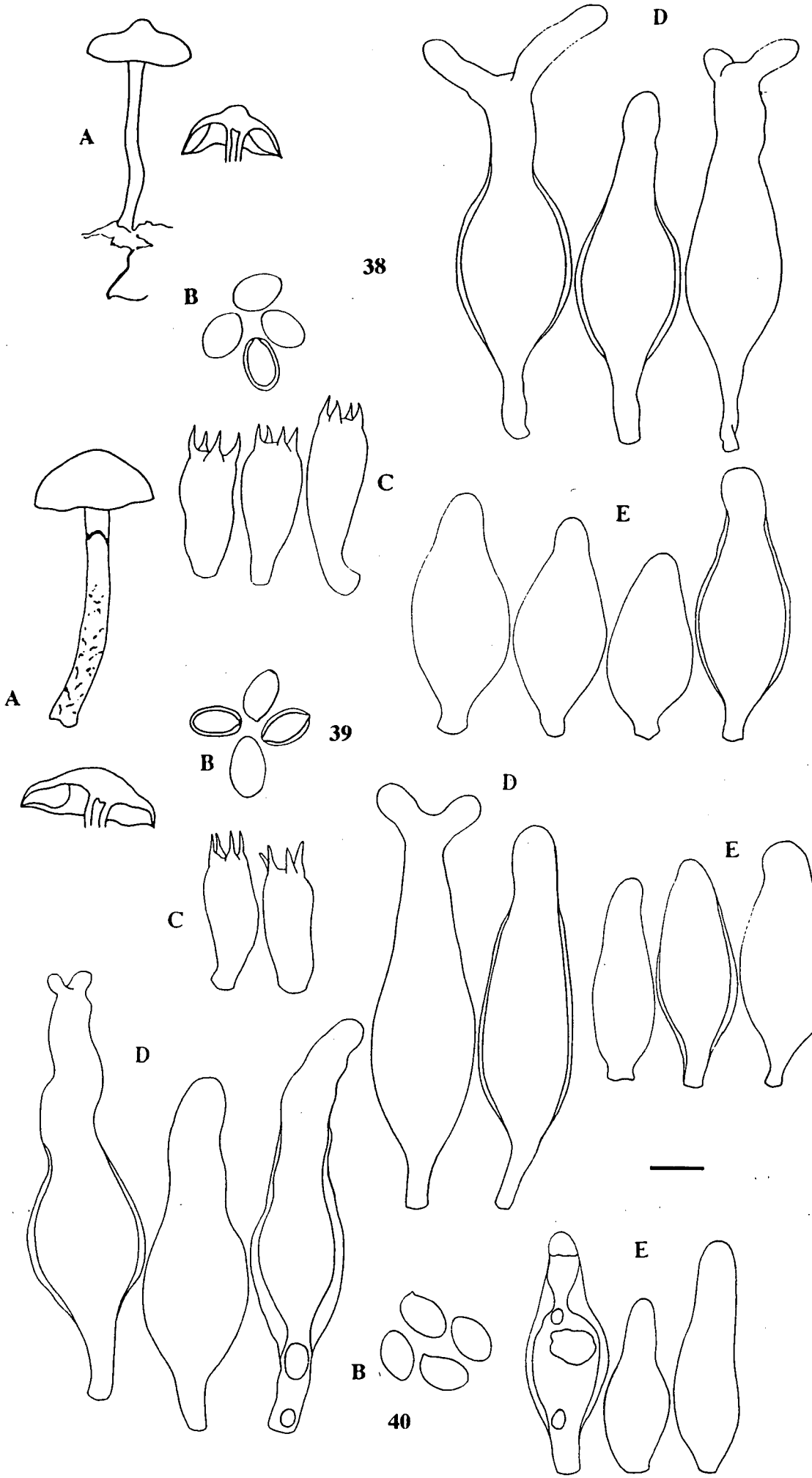
Figs. 9.35 - 36. Habit of *Pholiota visco-fumosa*. 35. CYS520 (type). 36. CYS256.

Fig. 9.37. Pholiota *visco-fumosa* in natural habitat (photo courtesy of B. Fuhrer).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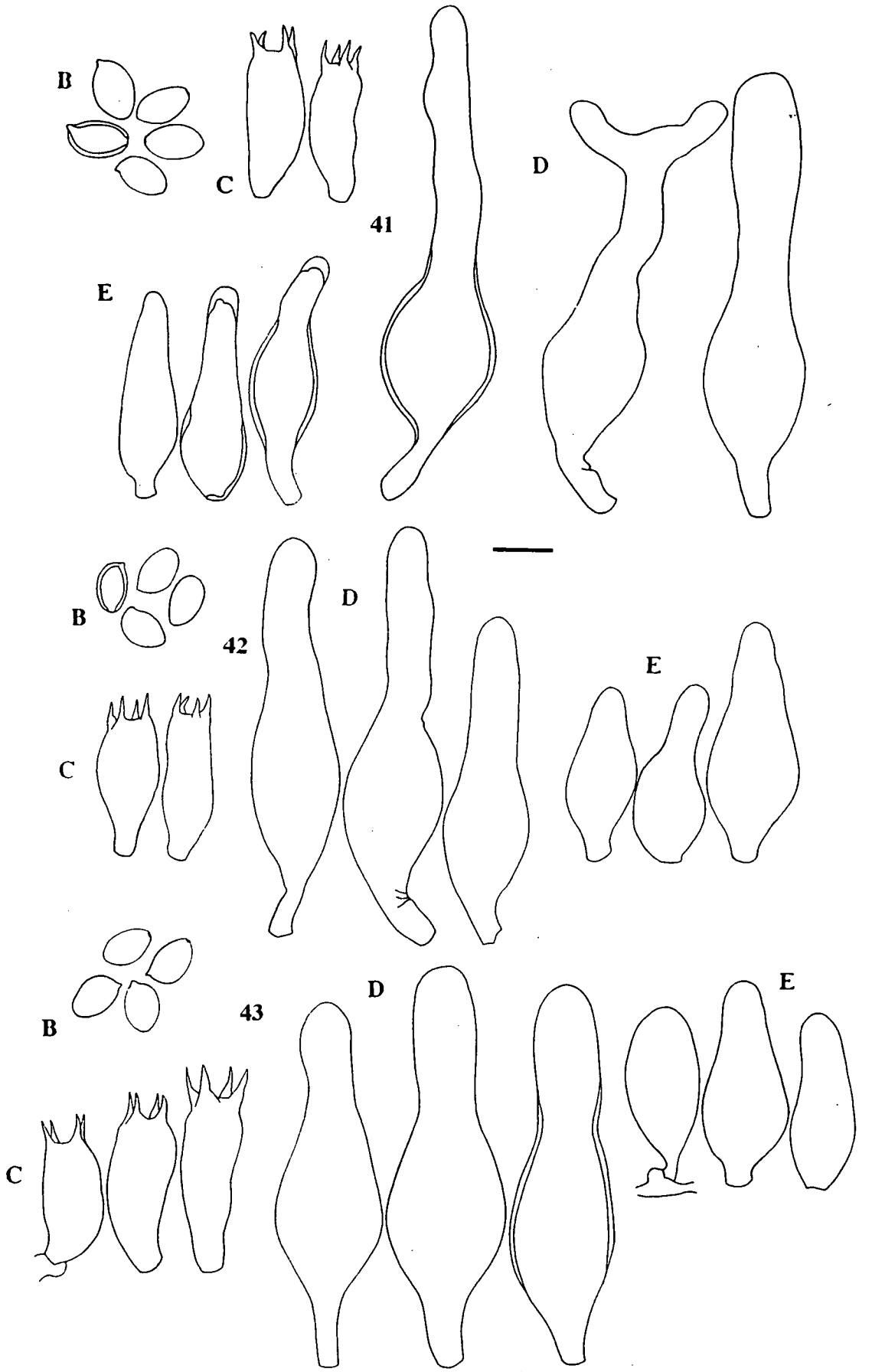

Figs. 9.41 - 43. *Pholiota pallidocaulis* sp. nov. A: habit, B: spores, C: basidia, D: pleurocystidia, and E: cheilocystidia. 41. CYS289. 42. CYS411. 43. CYS533.

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Figs. 9.44 - 45. Habit of *Pholiota pallidocaulis*. 44. CYS482 (type). 45. CYS289.

References

Ainsworth, G. C. (1976). *Introduction to the history of mycology.* Cambridge University Press: Cambridge.

Anderson, J. B. & Ullrich, R. C. (1979). Biological species of *Armillaria mellea* in North America. *Mycologia* **71**: 402-414.

Arora, D. (1986). *Mushrooms Demystified.* Ten Speed Press: Berkeley.

Avise, J. C. (1974). Systematic value of electrophoretic data. *Syst. Zool.* **23**: 465-481.

Axelrod, I. & Raven, P. H. (1982). Paleobiogeography and origin of the New Guinea flora. In *Biogeography and ecology of New Guinea* edited by J. L. Gressitt. W. Junk: The Hague.

Ayala, F. J. (1983). Enzymes as Taxonomic Characters. In *Protein Polymorphism: Adaptive and taxonomic significance.* The Systematics Association Special Volume No. 24. Academic Press: London & New York.

Backhouse, D., Willets, H. J. & Adam, P. (1984). Electrophoretic studies of *Botrytis* species. *Trans. Br. mycol. Soc.* **82**: 625-630.

Bas, C (1969). Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella. Persoonia* **5**: 285-579.

——— (1977). Species concept in *Amanita* Sect. Vaginatae. In *The Species Concept in Hymenomycetes* (ed. H. Clémençon). J. Cramer: Vaduz, Leichtenstein.

Berkeley, M. J. (1860). *Outlines of British Fungology.* Lovell Reeve: London.

Benedict, R. C. (1970). Chemotaxonomic relationships among the basidiomycetes. *Advances App. Microbiol.* **13**: 1-23.

Benedict, R. G., Brady, L. R., Smith, A. H. & Tyler, V. E., Jr. (1962). Occurrence of psilocybin and psilocin in *Conocybe* and *Psilocybe* species. *Lloydia* **25**: 156-159.

Bisby, F. A., Vaughan, J. G. & Wright, C. A. (Eds.) (1980). *Chemosystematics: principles and practice.* The Systematic Association Special Volume No. 16. Academic Press: London & New York.

Blaich, R. (1977). Enzymes as an aid in taxonomy of higher basidiomycetes. In *The Species Concept in Hymenomycetes* edited by H. Clémençon. Proc. Herbette Symp. Lausanne, Swtizerland, 1976. Bibliotheca Mycologica 61: 215-228. J.

Cramer: Vaduz.

Blaich, R. & Esser, K. (1975). Function of enzymes in wood destroying fungi. II. Multiple forms of laccase in white rot fungi. *Arch. Microbiol.* **103**: 271-277.

Boidin, J. (1977). Intéret descultures dans la delimitation des especes chez les Aphyllorales et les Auriculariales. In *The Species Concept in Hymenomycetes* edited by H. Clémençon. Bibl. Mycol. 61: 277-321. J. Cramer: Vaduz.

——— (1986). Intercompatibility and the species concept in the saprobic basidiomycotina. *Mycotaxon* **26**: 319-336.

Bonde, M. R., Peterson, G. L., Dowler, W. M. & May, B. (1984). Isozyme analysis to differentiate species of *Perenosclerospora* causing downy mildews of maize. *Phytopathology* **74**: 1278-1283.

Bosland, P. W. & Williams, P. H. (1987). An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. *Can. J. Bot.* **65**: 2067-2073.

Brasier, C. M. (1987). The dynamics of fungal speciation. In *Evolutionary Biology of the Fungi* edited by A. D. Rayner, C. M. Brasier & D. Moore, pp 231-260. Reprinted 1989. Cambridge University Press: Cambridge.

Bresadola, J. (1927). *Iconographia Mycologica* Vol. II. 1981 reprint. Mediolani. Italy.

Brown, A. H. (1990). The role of isozyme studies in molecular systematics. *Aust. Syst. Bot.* **3**: 39-46.

Browne, A. (1983). *Experimental Taxonomy in the genus* Hypholoma. Honours Thesis. Unpublished. University of Tasmania, Hobart.

Burnett, J. H. (1983). Speciation in fungi. *Trans. Br. mycol. Soc.* **81**: 1-14.

Buth, D. G. (1984). The application of electrophoretic data in systematic studies. *Ann. Rev. Ecol. Syst.* **15**: 501-522.

Carder, J. H. & Barbara, D. J. (1991). Molecular variation and restriction fragment length polymorphisms (RFLPs) within and between six species of *Verticillium*. *Mycol. Res.* **95**: 935-942.

Carlile, M. J. (1987). Genetic exchange and gene flow: their promotion and prevention. In *Evolutionary biology of the fungi* edited by A. D. M. Rayner, C. M. Brasier & D. Moore. pp. 203-214. Reprinted 1989. Cambridge

University Press: Cambridge.

Chang, L. O., Srb, A. M. & Steward, F.C. (1962). Electrophoretic separations of the soluble proteins of *Neurospora*. *Nature* **193**: 756-759.

Chang, Y. S. & Mills, A. K. (1992) Re-examination of *Psilocybe subaeruginosa* Clel. through comparative morphology, isozymes and mating compatibility studies. *Mycol. Res.* **96**: 429-441.

Clare, B, G, Flentje, N. T. & Atkinson, M. R. (1968). Electrophoretic patterns of oxidoreductases and other proteins as criteria in fungal taxonomy. *Aust. J. Biol. Sci.* **21**: 275-295.

Cleland, J. B. (1933). Australian fungi: Notes and descriptions No. 9. *Trans. Roy. Soc. S. Aust.* **57**: 187-194.

——— (1934). *Toadstools and Mushrooms and Other Larger Fungi of South Australia. Part I.* Government Printer: South Australia.

Cleland, J. B. & Cheel, E. (1918).Australian fungi: Notes and descriptions No. 1. *Trans. & Proc. Roy. Soc. South. Australia* **42**: 88-138.

Cleland, J. B. & Rodway, L. (1929). Notes on the genus *Poria*. *Pap. Proc. Roy. Soc. Tasm.* **1928**: 31-86.

Clemençon, H (1972) Die Wandstruktur der Basidiosporen. II: *Kuehneromyces mutabilis*. *Schweiz. Zeitschr. Pilzk.* **50**, 20-25.

Clémençon, H (Ed.) (1977). *The species concept in Hymenomycetes*. J. Cramer: Vaduz.

Cole, F. Mary, Fuhrer, B. A. & Holland, A. A. (1978). *A Field Guide to the common Genera of Gilled Fungi in Australia*. First Edition. Inkata Press Pty. Ltd.: Melbourne.

Cole, F. Mary, Fuhrer, B. A. & Holland, A. A. (1984). *A Field Guide to the common Genera of Gilled Fungi in Australia*. Revised Edition. Inkata Press Pty. Ltd.: Melbourne.

Cooke, M. C. (1892). *Handbook of Australian fungi*. Williams & Norgate: London.

Cruickshank, R. H. (1983). Distinction Between *Sclerotinia* species by their pectic zymograms. *Trans. Br. mycol. Soc.* **80**: 117-119.

——— (1989). *Zymograms as criteria in the taxonomy of fungi*. Unpublished Ph. D. thesis. University of Tasmania, Hobart.

Cruickshank, R. H. & Wade, G. C. (1980). Detection of pectic enzymes in pectin-

acryalamide gels. *Analytical Biochemistry* **107**, 177-181.

Cruickshank, R. H. & Pitt, J. I. (1987). Identification of species in *Penicillium* subgenus *Penicillium* by enzyme electrophoresis. *Mycologia* **79**: 614-620.

Crawford, D J. (1983). Phylogenetic and systematic inferences from electrophoretic studies in Isozymes in *Plant Genetics and Breeding. Part A* edited by S. D. Tanksley and T. J. Orton. Elsevier Science Publishers B. V.: Amsterdam.

Dennis, R. W. G. (1961). Fungi venezuelani: IV. Agaricales. *Kew Bull.* 15: 67-156.

Dennis, R. W. G., Orton, P. D. & Hora, F. B. (1960). New check list of british agarics and boleti. *Trans. Br. mycol. Soc. Suppl.* **43**.

Donk, M. A. (1962). *The generic names proposed for Agaricaceae.* Beih. Nova Hedwigia Heft 5. J. Cramer: Weinheim.

Edwards, R. & Kennedy, L. L. (1973). Biosystematics of *Coriolus hirsutus* and *C. pubescens*. I. Interfertility studies. *Can. J. Bot.* **51**: 2385-2393.

Eger, G., Li, S. F. & Leal-Lara, H. (1979). Contribution to the discussion on the species concept in the *Pleurotus ostreatus* complex. *Mycologia* **71**: 577-588.

Esser, K. (1966). Incompatibility. In *The Fungi Vol 2* edited by G. C. Ainsworth & A. S. Sussman, pp. 661-676. Academic Press: New York.

Esser, K. & Blaich, R. (1973). Heterogenic incompatibility in plants and animals. *Adv. in Genet.* **17**: 107-152.

Eygelsheim, W. F. T. (1981). *Tasmanian Mushrooms and Toadstools.* Burnie Adult Education Publ. Group: Burnie, Tasmania.

Farr, D. N. (1980). The acanthocyte, a unique cell type in *Stropharia* (Agaricales). *Mycotaxon* **11**: 241-249.

Farr, Ellen R., Miller, O. K. Jr. & Farr, D. F. (1977). Biosystematic studies in the genus *Pholiota* stirps *Adiposa*. *Can. J. Bot.* **55**, 1167-1180.

Ferguson, A. (1980). *Biochemical sysematics and evolution.* Wiley & Sons: New York, N. Y.

Filtenborg, O. & Frisvad, J. C. (1980). A simple screening-method for toxigenic moulds in pure cultures. *Lebensm.-Wiss. u. Technol.* **13**: 128-130.

Filtenborg, O., Frisvad, J. C. & Svendsen J. A. (1983). Simple screening method for molds producing intracellular mycotoxins in pure cultures. *Appl. Environ. Microbiol.* **45**: 581-585.

Flynn, T. & Miller, O. K. Jr. (1990). Biosystematics of *Agrocybe molesta* and sibling species allied to *Agrocybe praecox* in North America and Europe. *Mycol. Res.* **94**: 1103-1110.

Fries, E. (1821). *Systema Mycologicum* **I**: 1-508. Lund.

Fries, N. (1985). Intersterility groups in *Paxillus involutus*. *Mycotaxon* **24**: 403-409.

Frisvad, J. C. & Filtenborg, O. (1983). Classification of terverticillate Penicillia based on profiles of mycotoxins and other secondary metabolites. *Appl. Environ. Microbiol.* **46**: 1301-1310.

Fuhrer, B. (1985). *A field companion to Australian fungi.* The Five Mile Press: Melbourne.

Fuhrer, B. and Robinson, R. (1992). *Rainforest fungi of Tasmania and south-east Australia.* CSIRO Australia and the Forestry Commission, Tasmania.

Galland, M. C., Kemp, R. F. O. & Jurand, M. K. (1979). The speices problem in the *Psathyrella candolleana* complex. *Mycotaxon* **8**: 329-332.

Garber, E. D. (1973). Electrophoresis as a taxonomic and genetic tool for fungi. *Bull. Torrey Bot. Club* **100**: 308-312.

Gardes, M., Mueller, G. M., Fortin, J. A. & Kropp, B. P. (1991). Mitochondrial DNA polymorphisms in *Laccaria bicolor, L. laccata, L. proxima & L. amethystina. Mycol. Res.* **95**: 206-216.

Gill, M. & Steglich, W. (1987). Pigments of Fungi (Macromycetes). pp. 88-98. Springer-Verlag.

Ginns, J. (1985). *Hericium* in North America: cultural characteristics and mating behaviour. *Can. J. Bot.* **63**: 1551-1563.

Gottlieb, L. D. (1977). Electrophoretic evidence and plant systematics. *Ann. Mo. Bot. Gdn.* **64**: 161-180.

——— (1981). Electrophoretic evidence and plant populations. *Prog. Phytochem.* **7**: 1-46.

——— (1984). Conservation and duplication of isozymes in plants. *Science* **216**: 373-380.

Greville, R. K. (1827). *Scottish Cryptogamic Flora.* Maclachlan & Stewart: Edinburgh.

Griffith, K. (1985). *A Field Guide to the Larger Fungi of the Darling Scarp & South West of Western Australia.* K. Griffith.

Groves, J. W. (1979). *Edible and poisonous mushrooms of Canada.* Research Branch Agriculture Canada. Publication 1112.

Grgurinovic, C. A & Holland, A. A. (1982). A field identification key of some Victorian species of the genus *Mycena. Vic. Nat.* **99**: 102-109.

Guzmán, G. (1983). *The Genus* Psilocybe. Beihefte zur *Nova Hedwigia* Heft 74. J. Cramer: Vaduz, Liechtenstein.

Guzmán, G. & Watling, R (1978). Studies in Australian agarics and boletes I: Some species of *Psilocybe. Notes Roy. Bot. Gard. Edinb.* **36**: 199-210.

Guzmán, G. & Vergeer, P. P. (1978). Index of taxa in the genus *Psilocybe. Mycotaxon* **6**: 464-476.

Hall, R. (1973). Electrophoretic protein profiles as criteria in the taxonomy of fungi and algae. *Bull. Torrey Bot. Club* **100**: 253-259.

Hall, R., Zentmyer, G. A. & Erwin, D. C. (1969). Approach to taxonomy of *Phytophthora* through acrylamide gel-electrophoresis of proteins. *Phytopathology* **59**: 770-774.

Hallenberg, N. (1984). Compatibility between species of Corticiaceae s. l. (Basidiomycetes) from Europe and North America. *Mycotaxon* **21**: 335-388.

——— (1985). Compatibility between species of Corticiaceae s. l. (Basidiomycetes) from Europe and North America. II. *Mycotaxon* **24**: 437-443.

Harris, H & Hopkinson, D. A. (1976). *Handbook of Enzyme Electrophoresis in Human Genetics.* Oxford American Elsevier Publishing Co.: New York.

Hillis, D. M. (1987). Molecular versus morphological approaches to systematics. *Ann. Rev. Ecol. & Syst.* **18**: 23-42.

Hillis, D. M. & Moritz, C. (Eds.) (1990). *Molecular Systematics.* Sinauer Associates, Inc.: Massachusetts.

Hilton, R. N. (1988). A census of the larger fungi of Western Australia Part II. *J. R. Soc. W. Aust.* **70**: 111-118.

Ho, I. & Trappe,J. M. (1987). Enzymes and growth substances of *Rhizopogon* species in relation to mycorrhizal hosts and intrageneric taxonomy. *Mycologia* **79**, 553-558.

Hoffmann, P. & Esser, K. (1978). Genetics of speciation in the basidiomycetous

genus *Polyporus*. *Theor. Appl. Genet.* **53**: 273-282.

Holmgren, P .K., Keuken, W. & Schofield, E. K. (1981). *Index Herbariorum.* Seventh edition. Bohn, Scheltema & Hilkema: Utrecht.

Hongo,T. (1960). The Agaricales of Japan I-3. *Acta Phytotax. Geobot.* **18**: 129-146. Reprinted In *Selected mycological papers of Dr. Tsugo Hongo*, 1989, by Shiga University.

——— (1976). Agarics from Papua-New Guinea. *Mem. Shiga Univ. Nat. Sci.* **26**: 26-35. Reprinted in *Selected mycological papers of Dr. Tsuguo Hongo*, 1989, by Shiga University.

Hongo, T & Mills, A.K. (1988) Five noteworthy larger fungi new to Tasmania, Australia. *Trans. mycol. Soc. Japan* **29**, 351-357.

Horak, E. (1971). A contribution towards the revision of the Agaricales (fungi) of New Zealand. *New Zealand J. Bot.* **9**: 403-462.

——— (1977). The genus *Melanotus* Pat. *Personnia* **9**: 305-327.

——— (1983). Mycogeography in the south Pacific region: Agaricales, Boletales. *Aust. J. Bot. Suppl. Ser.* **10**: 1-41.

——— (1983). Mycogeography in the South Pacific region: Agaricales, Boletales. In Pacific Mycogeography: A Preliminary Approach. Edited by K. A Piraynski and J. Walker. *Aust. J. Bot. Supp. Series* **10**, 1-42.

——— (1990). Monograph of the New Zealand Hygrophoraceae (Agaricales). *New Zealand J. Bot.* **28**: 255-309.

Imazeki, R & Hongo, T. (1987). *Colored Illustrations of Mushrooms of Japan Vol. I.* Hoikusha Publ. Co. Ltd.: Osaka, Japan.

Itävaara, M. (1988). Identification of shiitake strains and some other basidiomycetes: protein profile, esterase and acid phosphatase zymograms as an aid in taxonomy. *Trans. Br. mycol. Soc.* **91**: 295-304.

Jacobsson, S. (1986). A taxonomic survey of the *Pholiota alnicola* group in Europe. *Windahlia* **16**: 129-143.

——— (1987) On the correct interpretation of *Pholiota adiposa* and a taxonomic survey of section Adiposae. *Windahlia* **17**, 1-18.

——— (1989). Studies on *Pholiota* in culture. *Mycotaxon* **36**: 95-145.

——— (1990). Studies on *Pholiota* in northern Europe. *Windahlia* **19**: 1-86.

Jülich, W. (1981). *Higher taxa of Basidiomycetes*. Bibliotheca Mycologica 85. J. Cramer: Vaduz.

Karlsson, J. & Stenlid, J. (1991). Pectic isozyme profiles of intersterility groups in *Heterobasidion annosum*. *Mycol. Res.* **95**: 531-536.

Kaosiri, T. & Zentmyer, G. A. (1980). Proteins, esterase and peroxidase patterns in the *Phytophthora palmivora* complex from *Cacao*. *Mycologia* **72**: 988-1000.

Kemp, R. F. O. (1975). Breeding biology of *Coprinus* species in the section *Lanatuli*. *Trans. Br. mycol. Soc.* **65**: 375-388.

Kerrigan, R. W. & Ross, I. (1988). Extracellular laccases: biochemical markers for *Agaricus* systematics. *Mycologia* **80**: 689-695.

——— (1989). Allozymes of a wild *Agaricus bisporus* population: new alleles, new genotypes. *Mycologia* **81**: 433-443.

Kile, G. A. & Watling, R. (1983). *Armillaria* species from south-eastern Australia. *Trans. Br. mycol. Soc.* **81**: 129-140.

——— (1988). Identification and occurrence of Australian *Armillaria* species, including *A. pallidula* sp. nov. and comparative studies between them and non-Australian tropical and Indian *Armillaria*. *Trans. Br. mycol. Soc.* **91**: 305-315.

Kornerup, A. & Wanscher, J. H. (1978). *Methuen Handbook of Colour*. Eyre Methuen: London.

Kohn, L. M. (1992). Developing new characters for fungal systematics: an experimental approach for determining the rank of resolution. *Mycologia* **84**: 139-153.

Koltin, Y., Stamberg, J. & Lemke, P. A. (1972). Genetic structure and evolution of the incompatibility factors in higher fungi. *Bacteriol. Rev.* **36**: 156-171.

Korhonen, K. (1987). Breeding units in the forest pathogens *Armillaria* and *Heterobasidion*. In *Evolutionary Biology of the Fungi* edited by A. D. Rayner, C. M. Brasier & D. Moore, pp 301-323. Reprinted 1989. Cambridge University Press.

Kühner, R. (1980). Les Hyménomycétes agaricoïdes. *Bull.Soc. Linn. Lyon* **49**. Numero spécial.

——— (1984). Some mainlines of classification in the gill fungi. *Mycologia* **76**: 1059-1074.

Kühner, R. & Romagnesi, H. (1978). *Flore Analytique des Champignons Supérieurs*. Priméire edition. Masson: Paris.

Kuyper, T. W. (1988). Specific and infraspecific delimitation. In *Flora Agaricini Neerlandica Volume I.* A. A. Balkema: Rotterdam.

Kuyper, TH. W. & Tjallingii-Beufers, G. (1986). Notes on *Pholiota. Persoonia* **13**, 77-82.

Lange, M. & Hora, F. B. (1965). *Collins Guide to Mushrooms & Toadstools.* Second Edition. 1975 reprint. Collins: London.

Lincoff, G. H. (1981). *The Audubon Society Field Guide to North American Mushrooms.* Chanticleer Press Edition. Alfred A. Knopf: New York.

Macrae, R. (1967). Pairing incompatibility and other distinctions among *Hirchioporus* (*Polyporus*) *abietinus, H. fuscoviolaceus* and *H. laricinus. Can. J. Bot.* **45**: 1371-1398.

McAlpine, D. (1895). *Systematic arrangement of Australian fungi.* Department of Agriculture: Victoria.

Manicom, B. Q., Bar-Joseph, M., Rosner, A., Vigodsky-Haas, H. & Kotze, J. M. (1987). Potential applications of random DNA probes and restriction fragment length polymorphisms in the taxonomy of the fusaria. *Phytopathology* **77**: 669-672.

Margot, P. & Watling, R. (1981). Studies in Australian agarics and boletes. II. Further studies in *Psilocybe. Trans. Br. mycol. Soc.* **76**: 485-489.

Marmeisse, R. Deband, J. C. & Casselton, L. A. (1992). DNA probes for species and strains identification in the ectomycorrhizal fungus *Hebeloma. Mycol. Res.* **96**: 161-165.

Massee, G. (1898). Fungi Exotici I. *Kew Bull. Misc. Inf.* **1898**: 113-136.

——— (1899). Fungi Exotici II. *Kew Bull. Misc. Inf.* **1899**: 164-185.

——— (1901). Fungi Exotici III. *Kew Bull. Misc. Inf.* **1901**: 150-169.

May, B. & Royse, D. J. (1988). Interspecific allozyme variation within the fungal genus *Pleurotus. Trans. Br. mycol. Soc.* **90**: 29-36.

May, T. (1990). History of the study of Australian agaricales. In *History of Systematic Botany in Australasia.* Edited by P. S. Short. Proceedings of a symposium held at the Univ. of Melbourne, 25-27 May 1988. Australian Systematic Botany Society Inc.

Micales, J. A., Bonde, M. R. & Peterson, G. L. (1986). The use of isozyme analysis

in fungal taxonomy and genetics. *Mycotaxon* **27**: 405-449.

Michelmore, R. W. & Hulbert, S. H. (1987). Molecular markers for genetic analysis of phytopathogenic fungi. *Ann. Rev. Phytopath.* **25**: 383-404.

Miller, O. K., Jr. (1975). *Mushrooms of North America.* E. P. Dutton: New York.

Mills, A.K. & Crowden, R. K. (1968). Distinction of soluble proteins and enzymes during early development of *Pisum sativum. Aust. J. Biol. Sci.* **21**: 1131-1141.

Monks, A. (1989). *A preliminary survey of the Hygrophoraceae of Tasmania.* Honours Thesis. Unpublished. University of Tasmania, Hobart.

Monks, A. & Mills, A. K. (1991). *Camarophyllus rodwayi* (Hygrophoraceae), a rediscovered fungus. In *Aspects of Tasmanian Botany. A Tribute to Winifred Curtis.* Edited by M. R. Banks, S. J. Smith, A. E. Orchard & G. Kantvilas. Royal Society of Tasmania: Hobart.

Moser, M. (1983). *Keys to agarics and boleti* (English translation of *Die Rohrlinge und Blatterpilze*, II, 1978) Roger Phillips: London.

——— (1985). The relevance of chemical characters for the taxonomy of Agaricales. *Proc. Indian Acad. Sci. (Plant Sci.)* **94**: 381-386.

Mounce, I & Macrae, R. (1938). Interfertility phenomena in *Fomes pinicola. Can J. Research, C* **16**: 354-376.

Mueller, G. M. (1991). *Laccaria laccata* complex in North America and Sweden: incollection pairing and morphometric analyses. *Mycologia* **83**: 578-594.

Murphy, R. W., Sites, J. W., Jr., Buth, D. G. & Haufler, C. H. (1990). Proteins I: Isozymes electrophoresis. In *Molecular Systematics* edited by D. M. Hillis & C. Moritz. p45-126. Sinauer Inc.: Massachusettes.

Neate, S. M., Cruickshank, R. H. & Rovira, A. D. (1988). Pectic enzyme patterns of *Rhizoctonia solani* isolates from agricultural soils in South Australia. *Trans. Br. mycol. Soc.* **90**: 37-42.

Neuhauser, K. S. & Gilbertson, R. L. (1971). Some aspects of bipolar heterothallism in *Fomes cajanderi. Mycologia* **63**: 722-735.

Orton, P. D. (1960). New Check List of British Agarics and Boleti. Part III. Notes on Genera and Species in List. *Trans. Brit. mycol. Soc.* **43**: 159- 439.

——— (1984). Notes on British agarics VIII. *Notes Roy. Bot. Gard. Edinb.* **41**: 565-

624.

Parker, C. S. (1933). A taxonomic study of the genus *Hypholoma* in North America. *Mycologia* **25**, 160-211.

Parmasto, E. (1985). The species concept in Hymenochaetaceae (Fungi, Hymenomycetes). *Proc. Indian Acad. Sci. (Plant Sci.)* **94**: 369-380.

Pegler, D. N. (1965). Studies on Australasian Agaricales. *Aust. J. Bot.* **13**: 323-356.

Pegler, D. N. & Young, T. W. K. (1972) Basidiospore form in the British species of *Galerina* and *Kuehneromyces*. *Kew Bull.* **27**, 483-500.

Pegler, D. N., Spooner, B. M. & Smith, R. L. (1981). Higher fungi of Antarctica, the subantarctic zone and Falkland Islands. *Kew Bull.* **35**: 499-562.

Petersen, R. (1992). Mating systems of three New Zealand agarics. *New Zealand J. Bot.* **30**: 189-197.

Perkins, D. D., Turner, B. C. & Barry, E. G. (1975). Strains of *Neurospora* collected from nature. *Evolution* **30**: 281-313.

Phillips, R. (1981). *Mushrooms and other fungi of Great Britain and Europe*. Pan Books Ltd.: London

Priest, M. J. (1980). *Studies in Basidiomycetes*. Honours Thesis. Unpublished. University of Tasmania, Hobart.

Pukkila, P. J. & Cassidy, J. R. (1987). Varying patterns of ribosomal RNA gene organization in basidiomycetes. In *Evolutionary biology of the fungi* edited by A. D. M. Rayner, C. M. Brasier & D. Moore. pp. 75-82. Reprinted 1989. Cambridge University Press: Cambridge.

Radford, A. E. (1986). *Fundamentals of Plant Systematics*. Harper & Row Publ. Inc.: New York.

Raper, J. R. (1966). Life cycles, basic patterns of sexuality, and sexual mechanisms. In *The Fungi Vol 2* edited by G. C. Ainsworth & A. S. Sussman, pp. 473-511. Academic Press: New York.

Rayner, A. D. M., Brasier, C. M. & Moore D. (Eds.) (1987). *Evolutionary biology of the fungi*. Reprinted 1989. Cambridge University Press: Cambridge.

Rea, C. (1922). *British Basidiomycetaceae*. Bibliotheca Mycologica 15. Reprinted 1980. J. Cramer: Vaduz.

Reddy, M. N. & Stahmann, M. A. (1972). Isozyme patterns of *Fusarium* species and

their significance in taxonomy. *Phytopath. Z.* **74**: 115-125.

Reid, D. A. (1955). New or interesting records of Australasian Basidiomycetes. *Kew Bull.* **10**, 631-648.

Reid, D. A. (1980). A monograph of the Australian species of *Amanita* Pers. ex Hook. (Fungi). *Aust. J. Bot. Suppl. Ser.* **8**: 1-96.

Rodway, L. (1898). Tasmanian Fungi. *Pap. Proc. R. Soc. Tasm.* **(1897)**: 129-144.

——— (1900). Additions to the fungus flora of Tasmania. *Pap. Proc. R. Soc. Tasm.* **(1898-1899)**: 97-99.

——— (1918). Botanical Notes. *Pap. Proc. Roy. Soc. Tasm.* **1917**: 105-110.

——— (1920). Notes and additions to the fungus flora of Tasmania. *Pap. Proc. Roy. Soc. Tasm.* **1919**: 110-116.

——— (1921). Additions to the fungus flora of Tasmania. Part 3. *Pap. Proc. Roy. Soc. Tasm.* **1920**: 153-159.

Rodway, L. & Cleland, J. B. (1930). Notes on the genus *Poria* No. 3. *Pap. Proc. Roy. Soc. Tasm.* **1929**: 7-24.

Royse, D. J. (1985). Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. *Mycologia* **77**: 756-762.

Royse, D. J. & May, B. (1982a). Use of isozyme variation to identify genotypic classes of *Agaricus brunnescens*. *Mycologia* **74**: 93-102.

——— (1982b). Genetic relatedness and its application in selective breeding of *Agaricus brunnescens*. *Mycologia* **74**: 569-575.

Saccardo, P. A. (1887). *Syll. Fung.* 5, 1021-1022. Reprinted 1966.

SAS Institute INC (1988). *SAS/STAT™ User's Guide, Release 6.03 Edition.* SAS Institute Inc.:Cary, NC.

Sen, R. (1990). Intraspecific variation in two species of *Suillus* from Scots pine (*Pinus sylvestris* L.) forests based on somatic incompatibility and isozyme analyses. *New Phytol.* **114**: 607-616.

Seviour, R. J., Chilvers, G. A. & Crow, W. D. (1972). Electrophoretic characterization of eucalypt mycorrhizas. *New Phytol.* **71**: 1107-1110.

Shaw, C. R. & Prasad, R. (1970). Starch gel electrophoresis of enzymes. A compilation of recipes. *Biochem Genet.* **4**: 297-320.

Shechter, Y., Landau, J. W. & Dabrowa, N. (1972). Comparative electrophoresis and numerical taxonomy of some *Candida* species. *Mycologia* **64**: 841-853.

Shepherd, C. J. & Totterdell, C. J. (1988). *Mushrooms and Toadstools of Australia.* Inkata Press: Melbourne & Sydney.

Singer, R. (1951). Type studies on Basidiomycetes V. *Sydowia* **5**: 445-475.

——— (1958). New genera of fungi IX. The probable ancestor of the Strophariaceae: *Weraroa* gen. nov. *Lloydia* **21**: 45-47.

——— (1962). *The Agaricales in modern taxonomy.* Second edition. J. Cramer: Weinheim.

——— (1969). *Mycoflora Australis.* Beih. Nova Hedwigia Heft. 29. J. Cramer.

——— (1983). Acanthocytes in *Amparoina* and *Mycena. Cryptogamie Mycologie* **4**: 111-115.

——— (1986). *The Agaricales in modern taxonomy.* Seventh edition. J. Cramer: Vaduz.

Singer, R & Moser, M (1965). Forest mycology and forest communities in South America I. *Mycopath. & Mycol. Appl.* **26**: 129-191.

Singer, R. & Smith, A. H. (1946). The taxonomic position of *Pholiota mutabilis* and related species. *Mycologia* **38**: 500-523.

Singer, R. & Smith, A. H. (1958). Mycological investigations on teonanácatl, the Mexican mushroom. Part II. A taxonomic monograph of *Psilocybe*, section *Caerulescentes. Mycologia* **50**: 262-303.

Smith, A. H. (1951). The North American species of *Naematoloma. Mycologia* **43**: 389-465.

Smith, A. H. & Hesler, L. R. (1968) *The North American Species of* Pholiota. Hafner Publ. Co.: New York & London.

Smith, M. L. & Anderson, J. B. (1989). Restriction fragment length polymorphisms in mitochondrial DNAs of *Armillaria*: identification of North American biological species. *Mycol. Res.* **93**: 247-256.

Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy: The principles and Practice of Numerical Classification.* W. Freeman & Co.: San Franscisco.

Stasz, T. E., Nixon, K., Harman, G. E. & Weeden, N. F. (1989). Evaluation of phenetic species and phylogenetic relationships in the genus *Trichoderma* by cladistic analysis of isozyme polymorphism. *Mycologia* **81**: 391-403.

Stearn, W. T. (1973). *Botanical Latin.* Second edition. David & Charles: Newton,

Abbot.

Steglich, W. (1980). Pigments of higher fungi (Macromycetes). In *Pigments in Plants* edited by F. C. Czygan. Second edition. G. Fischer: Stuttgart. pp.393-412.

Stenlid, J & Karlsson, Jan-Olof (1991). Partial intersterility in *Heterobasidion annosum*. *Mycol. Res.* **95**: 1153-1159.

Sweetingham, M. W., Cruickshank, R. H. & Wong, D. H. (1986). Pectic zymogram and taxonomy and pathogenicity of the Ceratobasidiaceae. *Trans. Br. mycol. Soc.* **86**: 305-311.

Tyrrell, D. (1969). Biochemical systematics and fungi. *Bot. Rev.* **35**: 305-316.

Wasfy, E. H., Farag, S. A., Tarabieh, M. A. & Abd-Elmoety, S. H. (1978). Studies on enzymes of different strains of *Botrytis cinerea*. *Phytopath. Z.* **92**: 168-179.

Watling, R. (1973). *Identification of the larger fungi*. Edited by A. R. Kenney. Hulton Educational Publications. Ltd.: Amershem.

Watling, R. & Gregory, Norma M. 1987. *British fungus flora Agarics and Boleti, 5. Strophariaceae & Coprinaceae* : Hypholoma, Melanotus, Psilocybe, Stropharia, Lacrymaria & Panaeolus (ed. D. M. Henderson, P. D. Orton and R. Watling). Royal Botanic Garden, Edinburgh.

Weber, C. A. Hudspeth, M.E. S., Moore, G. P. & Grossman, L. I. (1986). Analysis of the mitochondrial and neclear genomes of two basidiomycetes, *Coprinus cinerus* and *Coprinus stercorarius*. *Current Genet.* **10**: 515-525.

Whitehouse, H. L. K. (1949). Multiple-allelomorph heterothallism in the fungi. *New Phytol.* **48**: 212-244.

Willis, J. H. (1950). *Victorian Mushrooms & Toadstools*. Field Naturalists Club of Victoria.

Wong, A. L. & Willets, H. J. (1973) Electrophoretic studies of soluble proteins and enzymes of *Sclerotinia* species. *Trans. Br. mycol. Soc.* **61**: 167-178.

——— (1975). Electrophoretic studies of Australasian, North American and European isolates of *Sclerotinia sclerotiorum* and related species. *J. Gen. Microbiol.* **90**: 355-359.

Young, A. (1982). *Common Australian fungi*. Reprinted 1986. New South Wales University Press.

Appendix I

Fruiting medium used in the fruiting trial

Recipe of the artificial fruiting medium used in the fruiting trial modified from Royse (1985):–

sawdust (sassafras[1])	1590g.
white millet	170g.
wheat bran	170g.
malt extract	20g.
deionised water	1L.

[1] *Atherosperma moschatum*, one of the host plants of Tasmanian *Pholiota aurivella*.

Appendix II

Results of fruiting trials

Successful fruiting was achieved. As a result of inadequate light conditions, the basidiomes were rather elongated in the stipe (Fig. A). It was noted that the stipe was dry right from the button stage through to the later stages of development (Fig. B). Spores from spore print of this F_1 generation germinated under the same conditions as did the spores from the parental spore print.

Fig. A

Fig. B

Appendix III

Details of collections included in the study

A. Genus *Stropharia*

Collection	Locality	Altitude	Habitat	Date
Stropharia aurantiaca				
CYS202	Garden, Taroona, Hobart.	100m.	on lawn	21. v. 1989.
CYS230	Garden, Taroona, Hobart.	100m.	under cut grass	5. vi. 1989.
CYS281*	Garden, Taroona, Hobart.	100m.	amongst straw	1. vii. 1989.
CYS527*	Garden, Cascades, Hobart.	~125m.	on eucalypt bark mulch, under *Grevillea biternata*	24. iv. 1991.
S. coronilla				
CYS556	Sandy Bay, suburb of Hobart		on ground amongst grass	24. xii. 1991.
CYS557	Sandy Bay, suburb of Hobart		on ground amongst grass	30. xii. 1991.
CYS558	Sandy Bay, suburb of Hobart		on ground amongst grass	1. i. 1992.
***Stropharia* sp A**				
CYS137*	Mt Field National Park	275m.	on ground litter	2. v. 1989.
CYS160*	Lady Barron Falls Track Mt Field National Park	250m.	on ground litter	2. v. 1989.
CYS327*	Fortesque Bay, Tasman Peninsula	~75m.	on ground	25. ix. 1989.
CYS341*	Tahune Forest Reserve	80m.	on ground litter	1. v. 1990.
CYS358*	Tahune Forest Reserve	80m.	on ground litter	7. v. 1990.
***S. stercoraria* (LF)**				
CYS351**	Arve Road, Geeveston	240m.	on wallaby dung	1. v. 1990.
CYS382**	Geeveston	240m.	on cow dung	23. v. 1990.
AKM1013	on slopes of Mt Read	775m.	on very old wombat dung	iv. 1991.
***S. stercoraria* (MF)**				
CYS200**	Ellendale	~350m.	on wallaby dung	22. v. 1989.
CYS269	Snug Falls Track	280m.	on cow dung	26. vi. 1989.
CYS270	Snug Falls Track	280m.	on wombat dung	26. vi. 1989.
CYS273	Snug Falls Track	280m.	on cow dung	26. vi. 1989.
CYS343**	Mt Field National Park	200m.	on wallaby dung	2. v. 1990.
CYS386**	Snug Falls Track	280m.	on cow dung	30. v. 1990.
CYS387	Snug Falls Track	280m.	on horse dung	30. v. 1990.
CYS390**	Snug Falls Track	280m.	on horse dung	30. v. 1990.
CYS431	Snug Falls Track	280m.	on cow dung	13. vi. 1990.
CYS437**	Snug Falls Track	280m.	on cow dung	13. vi. 1990.
CYS443	Snug Falls Track	280m.	on cow dung	13. vi. 1990.
CYS444**	Snug Falls Track	280m.	on horse dung	13. vi. 1990.
CYS447	Snug Falls Track	280m.	on cow dung	13. vi. 1990.
CYS449	Snug Falls Track	280m.	on horse dung	13. vi. 1990.
CYS483**	Mt Field National Park	200m.	on wallaby dung	27. vi. 1990.
CYS523	Pandanus Walk Mt Field National Park	1050m.	on wallaby dung	16. iv. 1991.
CYS534	Hartz National Park	760m.	on wallaby dung	6. v. 1991.

Stropharia **sp C**				
CYS364**	Near Nugent	~280m.	on wallaby dung	15. v. 1990.
CYS486**	Mt Field National Park	200m.	on wallaby dung	27. vi. 1990.
CYS526	Past Robertson Bridge Sandspit River Forest Res.	~275m.	on wallaby dung	23. iv. 1991.
S. semiglobata **(MF)**				
HO124686	West coast, Tasmania	-	on wallaby dung	v. 1991.
H & S 1429	U.K.	-	on cow dung	26. viii. 1974.

* Collections with isolates included in electrophoretic studies.
** Collections with isolates included in both electrophoretic and mating compatibility studies.

B. Genus *Hypholoma*

Legend: Y = both pileus and lamellae yellow, AP = both pileus and lamellae apricot orange and *=isolates used in electrophoretic studies.

Collection	Locality	Habitat	Date
H. fasciculare **(Y)**			
CYS101	Campus, University of Tasmanis Hobart	on eucalypt wood chips	18. x. 1988
CYS102	Campus, University of Tasmania Hobart	on ground, amongst grass & mosses	19. x. 1988
CYS117	Fern Glades, slopes of Mt. Wellington, Hobart	on rotten wood	18. iv. 1989
CYS122	Myrtle Forest, Collinsvale, North of Hobart	on rotten wood	27. iv. 1989
CYS129	Myrtle Forest, Collinsvale, North of Hobart	on rotten wood	27. iv. 1989
CYS165	Scottsdale, NE Tasmania	on woody litter	9. v. 1989
CYS218	Balt Spur, Tasman Peninsula	on rotten wood	25. v.1989
CYS219	Adamsons Road, SE Tasmania	on rotten wood	30. v. 1989
CYS228	Peak Road, SE Tasmania	on ground	30. v. 1989
CYS234	Liffey Falls, NW Tasmania	on rotten wood	14. vi. 1989
CYS380	Arve Loop, near Geeveston	on rotten wood	23. v. 1990
CYS407*	Waterfalls Bay road, Tasman Peninsula	by roadside, on ground amongst grasses	6. vi.1990
CYS467	North Lune Road, near Hastings Caves	on wood	19. vi. 1990
Watling s.n.	Yorkshire, U. K.	-	ix. 1982
HDT40826	Tuolumne co., California, U. S. A.	on dead conifer log	17. v. 1980
H. fasciculare **(AP)**			
CYS13	Arve Loop, near Geeveston	on rotten wood	21. vi. 1988
CYS78	Balt Spur, Tasman Peninsula	on rotten wood	5. vii. 1988
CYS81	Balt Spur, Tasman Peninsula	on fallen log	5. vii. 1988
CYS108	Fern Glades, slopes of Mt Wellington	on rotten wood	18. iv. 1989
CYS124	Myrtle Forest, Collinsvale, north of Hobart	on rotten wood	27. iv. 1989
CYS133	Myrtle Forest, Collinsvale, North of Hobart	on rotten wood	17. iv. 1989

CYS141	Mt Field National Park	on rotten wood	2. v. 1989
CYS151	Mt Field National Park	on rotten wood	2. v. 1989
CYS169	Scottsdale, NE Tasmania	on woody litter	9. v. 1989
CYS215	Balt Spur, Tasman Peninsula	on rotten wood	25. v. 1989
CYS221	Adamsons Road, SE Tasmania	on rotten wood	30. v. 1989
CYS261	Milkshakes Hills Reserve, NW Tasmania	on rotten wood	16. vi. 1989
CYS274	Snug Falls Track, near Snug	on wood	26. vi. 1989
CYS333*	Fern Glades, slopes of Mt Wellington	on fallen log	19. iv. 1990
CYS344*	Myrtle Forest, Collinsvale, North of Habart	on rotten wood	3. v. 1990
CYS379	Arve Loop, near Geeveston	on rotten wood	23. v. 1990
CYS551	Tahune Forest Reserve	on tree stump	15. v. 1990
H. sublateritium			
CYS19	Arve Loop, near Geeveston	on mossy ground	21. vi. 1988
CYS82	Balt Spur, Tasman Peninsula	on ground	5. vii. 1988
CYS85	Balt Spur, Tasman Peninsula	on wood	5. vii. 1988
CYS125	Myrtle forest, Colinsvale, north of Hobart	on wood	17. iv. 1989
CYS138	Mt Field National Park	on wood	2. v. 1989
CYS166	Scottsdale, NE Tasmania	on ground, buried wood	9. v. 1989
CYS239	Track to Liffey Falls,	on ground, buried wood	14. vi. 1989
CYS337*	Arve Loop. near Geeveston	on ground, buried wood	1. v. 1990
CYS408*	Hyland Road, Spur 1, Forestier Peninsula	on ground	6. vi. 1990
CYS414	Balt Spur, Tasman Peninsula	on ground, buried wood	6. vi. 1990
Baroni 3642	Massachusetts, U. S. A.	on downed white birch log (*Betula papyriferus*)	? 1978
H. brunnea			
CYS176	Scotts Peak Road, SW World Heritage Area	on wood	17. v. 1989
CYS178	Scotts Peak Road, SW World Heritage Area	on wood	17. v. 1989
CYS182	Five Road, Little Florentine Valley	on wood	17. v. 1989
CYS185	Five Road, Little Florentine Valley	on wood	17. v. 1989
CYS207	Balt Spur, Tasman Peninsula	at base of dead tree stump	25. v. 1989
CYS210	Balt Spur, Tasman Peninsula	on dead tree stump	25. v. 1989
CYS211	Balt Spur, Tasman Peninsula	on dead tree stump	25. v. 1989
CYS223	Adamsons Road, SE Tasmania	on wood	30. v. 1989
CYS225	Adamsons Road, SE Tasmania	on rotten wood	30. v. 1989
CYS260	Milkshakes Hill Reserve, NW Tasmania	on wood	16. vi. 1989
CYS302	Keogh Road, off Arve Road, near Geeveston	on rotten wood	19. vii. 1989
CYS303	Keogh Road, off Arve Road, near Geeveston	on rotten wood	19. vii. 1989
CYS315*	picnic area, Tahune Forest Reserve	on wood	19. vii. 1989

CYS373*	Sandspit River Forest Reserve	on rotten wood	15. v. 1990
CYS550	Tahune Forest Reserve	on fallen trunk	15. v. 1991
***Hypholoma* sp A**			
CYS531*	c. 500m. from Arve Loop entrance, Arve Road	in sheltered gully, amongst *Sphagnum*	6. v. 1991
CYS543*	c. 500 m. from Arve Loop entrance, Arve Road	on boggy area, amongst *Sphagnum* and *Polytrichum*	15. v. 1991
AKM1001	on slope of Mt Read, west coast of Tasmania	wet, boggy ground in creek bed, on mud, mosses on the general area	iv. 1991
***Hypholoma* sp B**			
CYS491*	Hytten Hall, on the campus of University of Tasmania	on wood chips below *Melaleuca* bushes	1. vii. 1990
***Hypholoma* sp C**			
CYS259	Milkshakes Hill Reserve, NW Tasmania	on fallen manfern frond	16. vi. 1989
CYS262	Milkshakes Hill Reserve, NW Tasmania	on ground litter	16. vi. 1989
CYS287	Track to Lady Barron Falls, Mt Field National Park	on ground litter	3. vii. 1989
CYS292	Track to Lady Barron Falls, Mt Field National Park	on ground litter	3. vii. 1989
CYS332	Fern Glades, slopes of Mt Wellington	on dead tree stump	19. iv. 1990
CYS365	on the way to Sandspit River Forest Reserve, near Nugent	on ground	15. v. 1990
CYS427*	Fern Glades, slopes of mt. Wellington	on rotten wood	13. vi. 1990

C. Genus *Psilocybe*

Collection	Locality	Habitat	Date
Group I A. *Psilocybe australiana*			
CYS95*	Garden, Hobart, Tasmania	on woody litter	14. vii. 1988.
CYS112*^	Fern Glade, slopes of Mt. Wellington	on ground litter	18. iv. 1989.
CYS132*	Myrtle Forest, Collinsvale, north of Hobart	on ground litter	27. iv. 1989.
CYS135*^	Mt. Field National Park, Tasmania	on ground litter	2. v. 1989.
CYS139*^	Mt. Field National Park, Tasmania	on leafy litter	2. v. 1989.
CYS158*^	Lake Dobson Road, Mt. Field National Park	on ground litter	2. v. 1989.
CYS161*†^	Lady Barron Falls Track, Mt. Field National Park	on ground	2. v. 1989.
CYS170*^	Myrtle Forest, Collinsvale, north of Hobart	on rotten log	11. v. 1989.
CYS217*†^	Tasman Peninsula, SE Tasmania	on ground litter	25. v. 1989.

(=DAR66084)			
CYS233^	Liffey Falls, NW coast, Tasmania	on ground litter	14. vi. 1989.
CYS236†^	Liffey Falls, NW coast, Tasmania	on ground	14. vi. 1989.
(=DAR66085)			
CYS279^	Uni. of Tasmania campus, Hobart	on ground	29. vi. 1989.
CYS280^	Garden, Taroona, near Hobart	on mown lawn	1. vii. 1989.
CYS290	Lady Barron Falls Track, Mt. Field National Park	on leafy litter	3. vii. 1989.
CYS293^	Lady Barron Falls Track, Mt. Field National Park	on ground litter	3. vii. 1989.
CYS369	Sandspit River Forest Reserve, Tasmania	on mossy ground	15. v. 1990.
B. *P. eucalypta*			
CYS362† (=DAR63053)	Tidbinbilla Nature Reserve, near Canberra, A. C. T.	on leafy litter	5. v. 1990.
C *P. tasmaniana*			
AKM955	Colebrook, Tasmania	on pasture land	vii. 1990.
D *P. subaeruginosa*			
CYS515†^ (=DAR66086)	Jumping Creek Reserve, Warandyte, Victoria	on ground litter	vii. 1990.
Group II *P. semilanceata*			
CYS451†^ (=DAR66087)	Neika, Tasmania	on rich pasture	19. vi. 1990.
***Psilocybe* sp. A**			
CYS389^	Snug Falls Track, SE Tasmania	on horse dung	30. v. 1990.
CYS391^	Snug Falls Track, SE Tasmania	on cow dung	30. v. 1990.
CYS405	Snug Falls Track, SE Tasmania	on cow dung	30. v. 1990.
CYS448^	Snug Falls Track, SE Tasmania	on horse dung	13. vi. 1990.
CYS522^	Pandanis Walk, 1050 m., Mt. Field National Park.	on wallaby dung	16. iv. 1991.
Group III *Psilocybe* sp. B			
CYS381^	Arve Loop, off Arve Road, near Geeveston.	on wallaby dung	23. v. 1990.
Group IV *Psilocybe* sp. C			
CYS518^	Myrtle Forest, collinsvale, north of Hobart.	on rotten wood	3. iv. 1991.
CYS552	Tahune Forest Reserve, near Geeveston	on woody debris on bank of creek	5. vi. 1991.
AKM968	Julius River Reserve, NW coast	on very rotten myrtle beech log	9. iv. 1991.

* Part of collection lodged at Royal Botanic Garden, Edinburgh (E).

† Isolates and part of collection lodged at Biology Branch Herbarium, Rydalmere, New South Wales (DAR).

^ Isolates used in cultural studies.

ns No isolate, but specimens used in morphological study.

D. Genus *Melanotus*

Collection	Locality	Habitat	Date
Melanotus hepatochrous			
CYS254*	Blackwater Road, NW coast Tasmania	on recently burnt wood	15. vi. 1989.
CYS311	Keogh Road, Geeveston, SE Tasmania	on twigs	19. vii. 1989.
CYS326*	Fern Glades, Mt Wellington, near Hobart, SE Tasmania	on trachis of fallen manfern frond	2. viii. 1989.
CYS349*	Hartz Road, Hartz Mt National Park, SE Tasmania	on twig	7. v. 1990.
CYS367*	Sandspit River Forest Reserve, E coast, Tasmania	on wood	15. v. 1990.
CYS370*	Sandspit River Forest Reserve, E coast, Tasmania	on *Eucalyptus* log	15. v. 1990.
CYS425*	Balt Spur, Tasman Peninsula, SE Tasmania	on dead stump	6. vi. 1990.
CYS461	North Lune Road, near Hastings Caves, SE Tasmania	on dead tree stump	19. vi. 1990.
Crepidotus cassiaecolor			
Rdw166	Cascades, Hobart	-	v. 1898.
Crepidotus hepatochrous			
Rdw670	Cascades, Hobart	on scored bark of *Eucalyptus*	viii. 1898.

Collections marked with an asterisk are included in morphological, electrophoretic and mating studies.

E. Genus *Pholiota*
Subgenus *Phaeonaematoloma*

Collection	Locality	Habitat	Date
***Pholiota* sp. A**			
CYS284	Nature Trail, 660 m., Mt Field National Park	on ground, amongst moss	3. vii. 1989.
CYS509	Nature Trail, 660 m., Mt Field National Park	on ground, amongst moss	2. viii. 1990.
***Pholiota* sp. B**			
CYS256	Pine Walk, off Tayatea Road, NW coast	on ground, amongst moss	16. vi. 1989.
CYS285	Nature Trail, 660 m., Mt. Field Ntional Park	on ground litter	3. vii. 1989.
CYS342	Nature Trail, 660 m., Mt. Field National Park	on ground litter	2. v. 1990.
CYS410	Balt spur, Tasman Peninsula	on moss-covered ground	6. vi. 1990.
CYS487	Nature Trail,660 m., Mt. Field National Park	on ground litter	27. vi. 1990.
CYS520	Nature Trail,660 m., Mt. Field National Park	on ground litter and rotten wood	16. iv. 1991.
CYS521	Pandanus Walk, 1050 m.,	on ground amongst moss	16. iv. 1991.

Mt. Field National Park

Subgenus *Flammula*

Collection	Locality	Habitat	Date
P. malicola			
CYS20	Near Geeveston, Arve Loop, off Arve Road	on ground by roadside	21. vi. 1988.
CYS171	Near Geeveston, Arve Loop, off Arve Road	on ground by roadside	13. v. 1989.
CYS177	SW World Heritage Area, Scotts Peaks Road	on mossy ground	17. v. 1989.
CYS180	SW World Heritage Area, Scotts Peaks Road, carpark opposite Nature Walk	on ground in sheltered area	17. v. 1989.
CYS226	SE Tasmania, Adamsons Road.	on ground among grass	31. v. 1989.
CYS334	Mt. Field National Park, along Dobson Road	on ground, under *Eucalyptus delegatensis.*	24. iv. 1990.
CYS335	near Geeveston, Arve Loop, off Arve Road	on ground	1. v. 1990
CYS355	near Geeveston, Arve Loop Spur 1	on ground	7. v. 1990
CYS337	HartzRoad, just outside the boundary of Hartz Mountain National Park	on ground	7. v. 1990
CYS383	Near Geeveston, Arve Loop, off Arve Road	on ground by roadside	23. v. 1990.

Subgenus *Pholiota*

Collection	Locality	Habitat	Date
P. aurivella			
CYS116*	Jacksons Bend, near Mt. Wellington.	on sassafras stump	18. iv. 1989.
CYS128*	Myrtle Forest, Collinsvale, North of Hobart	on fallen sassafras log	27. iv. 1989.
CYS154*	Nature Trail, Mt. Field National Park	on dead sassafras stump	2. v. 1989.
CYS157*	Nature Trail, Mt. Field National Park,	on fallen myrtle trunk	5. v. 1989.
CYS159*	Nature Trail, Mt. Field National Park	on dead myrtle stump	5. v. 1989.
CYS181	Five Road, Little Florentine Valley	on myrtle trunk	17. v. 1989.
CYS324*	Near entrance to track Tahune Forest Reserve	on damaged but living sassafras	19. vii. 1989.
CYS325*	Tahune Forest Reserve	on trunk of dead sassafras	19. vii. 1989
CYS361	Track along stream, Sandspit	on fallen sassafras trunk	4. v. 1990

	River Forest Reserve			
CYS372	Past Robertson Bridge, near Picnic area, SandspitRiver Forest Reserve	on wood	15.	v. 1990
Smith13193	Washington, U.S. A.	on alder		
Hesler12508	Tennessee, U. S. A.	on elm		
P. squarrosipes				
CYS8*	Arve Loop, off Arve Rd, near Geeveston	on bare ground	21.	vi. 1988.
CYS11*	Arve Loop, off Arve Rd, near Geeveston	on woody debris	21.	vi. 1988.
CYS14*	Arve Loop, off Arve Rd, near Geeveston	on leafy debris	21.	vi. 1988.
CYS16*	Arve Loop, off Arve Rd, near Geeveston	on leafy debris and amongst *Polytrichum*	21.	vi. 1988.
CYS55*	Myrtle Forest, Collinsvale, north of Hobart	on ground, amongst grasses	28.	vi. 1988.
CYS90	Balt Spur, Tasman Peninsula	on ground	5.	vii. 1988.
CYS106	Hartz Mt Rd	on clayey soil, fairly	11.	iv. 1989.
CYS136	Mt Field National Park, grassy area near creek	on ground, amongst grasses & mosses	2.	v. 1989.
CYS163	Scottsdale, NE Tasmania	on ground litter, at altitude of c.650m.	9.	v. 1989.
CYS179	Scotts Peak Rd, SW World Heritage Area, carpark opposite Nature Walk	on ground, amongst grasses & mosses	19.	vii. 1989.
CYS246	Julius Reserve, picnic area, NW Tasmania	on mossy ground, slightly sheltered	15.	vi. 1990.
CYS247	Julius Reserve, picnic area, NW Tasmania	on ground amongst grasses & mosses	15.	vi. 1990.
CYS267*	University of Tasmania campus, Hobart	on eucalypt wood chips	20.	vi. 1989.
CYS318	Tahune Forest Reserve, picnic area	on ground amongst grasses & mosses	19.	vii. 1989.
CYS320	Tahune Forest Reserve, picnic area	on ground amongst grasses & mosses	19.	vii. 1989.
CYS345	Myrtle Forest, Collinsvale, north of Hobart	on ground amongst grasses	3.	v. 1990.
CYS363	Near Nugent	on ground litter	15.	v. 1990.
CYS366*	Near Nugent	on ground amongst grass	15.	v. 1990.
CYS371	Sandspit River Forest Reserve	on ground amongst grass	15.	v. 1990.
CYS377*	Hartz Mt Rd	on bare ground, by roadside, very exposed	23.	v. 1990.
CYS398*	North Lune Rd, near Hastings Caves	on bare ground	30.	v. 1990.
CYS400	North Lune Rd, near Hastings Caves	on ground	30.	v. 1990.
CYS422	Fortesque Rd, Tasman Peninsula	on ground	6.	vi. 1990.
CYS424*	Balt Spur, Tasman Peninsula	on ground amongst grasses & mosses	6.	vi. 1990.
CYS456*	North Lune Rd, near Hastings Caves	on ground amongst mosses in burnt area	19.	vi. 1990.
CYS457	North Lune Rd, near Hastings Caves	on ground amongst mosses in burnt area	19.	vi. 1990.
CYS458*	North Lune Rd, near Hastings Caves	on ground amongst	19.	vi. 1990.

		mosses in burnt area	
CYS472	End of North Lune Rd, near Hastings Caves	on bare ground amongst rocks, very exposed	19. vi. 1990.
CYS489*	Mt Field National Park, near car park	on ground amongst grasses	27. vi. 1990.
CYS496*	Tahune Forest Reserve, picnic area	on ground amongst grasses & mosses	4. vii. 1990.
CYS504	Keoghs Rd, off Arve Rd, south of Geeveston	on ground amongst grasses & mosses, by roadside	17. vii. 1990.
Syntype collections			
AD12142	Encounter Bay, South Australia	on the ground	9. v. 1931.
AD11958	Encounter Bay, South Australia	on ground	9. v. 1931.
AD12200	Back Valley, Encounter Bay, South Australia	on bare ground & rocks	24. v. 1933.
AD12050	Upper Tunkahlla Creek, South Australia	on wet ground, often near	4. vi. 1930.
AD11954	Encounter Bay, South Australia	on ground & woody debris	25. v. 1928.

* Collections included in cultural studies.

Subgenus *Flammuloides*

Collection	Locality	Habitat	Date
P. highlandensis			
CYS453	Arve Road, near Geeveston	on recently burnt ground	19. vi. 1990.
CYS497	Arve Road, near Geeveston	on recently burnt ground	4. vii. 1990.
CYS528	Arve Road, near Geeveston	on recently burnt ground	6. v. 1991.
CYS529	Arve Road, near Geeveston	on recently burnt groumd	6. v. 1991.
CYS530	Arve Road, near Geeveston	on recently burnt ground	6. v. 1991.
CYS532	Arve Road, near Geeveston	on recently burnt ground	6. v. 1991.
CYS538	Arve Road, near Geeveston	on recently burnt ground	15. v. 1991.
Flammula highlandensis			
Type	U. S. A.		1897.
***Pholiota* sp C**			
CYS257	Milkshakes Hill Reserve, NW coast	on fallen manfern	16. vi. 1989.
***Pholiota* sp D**			
CYS4*	Arve Loop, off Arve Rd, near Geeveston	on ground	21. vi. 1988.
CYS30	Arve Loop, off Arve Rd, near Geeveston	on ground, slightly mossy	21. vi. 1988.
CYS43	Arve Loop, off Arve Rd, near Geeveston	on ground	21. vi. 1988.
CYS54*	Myrtle Forest, Collinsvale,	on fallen rotten log	28. vi. 1988.

	north of Hobart		
CYS86*	Balt Spur, Tasman Peninsula	on ground, amongst mosses	5. vii. 1988.
CYS271*	Snug Falls Track, near Snug	on ground	26. vi. 1989.
CYS289*	Track to Lady Barron Falls, Mt Field National Park	on fallen manfern trunk	3. vii. 1989.
CYS411*	Balt Spur, Tasman Peninsula	on ground, amongst mosses	6. vi. 1990.
CYS433	Snug Falls Track, near Snug	on ground, buried wood	13. vi. 1990.
CYS482*	University of Tasmania campus, Hobart	on eucalypt wood chips	26. vi. 1990.
CYS488*	Nature Trail, Mt Field National Park	on ground, amongst mossy, sheltered	27. vi. 1990.
CYS494	Arve Rd, near Geeveston	on wood	14. vii. 1990.
CYS502*	Arve Rd, near Geeveston	on ground litter	17. vii. 1990.
CYS510	Nature Trail, Mt Field National Park	on ground, buried wood	2. viii. 1991.
CYS533*	c. 2/3 up Hartz Rd, Hartz Mt National Park	on fallen log, in rainforest area	6. v. 1991.
***Pholiota* sp E**			
CYS266	Campus, University of	on eucalypt wood chips	20. vi. 1989.
Pholiota multicingulata			
CYS49	Hobart, University of Tasmania campus	on eucalypt wood chips	24. vi. 1988.
CYS100	Hobart, University of Tasmania campus	on eucalypt wood chips	29. vii. 1988.
CYS144	Mt Field National Park	on wood	2. v. 1989.
CYS231	Hobart, University of Tasmania campus	on ground litter or pine bark mulch & eucalypt wood chips	7. vi. 1989.
CYS227	Near Dover, Adamsons Rd	on wood in young regenerated forest	30. v. 1989
CYS253	NW Tasmania, 30km south of Smithton, Blackwater Rd 1-1	on recently burnt wood & ground	15. vi. 1989.
CYS299	Mt Field National Park, Nature Trail	on ground litter	10. vii. 1989.
CYS300	Mt Field National Park, Nature Trail	on mossy ground	10. vii. 1989.
CYS331*	Fern Glades, at the foot of Mt Wellington	on wood	19. vii. 1990.
CYS376	NW Tasmania,Liffey Falls	on ground litter	v. 1990
CYS385*	Hobart, University of Tasmania campus	on pine bark mulch	24. v. 1990.
CYS397	North Lune Rd, near Hastings Caves, SE Tasmania	on burnt wood	30. v. 1990.
CYS413	Balt Spur, Tasman Peninsula	on ground	6. vi. 1990.
CYS426*	Fern Glades, at the foot of Mt Wellington	on twig	13. vi. 1990.
CYS440*	Snug Falls Track, near Snug	on ground litter	13. vi. 1990.
CYS475*	Near Pine Track entrance, Tahune Forest Reserve	on ground	19. vi. 1990.

CYS485*	Track to Lady Barron Falls, Mt Field National Park	on wood	27. vi. 1990.
CYS493	Arve Rd, near Geeveston	on ground	4. vii. 1990.
CYS501*	Keoghs Rd, off Arve Rd, near Geeveston	on wood	17. vii. 1990
CYS512*	University of Tasmania campus, Hobart	on eucalypt wood chips	12. viii. 1990.
CYS524	Nature Trail, Mt Field National Park	on ground litter	16. iv. 1990
CYS536*	off Arve Rd, near Geeveston	on ground or burnt wood	15. v. 1991.
CYS540*	off Arve Rd, near Geeveston	on ground in burnt area	15. v. 1991.
Pholiota (Flammula) spumosa			
PDD24372	Canada		
PDD47290	Germany		
Smith64712	Reese's bog, north end of Burt Lake, Cheboygan Co., Michigan, U. S. A.	on conifer saedust	17. x. 1961.
Phliota austrospumosa			
TNS-F-228300	Oksapmin, Papua New Guinea	on decaying wood	19. xii. 1971.
Pholiota stratosa			
Smith64684	Mud Lake Bog, Washtenaw Co., Michigan, U. S. A.	on decayed hardwood	14. x. 1961.
Pholiota bakerensis			
Smith16727	Washington, U. S. A.	on conifer sticks	
Pholiota iterata			
Smith9318	Cave Junction, Oregon, U. S. A.	on needle carpet under pine	1. xii. 1937.
Pholiota piceina			
Smith73446	Binarch Creek, Priest Lake, Idaho, U. S. A.	on or around old conifer logs	17. ix. 1966.

Appendix IV

Manuscript as accepted for publication

Re-examination of *Psilocybe subaeruginosa* Clel. and related species with comparative morphology, isozymes and mating compatibility studies

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Comparative morphology, isozyme analysis and mating compatibility approaches were used to investigate the relationships between *Psilocybe subaeruginosa* Clel. and three closely related taxa *P. australiana* Guzman & Watling, *P. eucalypta* Guzman & Watling and *P. tasmaniana* Guzman & Watling. The four names were found to represent one species and the use of microscopic features such as coloured pleurocystidia or neck length of cheilocystidia were shown to be inappropriate taxonomic discriminators in the separation of the four taxa. Zymograms of extracellular enzymes of laccase, peroxidase, acid phosphatase, pectinesterase and polygalacturonase were useful tools for delineation of taxa within the genus *Psilocybe*. The name *P. subaeruginosa* is retained and *P. australiana, P. eucalypta* and *P. tasmaniana* are reduced to synonyms. A lectotype is designated.

Investigation of isozyme bands previously reported to be pectinlyase activities showed that they were non-enzymic in nature and interpretation of these pectinlyase should be approached with caution.

Running title: *Psilocybe subaeruginosa* and related species

Suggested key words: *Psilocybe subaeruginosa*, comparative morphology, electrophoresis, extracellular enzymes, mating compatibility.

Psilocybe subaeruginosa Clel. is a widespread fungus in south-eastern Australia (Cleland, 1927, 1934). *Psilocybe australiana* Guzmán & Watling, *P. eucalypta* Guzmán & Watling and *P. tasmaniana* Guzmán & Watling are closely related to *P. subaeruginosa*(Guzmán & Watling, 1978; Guzmán, 1983) with the former two species occupying similar niches to *P. subaeruginosa*.

The four species exhibit a considerable overlap in both macroscopic and microscopic features. Guzmán & Watling (1978) separated the species using single discriminating characters. The feature which distinguished *P. subaeruginosa* (and also Section *Subaeruginosae* Guzmán) was the presence of coloured pleurocystidia, while the remaining three species have hyaline pleurocystidia (Guzmán & Watling, 1978; Guzmán, 1983). Similarly, *P. tasmaniana* was separated from the rest by its relatively long-necked cheilocystidia (>5µm) and coprophilous habitat, and *P. australiana* from *P. eucalypta* on the basis of spore size (Guzmán & Watling, 1978; Guzmán, 1983). In our experience, identification in the field is quite impossible.

Mating compatibility studies(Farr, Miller & Farr, 1977; Anderson, Korhonen & Ullrich, 1980; Fries, 1985; Boidin, 1986; Kile & Watling, 1988; Flynn & Miller, 1990) and isozyme analyses (Clare, Flentje & Atkinson, 1968; Franke, 1973; Garber, 1973; Blaich, 1977; Kerrigan & Ross, 1988) are techniques which have been used to clarify taxonomic problems in studies based on morphological and ecological data. Extracellular enzymes such as laccase, have been shown to be useful biochemical markers in the systematics of *Agaricus* (Kerrigan & Ross, 1988); pectinesterase and polygalacturonase have proved to be effective in species delineation in *Sclerotinia*, *Penicillium* and *Rhizoctonia* (Cruickshank, 1983; Cruickshank & Pitt, 1987; Cruickshank, 1990).

Examination of recently collected and syntype specimens has indicated that the discrete separations proposed may not be valid. Hence, a re-examination of these four taxa seemed necessary, supplementing morphological examination of fresh and dried collections with mating compatibility studies and electrophoresis of extracellular enzymes. This study, part of a study of the family Strophariaceae, focuses on three categories of investigation:(1) morphological examination of both fresh and dried collections; (2) electrophoresis of extracellular enzymes and (3) mating compatibility studies.

MATERIALS AND METHODS

Specimens were collected mainly from SE Tasmania with a few from NW Tasmania near Smithton and from type localities whenever possible. Comparisons were made with reliably identified or type material

(see under taxonomic conclusion for citation of type specimens). Dried specimens are lodged at Tasmanian Herbarium (HO), Royal Botanic Garden, Edinburgh (E) and Biology Branch Herbarium of New South Wales Department of Agriculture and Fisheries, in Rydalmere, NSW (DAR). All cultures utilised in the study are lodged at DAR. Abbreviations of herbaria follow Index Herbariorum (Holmgren, Keuken & Schofield, 1981). Table 1 lists all the collections with information on localities, isolates, habitat and date of collection.

Pure cultures were obtained from fresh spore deposits whenever possible. The non-quantitative dilution method used to obtain monosporous isolates was similar to that used by Farr *et al* (1977). All isolates used in the study were checked microscopically for the absence of clamps. All stock cultures were maintained on 2% malt extract agar (MA) incubated at 20°C and then stored at 4°C.

Morphological studies

Standard procedures (Guzmán, 1983) were followed for the examination of macroscopic and microscopic characters of both fresh and dried material. A single basidiome from each collection was used in the measurements of microscopic characters. Mean values were based on measurements of 25 for spores and 10 for pleurocystidia and cheilocystidia. A diagrammatic representation of the microscopic characters measured is shown in Fig1. The colour codes and description were according to Methuen (Kornerup & Wanscher, 1978).

Fresh collections were sorted into four groups A, B, C and D on the basis of macroscopic and microscopic morphology, in particular, spore size, colour of pleurocystidia and neck length of cheilocystidia. These four groups corresponded as nearly as possible to the putative species *P. australiana*, *P. eucalypta*, *P. tasmaniana* and *P. subaeruginosa*, respectively, although considerable difficulty was experienced in assigning some collections to a single group. Hyaline pleurocystidia were noted in most Tasmanian collections of fresh material, consequently they were grouped together as *P. australiana*. CYS95 was noted to have cheilocystidia of longer neck length than the other Tasmanian collections, however, it was not from a coprophilous habitat, consequently it was grouped tentatively with the rest of the Tasmanian collections. AKM955 was not collected by us but was known to have been collected from pasture land associated with animal grazing. The neck length of cheilocystidia was noted to be ≥5µm, plus the likely association with dung placed this collection closer to *P. tasmaniana* than any other Tasmanian collections. Unfortunately the specimens were not viable when we obtained them. The two collections from mainland Australia were initially identified as *P. eucalypta* (CYS362) and *P. subaeruginosa* (CYS515). CYS362 was collected from the type locality of *P. eucalypta* and the

habitat (*Eucalyptus* forest) corresponded to the proposed habitat of *P. eucalypta*. CYS515 contained some palely coloured pleurocystidia and was therefore tentatively placed in *P. subaeruginosa*.

Canonical discriminant function analysis was performed using the variables spore length, spore width (both face and side view), pleurocystidia length, pleurocystidia width and neck length of cheilocystiida. Normality was tested for each variable and appropriate transformation was applied before the variables were used in the analysis. The mean canonical variates generated were used in the scatter plots. These were also used in an Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) (Sneath & Sokal, 1973) cluster analysis using the CLUSTER subprogramme of SAS (SAS Institue Inc, 1988) to produce a dendogram.

Electrophoretic studies

Five extracellular enzyme systems were selected based on results from preliminary trials which also provided basic information on the incubation period for the production of various enzymes and ensuring as far as possible that the cultures were of equivalent physiological state. The enzymes used in electrophoresis were laccase, peroxidase, acid phosphatase, pectinesterase and polygalacturonase.

For the production of enzymes, cultures were grown in loosely capped 5 ml Bijou bottles, each containing 2ml of growth medium autoclaved at 121°C for 15 min and incubated at 20°C stationary in the dark. To accomplish this, isolates from stock cultures were transferred onto fresh MA plates and incubated at 20°C in the dark for 5 -7 d or longer in the case of slow growing strains. Discs of 8mm in diameter were cut from the actively growing edge of the colony and transferred to the selected growth medium.

A gel containing 13 monosporous isolates from one collection, CYS161 (in group A), was run for each enzyme system to establish a preliminary estimate of the range of internal variations. Whenever possible, thereafter, at least two to four isolates from other collections in the same or different group(s) were used when making comparisons and this included the isolates of a distinct outgroup species, *P. semilanceata*(Fr. ex Secr.)Kummer.

For laccase production, the growth medium consisted of 0.05% gallic acid in malate buffer (pH 4.0) (Cruickshank, per. comm.). Cultures were incubated for 3 d at 20°C stationary in the dark. Because of the short time required, laccase zymograms were used for the initial separation of species in addition to morphological characters.

For pectic enzymes, the growth medium was based on that used by Cruickshank & Pitt (1987) but modified by replacing $NH_4H_2PO_4$ with KH_2PO_4 and NH_4NO_3. Cultures were incubated at 20°C in the dark for 14 d.

For peroxidase and acid phosphatase production, the medium was a 20% potato decoction (20g chopped potato/100ml deionised water, simmered for 1.5h then sieved through two layers of muslin). The cultures were incubated for 10 d at 20°C in the dark.

Polyacrylamide gel electrophoresis followed the system of Cruickshank and Pitt (1987).

Pectic enzymes were examined by the method of Cruickshank and Wade (1980). Laccase and peroxidase were examined by the method of Mills and Crowden (1968). In these two oxidase enzyme systems, to increase the resolution of the bands, gels were stained for 30 min at room temperature and then overnight at 4°C in the staining solution. They were retained in water until photo records were taken. Acid phosphatase was examined by the method of Ho and Trappe (1987).

Photo records of gels were prepared by contact printing under water onto high contrast (No. 5). Ilfoprint paper.

All the isozyme data were analysed as phenetic characters. UPGMA (Sneath & Sokal, 1973) cluster analysis (using a SAS CLUSTER subprogramme) was performed based on the band frequency data to obtain a dendogram. Very faint bands were excluded.

Mating compatibility studies

For Group A, 12 isolates from one collection (CYS161) were paired in all possible combinations to determine the mating types. At least one isolate from each mating type was then crossed with monokaryons from other collections in the same group. Crosses were set up between the mating types of CYS161 and the monokaryotic isolates of CYS362 (Group B) and CYS515 (Group D). All crosses were mon-mon pairings. Monokaryotic isolates of CYS451 (*P. semilanceata*), a clearly separate species, were included in the crosses for comparison. The methodology described by Macrae (1967) was followed in the mating compatibility studies.

RESULTS

Morphological studies

The morphological comparisons between the four putative species were based on spores, pleurocystidia and cheilocystidia. Habitats were also included in the comparison. Table 2 shows the mean measurements of the microscopic characters used in the comparisons of the four groups.

On examination of fresh material designated as *P. subaeruginosa*(because of the presence of some coloured pleurocystidia), the majority of the pleurocystidia were found to be hyaline with a few basidiomes having pallid yellow pleurocystidia. Herbarium material of *P. subaeruginosa*(including all the syntype collections) had hyaline, pallid yellow to the occasional pale brown pleurocystidia. In all cases, when yellow colouration of cystidia was observed, basidia and hyphae in the subhymenium and trama were similarly coloured. The majority of pleurocystidia in the fresh collections designated as *P. australiana* were hyaline with some weakly coloured pleurocystidia noted in one collection.

Neck length of cheilocystidia was found to range from 3 to12.5µm in all putative species. The mean neck length of cheilocystidia was measured as >5µm in all four putative species (Table 2). Fig 2 illustrates the range of neck length noted in the cheilocystidia of the four groups.

Fig 3 illustrates the scatter plot using the mean canonical variates generated from the spore characters. There were no distinct clusters resolved along either of the axes. Collections with relatively broader spores tended to become the outliers as a result of variation along the second canonical axis resulted from contrast between spore length and spore width (side view).

Fig 4 shows the scatter plot of the mean canonical variates generated from the cystidia variables. Again no distinct clusters were resolved from the scatter plot using the first two mean canonical variates. Neck length of cheilocystidia which was earlier proposed to be diagnostic character for *P.tasmaniana* (Guzmán & Watling, 1978) contributed only slightly to variation along the first canonical axis.

The dendogram generated from the cluster analysis is shown in Fig 5. All four putative groups, A - D, were intermixed in their apparent relationships, i.e., none of the putative groups was separable on the selected parameters.

The range of habitat appeared quite varied for all the four putative species. For the group referred to as *P. australiana* alone, the habitat ranged from on ground (soil or mossy ground), ground litter (leafy

litter of mixed foliage of *Eucalyptus* , *Nothofagus* and manfern [*Dicksonia antarctica*Labill.] fronds or woody litter such as fallen twigs or branches), on rotten logs, dead stumps and manfern trunks to mown lawn. Both *P. eucalypta* and *P. subaeruginosa* shared similar habitats (see Table1).

Electrophoretic studies

Cruickshank and Wade (1980) have associated yellow stained bands with pectinlyase activities. Karlsson and Stenlid (1991) also found these yellow bands of 'pectinlyase' but for reasons of variable behaviour excluded them from their final analysis. These yellow bands were noted in the course of this electrophoretic study. Futher investigations into the enzymic nature of these bands revealed that they were non-enzymic and could be mimicked to a large extent by organic acids. Thus, extreme care should be exercised when interpreting these 'pectinlyase' activities. Cruickshank (per. comm.) has been consulted during the course of the investigation and agrees with this caution.

In each enzyme system, allelic designations were not assigned but observations regarding recognisable loci were noted. Each band was scored as an independent phenetic character and numbered from the cathodic end.

Laccase (Lac)

Seven bands were scored consistently (Fig 6i). Of these, Bands 1 and 4 were present exclusively in the isolates of CYS451 (*P. semilanceata*). Allelic designations were not assigned, though five loci were recognisable in the laccase activities. The first four bands corresponded to four monomorphic loci and Bands 5 to 7 appeared to be alleles of a polymorphic locus. Of the isolates in the three putative groups, Bands 2 and 3 were dominant (shared by 84.44% & 71.11% of isolates respectively).

Peroxidase (Per)

As a result of the influence of peroxide on some laccases (Blaich & Esser, 1975), only bands that appeared after addition of peroxide were included for comparison. Consequently two bands (Rf = 0.35 and 0.38) were excluded (Fig 6ii). The remaining seven bands were scored. These bands appeared to correspond to six loci. Bands 1 and 2 were alleles of a polymorphic locus and the remaining bands corresponded to five monomorphic loci. Band 4 was dominant (80%) in the isolates of the three putative groups. Band 7 was detected in only two isolates, as a result of its infrequent occurrence it was excluded from the cluster analysis. Bands 3, 5 and 6 occurred exclusively in the isolates of CYS451.

Acid phosphatase (AcP)

No extracellular AcP activity was detected in the isolates of CYS451. Of the remaining isolates, ten bands (Fig 6iii) were consistently scored. They appeared to correspond to seven loci. Three polymorphic loci (Rf = 0.11 & 0.13; Rf = 0.24 & 0.26 and Rf = 0.30 & 0.33) were noted while the rest were monomorphic. Band 5 was dominant throughout the isolates of the three putative groups (77.78%).

Pectinesterase (PE) and polygalacturonase (PG)

Variations were noted in the PE activities across the isolates of the three putative groups. A total of 16 bands were consistenetly scored and numbered from 1 to 14 from the cathodic end (Fig 6iv) and 1' and 2' for the two backrunners (of negative Rf values). Bands 1' and 2' were present exclusively in the isolates of CYS451, again all the bands detected in the isolates of CYS451 were not present in the remaining isolates. Of the isolates of the three putative groups, Band 3 was shared by 95% of the isolates followed by Bands 8 and 9 of 64.44% and 84.44% respectively.

No PG activities were detected in the isolates of CYS451. Five prominent bands were consistently noted in the remaining isolates (Fig 6v). Two bands of Rf 0.14 and 0.18 were noted in a single isolate and one of them may be alternating with the band of Rf 0.24. However, the present results could not confirm this. Seven bands were scored consistently. Band 3 was shared by 91.11% of the isolates in the three putative groups.

Figs 7 - 10 show representations of the zymograms of isolates of CYS161, 158 and 279 (group A, *P. australiana*), CYS362 (group B, *P. eucalypta*), CYS515 (group D, *P. subaeruginosa*) and CYS451 (*P.semilanceata*, an outgroup species) of the five enzyme systems. All the zymograms of isolates of *P. semilanceata* were very different.

Unfortunately, no isolate of *P. tasmaniana* was available for electrophoretic study.

The dendogram produced from the cluster analysis is shown in Fig 11. CYS451, being a distinct outgroup species, was clearly separated from all the collections of the three putative groups. A higher degree of affinity was expected between the Tasmanian collections and this was evident in the dendogram. The close link of the two mainland collections (CYS362 & 515) may indicate the effect of geographical distance on gene flow, however, they were not distinctly different from the Tasmanian collections.

Mating compatibility studies

The results indicated a tetrapolar incompatibility system for CYS161 (group A, putative *P. australiana*), and four mating types (A_1B_1: 1, 3, 4, 10,12, 15 &16; A_1B_2: 8 & 17; A_2B_1: 5 and A_2B_2: 2 & 11) were recovered from the polarity matrix. The four mating types were intercompatible with the monokaryotic isolates of all other collections in group A (Table 3) and indicated the involvement of a multiple allelic system. The results showed that isolates of both CYS362 (group B) and CYS515 (group D) were intercompatible with CYS161, 236 and 217 (all group A) as well as between themselves (Table 3). The isolates of collections from groups A, B and D were all interincompatible with *P. semilanceata*(Table 3).

DISCUSSION

Only one morphological species was identified and this corresponded to a single biological species from the results of morphological, electrophoretic and mating compatibility studies.

This re-examination has shown that the proposed use of coloured pleurocystidia as a taxonomic criterion is not valid when considering *P. subaeruginosa*. Results from this study show that pleurocystidia are hyaline in almost all the material examined, and only occasionally is pale yellow colouration noted in pleurocystidia of some material but then the rest of the gill tissue is also similarly coloured. None of the material examined has chocolate brown pleurocystidia as suggested by Guzmán (1983). Since this criterion, i.e., coloured (brown) pleurocystidia, also characterises Section *Subaeruginosae* Guzmán, the position of *P. subaeruginosa* in Section *Subaeruginosae* is not tenable, and the name should instead be transferred to Section *Cynaescens* Guzmán which is characterised by the presence of hyaline pleurocystidia.

Neck length of cheilocystidia of 5µm or more has been used by Guzmán & Watling (1978) to separate *P. tasmaniana* from *P. australiana* and *P. eucalypta*. In the same paper, it is noted that there was inconsistency between Table1 (p208) and drawings (Fig 1D and G, p205) of the cheilocystidia of *P. australiana* and *P. eucalypta*. The drawings show the neck length of cheilocystidia of both these species to be greater than 5µm and this contradicts the measurements given in their Table 1. Our study of the holotype and syntype specimens of these taxa supports the information contained in the drawings as neck length of cheilocystidia was found to be generally 5µm or more. Since all the four proposed species share similar neck length, the distinction based on neck length of cheilocystidia between *P. tasmaniana* and the remaining three species becomes untenable. Thus, neck length of cheilocystidia has little taxonomic value here. Results from the canonical discriminant function analysis enhanced the finding that these four putative groups were not separable on the proposed morphological criteria.

The coprophilous habitat has been used by Guzmán and Watling (1978) in addition to neck length of cheilocystidia to separate *P. tasmaniana* from *P. australiana* and *P. eucalypta*. Guzmán and Watling (1978) allude to a wider habitat preference, but list 'dung' as the habitat for *P. tasmaniana* in their Table 1 (p207). However, *P. subaeruginosa* has also been reported on dung (Cleland, 1927, 1934) and the New Zealand material of *P. tasmaniana* was not collected from dung (see Table 1). It appears that the habitat of *P. tasmaniana* is more varied than initially envisaged, thus, habitat is also not a valid criterion for the separation of species.

Morphological examination of material from New Zealand indicated from this study that *P. subaeruginosa* is not limited to Australia.

The electrophoretic data are particularly encouraging when considering wider studies in the family Strophariaceae. Isolates obtained from fresh collections and identified as *P. australiana*, *P. eucalypta* and *P. subaeruginosa* on the basis of morphological criteria all produced zymograms with a high degree of uniformity in all the selected enzymes and this was supported by the results of the UPGMA cluster analysis. This illustrates the potential of extracellular enzymes as biochemical markers in the genus *Psilocybe*. *P. semilanceata*, which is morphologically distinct from *P. subaeruginosa* produced zymograms which were distinctively different from those of *P. subaeruginosa* (Figs 6-9). Thus, species delineation for *Psilocybe* in Tasmania could be achieved through direct comparison of the zymograms. The results are reproducible and in agreement with Cruickshank's (1990) finding that 'intergel comparisons were meaningful and results could be presented as composites from several gels'.

The results of these isozyme analyses correlated closely with the results of morphological and mating studies. This electrophoretic technique may prove to be a very useful adjunct in taxonomic studies especially where spore germination is an intractable problem but wild isolates are more easily available.

TAXONOMIC CONCLUSION

Morphological examination, selected enzyme analyses (including cluster analysis) and mating compatibility experiments all indicate that a single species has been represented by four names. For reasons of nomenclatural priority, the name*Psilocybe subaeruginosa* Cleland must be retained with *P. australiana* Guzmán & Watling, *P. eucalypta* Guzmán & Watling and *P. tasmaniana* Guzmán & Watling reduced to synonymy. An emended description modified from Guzmán's is presented.

Psilocybe subaeruginosa Clel. Trans. & Proc. Roy. Soc. South Australia 51: 305, 1927.

Type citation: Cleland did not specify a type in his cited collections. Guzmán (1983) assumed Cleland 13251 (AD) to be the type and since this collection cannot be located, clearly lectotypification is required.

Lectotype (here chosen): South Australia, National Park, AD 5603! Isolectotype: South Australia, National Park, AD 5602! Allotypes: South Australia, Mount Lofty, AD 5604! New South Wales, Fitzroy Falls, AD 5599!; Victoria, Craigie, AD 5600!.

=*Psilocybe australiana* Guzmán & Watling, Notes from the Roy. Bot. Garden Edinburgh 36: 206, 1978. Holotype: New South Wales, near Canberra, Cotter Dam, Blue Range, Watling10617 (E!).

=*Psilocybe eucalypta* Guzmán & Watling, Notes from the Roy. Bot. Garden Edinburgh 36: 204, 1978. Holotype: A. C. T., near Canberra, Tidbinbilla Nature Reserve, Watling 10656(E!).

=*Psilocybe tasmaniana* Guzmán & Watling, Notes from the Roy. Bot. Garden Edinburgh 36: 207,1978. Holotype: Tasmania, NE of Hobart, Nugent, Buckland, Watling 10393 (E!).

Selected illustrations: Cleland (1934, Fig 25, p141); Cole, Fuhrer & Holland (1978, pl 5); Fuhrer (1985, p 75 as *Psilocybe* sp); Shepherd & Totterdell (1988, p 93 as *Psilocybe* sp).

Pileus 11-60 mm in diam, when young conic to convex, campanulate, then convex to plano-convex or expanded umbonate; greasy to tacky or more rarely subviscid when moist, surface glabrous, even, remnants of veil as white fibrils attached along the margin, margin slightly striate, hygrophanous, greyish yellow (4 A5-C5), then pale brownish (5C5, 5D7) to dull brown (6E5) when older, often with bluish-green tints, drying pallid brownish or straw colour. *Lamellae* adnate to adnexed. Pallid yellow (3A2) when veil breaks, becoming brownish-fuscous (5E5 - 6F7) with spores, edges pallid. *Stipe* 35-140 x 2-7.5 (10) mm, sometimes flexuos, equal, finely striate, mealy above, fine fibrils sometimes adherent below, base slightly swollen and passing sometimes into a broad mass of white mycelium, stuffed but sometimes hollow, cartilaginous. Surface whitish, streaked with dark greyish-brown, often blotched greenish-blue. *Veil* a whitish cobweb in young stages, occasionally leaving indefinite traces as somewhat superior annulus. *Context* white to pale yellow (3A2-3) in the pileus and stipe, but becoming brownish in the stipe. Turning blue when bruised or on drying.

Spore print violaceous black. *Spores* (9.6-) 10.8-15 (-15.8) x (6.4-) 6.6-8.8 x (5.6-) 6-7.5 (-8.7) µm, subellipsoid in face view, slightly inequilateral in profile, thick-walled, pale to dark yellowish-brown, with broad germ pore. *Basidia* (20.6-) 24-38.3 (-42.5) x 6.6-11.7 µm, 4-spored or rarely 2-spored, hyaline, or yellowish-brown, ventricose or subcylindric to subpyriform. *Cheilocystidia* (17-) 20-40.8 (-48) x (4-) 5.5-16.7 µm, similar to the pleurocystidia in form and colour, long-necked, 5µm or more, abundant, usually forming a sterile band, frequently with a hyaline drop at the apex. *Pleurocystidia*

(18.3-) 20-47.3 (-50) x (4.8-) 6-16.7 µm, fusoid-ventricose, subpyriform, mucronate or with a more or less elongated neck 2-4.5 µm broad, hyaline, some very pallid yellow, very rarely with brown contents or deeply coloured.

Subhymenium subcellular, hyaline to pale yellowish or brownish with diffused to irregularly incrusted pigment on the thick walls, sometimes diffused blue pigment is observed in KOH slides. *Trama* parallel, hyaline or brownish, with thick-walled hyphae (walls 1-1.5µm thick). *Epicutis* formed by a thin layer of gelatinised, repent hyphae, more or less 5µm diam, hyaline to brownish. *Hypodermium* hyaline to brownish, formed by subglobose to broad, elongate hyphae. Clamp connections very common and conspicuous.

Habitat and Distribution Solitary to gregarious, on rich soil among grass or mosses, or on dung, on leafy litter of mixed forest foliage of *Eucalyptus, Nothofagus* and manfern (*Dicksonia antarctica* Labill.) fronds, or woody litter such as fallen twigs, rotten logs, dead stumps and manfern trunks, mainly in deeply shaded places, occasionally in more exposed areas. Fruiting in April-August. Known only from Australasia.

AD5603 was chosen as the lectotype as it agrees most closely with Cleland's original description and has a range of microscopic features which are well within the range noted in the syntype and fresh collections.

SPECIMENS EXAMINED

P. subaeruginosa Clel. AUSTRALIA, South Australia, Morialta, AD 5606; New South Wales, National Park, AD 5598; Mt. Wilson, AD5597; near Robertson, Old Kangaloon Road, DAR 60750(as *P. eucalypta*); Victoria, Dandenong Ranges, AD5601; Jumping Creek Reserve, Warandyte, CYS515 (HO, DAR). Tasmania, Mount Field National Park, pathside to Russell Falls, Watling 10336, 10387(E); Colebrook, AKM 955(as *P. tasmaniana*); see Table 1 for other Tasmanian collections (as *P. australiana*).

NEW ZEALAND, Waikato, PDD 45554, 54315; Bay of Plenty, PDD 54517; Auckland, PDD 55207(all as *P. australiana*); New Plymouth, PDD 48120, PDD 48122 (all as *P. eucalypta*); Taranaki, PDD 46240 (as *P. tasmaniana*) and PDD 45296 and 45331 (as *Psilocybe* aff. *tasmaniana*).

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various loans. We are grateful to M. Priest (of DAR) and B. Fuhrer (Monash University) for the provision of fresh material from A.C.T. and Victoria respectively. YSC acknowledges B. Potts and G. Jordan for their help in the statiscal analyses. This work was carried out as part of a PhD degree (YSC) and is supported by a Postgraduate Research Award from the University of Tasmania.

REFERENCES

ANDERSON, J. B., KORHONEN, K. & ULLRICH, R. C. (1980). Relationships between Europe and North American biological species of *Armillaria mellea*. *Experimental Mycology* **4**, 87-95.

BLAICH, R. (1977). Enzymes as an aid in taxonomy of higher basidiomycetes in *The Species Concept in Hymenocycetes* edited by H. Clemençon. Vaduz, W. Germany: J. Cramer.

BLAICH, R. & ESSER, K (1975).Functions of Enzymes in Wood Destroying Fungi II. Multiple Forms of Laccase in White Rot Fungi. *Arch Microbiol* **103**, 271-277.

BOIDIN, J. (1986). Incompatibility and the species concept in the saprobic Basidiomycotina. *Mycotaxon* **26**, 319-336.

CLARE, B. G., FLENTJE, N. T. & ATKINSON, M. R. (1968). Electrophoretic patterns of oxidoreductases and other proteins as criteria in fungal taxonomy. *Australian Journal of Biological Science* **21**, 275-336.

CLELAND, J. B. (1927). Australian Fungi: Notes and descriptions-No. 6. *Transactions of the Royal Society of South Australia* **51**, 298-306.

CLELAND, J. B. (1934). *Toadstools and Mushrooms and other Larger Fungi of South Australia. Part 1.* Government Printer. Adelaide.

COLE, F. MARY, FUHRER, B. A. & HOLLAND, A. A. (1978). *A Field Guide to the Common Genera of Gilled Fungi in Australia.* Inkata Press.

CRUICKSHANK, R. H. (1983). Distinction between *Sclerotinia* species by their pectic zymograms. *Transactions of the British Mycological Society* **80**, 117-119.

CRUICKSHANK, R. H. (1990). Pectic zymograms as criteria in taxonomy of *Rhizoctonia*. *Mycological Research* **94**, 938-846.

CRUICKSHANK, R. H. & WADE, G. C. (1980). Detection of pectic enzymes in pectin-acrylamide gels. *Analytical Biochemistry* **107**, 177-181.

CRUICKSHANK, R. H. & PITT, J. (1987). Identification of species in *Penicillium* subgenus *Penicillium* by enzyme electrophoresis. *Mycologia* **79**, 614-620.

FARR, ELLEN R., MILLER, O. K., JR &FARR, D. F. (1977). Biosystematic studies in the genus *Pholiota* stirps *Adiposa*. *Canadian Journal of Botany* **55**, 1167-1180.

FLYNN, T. & MILLER, O. K. JR. (1990). Biosystematics of *Agrocybe molesta* and sibling species

allied to *Agrocybe praecox* in North America and Europe. *Mycological Research* **94**, 1103-1110.

FRANKE, R. G. (1973). Electrophoresis and the taxonomy of saprophytic fungi. *Bulletin of the Torrey Botanical Club* **100**, 287-296.

FRIES, N. (1985). Intersterility groups in *Paxillus involus*. *Mycotaxon* **24**, 403-409.

FUHRER, B. A. (1985). *A Field Companion to Australian Fungi.* The Five Mile Press. Melbourne.

GARBER, E. D. (1973). Electrophoresis as a taxonomic and genetic tool for fungi. *Bulletin of the Torrey Botanical Club* **100**, 308-312.

GUZMáN, G. (1983). *The Genus* Psilocybe. Beihefte zur Nova Hedwigia Heft 74. Vaduz, W. Germany: J. Cramer.

GUZMáN, G. & WATLING, R. (1978). Studies in Australian Agarics and Boletes 1: Some species of *Psilocybe. Notes from the Royal Botanic Garden, Edinburgh* **36**, 199-210.

HO, I. & TRAPPE, J. M. (1987). Enzymes and growth substances of *Rhizopogon* species in relation to mycorrhizal hosts and intrageneric taxonomy. *Mycologia* **79**, 553-558.

HOLMGREN, P. K., KEUKEN, W. & SCHOFIELD, E. K. (1981). *Index Herbariorum.* Seventh edition. Utrecht: Bohn, Scheltema & Hilkema.

KARLSSON, J & STENLID, J (1991). Pectic isozyme profiles of intersterility groups in *Heterobasidion annosum* . *Mycol. Res.* **95**, 531-536.

KERRIGAN, R. W. & ROSS, I. K. (1988). Extracellular laccases: biochemical markers for *Agaricus* systematics. *Mycotaxon* **80**, 689-695.

KILE, G. & WATLING, R. (1988). Identification and occurence of Australian *Armillaria*species, including *A. pallidula* sp. nov. and comparative studies between them and non-Australian tropical and Indian *Armillaria. Transactions of the British Mycological Society* **91**, 305-315.

KORNERUP, A. & WANSCHER, J. H. (1978). *Methuen Handbook of Colour.* London: Eyre Methuen.

MACRAE, R. (1967). Pairing incompatibility and other distinctions among *Hirchioporus* (Polyporus) *abietinus, H. fusco-violaceus,* and *H. laricinus. Canadian Journal of Botany* **45**, 1371-1398.

MILLS, A. K. & CROWDEN, R. K. (1968). Distinction of soluble proteins and enzymes during early development of *Pisum sativum. Australian Journal of Biological Science* **21**, 1131-1141.

SAS INSTITUTE INC (1988). *SAS/STAT™ User's Guide, Release 6.03 Edition.* Cary, NC : SAS Institute Inc,1028pp.

SHEPHERD, C. J. & TOTTERDELL, C. J. (1978). *Mushroom and Toadstools of Australia.* Melbourne, Sydney: Incata Press.

SNEATH, P. H. A. & SOKAL, R. R. (1973). *Numerical Taxonomy: The Principles and Practice of Numerical Classification.* San Francisco: W. Freeman & Co.

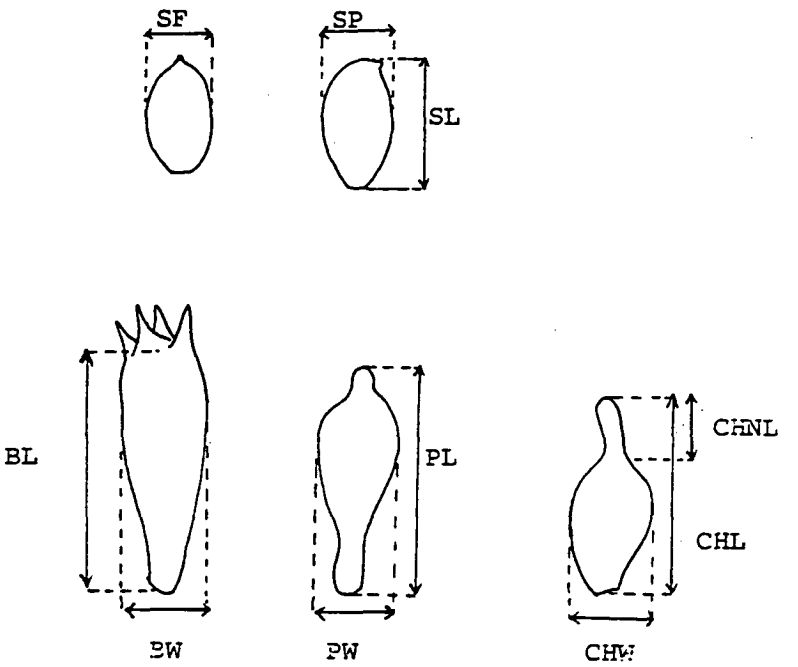

Fig 1. Diagrammatic representation of microscopic characters measured for the morphological studies. SL = spore length, SF = spore width in face view, SP = spore width in profile, BL = basidia length, BW = basidia width, PL = pleurocystidia length, PW = pleurocystidia width, CHL = cheilocystidia length, CHW = cheilocystidia width and CHNL = neck length of cheilocystidia.

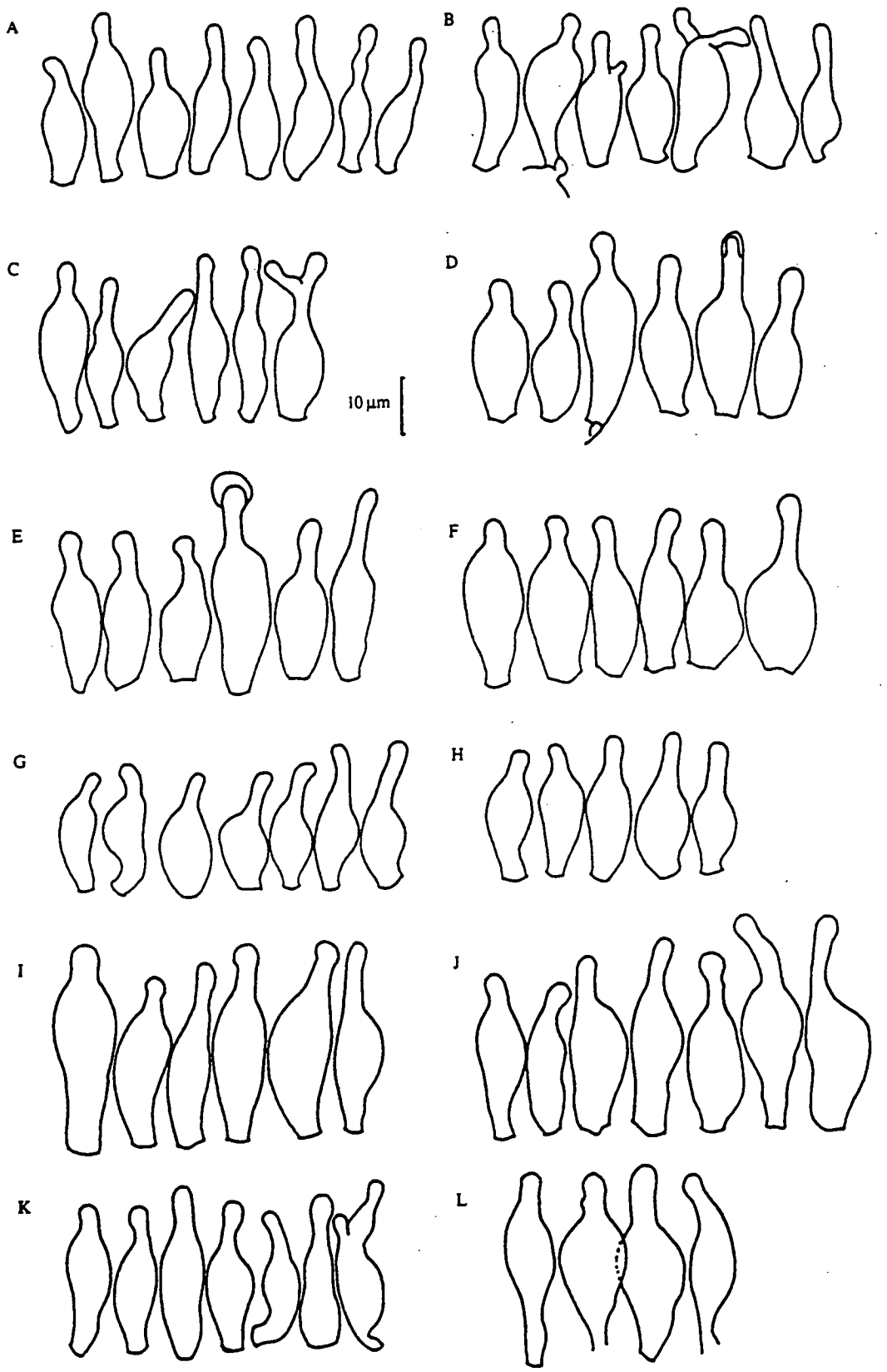

Fig. 2. The range of nexk length of cheilocystidia noted in both fresh and herbarium material of *Psilocybe australiana*: A CYS161, B CYS217, C CYS95, D CYS280, E CYS290, and F CYS369; *P. tasmaniana*: G Watling 10393 (holotype) and H AKM955; *P. eucalypta*: I CYS362: and *P. subaeruginosa*: J AD5603 (lectotype), K CYS515 and L AD5600 (syntype).

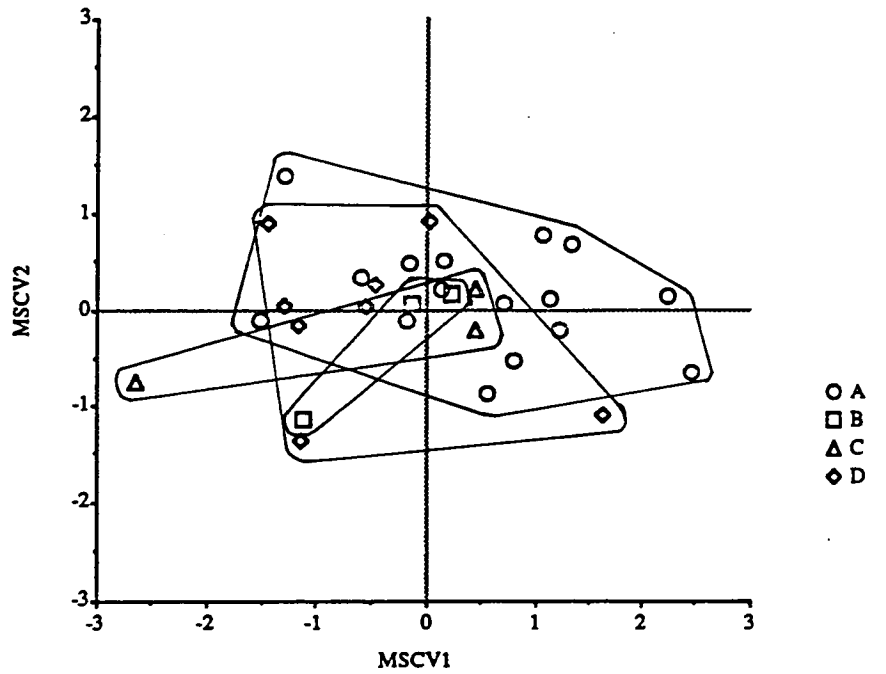

Fig 3. Plot of the mean canonical variates from canonical discriminant function analysis of spore variables (SL, SF & SP) of the four putative groups A - D.

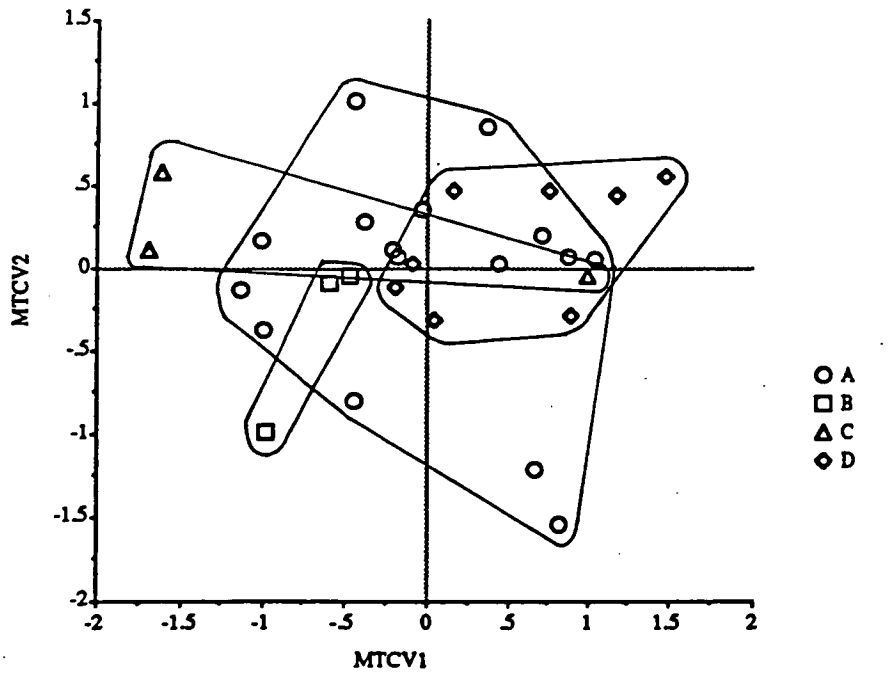

Fig 4. Plot of the first two canonical variates from the canonical discriminant function analysis of the cystidia variables (PL, PW & CHNL) of the four putative groups A - D.

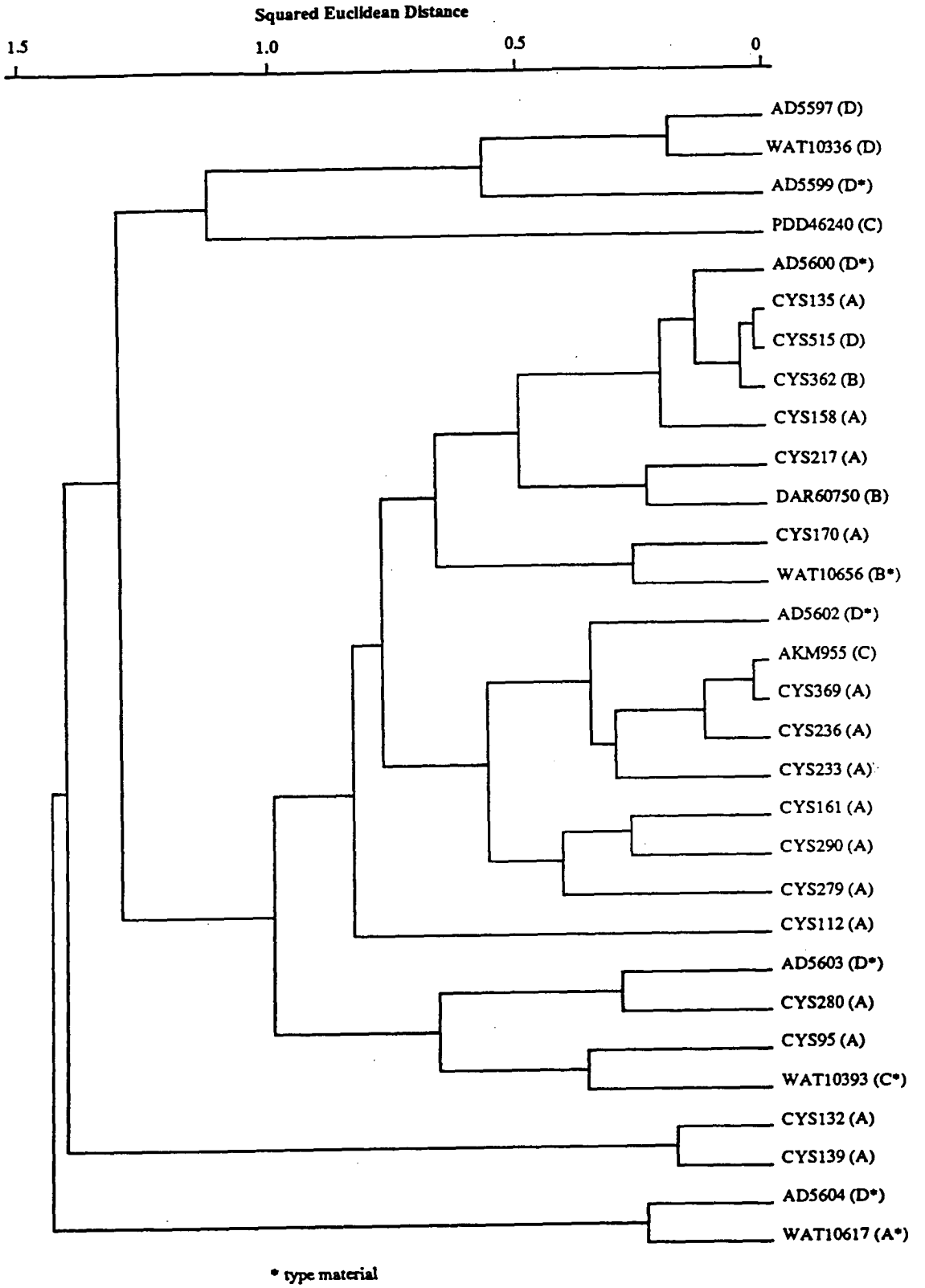

Fig 5. Dendogram from a cluster analysis based on all the mean canonical variates generated from the canonical discriminant function analysis.

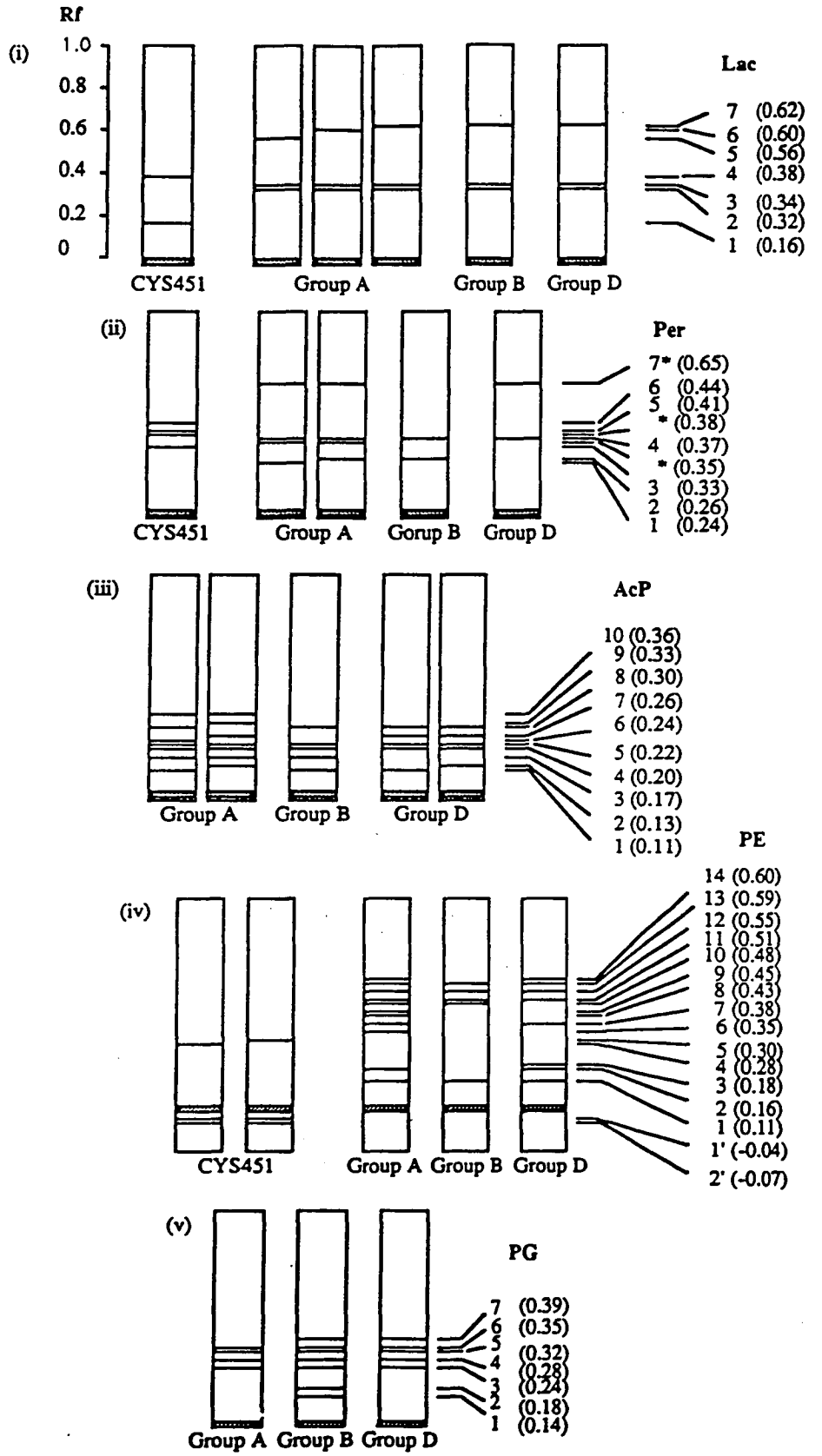

Fig 6. Schematic representations of isozyme patterns. Rf scale the same from (i) to (v). Bands marked with arrows were alleles of polymorphic loci. (i) Laccase (Lac) isozyme patterns of CYS451 (*P. semilanceata*), Group A (putative *P. australiana*), Group B (putative *P. eucalypta*) and Group D (*P. subaeruginosa*). Bands 1 - 7 numbered from the cathodic end. (ii) Peroxidase (Per) isozyme patterns of the same four groups as (i). Bands marked with an asterisk were excluded from cluster ananlysis. (iii) Acid phosphatase (AcP) isozyme patterns in the three putative groups A, B & D. (iv) Pectinesterase (PE) isozyme patterns of the same four groups as in (i). (v) Polygalacturonase (PG) isozyme patterns.

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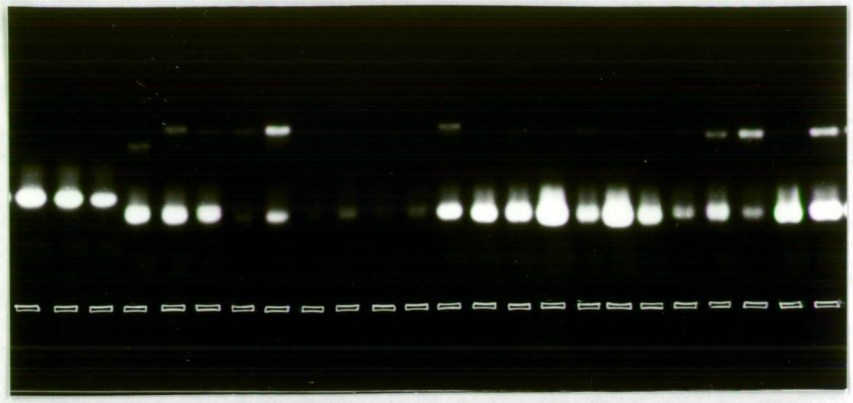

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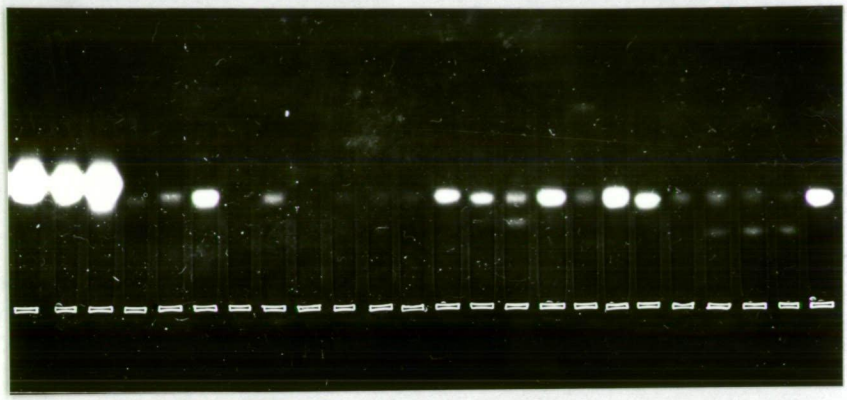

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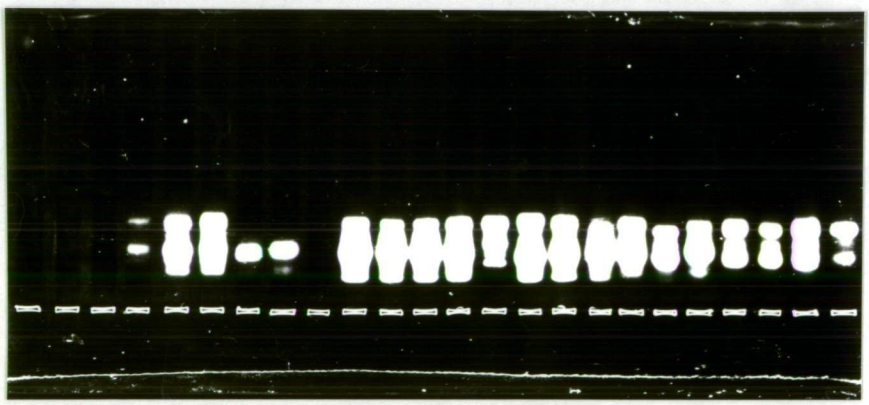

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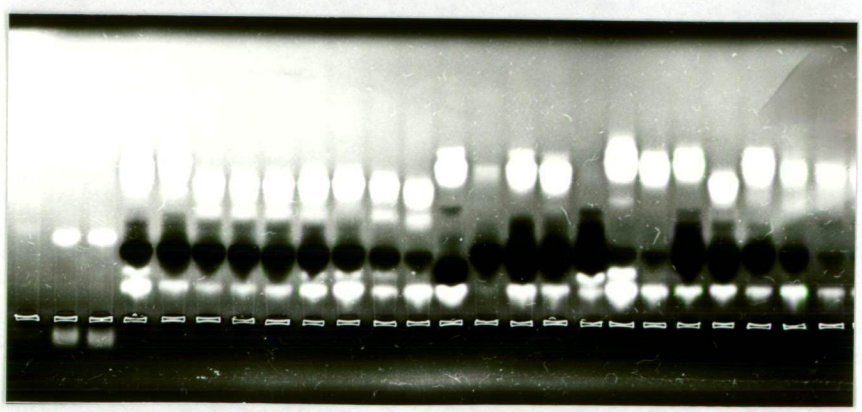

Figs 7 - 10. Representations of the zymograms of the five enzyme systems of the monokaryotic isolates of three of the four putative groups (A, B & D) and the outgroup species, *P. semilanceata*. In all figures from left: CYS451 (01, 03 & 04) (*P. semilanceata*), CYS158 (04), CYS279 (03), CYS217 (02, 03, 05 & 08), CYS236 (01 - 03) (all group A), CYS362 (01, 02, 04 & 05) (group B), CYS515 (01, 02 03 & 05) (group D) and CYS161 (01, 02, 10 & 11) (group A). **Fig 7.** Lac; **Fig 8.** Per; **Fig 9.** AcP and **Fig 10.** PE and PG.

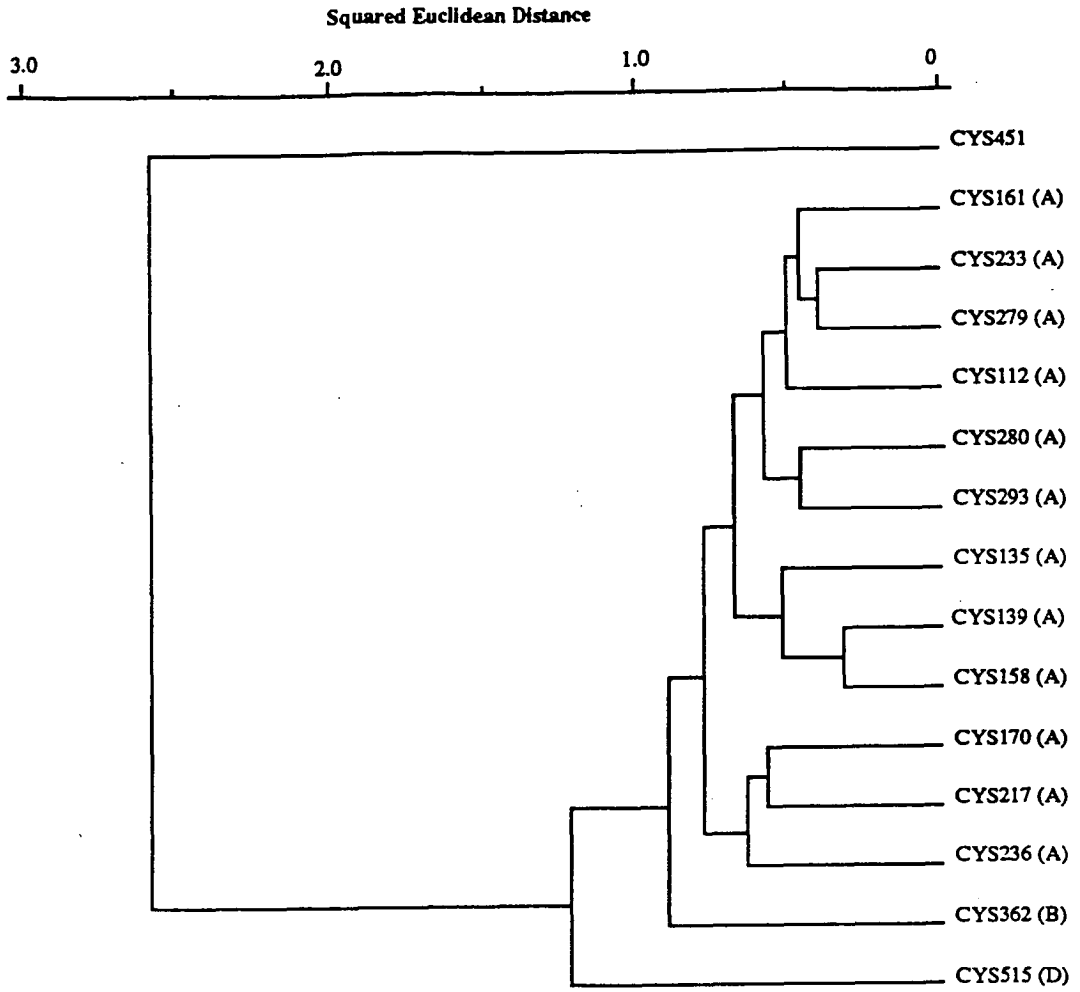

Fig 11. Dendogram from the UPGMA cluster analysis based on band frequencies. The number refers to collection (Table 1) and the putative group in parentheses.

Table 1. Sources of specimens and isolates used in the morphological, electrophoretic and mating compatibility studies. Number in brackets refers to the number of isolates used.

Collection	Locality	Isolate used	Habitat	Date
Psilocybe australiana				
CYS95*	Garden, Hobart, Tasmania	ns	on woody litter	14. vii. 88
CYS112*	Fern Glades, near Mt. Wellington, Tasmania	monokaryons (4)	on ground litter	18. iv. 89
CYS132*	Myrtle Forest, Collinsvale, Tasmania	monokaryons (4)	on ground litter	27. iv. 89
CYS135*	Mt. Field National Park, Tasmania	monokaryons	on ground litter (2)	2. v. 89
CYS139*	Mt Field National Park, Tasmania	monokaryons	on leafy litter (2)	2. v. 89
CYS158*	Lake Dobson Road, Mt. Field National Park	monokaryons (4)	on ground litter	2. v. 89
CYS161*	Lady Barron Falls Track, Mt. Field National Park	monokaryons** (13)	on ground	2. v. 89
CYS170*	Myrtle Forest, Collinsvale, Tasmania	monokaryons (4)	on rotten log	11. v. 89
CYS217 (=DAR66084)	Tasman Peninsular, Tasmania	monokaryons** (4)	on ground litter	25. v. 89
CYS233	Liffey Falls, on NW coast of Tasmania	monokaryons (2)	on ground litter	14. vi. 89
CYS236 (=DAR66085)	Liffey Falls, on NW coast of Tasmania	monokaryons** (4)	on ground	14. vi. 89
CYS279	Uni. of Tasmania campus, Hobart, Tasmania	monokaryons (2)	on ground	29. vi. 89
CYS280	Garden, Taroona, Tasmania	monokaryons (4)	on mown lawn	1. vii. 89
CYS290	Lady Barron Falls Track Mt. Field National Park	ns	on leafy litter	3. vii. 89
CYS293	Lady Barron Falls Track, Mt. Field National Park	monokaryons	on ground litter	3. vii. 89
CYS369	Sandspit River Forest Reserve, Tasmania	ns	on mossy ground	15. v. 90
Watling10617 (holo)	Cotter dam, Blue Range, near Canberra, A.C.T.	-	on ground debris	iv. 74
P. eucalypta				
CYS362 (=DAR63053)	Tidbinbilla Nature Reserve near Canberra, A. C. T.	monokaryons** (6)	on leafy litter (in *Eucalyptus* forest)	5. v. 90
DAR60750	near Robertson, Old Kangaloon Rd, N.S.W.	-	on ground	88
Watling10656 (holo)	Tidbinbilla Nature Reserve near Canberra, A.C.T.	-	on trackside	v. 74
P. tasmaniana				
AKM955	Colebrook, Tasmania	ns	on pasture land	vii. 90
PDD46240	Taranaki, New Zealand	-	on soil	84
Watling10393	NE Hobart, Nugent, Buckland, Tasmania	-	on dung	v. 74
P. subaeruginosa				
CYS515 (=DAR66086)	Jumping Creek Reserve, Warandyte, Victoria	monokaryons** (4)	on ground litter	vii. 90
AD5597	Mt Wilson, N.S.W.	-	at base of stump	5. vi. 15
AD5599 (syn)	Fitzroy Falls, N.S.W.	-	on ground litter	8. vi. 19
AD5600 (syn)	Craigie, Victoria	-	along creek near decaying leaves	vi. 17
AD5602 (syn)	National Park, South Australia	-	on ground	6. viii. 21
AD5603 (syn)	National Park, South Asutralia	-	on woody debris	19. v. 25
AD5604 (syn)	Mt Lofty, South Australia	-	on ground	vi. 23
P. semilanceata				
CYS451 (=DAR66087)	Neika, Tasmania	monokaryons** (3)	on rich pasture	vii. 90

* Part of collection lodged at Royal Botanic Garden, Edinburgh (E).
** Isolates and part of collection lodged at Biology Branch Herbarium, Rydalmere, New South Wales (DAR).
ns No isolate, but specimens used in morphological study.
holo Holotype
syn Syntype

Table 2. The mean measurements and standard deviations (s.d.) of microscopic characters for *Psilocybe subaeruginosa*, *P. australiana*, *P. eucalypta* and *P. tasmaniana*.

	P. subaeruginosa (n = 8)	*P. australiana* (n = 17)	*P. eucalypta* (n = 3)	*P. tasmaniana* (n = 3)
Spores				
length ± s.d. (µm)	12.56 ± 0.38	13.05 ± 0.35	12.72 ± 0.62	12.62 ± 0.65
width ± s.d. (f, µm)	7.66 ± 0.28	7.87 ± 0.27	7.49 ± 0.16	7.44 ± 0.49
± s.d. (p, µm)	7.13 ± 0.43	7.66 ± 0.27	7.47 ± 0.12	7.40 ± 0.39
Pleurocystidia	hyaline, rarely coloured	hyaline, rarely coloured	hyaline	hyaline
length ± s.d. (µm)	30.80 ± 1.95	29.99 ± 2.24	27.12 ± 2.23	29.15 ± 1.82
width ± s.d. (µm)	9.76 ± 0.74	9.47 ± 0.76	8.45 ± 0.15	8.30 ± 1.91
Cheilocystidia	hyaline, simple or bifurcate	hyaline, simple or bifurcate	hyaline, simple	hyaline, simple or bifurcate
neck length ± s.d. (µm)	6.39 ± 0.58	7.17 ± 0.89	6.68 ± 0.19	7.69 ± 0.68
length ± s.d. (µm)	28.34 ± 1.13	27.45 ± 2.21	26.29 ± 6.30	27.83 ± 6.41
width ± s.d. (µm)	7.89 ± 0.58	7.95 ± 0.78	8.27 ± 1.14	7.58 ± 0.44

Table 3. Mating tests between the tester strains of CYS161 and monokaryons of collections in putative groups A, B & D and CYS451 (*P. semilanceata*), as well as between putative groups B and D.

Species & isolate no.	No. of monokaryons		Species & isolate no.	No. of monokaryons	No. of pairings	Total no. positive	Total no. negative pairings
							pairings
P.australiana			*P.australiana*				
CYS161	4	X	CYS112	4	16	16	0
(1, 2, 5, 8)			(1, 2, 3, 4)				
		X	CYS132	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS158	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS170	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS217	4	16	12	4
			(1, 3, 5, 8)				
		X	CYS236	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS279	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS280	4	16	16	0
			(1, 2, 3, 4)				
P. eucalypta			*P. australiana*				
CYS362	5	X	CYS161	4	20	14	6
(1, 2, 3, 4, 5)			(2, 5, 10, 17)				
		X	CYS217	2	10	5	5
			(3, 8)				
		X	CYS236	2	10	10	0
			(1, 2)				
P. subaeruginosa			*P. australiana*				
CYS515	4	X	CYS161	4	16	16	0
(1, 2, 3, 5)			(2, 5, 10, 17)				
		X	CYS217	2	8	8	0
			(3, 8)				
		X	CYS236	2	8	8	0
			(1, 2)				
			P. eucalypta				
		X	CYS362	4	16	11	5
			(1, 2, 4, 5)				

Table 3. Mating tests between the tester strains of CYS161 and monokaryons of collections in putative groups A, B & D and CYS451 (*P. semilanceata*), as well as between putative groups B and D.

Species and isolate no.	No. of monokaryons		Species and isolate no.	No. of monokaryons	No. of pairings	Total no. positive pairings	Total no. negative pairings
P.australiana			*P.australiana*				
CYS161	4	X	CYS112	4	16	16	0
(1, 2, 5, 8)			(1, 2, 3, 4)				
		X	CYS132	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS158	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS170	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS217	4	16	12	4
			(1, 3, 5, 8)				
		X	CYS236	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS279	4	16	16	0
			(1, 2, 3, 4)				
		X	CYS280	4	16	16	0
			(1, 2, 3, 4)				
			P. semilanceata				
		X	CYS451	3	12	0	12
			(1, 3 & 4)				
P. eucalypta			*P. australiana*				
CYS362	5	X	CYS161	4	20	14	6
(1, 2, 3, 4, 5)			(2, 5, 10, 17)				
		X	CYS217	2	10	5	5
			(3, 8)				
		X	CYS236	2	10	10	0
			(1, 2)				
			P. semilanceata				
		X	CYS451	3	12	0	12
P. subaeruginosa			*P. australiana*				
CYS515	4	X	CYS161	4	16	16	0
(1, 2, 3, 5)			(2, 5, 10, 17)				
		X	CYS217	2	8	8	0
			(3, 8)				
		X	CYS236	2	8	8	0
			(1, 2)				
			P. eucalypta				
		X	CYS362	4	16	11	5
			(1, 2, 4, 5)				
			P. semilanceata				
		X	CYS451	3	12	0	12
P. semilanceata			*P. austraniana*				
CYS451	3	X	CYS217	2	6	0	6
		X	CYS236	3	9	0	9

Appendix V

Mean values of microcharacters of collections of *Pholiota squarrosipes*

	Spore l x f x p (µm.)			Basidia l x w (µm.)		Chrysocystidia l x w (µm.)		Cheilocystidia l x w (µm.)	
AD11958	7.06	x 4.61	x 4.37	20.54	x 6.25	39.58	x 12.16	24.96	x 5.62
	±0.28	±0.20	±0.21	±1.94	±0.43	±5.20	±2.08	±2.32	±0.86
AD12200	7.03	x 4.60	x 4.53	16.71	x6.04	34.18	x 10.08	27.37	x 6.08
	±0.44	±0.19	±0.25	±2.39	±0.56	±4.94	±1.20	±2.09	±0.71
AD12142	6.83	x 4.60	x 4.47	20.29	x5.79	42.40	x11.79	26.08	x 5.91
	±0.36	±0.22	±0.28	±2.79	±0.57	±5.72	±1.46	±2.45	±0.39
AD11954	6.95	x 4.45	x 4.37	20.02	x 6.71	41.58	x 13.37	27.48	x 5.71
	±0.37	±0.23	±0.24	±1.42	±0.86	±6.23	±0.77	±2.10	±0.74
AD10250	7.07	x 4.38	x 4.25	18.50	x 7.21	37.04	x 12.33	26.00	x 6.62
	±0.37	±0.24	±0.21	±2.06	±0.79	±3.09	±2.02	±2.42	±0.87
CYS8	6.38	x 4.47	x 4.35	23.08	x 7.29	43.58	x 11.33	28.29	x 6.37
	±0.27	±0.25	±0.27	±0.96	±0.82	±5.99	±1.19	±3.84	±0.83
CYS11	6.55	x 4.20	x 4.17	21.92	x 7.62	37.41	x 13.83	x 22.75	x 6.37
	±0.20	±0.17	±0.17	±2.15	±0.48	±4.52	±1.67	±2.89	±1.08
CYS14	7.22	x 4.51	x 4.33	23.67	x 8.00	48.83	x 15.17	29.83	x 6.75
	±0.41	±0.26	±0.21	±1.43	±0.43	±5.87	±1.56	±1.34	±0.73
CYS16	6.66	x 4.47	x 4.25	21.83	x 8.39	40.83	x 12.06	27.62	x 5.91
	±0.36	±0.30	±0.17	±1.75	±0.59	±2.24	±1.52	±3.58	±0.43
CYS55	7.40	x 4.71	x 4.67	21.17	x 7.91	32.25	x 13.00	25.00	x 6.50
	±0.25	±0.22	±0.20	±1.81	±0.44	±3.63	±1.05	±2.78	±0.56
CYS90	6.66	x 4.49	x 4.36	20.83	x 7.62	38.93	x 13.59	30.92	x 7.62
	±0.32	±0.22	±0.18	±3.79	±0.48	±3.59	±1.27	±2.37	±1.13
CYS106	6.57	x 4.51	x 4.27	19.04	x 6.46	31.04	x 12.18	31.21	x 6.23
	±0.30	±0.24	±0.14	±1.18	±0.39	±4.67	±1.09	±6.04	±1.10
CYS136	6.93	x 4.58	x 4.53	21.71	x 7.21	31.71	x 11.79	29.77	x 5.39
	±0.29	±0.29	±0.22	±2.49	±0.55	±2.98	±0.79	±2.98	±0.54
CYS163	6.71	x 4.59	x 4.47	22.29	x 7.04	36.42	x 11.77	27.29	x 5.50
	±0.28	±0.19	±0.21	±2.03	±0.77	±0.77	±1.43	±2.81	±0.61
CYS179	6.60	x 4.70	x 4.50	19.17	6.79	42.79	x 11.71	29.04	x 5.93
	±0.31	±0.21	±0.26	±1.52	±0.40	±3.46	±1.90	±3.95	±0.47
CYS246	6.99	x 4.47	x 4.41	21.96	x 6.66	40.71	x 12.27	28.45	x 5.64
	±0.30	±0.17	±0.18	±1.82	±0.43	±5.11	±1.60	±2.80	±0.56
CYS247	6.39	x 4.37	x 4.28	19.25	x 6.23	35.71	x 12.58	29.73	x 6.77
	±0.27	±0.20	±0.14	±1.64	±0.61	±2.52	±1.13	±4.32	±0.41
CYS267	6.51	x 4.45	x 4.40	24.42	x 8.58	40.50	x 10.17	24.46	x 7.66

	±0.47	±0.26	±0.27	±2.72	±0.60	±3.71	±1.10	±3.55	±1.04
CYS318	6.77	x 4.46	x 4.36	23.50	x 7.56	38.23	x 13.62	29.21	x 6.62
	±0.35	±0.25	±0.25	±2.60	±0.52	±4.80	±1.27	±3.55	±0.77
CYS320	6.61	x 4.71	x 4.51	22.46	x 6.37	44.50	x 9.08	24.25	x 5.66
	±0.56	±0.31	±0.31	±2.16	±0.62	±6.65	±1.37	±2.75	±0.53
CYS345	6.27	x 4.63	x 4.43	21.29	x 6.77	36.69	x 12.25	26.91	x 5.45
	±0.35	±0.26	±0.23	±1.37	±0.17	±5.32	±0.96	±2.70	±0.56
CYS363	6.87	x 4.70	4.51	20.98	x 8.02	37.83	x 17.50	29.04	x 7.37
	±0.29	±0.23	±0.30	±1.79	±0.55	±3.64	±1.34	±2.60	±0.76
CYS366	6.97	x 4.64	x 4.55	21.33	x 6.63	36.71	x 9.87	23.33	x 6.58
	±0.43	±0.20	±0.27	±2.58	±0.42	±8.11	±1.39	±2.75	±0.51
CYS371	6.96	x 4.50	x 4.40	22.42	x 7.66	38.44	x 11.87	25.25	x 7.27
	±0.46	±0.23	±0.23	±2.10	±0.82	±3.28	±1.32	2.86	±0.53
CYS377	6.34	x 4.42	x 4.37	21.83	x 9.00	40.00	x 11.96	22.33	x 6.50
	±0.30	±0.24	±0.21	±1.92	±0.53	±5.20	±0.79	±2.25	±0.77
CYS398	6.87	x 4.56	x 4.39	23.42	x 6.39	34.87	x 11.21	30.18	x 6.43
	±0.35	±0.27	±0.19	±1.94	±0.57	±3.26	±1.16	±2.50	±0.81
CYS400	6.68	x 4.40	x 4.33	16.67	x 6.33	34.08	x 12.92	27.08	x 6.41
	±0.49	±0.24	±0.24	±0.88	±0.58	±4.70	±1.19	±3.10	±0.71
CYS422	6.81	x 4.18	x 4.15	21.33	x 6.68	44.50	x 11.50	27.75	x 6.68
	±0.36	±0.19	±0.26	±1.83	±0.70	±7.21	±2.54	±3.72	±0.91
CYS424	6.56	x 4.46	x 4.45	28.00	x 6.12	52.58	x 9.88	32.04	x 6.04
	±0.27	±0.22	±0.23	±2.36	±0.49	±4.59	±1.49	±5.99	±0.88
CYS456	6.74	x 4.53	x 4.42	20.92	x 6.67	38.33	x 10.21	27.00	x 5.04
	±0.42	±0.27	±0.27	±1.07	±0.52	±3.89	±0.53	±3.00	±0.50
CYS457	6.60	x 4.47	x 4.31	19.92	x 6.66	35.66	x 12.79	25.37	x 6.10
	±0.39	±0.37	±0.23	±1.59	±0.71	±3.12	±1.60	±1.85	±0.69
CYS458	6.53	x 4.45	x 4.40	19.93	x 6.33	48.33	x 11.46	25.04	x 5.33
	±0.34	±0.26	±0.27	±1.52	±0.68	±8.35	±2.09	±3.30	±0.73
CYS472	7.09	x 4.66	x 4.35	19.97	x 6.4	34.96	x 12.32	27.57	x 6.33
	±0.41	±0.30	±0.23	±1.18	±0.75	±4.24	±1.39	±3.09	±0.88
CYS489	7.15	x 4.76	x 4.53	23.08	x 7.92	39.21	x 13.12	25.71	x 6.37
	±0.44	±0.24	±0.25	±2.15	±0.81	±4.25	±1.75	±2.31	0.40
CYS496	6.51	x 4.56	x 4.43	23.92	x 7.29	44.75	x 12.33	29.29	x 6.08
	±0.37	±0.27	±0.25	±3.12	±0.45	±4.25	±0.76	±2.82	±0.86
CYS504	6.77	x 4.35	4.25	20.71	x 6.72	38.00	x 12.00	28.83	x 6.69
	±0.37	±0.24	±0.24	±0.79	±0.55	±3.62	±1.25	±3.89	±0.59