The utility of morphological, *ITS* molecular and combined datasets in estimating the phylogeny of the cortinarioid sequestrate fungi

by

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

Molecular technology has shown the classical, morphologically defined groupings of sequestrate cortinarioid fungi to be artificial and in need of revision. However, these same molecular studies have highlighted morphological characters, such as spore shape and ornamentation, that have proved useful for distinguishing phylogenetically informative groups. This observation underpins the hypothesis of this study: that the numeric analysis of selected morphological characters can provide the same picture of the diversity of, and relationships among, sequestrate cortinarioid fungi as that recovered from phylogenetic analysis of rDNA sequence data.

Sequestrate fungi are those in which the spores mature inside an enclosed fruit body, remaining there until the fruit body decomposes or is eaten. For the purposes of this thesis the following genera are considered to contain cortinarioid sequestrate fungi: *Auritella*, *Cortinarius*, *Dermocybe*, *Descomyces*, *Hymenogaster*, *Hysterogaster*, *Inocybe*, *Protoglossum*, *Quadrispora*, *Setchelliogaster* and *Timgrovea*. This thesis focussed on Australian representatives of these fungi to address the hypothesis outlined above.

Four analysis methods were applied to each of three datasets (morphological, rDNA and combined data) in a comparative approach to test the stated hypothesis. The four analysis methods were two multivariate methods: cluster analysis and ordination (by principal coordinates analysis), and two phylogenetic methods: maximum parsimony and Bayesian analysis.

Low bootstrap support and Bayesian partition probabilities for phylogenetic analyses of the morphological data indicated this dataset had little to no phylogenetic signal

discernable by parsimony and Bayesian analyses. Different analyses of the morphological data differed in the way they grouped the collections. The type of clustering method used affected the pattern of relationships recovered. The coding of the data had a much more substantial effect on the patterns of relatedness suggested by the multivariate analyses. Despite the low level of phylogenetic information and agreement between analyses of the morphological data it was found that some collections were consistently grouped together. This included the separation of the *Cortinarius*-like collections from the *Descolea*-like collections and the relatively consistent grouping of some pairs of collections and some larger groups. Thus, despite the limited phylogenetic signal of the small morphological dataset and the artefacts of coding, some relatively consistent groups were recovered.

Separate analyses of the *Cortinarius*-, *Descolea*- and *Hebeloma*-like ITS sequences recovered similar patterns to published phylogenies. The inclusion of more sequestrate taxa and a greater sample of Australian collections than previous studies, indicated that both *Timgrovea* subgenera nest among the *Descolea*-like collections and that hitherto undiscovered lineages of *Descolea*-like fungi are represented among the collections in Australian herbaria. The *Cortinarius*-like fungi fall within clades recognised by published phylogenies. Similar topologies were supported by both Parsimony bootstrap and Bayesian partition probability values for analyses of the molecular data including the separation of *Cortinarius*-like collections from *Descolea*-like collections. However neither of these methods of analysis and evaluation yielded well-resolved deeper nodes for either of these two major clades. Comparable clades/clusters of *Cortinarius*-like and *Descolea*-like collections were found in all analyses of the molecular data. Thus phylogenetically distinct groups of cortinarioid sequestrate fungi could be consistently distinguished using ITS molecular data, but not confidently related to one another.

The ratio of molecular to morphological characters (741:16) meant the patterns observed for the combined analyses were more similar to those observed in analyses of the molecular data than those of the morphological data. This included the recovery of substantially similar clades/clusters to those recovered by analyses of the molecular data alone. The value of combining the morphological and molecular data as analysed is questioned despite the congruence of the datasets according to the Incongruence-Length Difference test. Differences between the molecular and combined datasets arose primarily where the molecular data grouped collections that were also grouped by the morphological data.

The numeric analysis of the selected morphological characters as carried out in this study did not recover the same pattern of groups and relationships among the cortinarioid sequestrate fungi as phylogenetic analyses of ITS data. The composition of groups recovered using the morphological data alone or as part of the combined dataset, and the relationships between those groups, differed from those recovered from the molecular data alone; although there were similarities between groups recovered from different datasets. The ability of this thesis to conclusively address its fundamental hypothesis was compromised by limitations of the study such as taxon sampling, character selection, character coding and the poor resolution of the ITS phylogeny. Acknowledging these limitations, and that some similar groups were recovered, the results of this thesis do not support its stated hypothesis that the numeric analysis of selected morphological characters can provide the same picture of the diversity of, and relationships among, sequestrate cortinarioid fungi as recovered from phylogenetic analysis of rDNA sequence data.

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Table of Contents

Section ***Page***

Table of Figures

Page

Table of figures

List of Tables

Page

Table of tables

Publications arising from the thesis

Reprints of both of these articles are included in Appendix 8.

Francis, A.A. and Bougher, N.L. (2003). Historical and current perspectives in the systematics of Australian cortinarioid sequestrate (truffle-like) fungi. *Australasian Mycologist* **21**: 81-92.

Francis, A.A. and Bougher, N.L. (2004). Cortinarioid sequestrate (truffle-like) fungi of Western Australia. *Australasian Mycologist* **23**: 1-26.

Abbreviations

Abbreviation	*Definition*
ABRS	Australian Biological Resources Study.
CANB	Australian National Herbarium, Canberra
CSIRO	Commonwealth Scientific and Industrial Research Organisation (Australia)
DAR	New South Wales Plant Pathology Herbarium, Orange Agricultural Institute, Orange, New South Wales, Australia
DNA	Deoxyribonucleic acid
ITS	Internal Transcribed Spacer. Non coding region between ribosomal DNA genes. May refer to spacer 1, spacer 2 or both of these along with the 5.8S rDNA gene *i.e.* ITS region.
K	Herbarium Royal Botanic Gardens Kew
MEL	Herbarium, Royal Botanic Gardens, Melbourne
nLSU	Nuclear large-subunit rRNA (28S) gene.
nSSU	Nuclear small-subunit rRNA gene, the 18S rRNA gene.
OSC	Herbarium, Oregon State University, Corvalis, Oregon, USA.
OTU	Operational taxonomic unit
PCR	Polymerase Chain Reaction

Abbreviations

PERTH	Western Australian Herbarium, Perth
rDNA	Genes coding for rRNA (also used in reference to the regions containing these genes)
RNA	Ribonucleic acid
RPB1 and RPB2	Largest and second largest subunits of RNA polymerase II respectively.
rRNA	Ribosomal RNA
s.s.	*Sensu stricto*. Latin for 'in the strict/restricted sense'
subg.	Subgenus

Chapter 1

General introduction

1.1 Aim of this thesis

This thesis seeks to increase knowledge of the diversity of, and relationships among, cortinarioid (allied to *Cortinarius* and related agaricoid fungi) sequestrate (truffle-like) fungi, with a particular focus on the Australian assemblage. Specifically this thesis aims to test the hypothesis that numeric analysis of selected morphological characters can provide the same picture of the diversity of, and relationships among, these fungi as that recovered from phylogenetic analysis of rDNA sequence data.

1.2 The cortinarioid sequestrate fungi

A wide variety of sequestrate (truffle-like) macrofungi (fungi producing large fruit bodies) have been aligned with *Cortinarius* and related agaricoid (mushroom-like) fungi. These phylogenetic affinities have not always been recognised and, in a number of cases, remain uncertain. Early fungal taxonomists placed the sequestrate fungi together under polyphyletic groupings such as the Gasteromycetes, based on the common trait of having enclosed hymenia through most or all stages of development including maturity. As a result, the cortinarioid sequestrate fungi largely share the same early history of study as the sequestrate fungi in general. For Australian sequestrate fungi this shared history of classification and collection is detailed in Castellano & Bougher (1994), May & Wood (1997), Lebel & Castellano (1999), May (2001) and Bougher & Lebel (2001). As different morphological, chemical and developmental characters were considered, sequestrate fungi were progressively separated into new

families and orders, including the Cortinariales, that more closely reflected their relationships with other fungi. Research employing molecular technology has affirmed the polyphyletic nature of many traditional cortinarioid taxa, and provided additional support for a number of postulated links between sequestrate and non-sequestrate forms.

Sequestrate fungi are those in which the spores, commonly statismospores (not forcibly discharged), mature inside an enclosed, underground, semi-underground or less often emergent fruit body, remaining there until the fruit body decomposes or is eaten. The cortinarioid sequestrate fungi exhibit a variety of basidiome forms, differing in the degree of gasteromycetation (pileus, stipe, veil, and hymenophore development) found independently or together with characteristics such as statismospory (Table 1). Particular sequestrate genera have been affiliated with *Cortinarius* on the basis of spore structure, pigmentation and ornamentation; basidiome pigmentation and development; and similarity in molecular sequence data (e.g. Singer 1975, Bougher & Castellano 1993, Peintner *et al.* 2001, Moncalvo *et al.* 2002). However, opinions differ on the significance of the various characters used to determine the phylogenetic relationships of the cortinarioid fungi, and thus which taxa should be included.

This thesis begins with a history of the collection and classification of sequestrate fungi currently considered related to *Cortinarius* and closely allied agarics, with a particular focus on Australia. The Australian assemblage of cortinarioid sequestrate fungi, though only partially known, is particularly diverse and represents a wide range of sequestrate forms, ecological associations and interactions (Bougher & Lebel 2001).

Table 1: Some broad groupings of morphological forms found among the cortinarioid sequestrate fungi [adapted from Peintner *et al.* (2001)].

CHARACTER	CORTINARII SEQUESTRATE BECAUSE OF PERSISTENT VEILS	SECOTIOID[1]	GASTEROID[2]
PILEATE	Yes	Yes or no	No
HYMENOPHORE STRUCTURE	Straight to more or less anastomosed lamellae	Anastomosed lamellae or a loculate gleba	Anastomosed lamella or a loculate gleba
STIPE/COLUMELLA	Generally possessing a well developed stipe/columella	With a stipitate to very short columella traversing the gleba	With or without a columella or internal sterile tissue
HABIT	Epigeous to hypogeous	Epigeous to hypogeous	Hypogeous
SPORE RELEASE	Ballistosporic or statismosporic	Commonly statismosporic	Statismosporic
REFERENCES AND EXAMPLES	Described as hypogeous *Cortinarii* by Thiers & Smith (1969)	*Thaxterogaster* Singer (1951) synonymised with *Cortinarius* Peintner *et al.* (2002)	*Protoglossum* Massee (1891)

[1] Secotioid. Sequestrate by means of peridium which is essentially a non-expanding pileus. Often with a "ring hole" formed around the stipe by the peridial/pileal margin. Indicating a similar basidiome form to the sequestrate fungus *Secotium* Kunze.

[2] Gasteroid. Sequestrate by means of a continuous (or nearly so) peridium. From the Greek, *gaster*-stomach-, *-oid* like. In searching the literature, the spellings "gast***e***roid" and "Gast***e***romycetes" appear more commonly than "gastroid" or "Gastromycetes"; Gast***e***romycetes is used in the Dictionary of the Fungi (Kirk *et al.* 2001); and Stearn (1992) writes on the matter "*Gaster* (f. gen. sing. *gasteris* or *gastri*) may be declined like *tuber* [p. 75, third decension, group 7 according the groupings of Wikén, E. (1951) *Latin för Botanister och Zoologer*, *fide* Stearn (1992), where the root retains the "-er" ending e.g. gasteroid] or like *ager* (p. 70) [second declension, where the e is removed from the root e.g. gastroid]". Consequently the spellings gasteroid and Gasteromycetes will be used throughout this thesis exept when citing taxa or publications by authors who did otherwise.

Recognition of sequestrate members of the Cortinariaceae and Cortinariales has been a relatively recent development. For much of the time between the naming of *Cortinarius* by Persoon (1801) and the naming of *Thaxterogaster* by Singer (1951), it was thought that the Gasteromycetes were a separate lineage from the Hymenomycetes, rather than a polyphyletic assemblage arising more than once from among Hymenomycete ancestors. This stems from the fact that taxonomic concepts have, classically, been based on basidiome morphology, the genetic control of which appears relatively simple and potentially subject to frequent mutation (Bruns *et al.* 1989, Hibbett *et al.* 1994, Peintner *et al.* 2001) and sometimes expressed as a high capacity for morphological plasticity (*e.g.* Chiu *et al.* 2000, Lago *et al.* 2001). Reassessments of morphological and molecular characteristics uniting sequestrate taxa to *Cortinarius* and allied agarics have supported the view that both the sequestrate Cortinariaceae and the Hymenogasteraceae are polyphyletic (Bougher & Castellano 1993, Peintner *et al.* 2001). However, the classical conceptual framework and historical taxonomic legacy continues to influence the classification of cortinarioid sequestrate genera (*c.f.* Bougher & Castellano 1993, and Kirk *et al.* 2001).

Table 2: World and Australian numbers of published species considered to be cortinarioid sequestrate fungi for the purposes of this thesis, based on references as provided and modified from Francis & Bougher (2003). World numbers from CABI Bioscience Databases (http://194.131.255.4), Australian numbers from May & Wood (1997) and May *et al.* (2004). Darker grey shading indicates genera excluded because of affinities to genera outside the Cortinariaceae or Hymenogasteraceae suggested by published or unpublished phylogenies since 2003. Light grey shading indicates genera excluded on the basis of morphological similarities to excluded genera. Totals in grey are for all taxa in the table, totals in bold are for only those taxa recognised as cortinarioid for the purposes of this thesis.

GENUS	COMMENTS/REFERENCES	WORLD	AUSTRALIA
Aroramyces Castellano & Verbeken	Australian and African, (Castellano *et al.* 2000). Placed in the Phallomycetidae by Hosaka *et al.* (unpublished)	2	**1**
Auritella Matheny & Bougher	This genus too is only known from Australia and Africa. One sequestrate species *A. geoaustralis* Matheny & Bougher listed as '*Genus B*' in Bougher & Lebel (2001), also cited when unnamed by Matheny (2005).	1	**1**
Cortinarius (Pers.) Gray (Sequestrate forms)	Worldwide. Includes strongly velar 'hypogeous *Cortinarii*' as coined by Thiers & Smith (1969). Watling (1980), Bougher & Malajczuk (1986) and ex *Thaxterogaster* Sing. species synonymised with *Cortinarius* by Peintner *et al.* (2002).	10 + 58 (ex *Thax.*)	**3 + 13** (ex *Thax.*)
Cribbea A. H. Smith & D. A. Reid	Australian and Argentinean, Smith & Reid (1962). Allied to *Xerula* Maire (Lebel 2006)	4	**3**
Dermocybe (Fr.) Wünsche	The only known sequestrate member of this genus is Australian (Bougher & Trappe 2002). Monophyletic group nested within *Cortinarius* (Peintner *et al.* 2001).	1	**1**
Descomyces Bougher & Castellano	Australasian (Bougher & Castellano 1993). Affinities with the Bolbitiaceae Sing. (Singer & Smith 1959, Matheny *et al.* 2006)	5	**4**
Destuntzia Fogel & Trappe	One unnamed species occurs in Australia. (Castellano & Bougher 1994, Fogel & Trappe 1985). Placed by Fogel & Trappe (1985) as the most reduced member in an evolutionary series spanning *Cortinarius*, *Thaxterogaster*, *Hymenogaster* and *Destuntzia*, *c.f.* Bougher & Castellano (1993). Questionable affinity – similar in macroscopic appearance to *Kjeldsenia*	5	**0**
Hymenogaster Vittad. [in the restricted sense of Bougher & Castellano (1993)]	Worldwide; many of the Australian species have been recombined (Bougher & Castellano 1993), allied to *Hebeloma* (Fr.) P. Kumm. (Peintner *et al.* 2001) and *Alnicola* (Moreau *et al.* 2006)	95	**5**
Hysterogaster Zeller & C.W. Dodge	Named species from Australia, perhaps also including *Hymenogaster luteum* Vittad. (Bougher & Castellano 1993)	2	**2**

Table 2 continued: World and Australian numbers of published species considered to be cortinarioid sequestrate fungi for the purposes of this thesis, based on references as provided and modified from Francis & Bougher (2003). World numbers from CABI Bioscience Databases (http://194.131.255.4), Australian numbers from May & Wood (1997) and May *et al.* (2004). Darker grey shading indicates genera excluded because of affinities to genera outside the Cortinariaceae or Hymenogasteraceae suggested by published or unpublished phylogenies since 2003. Light grey shading indicates genera excluded on the basis of morphological similarities to excluded genera. Totals in grey are for all taxa in the table, totals in bold are for only those taxa recognised as cortinarioid for the purposes of this thesis.

GENUS	COMMENTS/REFERENCES	WORLD	AUSTRALIA
Inocybe (Fr.) Fr.	At least one unpublished sequestrate form with metuloids from Australia mentioned in Matheny & Bougher (2006a) on the basis of unpublished observations by P.B. Matheny and J. Trappe.	-	-
Kjeldsenia Colgan et al.	North American, (Colgan *et al.* 1995). Placed in the Phallomycetidae by Hosaka *et al.* (unpublished)	1	**0**
Mackintoshia Pacioni & C. Sharp	African. Pacioni & Sharp (2000) in the paper describing this genus suggested affinities with *Galerina* and *Mycoamaranthus*. Questionable affinity.	1	**0**
Mycoamaranthus Castellano *et al.*	Australasian and African, (Trappe *et al.* 1992, Castellano *et al.* 2000) no familial affinity was proposed in the original generic description, questionable affinity.	2	**1**
Protoglossum Massee	Worldwide, formerly *Cortinomyces* (Bougher & Castellano 1993, May 1995). Nested within *Cortinarius* (Peintner *et al.* 2001, Garnica *et al.* 2005).	8	**6**
Quadrispora Bougher & Castellano	Australian endemic (Bougher & Castellano 1993). Nested within *Cortinarius* (Peintner *et al.* 2001, Garnica *et al.* 2005).	3	**3**
Setchelliogaster Pouzar	Worldwide. In association with *Eucalyptus* (Bougher & Lebel 2001, Beaton *et al.* 1985a, Pouzar 1958). Affinities with the Bolbitiaceae, (Singer & Smith 1959, Matheny *et al.* 2006)	7	**2**
Timgrovea Bougher & Castellano	Australian and Chinese, (Bougher & Castellano 1993).	5	**4**
TOTAL[3]		**(210) 195**	**(49) 44**

[3] As this thesis was in the final stages of production, a paper by Trappe *et al.* (2006), was published that has included the provisionally named sequestrate cortinarioid genus with affinities to *Cortinarius* subgens *Telamonia*, *Geotelamonia nom. prov.* containing four provisionally listed species.

The history of collection for the cortinarioid sequestrate fungi has been influenced by the nature of these organisms and the changing focus of human investigations. The collection and study of these fungi in Australia has been sporadic, and remains far from complete. It is probable that Australia has many more cortinarioid sequestrate fungi than the 44 species currently recorded (Table 3). They may be substantial components of important ecological guilds within many Australian ecosystems as mycorrhizal associates and food sources for animal mycophagists in addition to providing other environmental services such as decomposition, nutrient capture and cycling (Bougher & Lebel 2001). Further research is needed to fill the large gaps in our knowledge concerning the interrelationships of sequestrate and agaricoid cortinarioid genera, their distribution, associations and function in order to adequately assess their role in Australian ecosystems.

Despite the relatively large body of information regarding the cortinarioid sequestrate fungi compared with that available for some other groups of sequestrate fungi, their taxonomy is currently in a state of flux. Accordingly this thesis has used the informal grouping 'cortinarioid sequestrate fungi', acknowledging that different authors have placed these fungi in a variety of genera and suprageneric taxa. For the purposes of this thesis then, cortinarioid sequestrate genera are considered to be those that at some time, have been placed in either the Cortinariaceae or the Hymenogasteraceae and, as yet, have not been shown to have stronger affinities with taxa outside these families. Under this definition eleven genera are accepted as cortinarioid sequestrate fungi (unshaded entries in Table 2).

For many taxa inclusion or exclusion from the cortinarioid sequestrate fungi is, however, inconclusive. For example, Bougher & Castellano (1993) originally excluded

Timgrovea from the Cortinariaceae because it possessed Type I spore ornamentation (Singer 1975), suggesting instead a possible relationship to reticulate-spored *Strobilomyces* Berk. (Boletales). However, two subgenera of *Timgrovea* were recognised when the genus was coined, subgenus *Timgrovea* for species with spores bearing an ornamentation of polygonal alveoli and subgenus *Subreticulata* for those species whose spores bear a broken reticulate ornamentation but not polygonal alveoli. This second form of ornamentation falls into Type II or Type IIIb "Ridges and fine lines and warts form a reticulated surface" or "Warts or spines connected to form...an incomplete network" respectively (Singer 1975). Thus spore ornamentation in *Timgrovea* actually spans these three groups. Type IIIb ornamentation is now known to occur in the Cortinariaceae in *Cortinarius reticulisporus* Miyauchi (2001). With regard to the use of spore ornamentation types to include or exclude genera from a family, (Singer 1975) points out that his ornamentation types are not anatomical-ontogenetic and do not take into account possible multiple independent origins of particular types. Indeed Singer's use of genera now known to belong to only distantly-related homobasidiomycete clades as examples of Type I ornamentation [*Strobilomyces*, in the /bolete clade and *Lactarius*, in the /russuloid clade (Hibbett & Thorn 2001)] highlights the diverse, multiple origins for this ornamentation type. Two observations on Bougher & Castellano (1993) also support the inclusion of *Timgrovea* within the cortinarioid sequestrate fungi presumably with a close association to *Descomyces*. The first is the anastomosing short ridges of the ornamentation illustrated by those authors for *Descomyces javanicus* (Hohnel) Bougher & Castellano. These anastomoses may represent an intermediate step between the isolated warts and short ridges characteristic of *Descolea* ornamentation, and the broken reticulum of *Timgrovea* subgenus *Subreticulata*. The second is the mention of "golden" hyphae in the Type description of *Timgrovea reticulata*. Giachini *et al.* (2000) have considered the presence of golden,

thick-walled hyphae as characteristic of *Descomyces*. Consequently they named the novel alveolate-spored species with such hyphae they reported from *Eucalyptus* plantations in Brazil *Descomyces giachinii* rather than placing it in *Timgrovea*. If, as observations made for this study suggest, there is variability in the thickness of the walls of these golden, veil-remnant hyphae the "golden" hyphae observed in the Type of *Timgrovea reticulata* may well be homologous with those of *Descomyces*. If this it the case, it is not difficult to imagine a series whereby isolated *Descomyces* type spore ornamentation anastomoses to form *Timgrovea* subgenus *Subreticulata* type ornamentation which is then augmented to produce the full alveolate reticulation characteristic of *Timgrovea* subgenus *Timgrovea*. As there seems to be no grounds to exclude *Timgrovea* from the Cortinariaceae on the basis of spore morphology, and the spores of *Timgrovea* subgenus *Subreticulata* are quite similar to those of *Descomyces*, *Timgrovea* has been included within the definition of cortinarioid sequestrate fungi employed in this study.

As a second example of dubious inclusion in the cortinarioid fungi, Smith (1965) first described the 'amyloid'-spored *Mycolevis*, tentatively as a second genus in the Cribbeaceae Sing.. Smith (1965) also demonstrated, regarding mounts made in Melzer's solution, that 'not all violet or blue-black material (especially granules) are necessarily "truly amyloid". In the case of *Mycolevis*, Smith appears to have considered the structure of the spore ornamentation (*e.g.* the presence of a conspicuous perisporium), at least possibly, more phylogenetically informative than the amyloid Melzer's reaction. No published study has rigorously examined this possibility, and yet this genus has been aligned with the cortinarioid fungi by the reduction of the Cribbeaceae to synonymy with the Cortinariaceae (*e.g.* Kirk *et al.* (2001). It is unlikely that the *Mycolevis* amyloid reaction is significantly different from that observed in the

Russulaceae Maire, and molecular data apparently aligns *Mycolevis* with this family (Prof. J. Trappe pers. comm.). *Mycolevis* is therefore excluded from the concept of the cortinarioid sequestrate fungi applied in this thesis. *Cribbea* has recently been demonstrated to have affinities with *Xerula* Maire (Lebel 2006). *Aroramyces* and *Kjeldsenia* have affinities with the Phallomycetidae (Hosaka *et al.* unpublished). The remaining genera included in Table 2, *Destuntzia*, *Mackintoshia* and *Mycoamaranthus* are excluded from the cortinarioid sequestrate fungi for the purposes of this thesis on the basis of morphology being both considerably different from other included cortinarioid sequestrate fungi and similar to the excluded genera placed in the Phallomycetidae. All the eleven remaining genera fit the limited definition of cortinarioid as applied in this thesis. It is important to note however that Matheny *et al.* (2006) place these nine included genera in four separate clades recognised at family rank, Hymenogastraceae (*Hymenogaster*, *Hysterogaster*), Inocybaceae (*Auritella* and *Inocybe*), Cortinariaceae *sensu stricto* (*Cortinarius*, *Dermocybe*, *Protoglossum* and *Quadrispora*) and the Bolbitiaceae (*Descolea*, *Setchelliogaster*, *Timgrovea* and *Descomyces*).

A synthesis of hypotheses as to the phylogenetic/taxonomic placement of the various genera and families encompassed within the concept of the cortinarioid sequestrate fungi is presented as Figure 1 [from Francis & Bougher (2003)]. The representation in Figure 1 does not take into account the six gene phylogeny of Matheny *et al.* (2006) which proposes new relationships between taxa formerly classified in the Cortinariaceae and other families. In particular the Cortinariaceae *sensu stricto* and the Bolbitiaceae (including the only representative of the *Descolea*-like fungi) are sister taxa and are basal to the clade containing the Gymnopileae, and two larger clades. One of these larger clades contains the Hymenogastraceae (including *Galerina* and *Psilocybe* species) as sister to the *Strophariaceae*. The other contains the Inocybaceae as sister to

the Crepidotaceae and subtended by the Tubariae and Panaeolae. These relationships separate families formerly united by spore characteristics, particularly spore colour. The findings of Matheny *et al.* (2006) and Hibbett *et al.* (2000) also challenge the definition of groups of cortinarioid fungi on the basis of ectomycorrhizal status [a character cited as supporting the choice of *Gymnopilus* P. Karst. as outgroup for the Cortinariaceae in Peintner *et al.* (2001)]. A revised figure including these systematic changes is included as Figure 2.

1.2.1 Characters used to classify sequestrate cortinarioid fungi

Molecular and morphological characters unite the cortinarioid sequestrate fungi. Seven of the eleven genera included as sequestrate cortinarioid fungi in this work fall within the Cortinariaceae as examined molecularly by Peintner *et al.* (2001) (*Auritella*, *Hysterogaster*, *Inocybe* and *Timgrovea* were not included in that work). The inclusion of *Setchelliogaster* and *Descomyces* in the current work is further supported by the observation *Descolea* and *Cortinarius* lack the true germ pore characteristic of the Bolbitiaceae. For example in a 'true germ pore', such as that of *Pholiota filaris* (Fr.) Singer, the outer coriotunica is thinner in the concave pore region and the inner coriotunica thickens to form the germ pore medulla, whereas in *Descolea flavo-annulata* (Vasilieva) Horak the outer coriotunica is thicker, and the inner coriotunica truncated, at the distended spore apex (Bougher 1987). However the lack of other representatives of the Bolbitiaceae in molecular studies linking *Descolea*-like fungi with *Cortinarius* (e.g. Martin & Moreno 2001, Peintner *et al.* 2001) suggests that a reinvestigation of this character would be appropriate in light of the findings of Matheny *et al.* (2006). However, the phylogeny of Matheny *et al.* (2006) (summarised emphasising the position of the sequestrate cortinarioid fungi in Figure 2), though

showing high Bayesian partition probabilities for many branches including deeper nodes within their /Agaricoid clade, has many branches with poor bootstrap support values, including the branch uniting *Descolea* with *Bolbitius* etc. Alfaro *et al.* (2003) using a simulated analysis reinforce the different meanings of these two 'confidence measures'; that high Bayesian partition probabilities suggest that the tree is an accurate estimate of the phylogeny *given the data and the model used* and that low bootstrap values indicate that the tree topology is highly dependant on a small proportion of the underlying structure of the dataset used and may prove unstable if more data were to be added. Given this qualification of even the multi-gene phylogeny presented by Matheny *et al.* (2006), the taxonomic position of *Descolea* and related genera is not yet conclusively settled. The genus *Thaxterogaster* was reduced to synonymy under *Cortinarius* by Peintner *et al.* (2002)[4]. Similarities between these two genera had been noted since the first description of *Thaxterogaster* (Singer 1951) and the polyphyly of *Thaxterogaster* had also been suspected (*e.g.* Horak & Moser 1965, Moser 1964). Bougher & Castellano (1993) discuss the links between *Protoglossum* (*Cortinomyces*) and *Cortinarius*. They made no conclusion in that paper as to the placement of *Quadrispora*, however, basidiome form and spore structure (in all but symmetry) and molecular evidence (Peintner *et al.* 2001, Garnica *et al.* 2005) support the inclusion of *Quadrispora* within *Cortinarius*. *Dermocybe* was initially described as a subgenus of *Cortinarius* (chiefly distinguished by pigment characteristics) and forms a monophyletic group of fungi nested within *Cortinarius* (Chambers *et al.* 1999, Peintner *et al.* 2001). Further discussion on membership within the concept of cortinarioid sequestrate fungi employed here is provided in Francis & Bougher (2003) and Francis & Bougher (2004).

[4] For the purposes of this thesis, though acknowledging Peintner's synonymy, I continue to refer to *Thaxterogaster* species or *Cortinarius* (ex *Thaxterogaster*) speceis to distinguish these forms from the many agaricoid *Cortinarius* species not dealt with in this thesis.

Thus the main characters uniting the sequestrate cortinarioid fungi are spore characteristics (most commonly brown, ornamented spores which lack a true germ pore) coupled with basidiome characteristics (including pigmentation and peridiopellis structure) the interpretation of which are supported by molecular characteristics – similar characters to those uniting the agaricoid *Cortinarius* species.

1.3 Collection and Study of Cortinarioid Sequestrate Fungi

Collections of cortinarioid sequestrate fungi provide the raw material for our knowledge and classification of them. The increasing effort being expended in making collections of sequestrate fungi, particularly in Australia, has been instrumental in bringing to light sequestrate fungal species that have played a significant part in the changing classification of the sequestrate cortinarioid fungi.

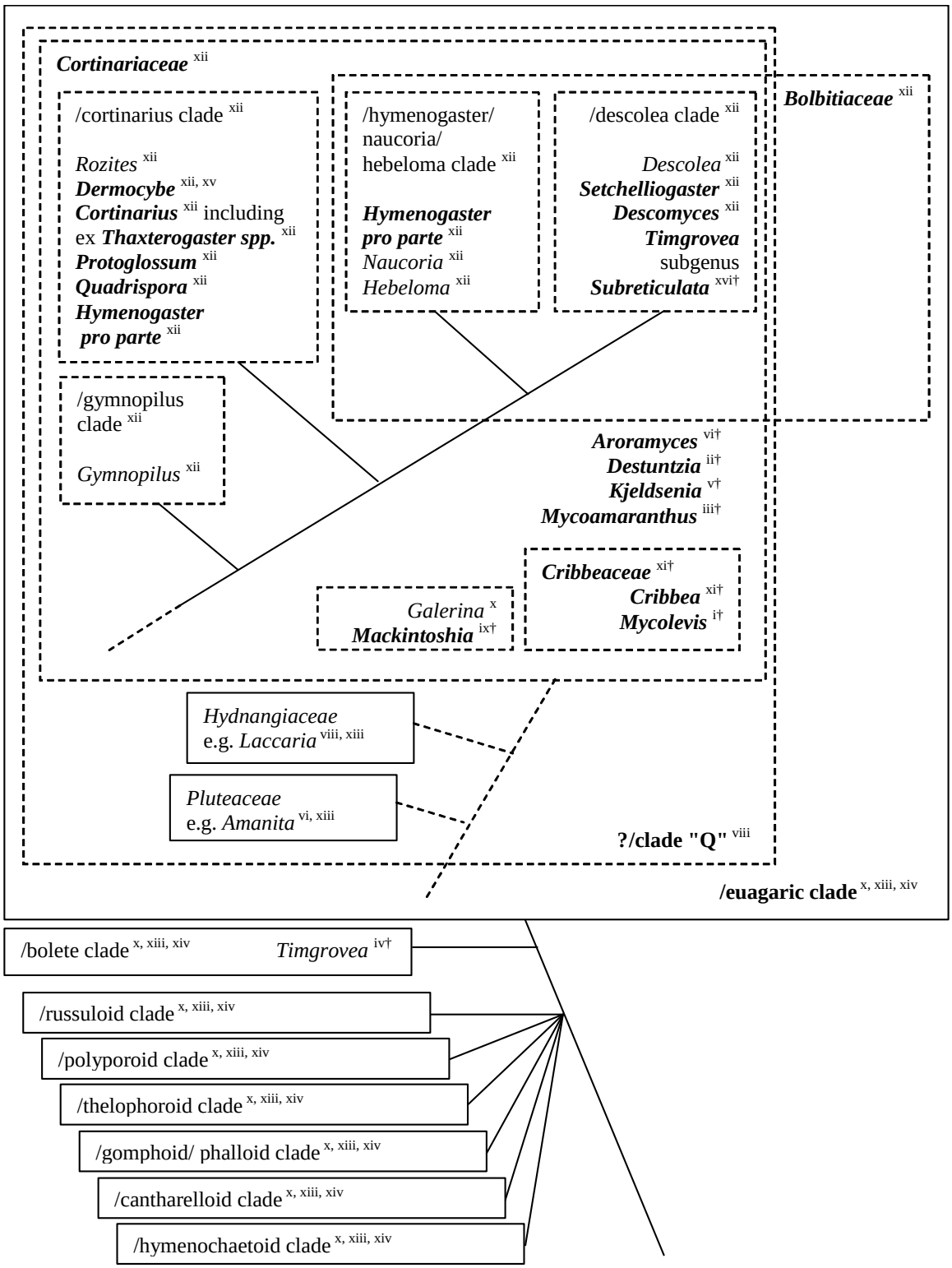

Cortinariaceae xii
/cortinarius clade xii
Rozites xii
Dermocybe xii, xv
Cortinarius xii including ex Thaxterogaster spp. xii
Protoglossum xii
Quadrispora xii
Hymenogaster pro parte xii
/hymenogaster/ naucoria/ hebeloma clade xii
Hymenogaster pro parte xii
Naucoria xii
Hebeloma xii
/descolea clade xii
Descolea xii
Setchelliogaster xii
Descomyces xii
Timgrovea subgenus Subreticulata xvi†
Bolbitiaceae xii
/gymnopilus clade xii
Gymnopilus xii
Aroramyces vi†
Destuntzia ii†
Kjeldsenia v†
Mycoamaranthus iii†
Cribbeaceae xi†
Cribbea xi†
Mycolevis i†
Galerina x
Mackintoshia ix†
Hydnangiaceae e.g. Laccaria viii, xiii
Pluteaceae e.g. Amanita vi, xiii
?/clade "Q" viii
/euagaric clade x, xiii, xiv
/bolete clade x, xiii, xiv
Timgrovea iv†
/russuloid clade x, xiii, xiv
/polyporoid clade x, xiii, xiv
/thelophoroid clade x, xiii, xiv
/gomphoid/ phalloid clade x, xiii, xiv
/cantharelloid clade x, xiii, xiv
/hymenochaetoid clade x, xiii, xiv

Figure 1. Diagrammatic representation of the postulated taxonomic position of sequestrate and selected agaricoid cortinarioid fungi amongst the Cortinariaceae and homobasidiomycete taxa as published in Francis & Bougher (2003). † indicates taxa that have not been included in molecular phylogenies at the time of publication. Taxa including known cortinarioid sequestrate forms are indicated in bold. From: i Smith (1965), ii Fogel & Trappe (1985), iii Castellano *et al.* (1992), iv Bougher & Castellano (1993), v Colgan *et al.* (1995), vi Hibbett *et al.* (1997), vii Castellano *et al.* (2000), viii Moncalvo *et al.* (2000), ix Pacioni & Sharp (2000), x Hibbett & Thorn (2001), xi Kirk *et al.* (2001), xii Peintner *et al.* (2001), xiii Binder & Hibbett (2002), xiv Moncalvo *et al.* (2002), xv Bougher & Trappe (2002), xvi Francis & Bougher (unpublished observations at 2003).

1.3.1 Early Collection and Study of Australian Cortinarioid Sequestrate Fungi

The infrequent collection of Australian sequestrate fungi in the early 19th century became more frequent with the increasing involvement of resident collectors, mycologists and Australian government departments and universities. The private collector J. Drummond, working in Western Australia from 1828 to 1863, collected at least one fragment of a *Hymenogaster* species. This was sent to Berkeley (and is now lodged at K), however, owing to poor preservation it cannot be identified (Hilton 1983). Working in the Victorian Department of Agriculture, McAlpine (1895) published a comprehensive bibliography and systematically arranged checklist of the known Australian fungi incorporating six orders of Gastromycetes, including the Hymenogasteraceae. The Hymenogasteraceae [*sensu* McAlpine (1895)] included three species *Hymenogaster* and four other genera. Collection and classification of Australian fungi was also advanced by the work of R. Rodway in Tasmania. Rodway (1912a & b) compiled all known Australian species considered as Hymenogasteraceae *s. lat.* including four other genera (13 spp.) alongside *Hymenogaster s. lat.* (six spp.). Rodway (1912b) refuted Cooke's statement (Cooke 1892) that Australia had few hypogeous sequestrate fungi and concluded that Australia indeed had a rich hypogeous fungal flora.

In subsequent publications Rodway added a further seven *Hymenogaster* species to this list (Rodway 1918, 1919). The increase in the number of species found is illustrated by comparing the number of species of Tasmanian Hymenogasteraceae in the work of 1912 with the species list from Rodway (1924). In this later work there were eight *Hymenogaster* and 28 other sequestrate species in five other genera, not including four suppressed names.

1.3.2 The 20th Century to 1970

During the 20th Century interest in fungi as pathogens, symbionts, and as of importance to medicine, overtook colonialist exploration and biodiversity inventory as the primary rationale behind mycological research in Australia (May 2001). J. B. Cleland, a resident collector and taxonomist, contributed significantly to the knowledge of sequestrate fungi in Australia with the publication of his *Toadstools and Mushrooms and other Larger Fungi of South Australia* in 1934. The section on Gastromycetes was based largely on the work of Cunningham and the author's own extensive writings (in particular the 'Australian Fungi' series *e.g.* Cleland & Cheel (1919), Cleland & Cheel (1923). Cleland incorporated in the order Hymenogastrales, 7 other genera (30 spp.) alongside *Hymenogaster* (10 spp.) and *Dendrogaster* (three spp.). In 1944, Cunningham published *The Gasteromycetes of Australia and New Zealand*. In this work, two families, the Hymenogasteraceae and the Secotiaceae, made up the Hymenogastrales, one of the 5 orders of the class Gasteromycetes. *Hymenogaster* (11 species) along with two other genera (14 spp.) made up the Hymenogastereae of the Hymenogastroideae. Cribb, working at the University of Queensland, wrote a series of papers on various genera considered to belong to the Hymenogastrales in which she included six *Hymenogaster* species (Cribb 1956).

1.3.3 Collection and Study of Cortinarioid Sequestrate Fungi Since 1970

Recognition of the role of fungi in ecosystems increased during the 1970s and 1980s. This provided a further impetus for research into the distribution, ecology, biology and systematics of fungi, including cortinarioid sequestrate forms. As awareness of the need to collect specimens of Australian fungi developed, and collections were made, the magnitude of Australia's sequestrate fungal flora became increasingly evident. Activity in collecting and defining sequestrate fungal taxa increased markedly during the 1980s. Systematic mycology and the collection of sequestrate macrofungi in Australia benefited from visits by such overseas mycologists as E. Horak, R. Petersen, G. Samuels, J. Trappe and co-workers, and R. Watling around this period (May 2001). This effort has lead to the acquisition of high quality herbarium collections including those held at PERTH (collections formerly housed at the CSIRO Mycology Herbarium, Floreat, Perth Western Australia), CANB, DAR, MEL, and OSC. Increased research also resulted in an expansion of the literature on cortinarioid sequestrate fungi. Both the 'Gasteroid Basidiomycota of Victoria state' and 'Australasian truffle-like fungi' series included papers on cortinarioid sequestrate fungi (Beaton *et al.* 1985a, Castellano & Trappe 1990, Trappe *et al.* 1992). In 1982 an extensive program of collecting sequestrate fungi was initiated by N. Malajczuk and J. Trappe in south-western Australia as a preliminary study of the diversity of ectomycorrhizal fungi with possible applications to mycorrhizal inoculation of *Eucalyptus* species in plantations and mine-spoil reclamation (Lebel & Castellano 1999). The team of scientists responsible for the study was later expanded to include M. Castellano, P. Reddell and N. Bougher to collect throughout Australia including the Northern Territory, Queensland, Tasmania and

Western Australia (Bougher pers. comm., Lebel & Castellano 1999). The CSIRO Forestry and Forest Products Mycology Herbarium, Perth, Western Australia, established as a result of this work and parallel studies on epigeous fungi, is now housed at PERTH and includes approximately 5000 collections of sequestrate fungi. Of these, ca. 20% are cortinarioid sequestrate forms which may be proportionate to the relative abundance of these fungi in Australian ecosystems. Beaton *et al.* (1985a) and Beaton *et al.* (1985b) dealt with the sequestrate Cortinariales of Victoria (Australia) including two *Setchelliogaster* and seven *Thaxterogaster* species in the Cortinariaceae and 10 *Hymenogaster* species in the Hymenogasteraceae. Growing interest in sequestrate fungi, and an increasing number of collections and publications produced on the subject, provided the impetus for the reassessment of *Hymenogaster s. lat.* by Bougher & Castellano (1993). Castellano & Trappe (1990) and Trappe *et al.* (1996) constitute a nomenclatural bibliography of Australian sequestrate fungi. In 1990 they included one sequestrate *Cortinarius*, four *Cribbea*, 37 *Hymenogaster*, one *Protoglossum*, one *Rozites* (originally described as a *Thaxterogaster*), five *Setchelliogaster* and 48 *Thaxterogaster* species. In 1996 they added a further four *Timgrovea*, three *Descomyces*, six *Cortinomyces*, and two *Quadrispora* species (including synonyms) (Castellano & Trappe 1990, Trappe *et al.* 1996). In recent years a focus on mycophagy among Australia's native marsupials has also provided significant stimulus to mycology, especially the study of sequestrate fungi, and raised the profile of these fungi among ecologists and forest managers (*e.g.* Claridge *et al.* 1996). Seven cortinarioid sequestrate genera were listed by Claridge *et al.* (1996) as providing food for animal mycophagists. Australian sequestrate fungi have featured in several other major works including Grgurinovic's *Larger fungi of South Australia* which was published in 1997 based on a re-examination of Cleland's specimens. This work included one *Setchelliogaster* species and two *Thaxterogaster* species (Cortinariaceae) and one

species now considered to be non-cortinarioid, in the Hymenogastrales. Bougher & Syme (1998) produced *Fungi of Southern Australia,* featuring 125 species of macrofungi and emphasising the relationship of sequestrate fungi to agaricoid forms. This work featured one *Cortinarius* (now *Dermocybe*), one *Descomyces*, and two *Thaxterogaster* species.

A recent study on Australian and southern hemisphere taxa of Inocybaceae has recognized the first sequestrate species of that family – *Auritella geoaustralis* from the Wheat Belt region of Western Australia (Matheny & Bougher 2006a, Matheny & Bougher 2006b). The occurrence of sequestrate inocyboid fungi in Australia had been flagged in several previous publications: as a separate, unpublished genus ["genus B" Bougher & Lebel (2001) and "Geoinocybe" Francis & Bougher (2003)] and as an unnamed sequestrate *Inocybe* species (accession H7344) (Matheny 2005). Matheny & Bougher (2006a) demonstrated, using RPB1, RPB2, and nLSU sequences, that the collection H7344 nested within the *Auritella* clade and named the fungus *A. geoaustralis* noting that recognition of a separate genus to accommodate this sequestrate species would make *Auritella* paraphyletic. Another Australian sequestrate fungus with metuloid cystidia showing affinities to the genus *Inocybe* is also noted by Matheny & Bougher (2006a), citing unpublished observations by P.B. Matheny and J. Trappe. Unfortunately it was not possible to sequence the ITS region of H7344 and there are no publicly available ITS sequences for *A. geoaustralis*, consequently it was not possible to include it in the analyses reported here.

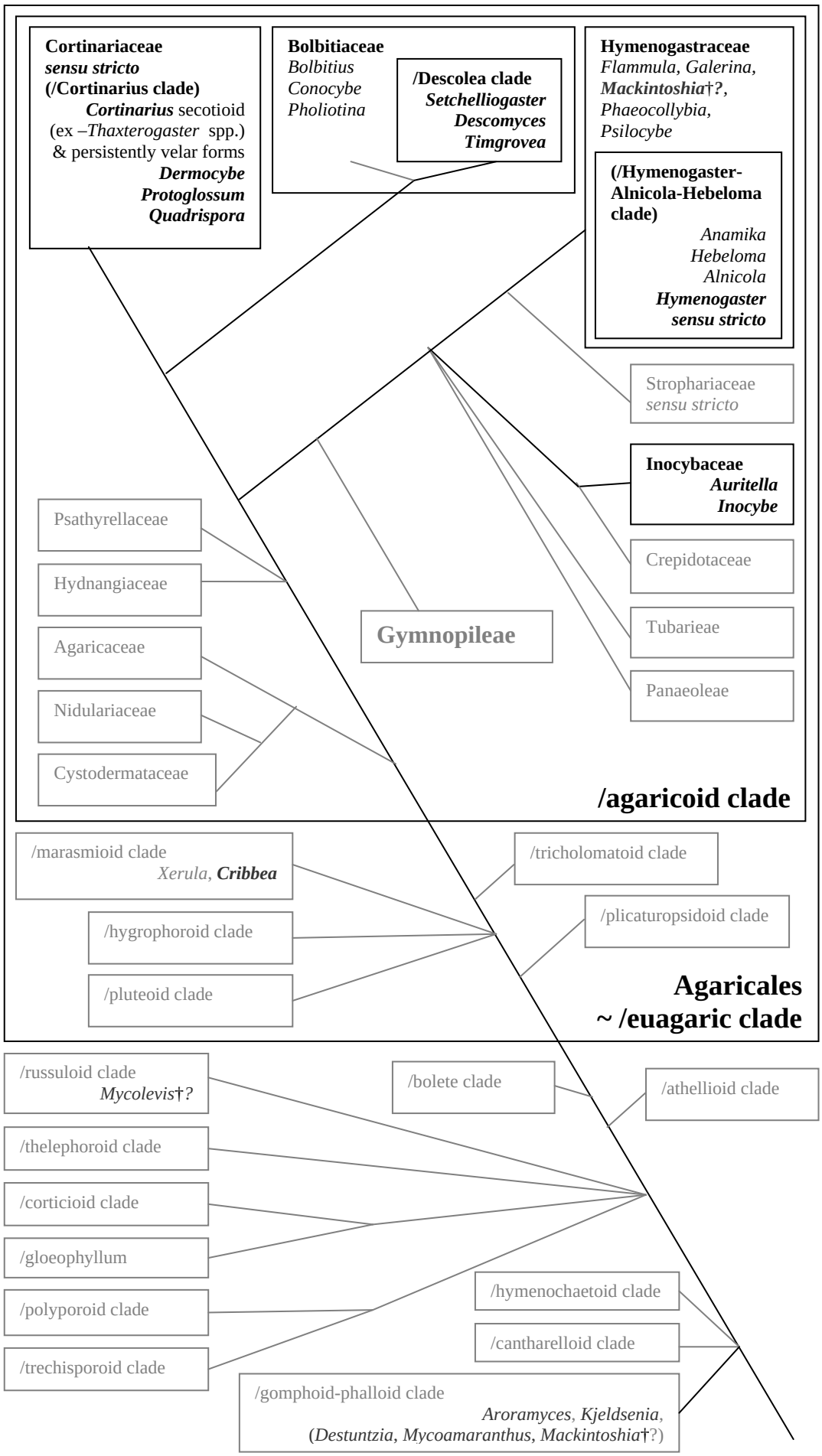

Cortinariaceae
sensu stricto
(/Cortinarius clade)
Cortinarius secotioid
(ex –Thaxterogaster spp.)
& persistently velar forms
Dermocybe
Protoglossum
Quadrispora
Bolbitiaceae
Bolbitius
Conocybe
Pholiotina
/Descolea clade
Setchelliogaster
Descomyces
Timgrovea
Hymenogastraceae
Flammula, Galerina,
Mackintoshia†?,
Phaeocollybia,
Psilocybe
(/Hymenogaster-Alnicola-Hebeloma clade)
Anamika
Hebeloma
Alnicola
Hymenogaster sensu stricto
Strophariaceae sensu stricto
Inocybaceae
Auritella
Inocybe
Psathyrellaceae
Hydnangiaceae
Agaricaceae
Nidulariaceae
Cystodermataceae
Gymnopileae
Crepidotaceae
Tubarieae
Panaeoleae
/agaricoid clade
/marasmioid clade
Xerula, Cribbea
/hygrophoroid clade
/pluteoid clade
/tricholomatoid clade
/plicaturopsidoid clade
Agaricales
~ /euagaric clade
/russuloid clade
Mycolevis†?
/thelephoroid clade
/corticioid clade
/gloeophyllum
/polyporoid clade
/trechisporoid clade
/bolete clade
/athellioid clade
/hymenochaetoid clade
/cantharelloid clade
/gomphoid-phalloid clade
Aroramyces, Kjeldsenia,
(Destuntzia, Mycoamaranthus, Mackintoshia†?)

Figure 2: Diagrammatic representation of the postulated taxonomic position of sequestrate and selected agaricoid cortinarioid fungi amongst the Homobasidiomycetes as at 2006 including changes proposed in this thesis. Sequestrate cortinarioid genera and clades containing these genera are shown in bold **type. The structure of the Agaricales is taken from Matheny *et al.* (2006) while the structure outside this clade is taken from the MOR website (Hibbett *et al.* (2005) http://mor.clarku.edu). † indicates taxa that have not been included in available molecular phylogenies at the current time. i. Pacioni & Sharp (2000), ii. Peintner *et al.* (2001), iii. Hibbett *et al.* (2005) http://mor.clarku.edu, iv. Matheny & Bougher (2006b), v. Matheny *et al.* (2006), vi. Hosaka *et al.* (unpublished) vii. This thesis.**

Francis & Bougher (2004) described four new sequestrate cortinarioid species. *Cortinarius sebosus*, *C. walpolensis*, *Descomyces angustisporus* and *Quadrispora tubercularis*. With the exception of *Q. tubercularis* (from which amplifiable DNA could not be obtained) these species have been included in the expanded molecular analyses presented in this study. Trappe & Claridge (2003) describe two further sequestrate cortinarioid fungi, *Cortinarius debbiae* and *Protoglossum niphophilum* from the Australian Alps and lower altitudes. A paper documenting the sequestrate fungi of Mt Wellington (Tasmania) is in preparation and further descriptions of novel sequestrate cortinarioid species are expected to arise from this and associated publications (Trappe *et al.* In preparation). It is anticipated that as environments previously unexplored for sequestrate fungi are surveyed more species will continue to be discovered for some time into the future.

Concurrently with rising awareness of the roles of fungi in ecosystems, molecular technology has emerged as a promising tool for the investigation of fungal taxonomy and ecology. Linking identified fungi to molecular sequences from mycelium in the soil or on roots is potentially a major tool for integrating molecular distribution/association data into our understanding of ecosystem functioning. This technology is already being applied for fungi of Australian ecosystems (*e.g.* Glen *et al.* 2001a & b). The ability to

identify fungi from vegetative mycelium is an especially promising development for the study of sequestrate fungi because of the difficulty of finding their often hypogeous fruit bodies.

Of the eleven genera containing sequestrate cortinarioid fungi as defined for the purposes of this thesis (Table 2), all occur in Australia, two genera (*Hysterogaster* and *Quadrispora*) and the sequestrate members of two others (*Auritella* and *Dermocybe*) are Australasian endemics. *Auritella* is currently known only from Australia and Africa Matheny & Bougher (2006a). There are currently 195 species of cortinarioid sequestrate fungi listed for the world (Table 2, CABI Bioscience Databases) of which 44 (approximately 20%) have been reported from Australia (Table 3). It is probable, given the richness of the Australian assemblage of these fungi, and the vast areas still relatively unexplored, that Australia possesses numerous undiscovered species of cortinarioid sequestrate fungi.

1.4 Classification of Cortinarioid Sequestrate Fungi

1.4.1 Early Developments

The classification of the sequestrate fungi builds on the raw material of the collections to hand using the technology, knowledge and theories current at the time. Early intuitive classifications grouped the sequestrate fungi now known to have affinities with genera among the Cortinariales, along with a variety of other sequestrate fungi in large polyphyletic groups (for some years also including ascomycetes). Vittadini (1831) proposed the first dedicated classification for sequestrate fungi, including the original description of the genus *Hymenogaster*. Vittadini (1831) classified the Tuberaceae of Fries' *Systema Mycologicum* into two subfamilies, the Hymenogastereae and the

Tubereae. The Hymenogastereae had three sections: Prototypi, Summi and Centrales which accommodated *Hymenogaster* (Vittadini 1831).

Berkeley (1840) pointed out that many of the Gastromycetes had basidia (not asci as had been generally supposed to that time). This observation, along with monographic works by Tulasne & Tulasne on the Nidulariaceae (Tulasne & Tulasne 1844) and the Hymenogastrales (Tulasne & Tulasne 1851) contributed to the exclusion of the sequestrate Ascomycetes, such as *Tuber*, from the Gasteromycetes (*c.f.* Cunningham 1944). Tulasne & Tulasne (1851) also proposed the existence of evolutionary links between hypogeous and epigeous ascomycetes (*i.e.* Tuberales and Pezizales). Parallel links among the Basidiomycota were not proposed until considerably later and the Gastromycetes, minus the ascomycetes, continued to be classified as a distinct basidiomycete lineage. In his *Outlines of British Fungology*, Berkeley (1860) produced a system of classification in accordance with this understanding of the Gastromycetes, including them at the rank of family. Within the Gastromycetes, Berkeley included six genera besides *Hymenogaster* in the Hypogaei.

In the later part of the 19th Century, continuing examination of the structure and development of fungi elucidated more characteristics uniting basidiomycete taxa than had been recognised previously. Microscopic features were also gradually incorporated into classifications of the Basidiomycetes (*e.g.* Fischer (1900) included basidial and hymenial characteristics in his system of classification). By the early 20th century, mycology and the study of sequestrate fungi had advanced to a point where some authors began to acknowledge the artificial nature of contemporary gastromycete classifications and anticipate major revisions in the taxonomy of the Hymenogasteraceae *s. lat.* (*e.g.* Rodway 1912a).

1.4.2 Bridging the gap between agaricoid and gasteroid fungi

During the 20th century micro-morphology, chemotaxonomy and developmental studies engendered new and increasingly natural fungal classifications, identifying sequestrate fungi with affinities to various agaricoid taxa, including *Cortinarius*. Sequestrate and agaricoid taxa united by obvious and consistent characters, such as the amyloid spore ornamentation and peridial sphaerocysts of the 'astrogastraceous series' (*e.g.* Heim 1934, Malençon 1931, Singer & Smith 1960), were first to be linked in basidiomycete 'evolutionary series'. Such theories strengthened the expectation that other sequestrate and agaricoid taxa would be related in a similar fashion. However, Cunningham's monographic work of Cunningham (1944), *The Gasteromycetes of Australia and New Zealand*, illustrates the continuing tendency around this time, despite such theories, to treat the Gasteromycetes as a single taxonomic unit. According to this work the order Hymenogastrales was comprised of two families, the Hymenogasteraceae and the Secotiaceae. The Hymenogasteraceae had two subfamilies and two named tribes including, alongside *Hymenogaster*, eight genera since found to be non-cortinarioid.

Recognition of the links between sequestrate fungi and agaricoid forms intensified in the mid to late 20th century. For example Singer (1951) incorporated a discussion on the similarities between *Cortinarius* and the new secotioid genus *Thaxterogaster*. Singer & Smith (1959) suggested a possible relationship between the secotioid *Setchelliogaster* and the agaricoid *Conocybe* (this connection to the Bolbitiaceae is noteworthy as Kirk *et al.* (2001) and Matheny *et al.* (2006) also classify *Descolea* in this family see Figure 2). Smith & Singer (1959) also detailed a series related to *Boletus* and *Suillus*, including *Rhizopogon* and *Chamonixia*, two genera formerly aligned with

Hymenogaster (*e.g.* Cunningham 1944). The discovery of sequestrate *Cortinarius* species (*e.g.* Thiers & Smith 1969) contributed to the understanding of the diversity of basidiome forms among the cortinarioid sequestrate fungi. A major factor contributing to the retention of sequestrate forms in artificial taxa, such as the Gastromycetes, were theories suggesting that agaricoid forms had developed from gasteroid ancestors (*e.g.* Singer 1975). These theories were challenged by discoveries concerning morphological plasticity of sequestrate basidiomes. In the late 1960s culturing techniques led to the observation of mating intercompatibility between secotioid and agaricoid forms (*e.g.* Rosinski & Robinson 1968). Similarly, evidences of morphological plasticity have been found among the cortinarioid sequestrate fungi. For example, Lago *et al.* (2001) demonstrated a high degree of phenotypic plasticity within single collections and axenic cultures of *Setchelliogaster* and *Descolea* species. Such observations blurred traditional taxonomic boundaries based on basidiome morphology. Fruit body development is thought to be under relatively simple genetic control and hence potentially frequently mutated (Bruns *et al.* 1989, Hibbett *et al.* 1994). This means that some sequestrate basidiome forms, previously used to distinguish taxa (*e.g.* secotioid *Thaxterogaster* from agaricoid *Cortinarius* species) appear to have arisen more than once, making taxa so defined polyphyletic (Peintner *et al.* 2001, Peintner *et al.* 2002). Such research into the sequestrate fungi continued to raise questions about the boundaries between agaricoid and sequestrate genera and, more broadly, the grounds for maintaining the Gastromycetes as a distinct taxonomic entity.

Significant changes to the way in which sequestrate fungi are classified were set in motion by discoveries of the 1970s. Increasing recognition of the multiple origins of the sequestrate fungi provided an impetus for the reassessment of polyphyletic groupings such as the Gastromycetes. Extensive collection of fungi in the Southern

Hemisphere begun during this period also highlighted the richness and uniqueness of the region's sequestrate fungal flora (Lebel & Castellano 1999, Bougher & Lebel 2001).

The 1980s was a period of seminal discussion on the links between agaricoid and sequestrate genera, including summations of the then known (or suspected) evolutionary series (*e.g.* Singer 1975, Thiers 1984). Thiers (1984) named *Hymenogaster* as the closest gasteroid form to the Cortinariaceae, as did Singer (1975), building on works by Singer, Smith and others (*e.g.* Singer 1958, Smith 1973). Jülich (1981) formulated a system of classification that, acknowledging the links between non-sequestrate and sequestrate forms, raised a number of groups to ordinal level, including the Cortinariales. Since this work, the cortinarioid sequestrate fungi have been placed either in the Cortinariales or retained in the order Hymenogastrales. For example, (Beaton *et al.* 1985a & b) dealt with the sequestrate Cortinariales of Victoria, including *Setchelliogaster* and *Thaxterogaster* in the Cortinariaceae and *Hymenogaster* in the Hymenogasteraceae.

Bougher & Castellano (1993) delimited *Hymenogaster s.s.* by excluding four of the eight species originally included by Vittadini (1831) and proposing four new genera, *Cortinomyces*, *Descomyces*, *Quadrispora* and *Timgrovea*. *Hymenogaster s. lat.* had encompassed a very mixed bag of species lumped together on the basis of having brown, ornamented and/or perisporial spores (Pegler & Young 1987). Bougher & Castellano (1993) reassessed the previously dominant view that the genus *Hymenogaster s. lat.* represented the most reduced form of the evolutionary series related to *Cortinarius*. Their paper demonstrated the polyphyletic nature of *Hymenogaster s. lat.* indicating that only a subset of species formerly included in this group (designated *Cortinomyces*) were likely to be closely aligned with *Cortinarius*.

Cortinomyces was later deemed invalid as the Type species chosen for it, *Protoglossum luteum*, had been used to typify *Protoglossum* by Massee in 1891 (May 1995).

The Australian Biological Resources Study (ABRS) commenced the *Fungi of Australia* series in 1996. This project has provided a major impetus to fungal taxonomy in Australia including Walker's classification of 1996. Walker (1996) is unusual among modern classifications in that, rather than attempting to develop the most natural classification possible, Walker maintains the artificial Euholobasidiomycete 'Group 1' (the Gastromycetes). Grgurinovic's *Larger Fungi of South Australia*, published in 1997, used the concept of the Cortinariales *sensu* Jülich (1981). Grgurinovic (1997) included the genera *Setchelliogaster* and *Thaxterogaster* (Cortinariaceae) in the Cortinariales but, in keeping with Cleland (1934) and Jülich (1981), the Hymenogastrales was reserved for gasteroid taxa.

Modern classifications of the higher fungi have treated *Cortinarius* and related genera in various ways. Higher taxa containing *Cortinarius* have been variously designated at the levels of order (Cortinariales Jülich), family (Cortinariaceae R. Heim ex Pouzar) and tribe (Cortinarieae *e.g.* Singer 1975). The degree to which these classifications integrate sequestrate forms into predominantly agaricoid higher taxa also varies. Some classifications maintain the sequestrate genera independently of their agaricoid relations but note their affinities (*e.g.* Singer 1975, Walker 1996). Following the lead of Jülich (1981) other classifications have further highlighted relationships between sequestrate and agaricoid cortinarioid taxa by including orders containing, for example, both *Hymenogaster* and *Cortinarius* in the Cortinariales (*e.g.* Pegler *et al.* 1993). Thus, largely based on morphological methods, traditional taxonomy has identified some

sequestrate taxa that appear to be more closely related to agaricoid Cortinariales than to other sequestrate or agaricoid genera.

1.4.3 The Molecular Age

Techniques for analysing DNA and amino acid sequence data have made additional characters available to systematic mycology and provided new insights into the evolutionary relationships of, and the diversity among, the cortinarioid sequestrate fungi. Molecular work on basidiomycete phylogeny has generally either focussed on elucidating the broad picture of evolutionary relatedness among the higher taxa, or the relationships among smaller components thereof. For example Hibbett & Thorn (2001) present a classification of the Homobasidiomycetes, synthesising the results of published molecular studies to eight major clades (monophyletic groups) derived from a previous study by Hibbett *et al.* (1997). The eight clades are the euagaric (which includes the cortinarioid taxa), bolete, russuloid, polyporoid, thelephoroid, gomphoid-phalloid, cantharelloid and hymenochaetoid clades. This work provided further support for the theory that a number of basidiome forms formerly used to distinguish taxa (*e.g.* the structure of the hymenophore: gilled, pored, toothed *etc*.) had arisen more than once. Binder & Hibbett (2002) supported the groups resolved in Hibbett & Thorn (2001) demonstrating that the bolete clade is the sister taxon of the euagaric clade.

Works examining higher taxonomic levels have used only a relatively few 'representative' taxa (sometimes only one) to represent higher taxonomic groupings. For example Binder & Hibbett (2002) use only *Cortinarius iodes* Berk. & M.A. Curtis to represent all the cortinarioid fungi, agaricoid or otherwise. Moncalvo *et al.* (2002) deals with the euagaric clade identified in Binder & Hibbett (2002) and Hibbett & Thorn

(2001), citing the work of Peintner *et al.* (2001) and others with regard to the position of sequestrate taxa nested within the euagaric clade. The '/cortinarioid clade' is poorly supported in Moncalvo *et al.* (2002), as are many other clades along the 'spine' of their tree. This means that their analysis could not confidently resolve the position of the /cortinarioid clade relative to any of the other euagaric clades, including those recovered by Peintner *et al.* (2001), and other cortinarioid agaric taxa. The large size of the data matrix used (number of characters multiplied by the number of taxa) is given as the primary reason for this. Nevertheless, these works support the contention that relationships exist between sequestrate and agaricoid fungi as suggested by morphology, and propose some links that were not previously suspected [*e.g.* the nesting of the Lycoperdales within the Agaricaceae Moncalvo *et al.* (2002)].

Within the euagaric clade, these 'larger scale' studies often place cortinarioid taxa close to clades containing representatives of the Hydnangiaceae and Pluteaceae (Hibbett *et al.* 1997, Binder & Hibbett 2002, Moncalvo *et al.* 2000, Figure 1). Other molecular studies, focussed more at the rank of family and below, indicate that sequestrate forms have been derived a number of times from within predominantly agaricoid clades including cortinarioid fungi, rather than representing an ancestral state (*e.g.* Peintner *et al.* 2001 *c.f.* Singer 1975). Molecular studies examining purportedly cortinarioid taxa have already effected changes in the taxonomy and circumscription of the cortinarioid sequestrate fungi. For example, molecular technology has shown the Gautieriaceae to have affinities to the Gomphales and Phallales, leading to the separation of this family from the Hymenogasteraceae (Humpert *et al.* 2001, Hosaka *et al.* unpublished). Lebel (2006) has also shown that *Cribbea* is more closely related to *Xerula* Maire than *Cortinarius* (*c.f.* Kirk *et al.* 2001). Bougher & Castellano (1993) could not relate *Hymenogaster s.s.* to any agaricoid genus based on morphology however molecular data

has linked this cortinarioid genus to *Hebeloma*/*Naucoria* Peintner *et al.* (2001) and more recently *Alnicola* Moreau *et al.* (2006). Arguments for the inclusion of the *Descolea-Setchelliogaster-Descomyces* complex among the cortinarioid fungi have also been influenced by molecular studies. Martin & Moreno (2001) and Peintner *et al.* (2001) include the complex, the /cortinarioid and /bolbitioid clades are not strongly associated in Moncalvo *et al.* (2002) however Matheny *et al.* (2006) indicates that *Descolea* and allies *are* in fact more closely related to *Bolbitius* and related genera – despite ultrastructural differences highlighted by Bougher (1987). These examples highlight the usefulness of ITS molecular data in distinguishing phylogenetically distinct lineages of cortinarioid fungi but raise questions about the reliability of relationships discerned using only this region when compared with multiple gene phylogenies.

Peintner *et al.* (2001) provides a molecular phylogeny of the cortinarioid sequestrate fungi as defined by Bougher & Castellano (1993), Singer (1951), Singer & Smith (1963), Thiers (1984) and Thiers & Smith (1969), based on nuclear rDNA sequences from the Internal Transcribed Spacer (ITS) region. The Peintner *et al.* (2001) phylogeny supports the delimitation of *Hymenogaster s. lat.* by Bougher & Castellano (1993). The phylogeny indicates that all the genera described in Bougher & Castellano (1993), except *Timgrovea* (which was not examined), were nested within their target group of cortinarioid taxa. However it should be noted that Peintner *et al.* (2001) defined their outgroup (*Gymnopilus* P. Karst.) on the basis of sequence alignability and an assumption that the ectomycorrhizal habit was monophyletic among the genera of the Cortinariaceae as defined. Matheny *et al.* (2006) and Rees *et al.* (2003) both disperse ectomycorrhizal cortinarioid taxa among non-ectomycorrhizal and even non-cortinarioid taxa supporting the contention that the ectomycorrhizal habit is

evolutionarily unstable Hibbett *et al.* (2000) and hence unsuitable as a criterion for defining an outgroup for the Cortinariaceae as defined by Peintner *et al.* (2001). Peintner *et al.* (2001) found *Hymenogaster s.s.* to be most closely related to species of *Hebeloma* and *Naucoria*. This supports statements by Bougher & Castellano (1993) indicating that *Hymenogaster* was not the most reduced form of the *Cortinarius-Thaxterogaster* complex. Peintner *et al.* (2001) also support the contention that *Cortinarius* itself is paraphyletic, and indicate that distinct lineages within *Cortinarius* (often representing components of morphologically defined subgenera *e.g.* /*myxacium1 etc.*), and related genera, have given rise to different sequestrate forms, as first suggested by Bougher & Castellano (1993). Peintner *et al.* (2002) collapsed *Thaxterogaster* into *Cortinarius* citing the ITS phylogenies of Peintner *et al.* (2001) that supported earlier theories that *Thaxterogaster* is polyphyletic (*e.g.* Moser 1964, Horak & Moser 1965). It now seems likely that a range of sequestrate taxa will be aligned to subgenera of *Cortinarius* and other cortinarioid agaricoid taxa. Indeed this process has already begun with the recombination of *Cortinarius globuliformis* as *Dermocybe globuliformis* (Bougher & Malajczuk 1986, Bougher & Trappe 2002). Integrating studies examining smaller numbers of taxa, with each other and with larger studies such as Moncalvo *et al.* (2002) and Matheny *et al.* (2006), should provide better resolution of interrelationships between diverse taxa, including the cortinarioid sequestrate fungi.

1.5 Experimental approach

This thesis sought to test the hypothesis that numeric analysis of selected morphological characters could provide the same picture of the diversity of, and relationships among, the cortinarioid sequestrate fungi as recovered from phylogenetic analysis of rDNA sequence data. The experimental approach chosen involved the application a set of four

analysis techniques to each of three datasets: morphological molecular and combined. Because of this approach, both the raw material – the herbarium collections examined – and the types of analyses applied are common between the different datasets (though parameters varied). These commonalities are dealt with in the following section.

Table 3 Published cortinarioid sequestrate fungi of Australia taken from May & Wood (1997), May *et al.* (2004) and subsequent publications as indicated.

NAME AND REFERENCE
Auritella geoaustralis Matheny & Bougher, *Mycol. Prog.* 5:6-8 (2006), *Mycotaxon* 97:231-233 (2006)
Cortinarius basipurpureus (Bougher) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 178 (2002)
Cortinarius campbelliae (Berk. & Broome ex Zeller & C.W.Dodge) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 179 (2002)
Cortinarius cunninghamii (E.Horak) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 179 (2002)
Cortinarius debbiae Trappe & Claridge, *Australas. Mycol.* 22: 32 (2003)
Cortinarius deminutus Peintner *in* Kuhnert-Finkernagel & Peintner, *Mycotaxon* 87: 119 (2003)
Cortinarius flavovelus (Grgur.) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 179 (2002)
Cortinarius fragilis (Zeller & C.W.Dodge) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 180 (2002)
Cortinarius leucocephalus (Massee) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 180 (2002)
Cortinarius levisporus (Massee & Rodway) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 180 (2002)
Cortinarius luteirufescens (Bougher) Peintner & M.M. Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 180 (2002)
Cortinarius orphinus (G.W.Beaton, Pegler & T.W.K.Young) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 181 (2002)
Cortinarius piriforme (Cleland & G.Cunn.) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 181 (2002)
Cortinarius porphyroideus Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 182 (2002)
Cortinarius scabrosus (Cooke & Massee) Peintner & M.M.Moser *in* Peintner, Moser & Vilgalys, *Mycotaxon* 81: 182 (2002)
Cortinarius sebosus Francis & Bougher, *Australas. Mycol.* 23: 6 (2004)
Cortinarius walpolensis Francis & Bougher, *Australas. Mycol.* 23: 8 (2004)
Dermocybe globuliformis (Bougher) Bougher & Trappe, *Australasian Mycologist* 21(1): 1-3 (2002)
Descomyces albellus (Massee & Rodway) Bougher & Castellano, *Mycologia* 85: 282 (1993)
Descomyces albus (Klotzsch) Bougher & Castellano, *Mycologia* 85: 280 (1993)
Descomyces angustisporus Francis & Bougher, *Australas. Mycol.* 23: 15 (2004)
Descomyces giachinii Trappe, V.L.Oliveira, Castellano & Claridge *in* Giachini et al., *Mycologia* 92: 1172 (2000)
Hymenogaster aureus Rodway, *Pap. & Proc. Roy. Soc. Tasmania* 1923: 152 (1924)
Hymenogaster fuligineus G.Cunn., *New Zealand J. Sci. Technol., ser. B*, 22: 299 (1941)
Hymenogaster lycoperdineus Vittad., *Monogr. Tuberac.* 22 (1831)

Table 3 Published cortinarioid sequestrate fungi of Australia taken from May & Wood (1997), May *et al.* (2004) and subsequent publications as indicated.

NAME AND REFERENCE
Hymenogaster monosporus E.L.Stewart & Trappe, *Trans. Brit. Mycol. Soc.* 65: 331 (1975)
Hymenogaster nanus Massee & Rodway *in* Massee, *Bull. Misc. Inform. Kew* 1899: 180 (1899)
Hysterogaster fusisporus (Massee & Rodway) Zeller & C.W.Dodge *in* Dodge, *Compar. Morph. Fungi* 488 (1928)
Hysterogaster tasmanicus (G.Cunn.) G.W.Beaton, Pegler & T.W.K.Young, *Kew Bull.* 40: 590 (1985)
Protoglossum cribbiae (A.H.Sm) T.W.May, *Muelleria* 8: 287 (1995)
Protoglossum luteum Massee, *Grevillia* 19: 97 (1891)
Protoglossum niphophilum Trappe & Claridge, *Australas. Mycol.* 22: 32 (2003)
Protoglossum purpureum (J.W.Cribb) T.W.May, *Muelleria* 8: 288 (1995)
Protoglossum violaceum (Massee & Rodway) T.W.May, *Muelleria* 8: 288 (1995)
Protoglossum viscidum (Massee & Rodway) T.W.May, *Muelleria* 8: 288 (1995)
Quadrispora musispora Bougher & Castellano, *Mycologia* 85: 286 (1993)
Quadrispora oblongispora (G.W.Beaton, Pegler & T.W.K.Young) Bougher & Castellano, *Mycologia* 85: 286 (1993)
Quadrispora tubercularis Bougher & Francis *in* Francis & Bougher, *Australas. Mycol.* 23: 23 (2004)
Setchelliogaster australiensis G.W.Beaton, Pegler & Young, *Kew Bull.* 40: 169 (1985)
Setchelliogaster tenuipes (Setch.) Pouzar, *Česká Mykol.* 12: 34 (1958)
Timgrovea ferruginea (J.W.Cribb) Bougher & Castellano, *Mycologia* 85: 290 (1993)
Timgrovea macrospora (G.Cunn) Bougher & Castellano, *Mycologia* 85: 289 (1993)
Timgrovea reticulata (G.Cunn) Bougher & Castellano, *Mycologia* 85: 289 (1993)
Timgrovea subtropica (J.W.Cribb) Bougher & Castellano, *Mycologia* 85: 290 (1993)

1.5.1 Collections

535 collections of fungal fruit bodies were examined to generate the datasets analysed for the purposes of this thesis. Some of these fruit bodies were collected fresh, annotated and preserved as air-dried herbarium specimens for this project; others were pre-existing herbarium collections prepared, and annotated to differing degrees, by various collectors in the last *ca.* 30 years.

Pre-existing collections were loaned from, or examined at: PERTH (including ex CSIRO Forestry and Forest Products Mycology Herbarium Perth, Australia collections coded as 'H' or 'E' numbers), MEL, OSC, CANB, HO, the working collection of Prof. J. Trappe (coded as 'TRAPPE' numbers, eventually to be lodged at PERTH or the

public herbarium indicated in brackets after the number) and the working collection of Dr. T. Lebel (coded as 'TL' numbers, eventually to be lodged at MEL). Primary accession numbers and codes used in the analyses can be seen in Appendix 1.

The quality of collection annotation varied not only between collectors, but also between collections made by the same collector. Factors contributing to this may have included variable technology and taxonomic/mycological knowledge, differing opinions as to the importance of various characters or merely the limited time available to annotate each collection while fresh (a common occurrence when sampling fungal biodiversity). Variability in data available for each collection limits the usefulness of pre-existing herbarium vouchers for creating a comprehensive and uniform morphological dataset for comparative and numerical analyses. For example the characters needed to distinguish a particular fungus may not have been recorded, or recorded in sufficient detail or uniformity.

The complete list of collections examined is included as Appendix 1. Due to several factors, including availability and variable number and quality of collections, difficulty in successfully amplifying DNA and time constraints, it was not possible to include collections from all taxa in all analyses. However, collections representing the genera, *Cortinarius* (ex *Thaxterogaster* species), *Descomyces*, *Protoglossum*, *Quadrispora*, *Setchelliogaster* and *Timgrovea* are included in all analyses. Unfortunately *Protoglossum*, in particular, is under-represented as I chose to focus on the *Descolea*-like taxa (*Descomyces*, *Setchelliogaster* and *Timgrovea*) and this limited the time I could allocate to examination of *Protoglossum* collections.

1.5.2 Types of analysis employed

The four different types of analysis employed in the current study were cluster analysis, ordination, maximum parsimony analysis and Bayesian Markov chain Monte Carlo sampling (referred to as Bayesian analysis).

Cluster analysis and ordination are multivariate statistical methods for visualising associations within a dataset (Sneath & Sokal 1973, Everitt *et al.* 2001). These two methods may be considered phenetic measures, relating the various operational taxonomic units (OTUs) to one another by the similarity of their characters without the imposition of explicit hypotheses as to the evolutionary reasonableness or probability of any given similarity. Both cluster analysis and ordination have been employed in taxonomic investigations incorporating a variety of data sources and taxonomic groups including the numerical taxonomic analysis of the *Cortinarius argutus* complex by Brandrud (1996).

Maximum parsimony and Bayesian analysis apply different criteria to determine the optimal phylogenetic estimate or tree, either parsimony or likelihood based Bayesian posterior probabilities. Parsimony has been used extensively in fungal systematics including analysis of morphological, molecular and combined datasets (e.g. Tehler 1995). Parsimony bootstrapping is also one of the most commonly applied indicators of branch support, even on maximum likelihood trees, because it is computationally less demanding than maximum likelihood bootstrapping (Alfaro *et al.* 2003). Maximum parsimony was used in estimating the phylogeny of the sequestrate cortinarioid fungi by Peintner *et al.* (2001). Bayesian analysis has been used for the analysis of morphological characteristics among the fungi (Machol & Singer 1971) and increasingly in analyses of large molecular datasets because of the speed with which it

can carry out likelihood based analyses compared to maximum likelihood analysis (Alfaro *et al.* 2003) e.g. Matheny *et al.* (2006).

Chapter 2

Morphological dataset

2.1 Introduction

Gross fruit body morphology has traditionally been the basis of fungal taxonomy in general and particularly so for the sequestrate cortinarioid fungi. From relatively early in the 19th Century, similarities between certain groups of sequestrate and agaricoid fungi were noted (e.g. Tulasne & Tulasne 1851). Various authors have expanded upon these relationships including Thiers & Watling (1971), Singer (1958), Bougher & Castellano (1993) and Bougher *et al.* (1993). However it has really only been since the advent of molecular technology that it has been recognised just how diverse the multiple origins of the sequestrate fungi are. This has lead to the observation that many characters formerly used to define genera and subgenera among the cortinarioid fungi appear to be the product of convergence. Peintner *et al.* (2001) and subsequent researchers (e.g. Garnica *et al.* 2003a & b, Garnica *et al.* 2005, Frøslev *et al.* 2005, Moreau *et al.* 2006) have consistently recovered the diverse lineages associated with different sequestrate cortinarioid fungi. This suggests that just as morphologically based subgenera and sections appear to require re-assessment, so morphologically based sequestrate generic concepts also require re-assessment. To this end, this project aimed to assess the potential of commonly scored morphological characters to discriminate between groups defined on the basis of ITS sequence data.

Despite the finding that morphology (as traditionally, broadly defined) has often proved inadequate for the definition of monophyletic taxa of euagarics, certain morphological characters have been used to accurately distinguish molecularly distinct lineages. Chief

among these have been characters of the spores and peridiopellis. Bougher & Castellano (1993) represents the last major morphological revision of the classification of the sequestrate cortinarioid fungi. In this paper spore morphology and the structure of the peridiopellis were used to distinguish *Cortinarius*-like and *Descolea*-like species from those belonging to *Hymenogaster sensu stricto* for which no agaricoid link was suggested by Bougher & Castellano (1993). The distinctness of these three lineages has been supported by the molecular analyses of Peintner *et al.* (2001). It is this agreement that underpins the hypothesis that by selecting an appropriate set of morphological characters, phylogenetic patterns may be discerned directly via morphology.

In fungal taxonomy the 'subjective' comparison of qualitative characteristics has traditionally been used to recognise taxa. 'Subjective' here is used in the sense of the opinions of mycologists comparing and weighing up the various characters of a particular fungus often based on their experience of a wide variety of fungi. Phyletic taxon recognition refers to the definition of taxa based on 'peaks' in continuously varying characters (May 1991) and are relatively common among the sequestrate fungi [e.g. *Thaxterogaster* subgenus *Microsporogaster* Singer & Smith (1963)]. Multivariate methods are useful in discerning, patterns in the variation of many characters simultaneously (e.g. the molecular support for phyletic species concepts applied to Australian *Laccaria* species May (1991), G. Mueller pers. com. (2006). Such methods have also been used successfully to distinguish groups among the *Cortinarius argutus* complex Brandrud (1996).

Cladistics is the other method commonly used for the numeric analysis of morphological data. Cladistics aims to discern the most reasonable pattern of character development via construction of 'trees' which show arrangements of between-taxa

relationships and then assessment of the reasonableness of the different trees, based on a pre-defined optimality criterion (such as parsimony analysis) or likelihood based methods (such as maximum likelihood analysis or Bayesian analysis).

Continuous morphological characters must be recoded for use in cladistic methods, as these calculate tree scores based on changes between discrete characters. Recoding demands that the continuous variation of the character must be broken up, ideally into meaningful 'bins' that retain as much as possible of the information in the structure of the continuous data. Bins could be defined with reference to patterning in the data or according to a set number of equally sized bins.

2.2 Materials and Methods

2.2.1 Data collected

The morphological dataset comprised two sub-datasets: a preliminary examination of the 535 collections and a more detailed secondary examination of 42 collections considered to represent morphologically well-defined taxa and for which it was possible to sequence the ITS region.

2.2.1.1 Macroscopic characteristics of fresh specimens

For the herbarium collections examined in this study, the characteristics that had been most commonly noted for fresh fruit bodies were size, shape, colour and texture of the fruit body and its various parts/organs. Some collectors had taken or made pictures or drawings – sometimes with size and/or colour standards, and other times not. Where

colour codes for macroscopic features were recorded by the collector these have been converted to those of Kornerup *et al.* (1978) as necessary.

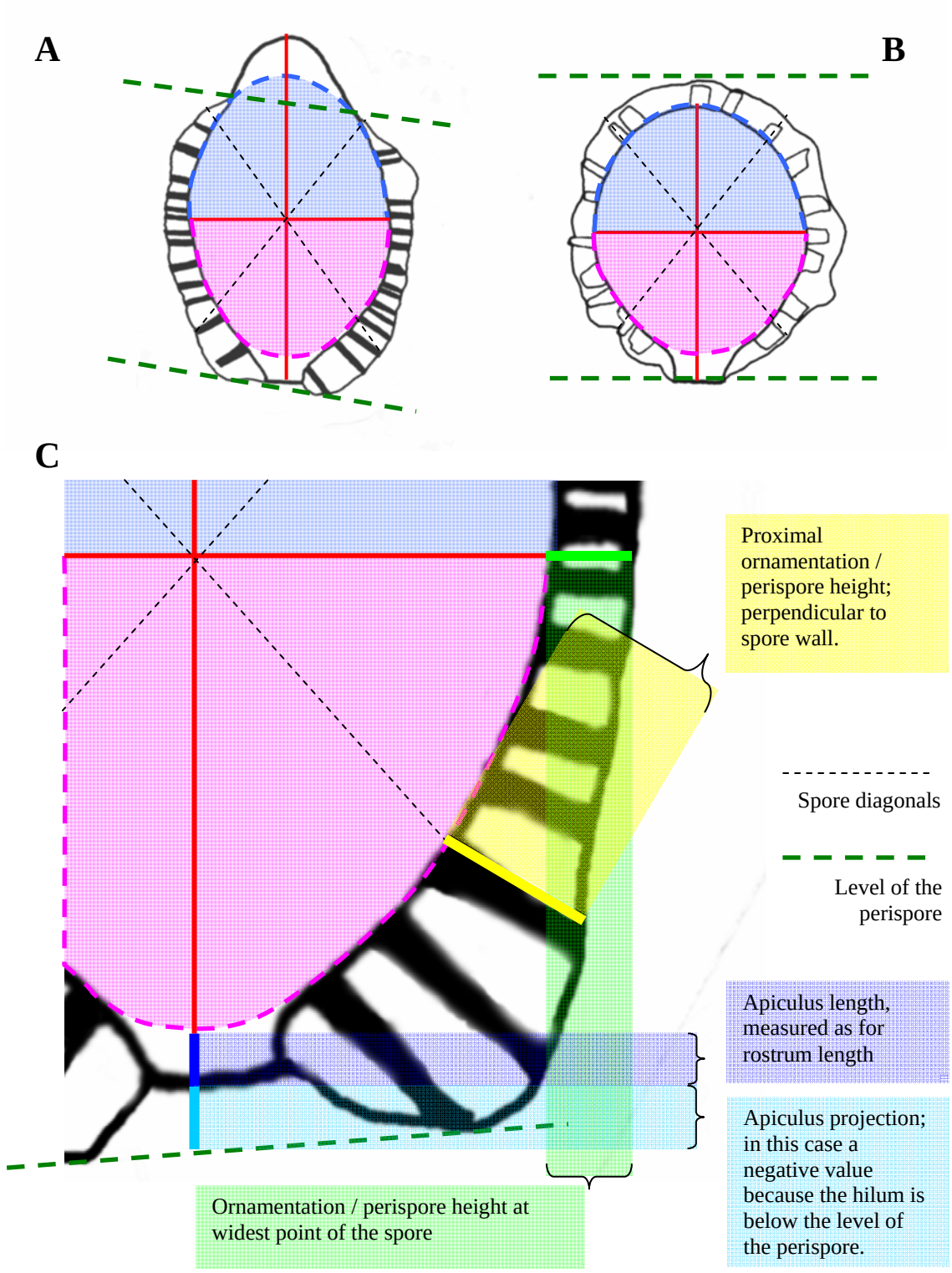


Figure 3: Diagrammatic representation of measurements made of microscopic characters of basidiospores

2.2.1.2 Macroscopic characters of air-dried specimens

Brief descriptions, and in some cases sketches, of the macroscopic characteristics of the air-dried specimens examined from each herbarium collection were made. The characteristics described were: peridium texture, colour and approximate thickness and the colour and structure of the gleba and any sterile tissue. The colours were recorded subjectively without the use of a colour standard because of anticipated artefactual variation due to the combination of the variable condition of specimens before drying, different drying times and temperatures, and artefacts of preservation such as adhering spore-mass or soil. These observations in combination with observations on the fresh specimens were used as an aid in developing the subjective morphological groupings the 'working genera' - used as labels in the figures for the various analyses.

2.2.1.3 Microscopic characteristics of air-dried specimens

For microscopic examination one or more specimens considered representative of each collection were cut transversely and several thin sections taken from the cut surface and edges to sample peridium, gleba and sterile tissues (e.g. columella). Detailed descriptions of measurements made are given in Table 4 and illustrated in Figure 3. The microscopic features of the sections (mounted in 3% KOH) were measured on 2000x scale line drawings prepared with an Olympus BH2–DA drawing attachment on an Olympus BHS microscope with 1.5x magnifier. Average spore dimensions were based on measurements of 20 or more spores. If the spores were asymmetrical 20 spores were measured in profile and 20 in face view. Average dimensions of basidia, hymenium elements or other hyphae were obtained from measurements of five of these elements and three peridial sections were measured to generate averages for peridial characters. Congo red was applied to visualise hyaline structures revived in 3% KOH (e.g. basidia)

either by immersing the section before mounting or by flushing the stain under the cover slip already mounted on the slide. Tissue and spore colours were determined in both water and Melzer's solution for sections mounted directly in these media. Spore length includes the hilar appendix but neither length nor width includes the ornamentation or perisporium. Shapes and terminology for spore ornamentation are according to Kirk *et al.* (2001). In this thesis and in a published taxonomic paper from the project (Francis & Bougher 2004), figures illustrating the characteristics of particular taxa show spores illustrated at 2000x, and other elements at 1000x magnification; figures illustrating both spores and other elements have separate bars indicating 10µm at the relevant scales.

Table 4: Details of measurements made of microscopic characters. Only those characters that have a bold code were included in the comparative analyses. Character codes shaded in grey indicate characters measured in the preliminary examination phase as well as the detailed examination phase

MICROSCOPIC CHARACTER	CODE	DESCRIPTION OF METHOD OF MEASUREMENT
AVERAGE SPORE LENGTH WITH APICULUS	SL	Mean spore length including the apiculus but excluding ornamentation and any visible perisporium.
AVERAGE SPORE WIDTH	**SW**	Mean spore width, measured at the widest point excluding ornamentation and any prominent perisporium.
RATIO OF SPORE LENGTH TO SPORE WIDTH	**QS**	SL/SW
AVERAGE ROSTRUM LENGTH	**RL**	Mean length of any apical distension (mucro/rostrum). This measurement was taken, excluding any ornamentation and prominent perispore, from the apex of the spore to the apex of an imaginary ellipse drawn such that its boundary continues the curve of the spore wall as shown by the blue shaded (apical) half-ellipse pictured in Figure 3A and B.
AVERAGE ROSTRUM PROJECTION	**RP**	Mean Rostral projection. This was the mean distance that any apical protrusion extends beyond the ornamentation/perispore measured from the spore apex to an imaginary line drawn across the spore joining most apical point on each side where the ornamentation/perispore appears to contact the spore wall. If there was negligible apical distension or the apex of the spore was heavily ornamented the measurement was the height of the perispore/ornamentation above the apex of the spore (see Figure 3A and B).

Table 4: Details of measurements made of microscopic characters. Only those characters that have a bold code were included in the comparative analyses. Character codes shaded in grey indicate characters measured in the preliminary examination phase as well as the detailed examination phase

MICROSCOPIC CHARACTER	CODE	DESCRIPTION OF METHOD OF MEASUREMENT
AVERAGE APICULUS LENGTH	**AL**	Mean length of the apiculus. The length of the apiculus was measured in the same way as the length of the rostrum, i.e. the distance from the hilum to the most basal point of an ellipse drawn to continue the curve of the spore wall (see the darker blue measurement in Figure 3C).
AVERAGE APICULUS PROJECTION	**AP**	Mean projection of the apiculus. This was measured essentially as for the rostral projection except that where the perispore flares around the apiculus (as in Figure 3A and C) the projection of the apiculus was considered negative and was the distance from the hilum to an imaginary line drawn between the most basal parts of the perispore/ornamentation on either side of the spore.
Distal ornamentation/perispore height		A measurement of the height of perispore/ornaments was taken on both sides of the spore drawing roughly at the 'corner of the spore' closest to the apex. This measurement varies with the attenuation of the spore and was essentially measured perpendicular to a tangent where the spore wall 'turns' (the 'shoulder' of rostrate spores corresponding with the 'corner' of non-rostrate spores) see Figure 3.
Proximal ornamentation/perispore height		A measurement of the height of perispore/ornaments was taken on both sides of the spore drawing at the 'corner of the spore' closest to the apiculus. This was measured as for distal ornamentation/perispore height but may be less indicative of the height of the ornamentation if the perisporium flares around the apiculus see Figure 3.
Ornamentation height at widest point		The height of the ornamentation at the widest point of the spore.
DISTRIBUTION OF PERISPORE	**PD**	Perispore distribution, an un-ordered, five-state categorical variable taking values: 0 – no perispore, 1 – proximal perispore or proximal perispore and perispore at widest point tallest, 2 – perispore at widest point tallest, 3 – distal perispore or distal perispore and perispore at widest point tallest, 4 – distal and proximal equal and greatest.
AVERAGE MAXIMUM HEIGHT OF PERISPORE	**MP**	Mean maximum perispore height. This was the maximum height of the perispore/ornamentation. This takes into account the possibility that the tallest ornaments may not always be measured by the other perispore measurements.
ORNAMENTATION TYPE	**OT**	Ornamentation an un-ordered categorical variable categorising the different types of ornamentation observed and taking values: 1 – smooth; 2 – isolated ornaments and isolated short ridges; 3 – partial reticulum; 4 – reticulate; or 5 – alveolate.
SPORES IN TETRADS	**SF**	An un-ordered categorical variable indicating if spores are released in tetrads and taking values: 0 – Spores not normally released in tetrads; or 1 – Spores normally released in tetrads.
AVERAGE BASIDIUM LENGTH	BL	Mean basidium length. The length of the basidia was measured from the base of complete basidia (i.e. where clamps or subtending cells were obvious) to the 'dome' of the basidium apex (around which the sterigmata are normally arranged).
AVERAGE BASIDIUM WIDTH	**BW**	The mean width of the basidia measured at the widest point.

Table 4: Details of measurements made of microscopic characters. Only those characters that have a bold code were included in the comparative analyses. Character codes shaded in grey indicate characters measured in the preliminary examination phase as well as the detailed examination phase

MICROSCOPIC CHARACTER	CODE	DESCRIPTION OF METHOD OF MEASUREMENT
RATIO OF BASIDIUM LENGTH TO BASIDIUM WIDTH	**QB**	BL/BW
AVERAGE NUMBER OF STERIGMATA	**SN**	Mean number of sterigmata per basidium
AVERAGE NUMBER OF PERIDIOPELLIS LAYERS	**PL**	Mean number of layers discerned in the peridiopellis. Treated as a seven state categorical variable for the range of values encountered in this study.
Width of peridiopellis layers		Drawn before major disruption of the section under the cover slip, usually at 200x. Layers numbered from closest to the hymenium outwards. Layer width taken as the distance from the 'average' boundary of the layer, taking into account any loose hyphae or visual artefacts of the thickness of the section that might artificially inflate the measurement.
AVERAGE PERIDIOPELLIS WIDTH	**PW**	Mean width of the peridiopellis normally measured on three sections, usually at 200x, as for width of peridiopellis layers and equalling the sum of individual layer widths. This measure was affected by artefacts of slide preparation despite being measured before major disruption of the section. The effects of any preparation-related artefacts may accentuate differences between different pellis structures.
Sub-hymenium		Measured as for peridiopellis layers. Thin, inner layers of the peridiopellis or the hymenophoral trama may in some cases be mistaken for sub-hymenial layers.
Hymenium		Measured as for peridiopellis layers. The length from the sub-hymenium to the layer of the spores or the height of the basidia if visible.
TYPE OF OUTER PERIDIUM LAYER	**OP**	An un-ordered categorical variable indicating the presence of particularly distinctive outer peridiopellis layers and taking values: 0 – gelatinised or non-gelatinised hyphae in a gelatinous hyaline matrix; 1 – gelatinised hyphae but no discernable hyaline matrix; 2 – Not distinctive; 3 – brown to golden, thin to thick-walled hyphae sometimes degraded (suspected degraded *Descomyces* type golden hyphae); or 4 – obvious *Descomyces* type "golden hyphae".

Table 5: Magnifications with a 10x ocular lens on an Olympus BHS microscope with an Olympus BH2–DA drawing attachment set at position "A" and with the magnifier set at 1.5.

OBJECTIVE LENS	MAGNIFICATION	EQUIVALENT
10x	200x	2mm ≡ 10µm
20x	400x	4mm ≡ 10µm
40x	800x	8mm ≡ 10µm
100x	2000x	20mm ≡ 10µm

2.2.2 Preparation of dataset

Measurements were made from drawings prepared at the scales indicated above in section 2.2.1.3, rather than directly under the microscope to facilitate the measurement of the thirteen measurements made on each spore (Table 4). These measurements, in millimetres, were converted to microns using the scale factors in Table 5. Histograms indicating the distribution of values for each quantitative character were developed using size classes ('bins'), the range of which was equal to the smallest interval between any two values for that character. The range of values for each character was then divided by ten to determine sizes for the 10 'bins' that would be used to recode the quantitative variables into a format that could be entered into both phylogenetic programs (ten being the maximum number of states the program MrBayes (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) can accept for "standard" characters). Histograms were constructed using the 10 bins developed in this way and compared with the original histograms to verify that they approximated the latter. To demonstrate the kind of approximations obtained, the initial histograms, and the '10-bin' approximations of these, for spore length (SL) and spore width (SW) are included in Appendix 6 as Figure 22a & b, and Figure 23a & b respectively. The quantitative characters were recoded as multi-state categorical variables using 10 bins for each quantitative character.

Spore length (SL) and basidium length (BL) were not included in the analysis as both the ratio of length to width, and the width of these organs were included. Spore width was chosen for inclusion rather than length because the length includes any apical elongation. Apical elongation appeared particularly associated with one of the

taxonomic groups under study and it was decided not to risk biasing the analysis by inclusion of this character. The other characters in Table 4 that were not included contributed to the values in one or more of the other characters for example ornamentation height at the distal end of the spore was incorporated into perispore distribution (PD).

All characters (including categorical variables such as ornamentation type) were standardised as a percentage of the maximum value for that character. This was to prevent the values of larger organs obscuring pattern in the values of the spores.

The morphological data was analysed either in a tab delimited text format or in a Nexus format file modified as appropriate for the programs PAUP*4.10b (Swofford 2001) or MrBayes (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003).

Two distance measures for morphometric analysis were used depending on the data being input. Euclidian distance was used with Ward's method of clustering to analyse continuous variables or 'standardised' (percentage of maximum) values. However, to account for missing values in the molecular data and any possible weightings, the "mean distance" as calculated by PAUP*4.10b (Swofford 2001) was used.

2.2.3 Analyses

2.2.3.1 Cluster analysis and Ordination

Cluster analysis was performed using the program PAST - *PA*laeontological *ST*atistics, ver. 1.53 (Hammer *et al.* 2001). The 'standardised' morphological data was clustered using the Euclidian pair-wise distances and both Ward's clustering method (the "error

sum of squares" method) and the Un-weighted Pair-Group Method using arithmetic Averaging (UPGMA) group-linkage method. The '10-bin' multi-state morphological data used in the phylogenetic methods was subjected to cluster analysis using the 'mean distances' calculated by PAUP*4.10b and UPGMA clustering. Trees were output in nexus format and arranged for display using TREEVIEW (Page 2001).

Ordination was also performed using PAST. The 'standardised' morphological data was first subjected to Principal Components Analysis (PCA) with a variance-covariance cross-products matrix and 'joint-plots' (biplots) of the 'coefficients' of the characters was superimposed on the centred scatter plots of both Axis one against Axis two and Axis three against Axis two. For comparison, Principal Coordinates analysis (PCO) using the Euclidian distance measure was also applied to the 'standardised' morphological data. PCO was carried out on the mean distances of the '10-bin' multi-state morphological data using the 'user distance' option in PAST.

2.2.3.2 Parsimony analysis

Parsimony analysis was carried out using PAUP*4.10b (Swofford 2001) on the '10-bin' multi-state morphological data with the following commands:

```
Set criterion=parsimony Maxtrees=250000 Increase=Auto
AutoInc=100 TaxLabels=full;
Hsearch start=stepwise addseq=random nreps=10 rstatus=yes;
```

The resulting trees were saved and strict and majority-rule consensus trees were calculated (Le50 was set to 'yes' so that groupings occurring in less than 50% of the trees would be included in the majority-rule tree provided they didn't contradict any groups already in the tree). Bootstrap support for the topology was determined by

Morphological dataset

10000 bootstrap replicates subjected to a heuristic "faststep" search where for each bootstrap replicate there is only one random-sequence-addition replicate and no branch swapping, using the following commands:

```
Bootstrap nreps=10000 brlens=yes format=nexus
treefile=filename.tre search=faststep;
```

2.2.3.3 Bayesian analysis

Bayesian analysis was carried out using the program MRBAYES (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). MRBAYES implements only one model for morphological ("standard") data (Ronquist *et al.* 2005) so except for the following commands the default settings were used:

```
Lset coding=variable
Mcmcp ngen=2000000 samplefreq=2000
```

The number of generations was increased by 2000000 or more until the standard deviation of split frequencies dropped below 0.01.

2.3 Results

2.3.1 Cluster analysis

The tree produced by Ward's clustering of the Euclidian distances of the 'standardised' morphological data is presented in Figure 4. Figure 4 shows the most prominent separation is between the *Cortinarius*-like collections (*Thaxterogaster*, *Cortinarius*, *Protoglossum* and *Quadrispora* specimens in clusters W, L, P, B and q1 and the single collections H5258 and H1486) and those that are more *Descolea*-like (*Setchelliogaster*,

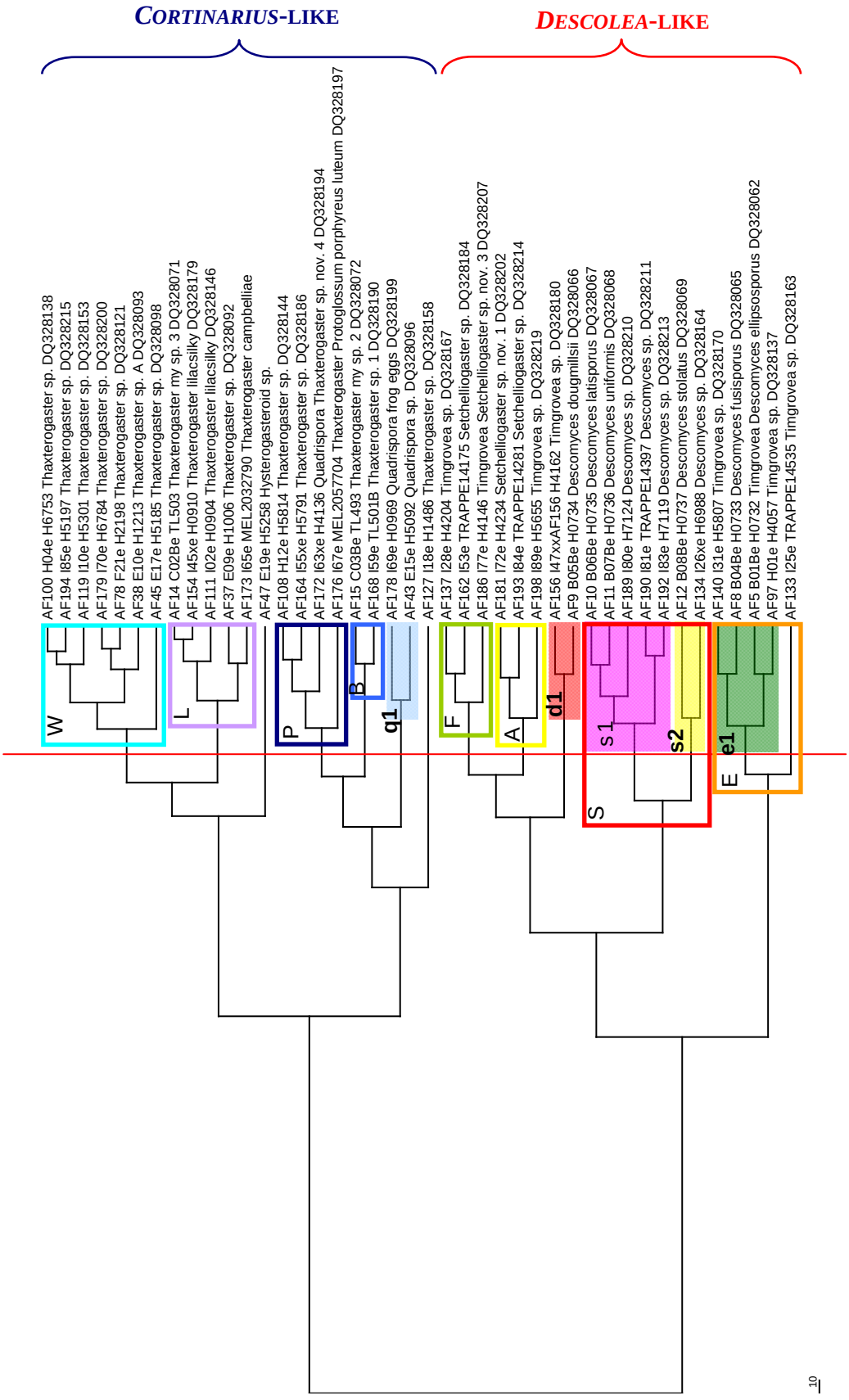
Timgrovea and *Descomyces* specimens in clusters F, A, d1, S and E) by more than 580 distance units.

There are two main clusters of *Cortinarius*-like collections in Figure 4, one characterised by smaller, elliptical, relatively lightly-ornamented spores and non-gelatinous peridia (clusters W and L) and the other by having larger spores, often having gelatinous peridia (clusters P, B and q1). Within the cluster of smaller-spored collections "hysterogasteroid" H5258 groups with two other clusters; one (W) comprising collections with dry whitish peridia resembling H5185 (with the exception of H6753, which has a purple peridium) and the second (L) comprising collections with silky, lilac to purple peridia (resembling H0904). The cluster of larger-spored collections comprises H1486, an unusually large-spored secotioid *Cortinarius* and three other clusters. The first (P) comprises collections with gelatinous peridia and more spherical spores (resembling H5791), though one of the collections (H4136), has more elongate-ellipsoid spores. The second two clusters had only two collections in each, the first (B) represented two collections with dry white peridia and quite large, coarsely ornamented, sub-spherical spores, while the other cluster (q1) was comprised of the two *Quadrispora* collections (H0969 and H0592).

The *Descolea*-like collections formed two major branches, one comprised of several smaller clades while the other contained only one cluster (E) of four collections (e1) and the single collection TRAPPE14535. Within cluster e1, two collections (H5807 and H0733) have elongate spores with isolated ornaments and the other two have reticulate to alveolate spore ornamentation as does TRAPPE14535 (*Timgrovea* subgenus *Timgrovea*). The sister branch to that containing E is divided in two, one branch comprised of three clusters (F, A and d1) and the other labelled S. Cluster F contains

broader-spored collections with isolated ornaments. Cluster A contains *Setchelliogaster* collections resembling *Setchelliogaster australiensis* and a small, partially-reticulate *Timgrovea* subgenus *Subreticulata* collection. The third cluster, d1, is marked in red and contains two squat-spored *Descomyces*. Cluster S has two major sub-clusters, s1 comprised of squat-spored collections with prominent rostra and s2 that contains *Descomyces* collections H0737 (with a prominent characteristic 'flaring' perispore) and H6988 (also squat-spored).

Figure 4: Cluster analysis dendrogram for Ward's clustering of the Euclidian pair-wise distances of the morphological data "standardised" as a percentage of the maximum value. Coloured boxes indicate clusters discussed in the text. The red line indicates the distance cut-off for this analysis


CORTINARIUS-LIKE
DESCOLEA-LIKE
AF100 H04e H6753 Thaxterogaster sp. DQ328138
AF194 I85e H5197 Thaxterogaster sp. DQ328215
AF119 I10e H5301 Thaxterogaster sp. DQ328153
AF179 I70e H6784 Thaxterogaster sp. DQ328200
AF78 F21e H2198 Thaxterogaster sp. DQ328121
AF38 E10e H1213 Thaxterogaster sp. A DQ328093
AF45 E17e H5185 Thaxterogaster sp. DQ328098
AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071
AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179
AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146
AF37 E09e H1006 Thaxterogaster sp. DQ328092
AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF47 E19e H5258 Hysterogasteroid sp.
AF108 H12e H5814 Thaxterogaster sp. DQ328144
AF164 I55xe H5791 Thaxterogaster sp. DQ328186
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF178 I69e H0969 Quadrispora frog eggs DQ328199
AF43 E15e H5092 Quadrispora sp. DQ328096
AF127 I18e H1486 Thaxterogaster sp. DQ328158
AF137 I28e H4204 Timgrovea sp. DQ328167
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214
AF198 I89e H5655 Timgrovea sp. DQ328219
AF156 I47xxAF156 H4162 Timgrovea sp. DQ328180
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF189 I80e H7124 Descomyces sp. DQ328210
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
AF192 I83e H7119 Descomyces sp. DQ328213
AF12 B08Be H0737 Descomyces stolatus DQ328069
AF134 I26xe H6988 Descomyces sp. DQ328164
AF140 I31e H5807 Timgrovea sp. DQ328170
AF8 B04Be H0733 Descomyces fusisporus DQ328065
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus DQ328062
AF97 H01e H4057 Timgrovea sp. DQ328137
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163
W
L
P
B
q1
F
A
d1
s1
s2
S
e1
E
10

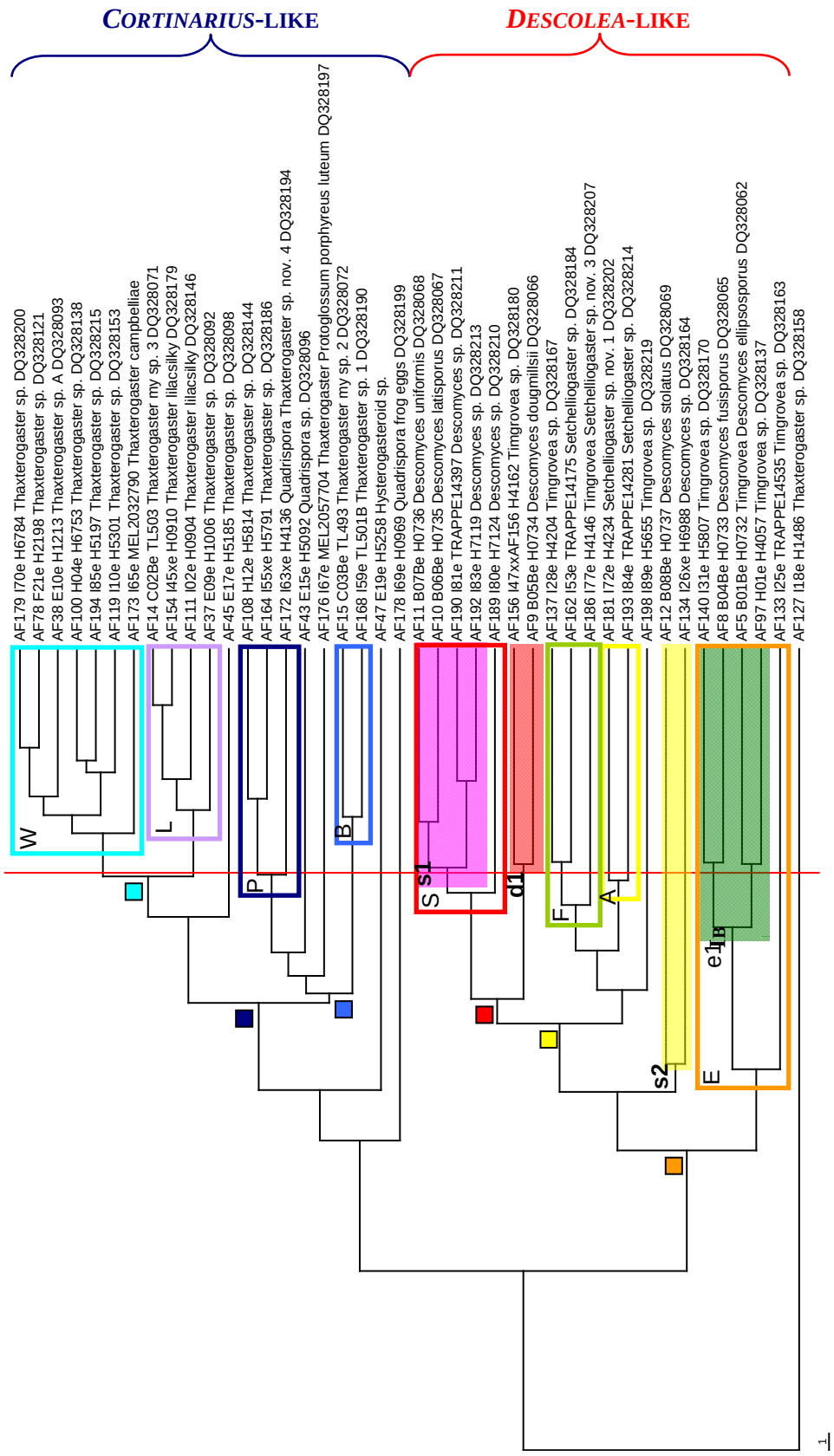

CORTINARIUS-LIKE
DESCOLEA-LIKE
AF179 I70e H6784 Thaxterogaster sp. DQ328200
AF78 F21e H2198 Thaxterogaster sp. DQ328121
AF38 E10e H1213 Thaxterogaster sp. A DQ328093
AF100 H04e H6753 Thaxterogaster sp. DQ328138
AF194 I85e H5197 Thaxterogaster sp. DQ328215
AF119 I10e H5301 Thaxterogaster sp. DQ328153
AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071
AF154 I45xe H0910 Thaxterogaster liliacsilky DQ328179
AF111 I02e H0904 Thaxterogaster liliacsilky DQ328146
AF37 E09e H1006 Thaxterogaster sp. DQ328092
AF45 E17e H5185 Thaxterogaster sp. DQ328098
AF108 H12e H5814 Thaxterogaster sp. DQ328144
AF164 I55xe H5791 Thaxterogaster sp. DQ328186
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194
AF43 E15e H5092 Quadrispora sp. DQ328096
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF47 E19e H5258 Hysterogasteroid sp.
AF178 I69e H0969 Quadrispora frog eggs DQ328199
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
AF192 I83e H7119 Descomyces sp. DQ328213
AF189 I80e H7124 Descomyces sp. DQ328210
AF156 I47xxAF156 H4162 Timgrovea sp. DQ328180
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
AF137 I28e H4204 Timgrovea sp. DQ328167
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214
AF198 I89e H5655 Timgrovea sp. DQ328219
AF12 B08Be H0737 Descomyces stolatus DQ328069
AF134 I26xe H6988 Descomyces sp. DQ328164
AF140 I31e H5807 Timgrovea sp. DQ328170
AF8 B04Be H0733 Descomyces fusisporus DQ328065
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus DQ328062
AF97 H01e H4057 Timgrovea sp. DQ328137
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163
AF127 I18e H1486 Thaxterogaster sp. DQ328158
W
L
P
B
S
s1
d1
F
A
s2
E
e1
1

Figure 5: Cluster analysis dendrogram for Un-weighted Pair-Group Method using arithmetic Averaging (UPGMA) clustering of the Euclidian pair-wise distances of the morphological data "standardised" as a percentage of the maximum value. Coloured boxes indicate comparable clusters to those found using Ward's clustering method and are discussed in the text. A paler box surrounding another indicates a cluster which occurred in the Ward's method clustering of the Euclidian distances but is not recognised by the distance cut-off in this analysis (the red line).

The tree produced by UPGMA clustering of the Euclidian distances of the 'standardised' morphological data is presented in Figure 5. The most prominent separation is that between collection H1486 (the abnormally large-spored secotioid *Cortinarius*) and all other collections by more than 60 distance units (note that the distance units are only for comparison *within* a particular analysis and not between analyses). The *Cortinarius*-like collections (clusters W, L, P, and B) are however well separated from the *Descolea*-like collections (clusters S, d1, F, A, s2 and E) by a distance of more than 15 distance units. The clusters produced under this clustering method cluster at relatively greater distances than under Ward's clustering method. Consequently a number of clusters are not recognised at the distance cut-off that maximises the recovery of clusters found by Ward's clustering method.

The two main clusters of *Cortinarius*-like collections found using Ward's clustering method are again visible in the dendrogram of the UPGMA clustering (Figure 5). Within the group characterised by dry peridia and relatively small lightly ornamented spores collection MEL2032790 is associated with cluster W of H5185-like collections with white peridia rather than being clustered with H1006 in cluster L of H0904-like collections with silky, lilac to purple peridia as in the Wards' method tree (Figure 4). The collection H5185 that was placed with cluster W in the Ward's method tree is basal to both clusters W and L in the UPGMA tree (Figure 5). The cluster of larger-spored collections comprises only two clusters in the UPGMA tree (Figure 5) and shows more

'chaining' (many single taxon clades all slightly more distant than the previous) and clusters at greater distances than in the Ward's method tree. Cluster P (resembling H5791 with gelatinous peridia and generally more spherical spores) including H4136 (elongate-ellipsoid spores) is represented in (Figure 5) with the addition of collection H5092 that was in cluster q1 in the Ward's method tree. However only collections H5814 and H5791 cluster within the distance cut-off (ca. 13 distance units) that recognises both clusters W and L in the smaller-spored cluster. Cluster B (representing two collections with dry white peridia and quite large, coarsely ornamented, sub-spherical spores) was recognised at the distance cut-off indicated in Figure 5. Cluster q1 seen in the Ward's tree (comprised of the two *Quadrispora* collections) was not present in Figure 5, these two collections being distributed among the other clusters. The "hysterogasteroid" collection H5258 occurs at the base of the cluster including clusters W, L, P, B and their associated single collections. *Quadrispora* collection H0969 subtends H5258 and is, in turn subtended by H1486 (the abnormally large-spored secotioid *Cortinarius*).

The *Descolea*-like collections form two major branches in the UPGMA tree (Figure 5). One branch is comprised of five smaller clades, while the other (labelled E) is composed of the same cluster of four collections, grouped in the same way as in the Ward's method tree. Collections H5807 and H0733 (elongate spores with isolated ornaments) and collections H0732 and H4057 (reticulate to alveolate ornamented *Timgrovea* subgenus *Timgrovea*) are within cluster E but the two groups do not cluster within the distance cut-off set for the UPGMA tree. TRAPPE14535 is within cluster E in both (Figure 5 and Figure 4). A branch grouping collections H6988 and H0737 (labelled s2) branches off basally to the sister cluster of E. This pair of collections was associated with cluster S in the Ward's method tree (*c.f.* Figure 4). The branch

subtended by s2 divides in two, each branch comprised of two clusters found in the Ward's method tree. One branch groups cluster F (broader-spored collections with isolated ornaments) and cluster A (*Setchelliogaster australiensis* collections and the partially-reticulate *Timgrovea* subgenus *Subreticulata* collection H5655). The other branch groups cluster d1 (squat-spored *Descomyces*) and cluster S of squat-spored collections with prominent rostra.

The dendrogram constructed using the 'mean distances' of the '10-bin' multi-state morphological data as calculated by PAUP*4.10b and clustered using the UPGMA method (Figure 6) had only 7 groups in common with either the Ward's or the UPGMA tree of the Euclidian distances of the 'standardised' data (Figure 4 and Figure 5). The longest branch however was that separating the *Cortinarius*-like collections (including clusters L, W and P) from the *Descolea*-like collections (including clusters S, E, F, e1a, d1 and A) by 0.13 distance units.

The *Cortinarius*-like collections can be considered to group into two main clusters similar to those observed in the other dendrograms, i.e. one group of smaller-spored collections with dry peridia (including clusters L and W) and a second of larger-spored collections more commonly with gelatinous peridia (the isolated collection H1486 and the cluster labelled P). Among the smaller spored collections, 'hysterogasteroid' collection H5258 is basal to two other clusters each containing one of the 7 groups common to this analysis and the other dendrograms. The first group contains a trichotomy including collections TL503 and H0910 that group together in cluster L in both the Ward's method and UPGMA trees of the Euclidian distances of the 'standardised' data. H6753 (the third member of the trichotomy) has a lilac peridium, as does H1213, which subtends the trichotomy but H5185 which subtends these four

collections has a white peridium. The other group of small-spored collections (labelled W) contains two groups, one consisting of another pair common to the other dendrograms: H6784 and H2198, both of which have white peridia as does H5031 that clusters with this pair. The other branch consists of H0904 with a lilac peridium and H5197 which has a white peridium. The cluster of collections with larger spores and commonly gelatinous peridia has one major cluster and five subtending single-taxon branches. The cluster contains two pairs of collections. One pair (H5814 and H5791) which have gelatinous peridia and sub-spherical spores, is found in cluster L in both the Ward's and the UPGMA tree of the Euclidian distances of the 'standardised' data and is marked in dark blue in Figure 6. The other pair in cluster P of Figure 6 is shown in lilac and contains a pair of collections with smaller spores and lilac peridia. These collections also belong to cluster L in Ward's method and UPGMA trees, and form a pair in the Ward's method tree though not in the UPGMA. The single taxon branches bearing collections H5092, MEL2057704, H4136, TL493, TL501B and H0969 were all grouped in the 'larger-spored' cluster in Figure 4 and Figure 5 (that containing clusters P, B and q1).

The *Descolea*-like collections are divided into five clusters and associated single-taxon branches at the distance cut-off chosen to maintain the division of the *Cortinarius*-like taxa into two major clusters (ca. 0.59 distance units). The first of these, S, is comprised of squat-spored collections with prominent rostra, as in the Ward's method tree of the Euclidian distances for the 'standardised' data. H6988 belongs to cluster S in Figure 6 and although it fits the above description it is clustered in s2 in Figure 4 and Figure 5. This cluster's sister group, labelled E, contains the other collection from the yellow cluster of the Ward's method tree (H0737), a collection with a characteristically flaring perispore and two collections (H5807 and H0733) with especially elongate spores.

These last two collections formed a pair in both the Ward's and UPGMA method trees for the standardised data, but not in the UPGMA tree of the '10-bin' data (Figure 6).

The third cluster of *Descolea*-like collections is made up of two of the seven pairs of collections found in more than one of the dendrograms. The first is found in cluster F in both Figure 4 and Figure 5 and contains broader-spored *Setchelliogaster* (TRAPPE14175) and putative *Timgrovea* (H4204). The other pair comprises H0732, an alveolate-spored *Timgrovea*/*Descomyces* (putative *Timgrovea* subgenus *Timgrovea*), and H4057, a quite broad-spored collection with irregular reticulate spore ornamentation. The pair (H0732 and H4057, labelled e1a) were in cluster E in both the preceding dendrograms. This clade is subtended by a single taxon H5655 (with the small, partially reticulate spores characteristic of *Timgrovea* subgenus *Subreticulata*). The following cluster is also found in both dendrograms of the Euclidian distances of the 'standardised' data as cluster d1 comprising squat-spored *Timgrovea* H4162 and *Descomyces* H0734. Two of the three collections labelled A in Figure 6 (H4234 and TRAPPE14175) are putative *Setchelliogaster australiensis* collections that form a pair in cluster A of both the other dendrograms though not in Figure 6. Instead H4234 forms a pair with broad, asymmetric-spored *Timgrovea*/*Setchelliogaster* collection H4146 within the distance cut-off indicated in Figure 6.

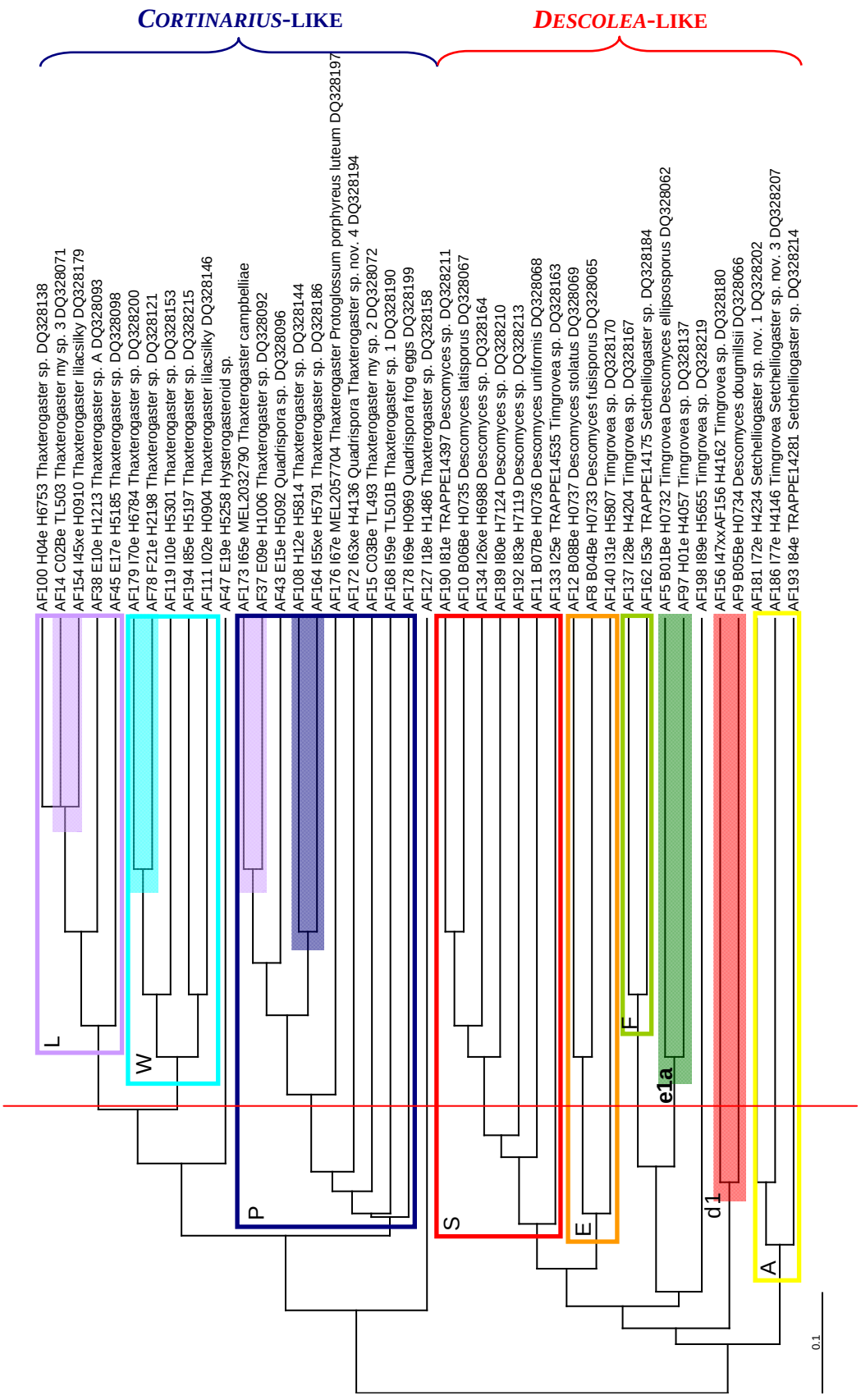
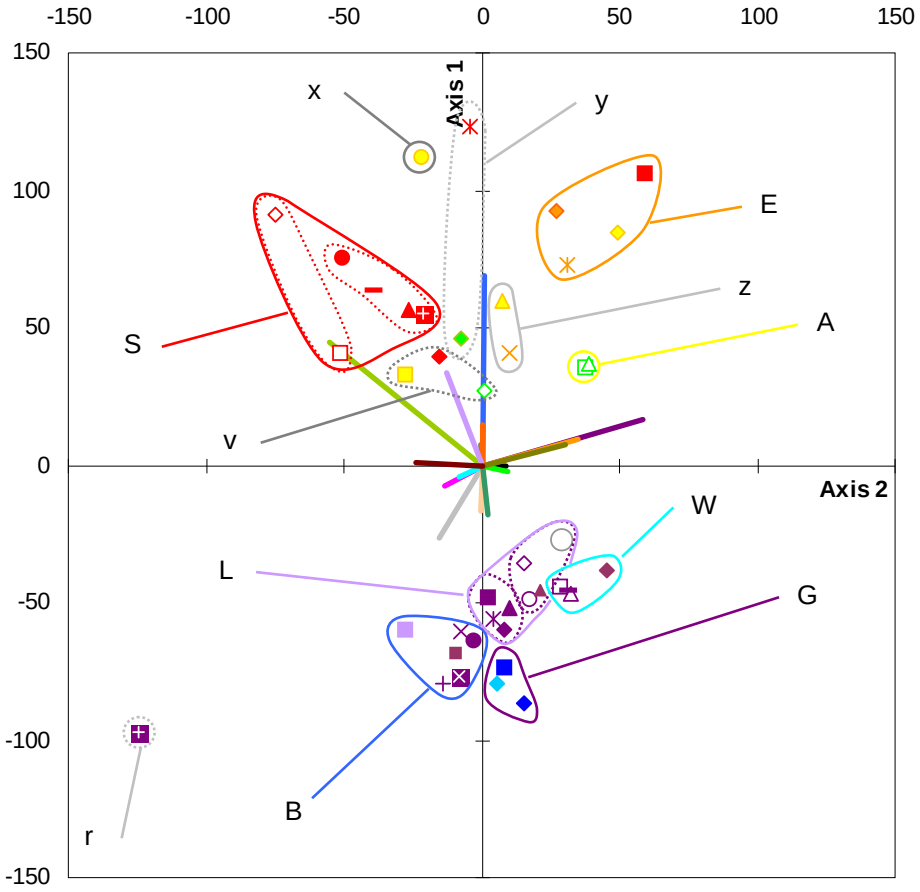


Figure 6: Cluster analysis dendrogram for UPGMA clustering of the Mean pair-wise distances of the '10 bin' multi-state morphological data. Coloured boxes and outlines indicate clusters discussed in the text. The red line indicates the distance cut-off for this analysis.

2.3.2 Ordination

The Principal Components Analysis (PCA) of the 'standardised' data produced the scatter and 'joint' plots shown in Figure 7. The percentages of the variance accounted for by each of the first three axes were: Axis one: 51.664%, Axis two: 14.058% and Axis three: 11.755%. The *Cortinarius*-like collections (purple, lilac, blue and grey symbols) are separated from the *Descolea*-like collections (red, orange yellow and green symbols) along Axis one though not along any other axis. The three variables with the largest coefficients for this axis are Rostrum Length (RL%), Rostrum Projection (RP%), and Outer Peridium type (OP%), remembering that the values used in this analysis are 'standardised' as percentages of the maximum value to minimise the influence of differences in scale of the characters (e.g. rostrum projection versus peridium width). The other major separation is of the unusually large-spored, secotioid *Cortinarius* H1486. The isolation of this collection from all others in ordination space is associated with large coefficients in the variables Apiculus Projection (AP%), Rostrum Projection (RP%), Maximum Perispore height (MP%), and number of Peridium Layers (PL%) and percentage ratio of basidium length to width (QB%). The distribution of *Cortinarius*-like collections and *Descolea*-like collections overlap in the scatter plot of Axis one against Axis two. The five variables with the greatest coefficients for these axes are Apiculus Projection (AP%), Apiculus Length (AL%), Spore Width (SW%), Peridiopellis Width (PW%) and Maximum Perispore height (MP%).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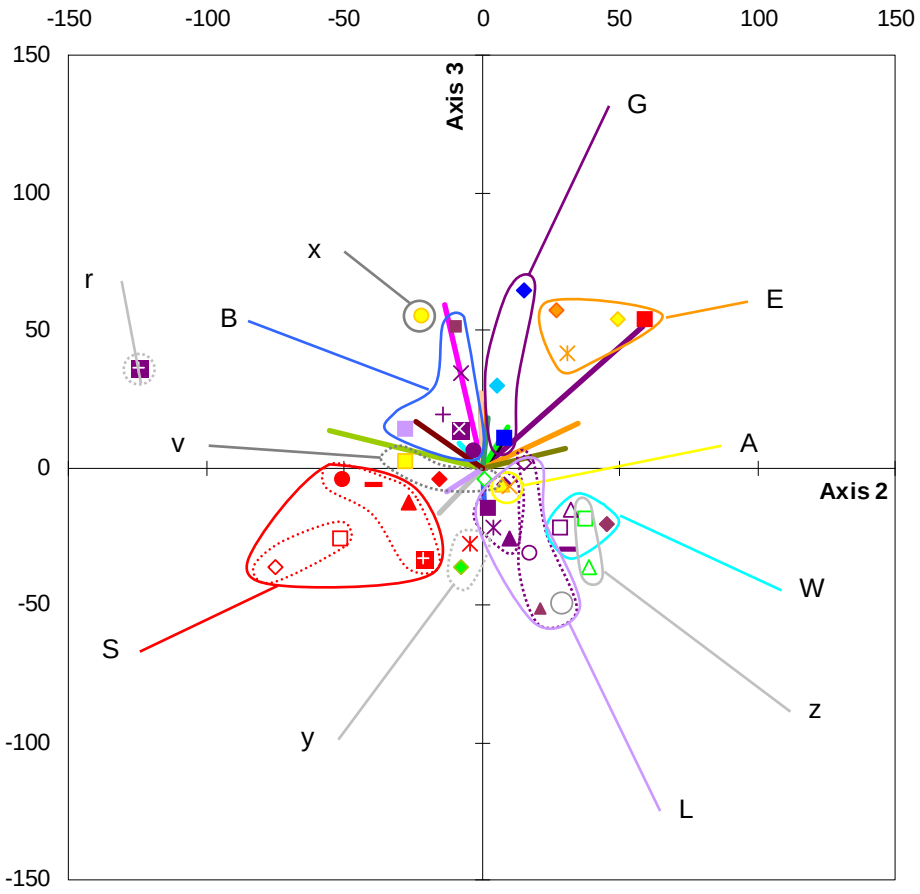
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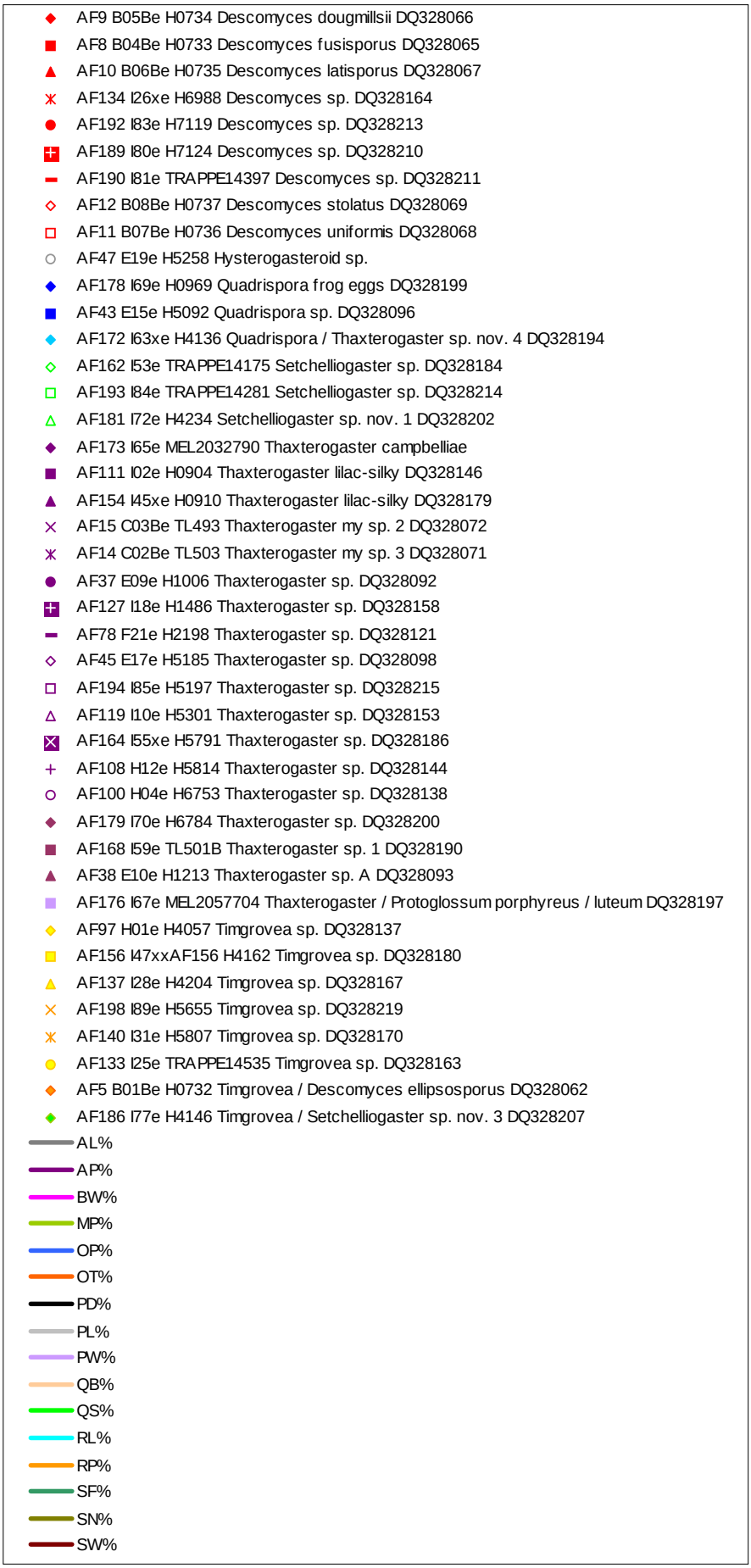


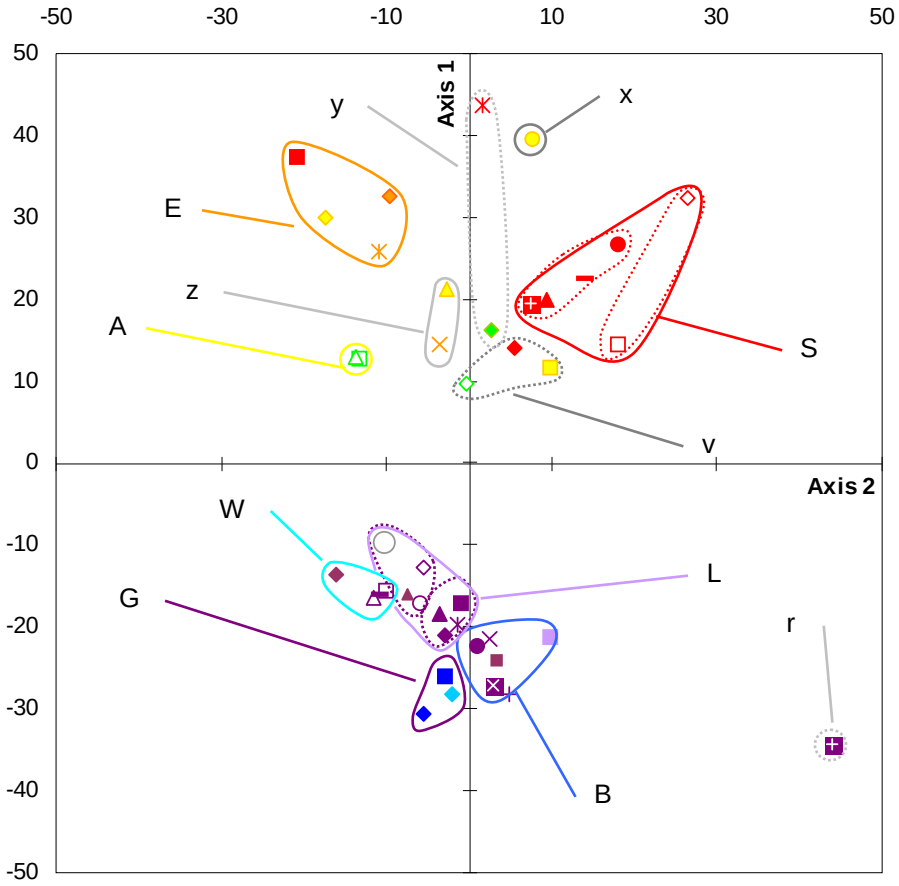
Figure 7: Principal Components Analysis (PCA) of the Euclidian pair-wise distances of the morphological data "standardised" as a percentage of the maximum value. Coloured lines are joint plot vectors of the coefficients of the axes multiplied by 100. The axes are in eigenvalue units.

Five groups of *Cortinarius*-like collections were discerned from examination of both scatter plots in Figure 7. The first is the isolated single collection H1486 as mentioned above (labelled r). The *Quadrispora* collections (H0969, H5092) and elongate spored '*Quadrispora*/*Thaxterogaster*' collection (H4136) group together (labelled G) as do '*Thaxterogaster*' collections TL493, H1006, H5791, H5814, TL501B and 'Thaxterogaster/Protoglossum' collection MEL2057704 (Figure 7 labelled B). Group B in Figure 7 comprises collections belonging to clusters B and P in Figure 4 with the exception of H1006 which belonged to L. These two groups have positive Axis three values and slightly more negative Axis one values than the next three groups that had negative Axis three values. Group L in Figure 7, contains two subgroups: the closest of these to groups B and G in Figure 7, contains four collections from cluster L of Figure 4 (MEL2032790, H0904, H0910 and TL503). In Figure 7 both the other subgroup of cluster L and the group labelled W are made up of collections belonging to cluster W of Figure 4 but not from particular sub-clusters of W. The second cluster L subgroup contains the 'hysterogasteroid' collection H5258 and '*Thaxterogaster*' collections H5185, H6753 and H1213. The last Cortinarius like group contains '*Thaxterogaster*' collections H2198, H5197, H5301 and H6784 (labelled W).

The *Descolea*-like collections were divided into seven groups on examination of the two scatter plots in Figure 7. One alveolate-spored *Timgrovea* subgenus *Timgrovea* collection, TRAPPE14535, did not appear to group with any of the other *Descolea*-like collections consistently between the two scatter plots in Figure 7 (labelled as x). Group S in Figure 7 comprises two quite closely related subgroupings of six *Descomyces* collections. H0736 and H0737 belonged to different sub-clusters in cluster S of Figure 4 (s1 and s2 respectively) though they group together in Figure 7. The other group of

four *Descomyces* collections included in S in Figure 7 all belong to cluster s1 of Figure 4 characterised by relatively squat spores with prominent rostra. The next group (v) contains three collections, a squat-spored *Descomyces* (H0734) and a broad-spored *Timgrovea* (H4162) from cluster d1 of Figure 4 and a broad-spored *Setchelliogaster* (TRAPPE14175) from the cluster F. The group labelled y groups a broad, asymmetric-spored *Timgrovea* (H4146) with a squat-spored *Descomyces* (H6988) from widely separated clusters in the Ward's tree of the Euclidian distances of the standardised data (Figure 4). Two *Timgrovea* subgenus *Subreticulata* collections, one (H4204) with quite broad spores and the other with small, partially-reticulate spores (H5655) were grouped together (z) as were *Setchelliogaster australiensis* collections TRAPPE14281 and H4234 (group A Figure 7) that also formed a pair in cluster A of Figure 4. The final *Descolea*-like group (E) comprised four collections; *Timgrovea* subgenus *Subreticulata* collection H4057 (quite broad spores with irregularly reticulated ornamentation), alveolate *Timgrovea*/*Descomyces* (putative *Timgrovea* subgenus *Timgrovea*) collection H0732 and two fusoid-spored *Descomyces* collections (H5807 and H0733).

The Principal Coordinates analysis (PCO) ordination using the Euclidian distance measure when applied to the 'standardised' morphological data produced scatter plots of Axis one against Axis two and Axis three against Axis two, that were mirror images of the plots obtained for the PCA but with a different scale. The groups are the same as those detailed above for the PCA. Indeed the boundaries in Figure 8 were generated with only very minimal resizing by copying those from Figure 7 and flipping them horizontally.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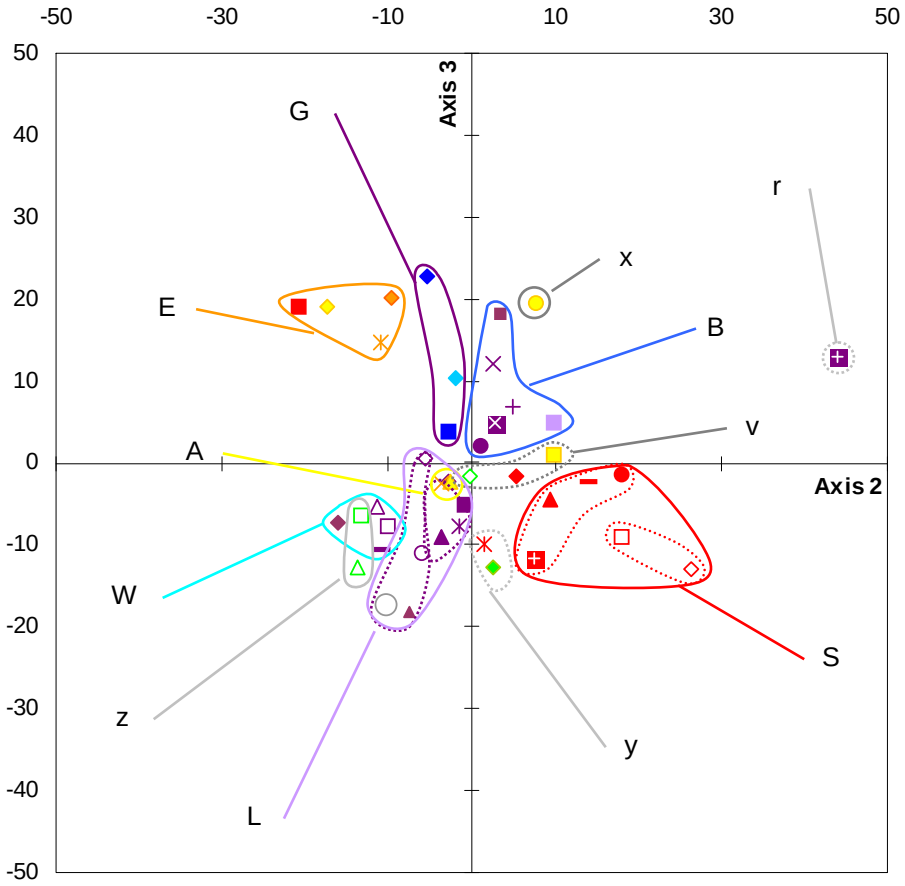
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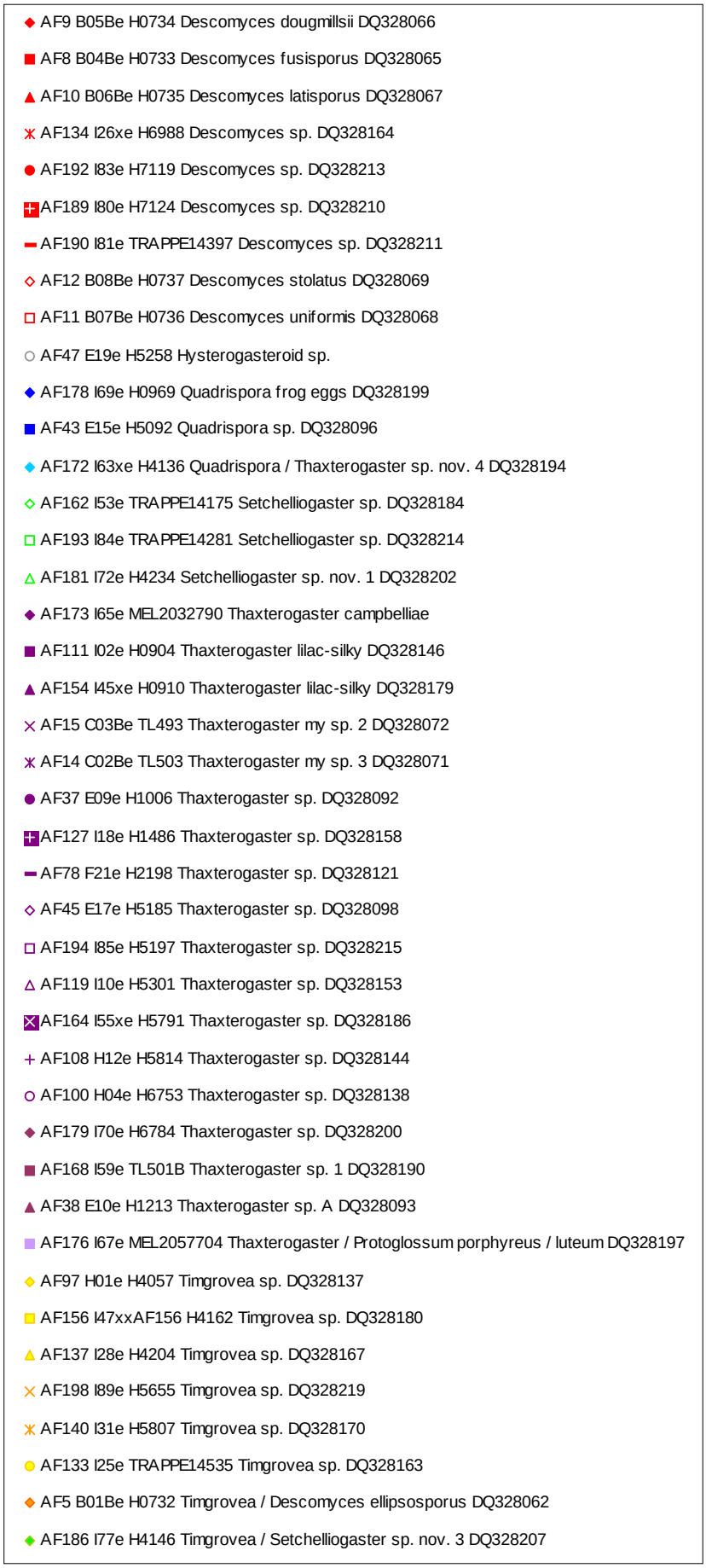
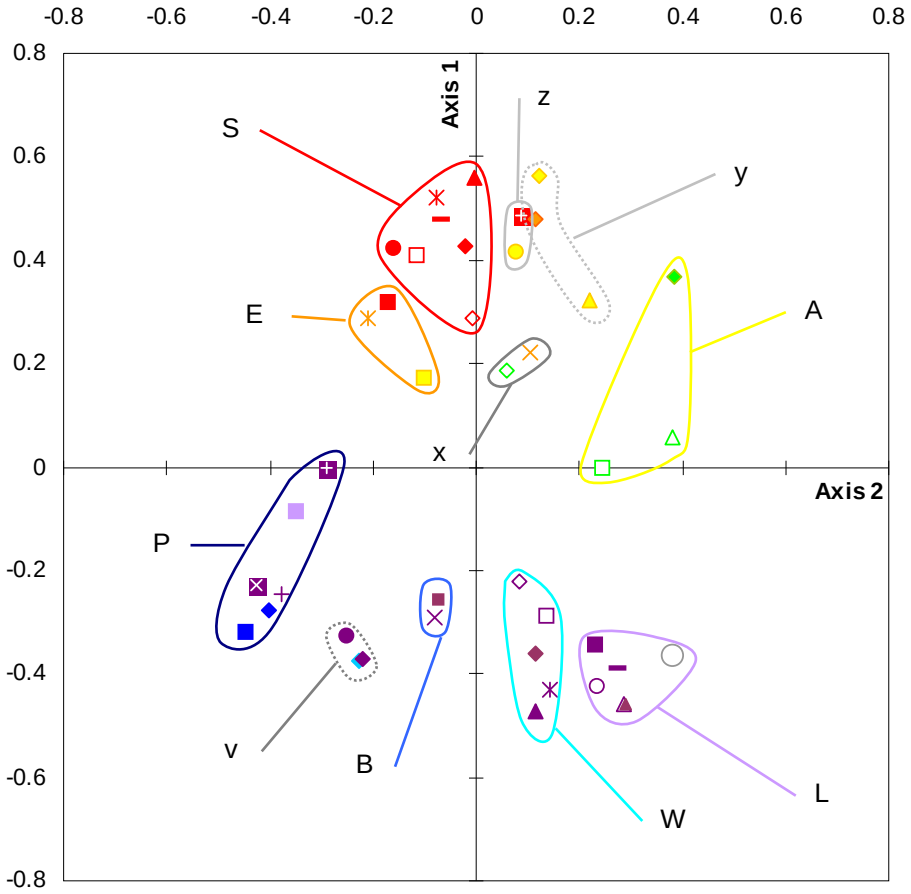


Figure 8: Principal COordinates analysis (PCO) of the Euclidian pair-wise distances of the morphological data "standardised" as a percentage of the maximum value. The axes are in eigenvalue units.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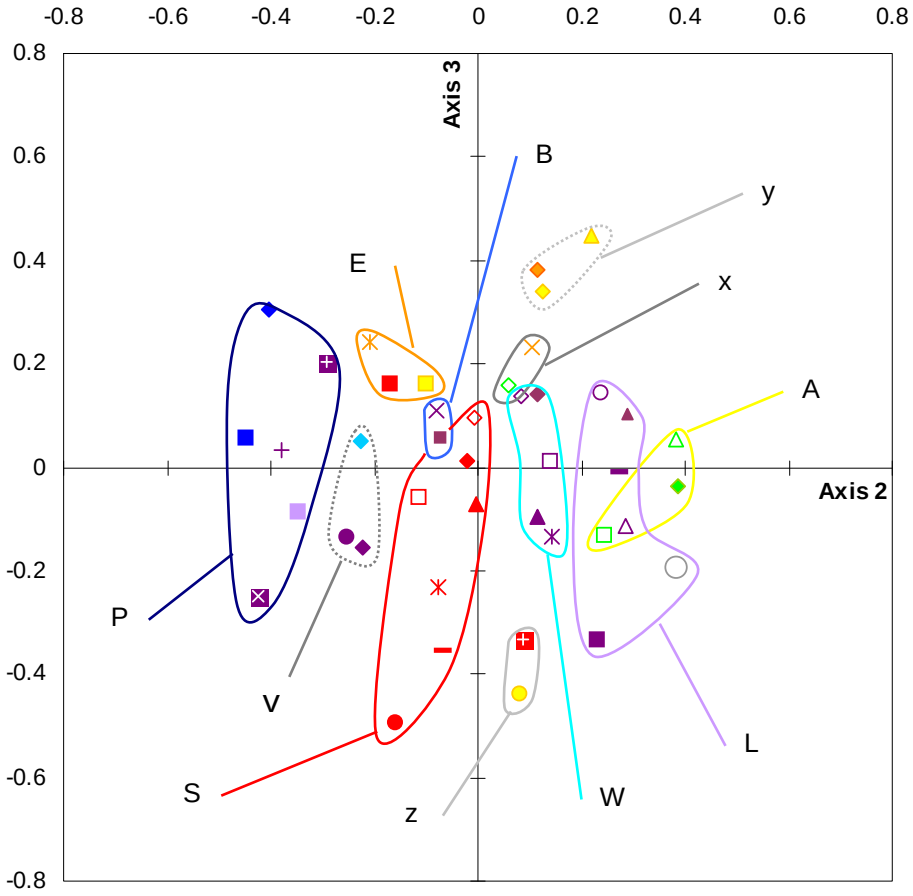
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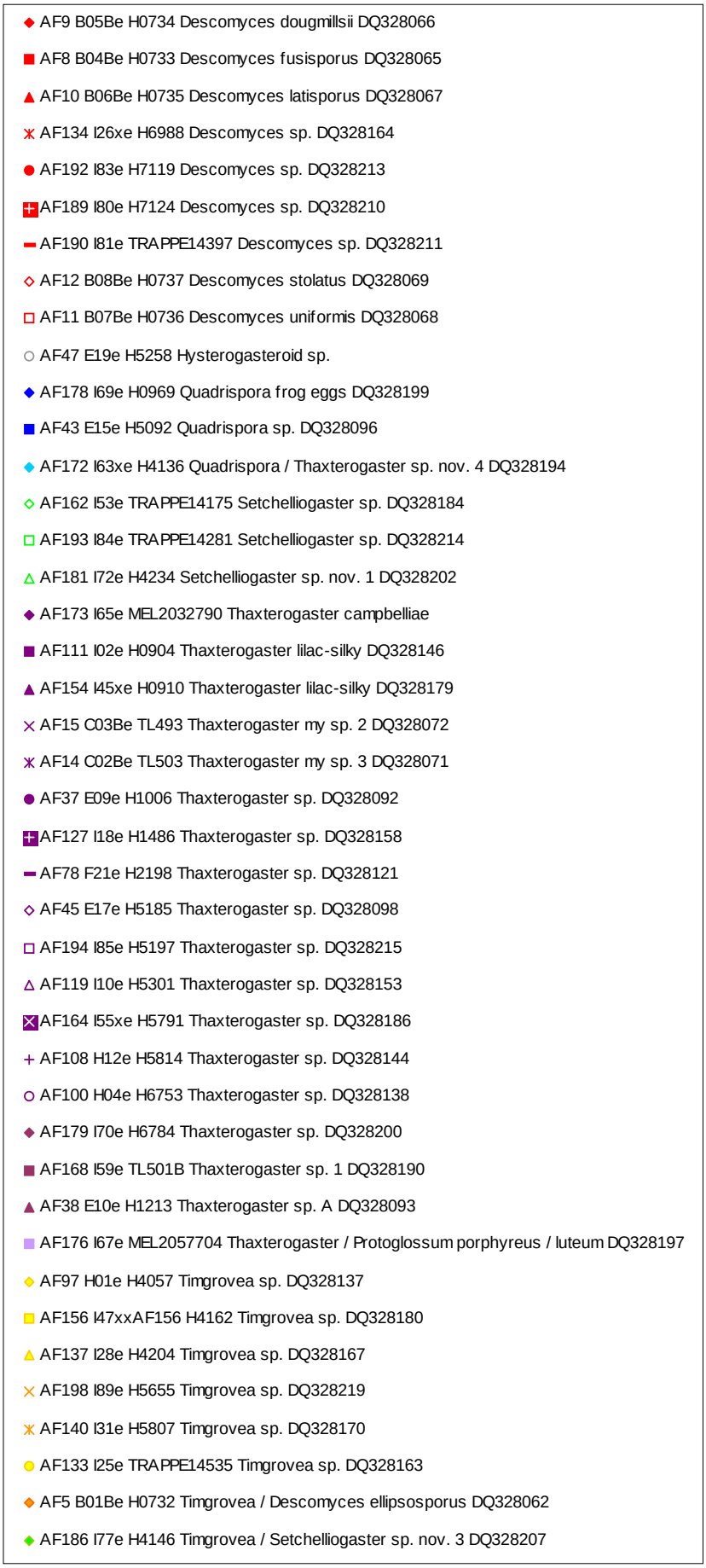


Figure 9: Principal COordinates analysis (PCO) of the Mean pair-wise distances of the recoded '10 bin' multi-state morphological data. The axes are in eigenvalue units. Please note that the symbol for H0910 obscures that of H5301 in the figure showing Axis one against Axis two.

Morphological dataset

When PCO was carried out on the mean distances of the '10-bin' multi-state morphological data quite different groups were recovered (Figure 9). The division between the *Cortinarius*-like collections and *Descolea*-like collections is apparent in the scatter plot of Axis one against Axis two, but not in that for Axis three against Axis two.

There were five groups of *Cortinarius*-like collections found in the scatter plots in Figure 9 that will be dealt with in order of increasing Axis two score (moving from left to right across the figure). One of the largest of these (labelled P) positioned furthest to the left, contains four '*Thaxterogaster*' collections, a *Protoglossum* and both *Quadrispora* collections (H0969 and H5092). Within this group, two '*Thaxterogaster*' collections (H5791 and H5814) with gelatinous peridia and coarsely ornamented spores, group closest to the *Quadrispora* collections on the scatter plot of Axis one against Axis two (Figure 9). H5791 and H5814 form a pair in all the cluster analysis dendrograms and the two *Quadrispora* collections are a pair in the Ward's method tree of the Euclidian distances of the 'standardised' morphological data (Figure 4). The other two collections are the unusually large-spored secotioid *Cortinarius* (H1486) and *Protoglossum luteum* collection MEL2057704, both have gelatinous peridia and broad, coarsely-ornamented spores but they do not form a pair in any of the cluster dendrograms. Two relatively small-spored '*Thaxterogaster*' collections (MEL2032790 and H1006) in the next group (v) also form a pair in Figure 4 and Figure 6 but in a different cluster to the third collection in this group, the elongate-spored '*Quadrispora/Thaxterogaster*' H4136. Two '*Thaxterogaster*' collections with similar broad-elliptical, coarsely ornamented spores (TL501B and TL493) form the next cluster (B) and also form a pair in Figure 4. The next group (W) consists of five relatively small-spored (with white or purple peridia) '*Thaxterogaster*' collections (H0910, TL503, H5185, H5197 and H6784), all in the small-spored cluster of Figure 6. The

final cluster of 6 collections (L) includes the smooth spored 'hysterogasteroid' collection H5258 and five '*Thaxterogaster*' collections. Of these one, H0904 (with a silky, lilac peridium and relatively small spores) groups quite closely with H5258 especially in the scatter plot of Axis three against Axis two. H5258 and all of the relatively small-spored (with white or purple peridia) '*Thaxterogaster*' collections (H0904, H2198, H5301, H6753 and H1213) were in the cluster of smaller-spored collections in Figure 6.

The *Descolea*-like collections were divided into six groups on the basis of the two scatter plots in Figure 9. Again moving from lower to higher Axis two values the first group (labelled E) contains three collections, a broad-spored *Timgrovea* (H4162) and two fusoid-spored *Descomyces* (H5807 and H0733) that form a pair in Figure 4. The second group (S) contains seven *Descomyces* collections, two of which are *Descomyces albus*-like collections in cluster s2 of Figure 4, one (H0734) has squat spores and is in cluster d1 in Figure 4 and the remaining four collections are also squat-spored but are all in cluster s1 in Figure 4. The next group (x) is formed by *Setchelliogaster tenuipes*-like TRAPPE14175 and the small, partially-reticulate spored *Timgrovea* subgenus *Subreticulata* H5655. Squat-spored *Descomyces* H7124 and alveolate-spored *Timgrovea* subgenus *Timgrovea* TRAPPE14535, which are both part of cluster S in Figure 6 form the next group (z). Three *Timgrovea* collections make up the next group (y); H4204 and two collections (H4057 and H0732) which form a pair (e1a) in all three cluster analysis dendrograms. The final *Descolea*-like group (A) is made up of *Setchelliogaster* collections, two of which (H4234 and TRAPPE14281) form a pair in all cluster analysis dendrograms and H4146 that groups clusters on the same branch as the other two in the UPGMA clustering of the mean distances of the multi-state morphological data.

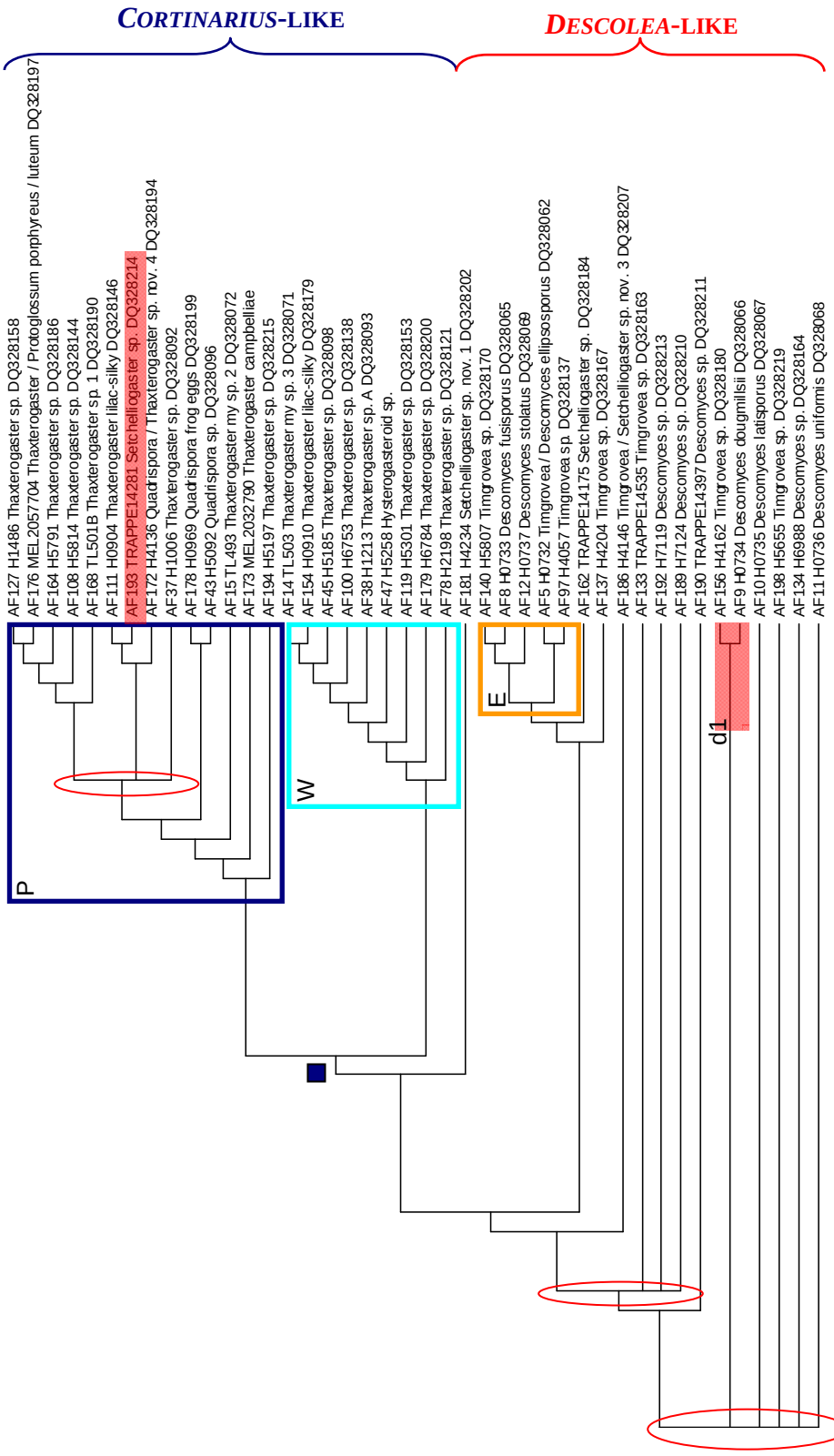


Figure 10: 50% majority rule consensus tree of the trees found by a heuristic parsimony search of the '10 bin' multi-state morphological data. Coloured boxes indicate clusters discussed in the text, red ellipses indicate polytomies.

2.3.3 Parsimony analysis

Parsimony bootstrap analysis of the '10 bin' morphological data did not support any branch with a value greater than 50%. The 50% majority rule tree (i.e. with LE50 set to no, so that only groups occurring in 50% or more of the trees would be included) is included as Figure 10 despite this lack of topological support. The *Cortinarius*-like collections, though forming a cohesive unit at the top of the tree (as shown in Figure 10), were not separated fully from the *Descolea*-like collections. For example *Setchelliogaster* collection TRAPPE14281 (highlighted in red) is positioned in a clade with a '*Thaxterogaster*' collection and a *Quadrispora* collection in the midst of this clade.

The *Cortinarius*-like collections form a clade comprised of two major sub-clades each with a relatively high degree of chaining. Clade P in Figure 10 has four major sub-clades (two nested in the third). Clade P contains one *Setchelliogaster* collection (TRAPPE14281), four small-spored '*Thaxterogaster*' collections with dry peridia and all nine collections that made up the group of *Cortinarius*-like collections in Figure 4, characterised by larger spores and often having gelatinous peridia. The most basal of these four sub-clades is a pair of *Quadrispora* collections H0969 and H5092. The next most apical 'sub-clade' contains the two other sub-clades (which include all the members of cluster P from Figure 4) in a polytomy with collection H1006 (one of the small-spored '*Thaxterogaster*'). The clade containing the unusually large-spored '*Thaxterogaster*' H1486, also contains one of the broad-spored '*Thaxterogaster*' with dry peridia (that form a pair in both Figure 4 and Figure 5) and three of the four collections from cluster P in the Ward's clustering of the Euclidian distances of the 'standardised' morphological data (Figure 4). The last of the sub-clades places *Setchelliogaster australiensis*-like collection TRAPPE14281 as sister to the small-

spored '*Thaxterogaster*' with a lilac peridium (H0904) and as sister to this pair, the elongate-spored '*Thaxterogaster*' H4136. The three other collections in sub-clade P, are all on single taxon branches subtending the collections and clades detailed above. Most apical is the other broad-spored '*Thaxterogaster*' with a dry peridium TL493 followed by two small-spored '*Thaxterogaster*' with dry, white peridia, MEL2032790 and the most basal collection in the sub-clade, H5197. The second major sub-clade of the *Cortinarius*-like clade (W) has only one pair, TL503 and H0910, two small-spored '*Thaxterogaster*' with lilac peridia that form a pair in all of the cluster analysis dendrograms (in cluster L in Figure 4). The other collections of sub-clade W subtend this pair, 'chained' one after the other, and were in cluster W in Figure 4 (H5185, H6753, H1213, H5301, H6781 and H2198) with the exception of the 'hysterogasteroid' collection H5258. The other *Setchelliogaster australiensis*-like collection (H4234) that forms a pair with TRAPPE14281 in both the PCA and PCO of the Euclidian distances of the 'standardised' morphological data is placed as sister to the whole *Cortinarius*-like clade.

The *Descolea*-like clade shows more polytomy than the *Cortinarius*-like clade (polytomies marked in red). The most basal five branches of Figure 10 all form a single polytomy. The only non-single taxon branch of this basal polytomy links two broad-spored collections (*Timgrovea* H4162 and *Descomyces* H0734) that form cluster d1 in Figure 4. The other collections of this polytomy (H0735, H5655, H6988 and H0736), do not group together in any of the cluster analysis dendrograms. The next collection above the basal polytomy is one of the *Descomyces albus*-like collections (TRAPPE14397) with slightly tapered spores and without a prominently flaring perispore. The next node is another polytomy, this time containing two *Descomyces* collections: H1724 and H7119 (the other *Descomyces albus*-like collections) from sub-

cluster s1 of Figure 4, and alveolate-spored *Timgrovea* subgenus *Timgrovea* TRAPPE14535. The next branch away from the basal polytomy contains only the broad spored '*Timgrovea*/*Setchelliogaster*' collection H4146. The final branch of the *Descolea*-like clade has three sub-clades (one containing the other two) and two other collections. The most basal collection on this branch is *Timgrovea* H4204 followed by *Setchelliogaster* TRAPPE14175, two relatively broad spored collections that form a pair in all the cluster dendrograms. The next most apical clade (E) contains the other two sub-clades, one of these grouping the alveolate spored *Timgrovea*/*Descomyces* H0732 (putative subgenus *Timgrovea* collection) and the irregularly-reticulate spored *Timgrovea* subgenus *Subreticulata* H4057 that form a pair in all of the cluster analysis dendrograms (e1a). The final *Descolea*-like clade pairs fusoid-spored *Descomyces* collections H5807 and H0733 which also form a pair in both the Ward's method and UPGMA cluster analysis dendrograms of the Euclidian distances of the 'standardised' data (e1b Figure 4 and Figure 5). This pair of collections is subtended by *Descomyces* collection H0737, characterised by a prominently proximally flaring perispore.

2.3.4 Bayesian analysis

Bayesian analysis of the '10 bin' multi-state morphological data found partition probabilities greater than 0.5 for only four branches of the 50% majority rule consensus tree (Figure 11). No separation was discerned between the *Cortinarius*-like and *Descolea*-like clades.

Two of the four clades with partition probabilities greater than 0.5 were pairs of collections that also occurred in all the cluster analysis dendrograms. One pair, of broad-spored *Timgrovea* (subgenus *Subreticulata*) collections from group E in the

cluster dendrograms (H0732 and H4057, 0.88 partition probability) is subtended by another broad-spored *Timgrovea* subgenus *Subreticulata* collection H4204 with a partition probability of 0.74. The other two collections that formed a pair in all the cluster analysis dendrograms as well as forming a pair in Figure 11 with a partition probability greater than 0.5 were '*Thaxterogaster*' collections H6784 and H2198 both with white peridia and relatively small spores. The final clade recognised by the Bayesian 50% majority rule tree grouped the fusoid-spored *Descomyces* H0733 with *Descomyces* H0737 characterised by its prominent proximally flared perispore. These two collections formed a pair in cluster E of the UPGMA cluster analysis of the Mean distances of the '10 bin' multi-state morphological data (Figure 6).

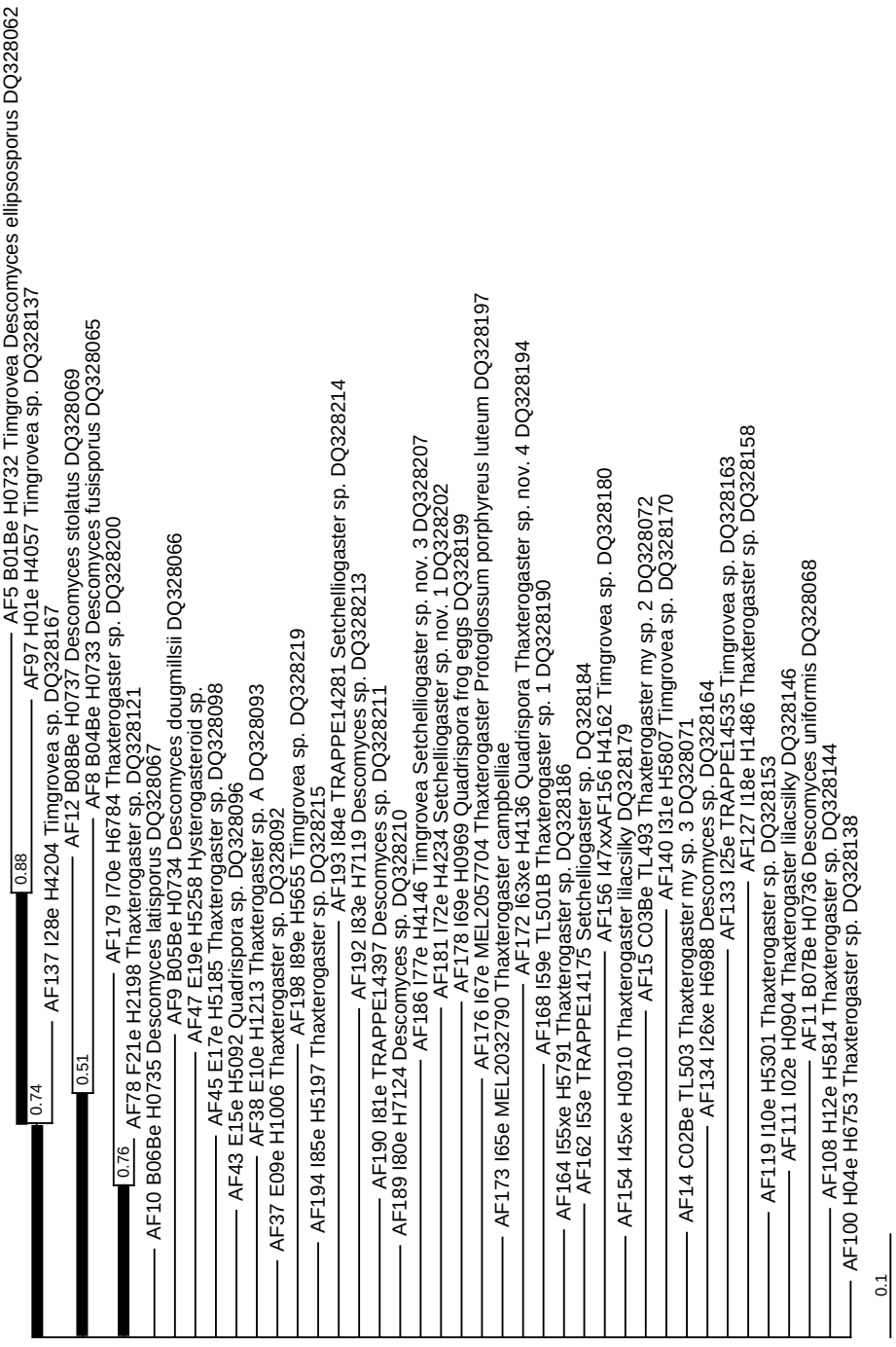


Figure 11: 50% majority rule consensus tree of 6750 of the trees produced by Bayesian analysis of the '10 bin' multi-state morphological data. Bold branches are those with partition probabilities ≥ 0.5.

2.4 Discussion

Bootstrap analysis found no support for the topology of the morphological data under parsimony. Partition probabilities of all of the four clades supported by Bayesian analysis fall below the 95% level suggested by Frøslev *et al.* (2005) for robust ITS defined clades for *Cortinarius* species. This suggests that the morphological data as analysed ('10 bin' multi-state data) has little to no phylogenetic signal[5] discernable by parsimony and Bayesian analyses as performed. Possible reasons for this include the low number of characters (only sixteen), the fact that quantitative variables were coded as multi-state categorical variables and character choice.

There is disagreement between the groupings recovered from different analyses of the morphological data. Disagreement between the Ward's method and UPGMA cluster analyses of the Euclidian distances of the 'standardised' morphological data indicate that the use of a different clustering method changes the pattern of relationships suggested. There are more substantial differences between the UPGMA cluster analysis of the mean distances of the '10 bin' multi-state morphological data and either of the preceding cluster analyses and the same is true of the ordinations. There were still further differences when clades found in the maximum parsimony 50% majority rule tree were compared to those found by cluster analysis and ordination. These observations indicate that the combination of a different distance measure coupled with the recoding of the data had a substantial effect on the patterns of relatedness suggested by the analyses. Additionally, because the 'mean' distance acts as a simple distance

[5] In this thesis 'phylogenetic signal' refers to features of / structure within a particular dataset that enable the phylogenetic relationships between the taxa in question to be confidently estimated by numeric analysis.

measure when no weightings are in place (i.e. distance = number of common characters/total number of characters), most of the differences between analyses of the morphological data can be attributed to effects of the recoding.

Both the Un-weighted Pair-Group Method using arithmetic Averaging (UPGMA Sneath & Sokal 1973) clustering method and Ward's minimum variance clustering method (Ward 1963) used in this study are sequential, polythetic, non-probabilistic, hierarchical, agglomerative clustering methods (Everitt *et al.* 2001). That is they group collections by iterative comparisons of similarity, based on multiple characters, without the use of a probabilistic model regarding the distribution of collections in character space, uniting clusters into still larger clusters and proceed by joining similar collections rather than splitting more divergent clusters/collections. UPGMA groups collections/clusters based on the average similarity between all collections in both collections/clusters while Ward's method groups those collections/clusters the fusion of which produces the smallest increase in the sum of the squared distances between collections and their centroids in character space. This later criterion is the same "squared error" measure as that used by the Multivariate Analysis of Variance (MANOVA) and it is this mathematical link to hypothesis testing among other factors that made this an attractive clustering option. Simple-linkage clustering (nearest neighbour) is also offered in the program PAST (Hammer *et al.* 2001) used to carry out the multivariate analyses in this study. However this simple clustering algorithm is highly subject to chaining in the presence of intermediate collections between clusters (anticipated to be a problem of the datasets analysed because of the small sample size and fact that continuous variables were both used and somewhat arbitrarily divided). The two methods chosen are less subject to this phenomenon. UPGMA however, is susceptible to distortions such as exaggerated similarity due to the excessive influence of large groups. This dataset had a

sampling bias towards the more common *Cortinarius* (ex *Thaxterogaster*) species with dry peridia in clusters/clades L and W and the squat-spored *Descomyces* (cluster/clade S). Weighted Pair-Group Method using arithmetic Averaging (WPGMA) would have been a better choice of clustering method because of this bias. This method corrects for group size when determining cluster similarity. WPGMA however is not currently offered in the PAST package. Thus Ward's method was used as it was anticipated to give the most readily interpretable clustering of collections with the least bias. It was tested for the Euclidian distances of the 'standardised' morphological dataset, but as the Euclidian distance could not be readily calculated for the molecular data (for which 'distances' are not expected to be symmetrical and which included gaps and ambiguity codes) UPGMA was used as a 'next-best-option' for the comparative analyses of all three datasets.

Some collections are grouped together consistently across a range of the analyses. The strength of such associations varied; some collections consistently formed pairs, others were only associated by joint membership in clusters/clades of varying sizes. Collections that formed pairs in any of the analyses are listed in Table 6. No collection had exactly the same combination of characters in the '10 bin' multi-state coding of the data and so none of the pairs listed was merely an artefact of duplicate data. Table 6 indicates that the pair H0732 and H4057 (highlighted in pale green) occurred in six of the seven analyses. This pair had a mean distance (as calculated by PAUP*4.10b) of 0.4375 and a Euclidian distance of 12.896. The most similar collections however were TL503 and H0910 (highlighted in lilac), which also grouped together in all but the Bayesian analysis (Mean distance = 0.1875, Euclidian distance = 3.3615). H6753 also had a mean distance of 0.1875. Pairs, rather than groups of more than two collections, were the most stable groupings across analyses. Eight of the 15 pairs identified from

the Ward's clustering of the Euclidian distances of the 'standardised' data belonged to the same or similar 'by-eye' groups (the 'FBE' groups as detailed in Appendix 2) defined on the basis of the preliminary morphological examinations. The major characters defining relationships between the taxa as indicated by the joint plot in Figure 7 were characteristics of the spore apex and peridium. Spore size appears to be more important in determining the grouping of sub-clades/-clusters within the major *Cortinarius*-like and *Descolea*-like clades/clusters.

Pairs of collections associated together for some analyses and not for others. It was observed that, in several analyses, only one pair found in the Ward's clustering of the Euclidian distances of the 'standardised' morphological data would be found in a clade in which all other members were joined on single-taxon branches (compare Figure 4 with Figure 6 and Figure 10). It can be seen however that the pattern of pairs recovered was not greatly affected by the use of UPGMA rather than Ward's method of clustering (*c.f.* Figure 4, Figure 5 and Figure 6). This reinforces the suggestion that differences observed in the UPGMA clustering of the mean distances of the '10-bin' multi-state data are largely due to the interaction of the conversion of the data to multi-state variables and the distance measure. Note that for this dataset the mean distance values above are whole numbers (the number of shared characters) divided by the total number of characters (16). The generation of fewer pairs and a corresponding increase in chaining probably results from the loss of information inherent in the conversion of quantitative variables (10 of the 16 characters included) to multi-state categorical variables.

Comparable groupings of several collections could be discerned between analyses. Firstly, the division between the *Cortinarius*-like collections and the *Descolea*-like

collections was evident in six of the analyses but not the parsimony analysis. Examining the character-state reconstruction for the consensus tree produced by PAUP*4.10b, several issues with the character coding and conversion of quantitative characters to multi-state variables contributed to the placement of *Setchelliogaster* TRAPPE14281 among the *Cortinarius*-like collections. For many characters (AL, AP, RL, RP, BW, PW, PL, PD, QS and QB), this collection belongs to rare and/or scattered states limiting the grouping value of these characters. This collection also has a number of character states found more commonly among, or only among, *Cortinarius*-like collections (QB, OT, SN, MP and AP) or even more importantly among the same clade (SW) or *Cortinarius*-like 'sister taxa' (AL, PD and QS). Such characters are less important in separating *Cortinarius*-like and *Descolea*-like collections (*c.f.* joint plot in Figure 7). The parsimony analysis was not sensitive to the patterns picked up in the cluster analyses and ordinations, because the characters were unordered, un-weighted, and equal substitution rates were employed. Finally because of the level of subdivision of characters such as rostrum projection (RP) and outer peridium type (OT) the relationship of these states to others is obscured (i.e. "has no rostrum" versus "has a rostrum") so that these 'more informative' characters had relatively less impact while presumably 'less informative' characters (such as apiculus projection) had relatively more impact on the patterns recovered in the analyses.

Groups of more than three collections from different analyses were considered comparable if they had more than half their collections in common. On this basis, within the *Cortinarius*-like and *Descolea*-like groups some collections tend to be similarly grouped together by different analyses. The larger-spored *Cortinarius*-like collections (often also those with gelatinous peridia) tend to group together as do the smaller-spored '*Thaxterogaster*' collections with dry white or lilac peridia, however in

Figure 6, Figure 7, Figure 9 and Figure 10 collections H1006 and MEL2032790 (small-spored collections) group with the larger-spored collections. Again examining the character state reconstruction of the parsimony consensus tree (Figure 10) it appears that slightly broader, more prominently-ornamented spores differentiate these two collections from the other members of the small-spored group. The bias of this analysis towards spore characters overwhelmed the relatively non-descript peridial characters of this group as coded. Molecular analyses by (Peintner *et al.* 2001) suggest that characteristics such as gelatinous peridia have arisen multiple times within *Cortinarius*. Given the likelihood of convergence in peridium structure and the limitations of the peridial characterisations presented here, any division along the lines of peridium characters is highly questionable. Within the *Descolea*-like group squat-spored *Descomyces* tend to group together (for example cluster s1 in Figure 4) as do fusoid and alveolate-spored collections (cluster E). The arrangement of the other pairs and individual collections around these two more stable groups varies substantially, however clade/cluster E group is usually more basal or distant to other *Descolea*-like collections than is clade/cluster S. *Timgrovea* collections with varying degrees of reticulation of spore ornamentation are generally distributed throughout the *Descolea*-like clusters/clades. This again could well be an artefact of the coding of the ornamentation type. The coding of the spore ornamentation ('OT' in Table 4) as three discrete, unordered character states (3 – partial reticulum; 4 – reticulate; or 5 – alveolate) meant that, in the unweighted analyses performed, there was no relationship between the differing degrees of ornamentation reticulation. Alternatively ornamentation type could have been split into several, fractionally weighted descriptive 'sub-characters' e.g. three characters: ornamentation present? Y/N, ornamentation of discrete units? Y/N, non-discrete degree of reticulation? 0/1/2; all weighted at 1/3 of the weight ornamentation type would otherwise have. This may have resulted in the

opposite problem of overweighting correlated characters e.g. whatever the "non-discrete degree of reticulation" value a *Timgrovea* had, they would, of necessity, already share the two other spore sub-characters in common, even if this similarity was down weighted. Such an alternative character division would also have introduced a complex weighting issue as spore ornamentation could be viewed as merely a component character among other spore related characters. It is possible that using measures such as "number of *ornaments* crossed" (with continuous ornamentation counted only once) and "number *of times* ornamentation is crossed" (in a 'transect across/along a spore) as applied to *Russula* spores by Adamcik & Marhold (2000) might provide a more phylogenetically informative, quantitative measure of ornamentation reticulation. It should be noted however that even such a method would still leave the challenge of dividing quantitative characters into discrete states for analysis and the relative weighting of the component measurements describing a 'single' character such as ornamentation type or spore size.

The small size of and suspected convergences among the morphological character set made it unlikely that phylogenetic analysis of the morphological dataset would yield reliable results (as indicated by simulation studies such as that of Givnish & Sytsma, 1997). It was postulated however, that the methods used might be a useful step towards discerning those characters that are associated with phylogenetic patterns. The reliability of the characters used and the assessments made of the importance of spore characters have been challenged as phylogenetic data became available for more taxa and molecular regions. An example of relevance to understanding the sequestrate cortinarioid fungi is the interpretation of the spore apex. Bougher (1987) used Transmission Electron Microscopy (TEM) to show that the rostrum of *Descolea flavoannulata* is occupied by a thickened/inflated episporium (outer coriotunica), while

the germ pore of *Pholiotina filaris* is associated with a reduction in the outer coriotunica. From these and other observations, Bougher (1987) interpreted that the *Descolea* spore apex resembled that of the papilla in *Cortinarius*. Matheny *et al.* (2006) have again raised an alternative explanation proposed by some earlier mycologists such as Rolf Singer that the thickened wall of the rostrum of *Descolea*-like fungi may be related to the germ-pore of the smooth-spored members of the Bolbitiaceae (e.g. Singer 1969). Other characters require further investigation and standardised observation – particularly peridium colour and peridiopellis structure. The use of a colour standard for dried herbarium specimens as well as for fresh colours could assist greatly in differentiating species if preservation conditions have been similar. This was noted in the course of morphological investigations carried out for this study as it was possible to distinguish microscopically distinct but macro-morphologically similar secotioid *Cortinarius* species (TL collections from FBE groups 4 and 41) in mixed collections without using a microscope on the basis of slight variations in peridium colour once these differences had been associated with the microscopic characters. Separation of species of *Descomyces* in particular is dependent on discerning the structure of the peridiopellis, a character subject to artefacts of slide preparation, collection condition and specimen maturity Bougher & Castellano (1993). Discerning the developmental origins of various tissues in the mature fruit bodies could prevent the incorporation of analogous characters and assist in the interpretation of the mature form. Ontological studies have great potential for solving taxonomic problems or finding the morphological common ground between morphologically divergent taxa allied by molecular technology (Reijnders 1991). The use of some characters, such as host plant association, is, as for colour, hampered by inconsistent and often subjective recording in the field. It may be that possible host plants have been overlooked because of their stature. For example *Cortinarius phalarus* appears to be associated with the myrtaceous

shrub genus *Astartea* in Western Australia however, such small plants are often overlooked in assessments of possible host plants (Bougher pers. com.). The incorporation of chemical characters has been successfully used by a number of authors for the Cortinariaceae especially so for *Dermocybe* (Kidd *et al.* 1985, Keller-Dilitz & Moser 1985, Gill 1995, Raisanen *et al.* 2000). However the small size and small proportion of the fruit body made up by sterile tissue may often make procedures such as thin layer chromatography not feasible for sequestrate taxa. More detailed examination of the fruit bodies, with particular reference to peridial hyphal types and the presence of KOH soluble peridial pigments, combined with a standardised assessment of fresh colour and peridial texture, could make at least the multivariate estimates of relatedness more phylogenetically robust.

Table 6: Collections grouping together in the various analyses of the morphological dataset: Ward's clustering of Euclidian distances of 'Standardised' morphological data (WES), UPGMA clustering of Euclidian distances of 'Standardised' morphological data (UES), UPGMA clustering of Mean distances of '10 bin' Multi-state morphological data (UMM), Principal Components Analysis of the 'Standardised' morphological data (PCAS), Principal COordinates analysis of the Mean distances of the '10 bin' multi-state morphological data, Parsimony analysis (PARS) and Bayesian analysis (BAYES). In the cells, a numeral one indicates that the pair was recovered in the analysis in question. A two indicates that though the pair was not recovered the collections were closely associated in a larger cluster/clade. Numbers in *italics* are pairs recovered in only one analysis. Colours indicate groups and sub-groups of Figure 4. Numbers on the left of a row indicate: on the top, the number of ones and on the bottom the number of twos. A '☑' symbol indicates that the pair in question were in the same or similar 'FBE' groups (Table 14, Appendix 2) generated from the subjective preliminary examination.

PAIR #		PAIR		WES	UES	UMM	PCAS	PCOM	PARS	BAYES
1	2	H6753 AF100	H5197 AF194	1	1					
2	4 1	H6784 AF179	H2198 AF78	1	1	1	2			1
3	5 1	TL503 AF14	H0910 AF154 ☑	1	1	1	2	1	1	
4	3	H1006 AF37	MEL2032790 AF173 ☑	1		1		1		
5	4 1	H5814 AF108	H5791 AF164	1	1	1	2	1		
6	3	TL493 AF15	TL501B AF168 ☑	1	1			1		
7	3 1	H0969 AF178	H0592 AF43 ☑	1			2	1	1	
8	3	H4204 AF137	TRAPPE14175 AF162	1	1	1				
9	4	H4234 AF181	TRAPPE14281 AF193 ☑	1	1		1	1		
10	5	H4162 AF156	H0734 AF9	1	1	1	1		1	
11	2 1	H0735 AF10	H0736 AF11 ☑	1	1		2			
12	2 1	H7119 AF192	TRAPPE14397 AF190 ☑	1	1		2			
13	2	H0737 AF12	H6988 AF134	1	1					
14	4 1	H5807 AF140	H0733 AF8 ☑	1	1		2	1	1	

Table 6 continued: Collections grouping together in the various analyses of the morphological dataset: Ward's clustering of Euclidian distances of 'Standardised' morphological data (WES), UPGMA clustering of Euclidian distances of 'Standardised' morphological data (UES), UPGMA clustering of Mean distances of '10 bin' Multi-state morphological data (UMM), Principal Components Analysis of the 'Standardised' morphological data (PCAS), Principal COordinates analysis of the Mean distances of the '10 bin' multi-state morphological data, Parsimony analysis (PARS) and Bayesian analysis (BAYES). In the cells, a numeral one indicates that the pair was recovered in the analysis in question. A two indicates that though the pair was not recovered the collections were closely associated in a larger cluster/clade. Numbers in *italics* are pairs recovered in only one analysis. Colours indicate groups and sub-groups of Figure 4. Numbers on the left of a row indicate: on the top, the number of ones and on the bottom the number of twos. A '☑' symbol indicates that the pair in question were in the same or similar 'FBE' groups (Table 14, Appendix 2) generated from the subjective preliminary examination.

PAIR #		PAIR		WES	UES	UMM	PCAS	PCOM	PARS	BAYES
15	6	H0732 AF5	H4057 AF97	1	1	1		1	1	1
16		H0904 AF111	H5197 AF194			*1*				
17		H4234 AF181	H4146 AF186			*1*				
18		H0736 AF11	H0737 AF12				*1*			
19		H6988 AF134	H4146 AF186				*1*			
20		H4204 AF137	H5655 AF198				*1*			
21		H0732 AF5	H5807 AF140				*1*			
22		H4057 AF97	H0733 AF8				*1*			
23		H1486 AF127	MEL2057704 AF176					*1*		
24		H5185 AF45	H5197 AF194					*1*		
25		H5258 AF47	H0904 AF111					*1*		
26		H7124 AF189	TRAPPE14535 AF133					*1*		
27		H5655 AF198	TRAPPE14175 AF162					*1*		
28		H1486 AF127	MEL2057704 AF176						*1*	
29		H0904 AF111	TRAPPE14281 AF193						*1*	
30		H0737 AF12	H0733 AF8							*1*

Chapter 3

rDNA sequence dataset

3.1 Introduction

Molecular data from a variety of regions is available for a relatively large number of cortinarioid taxa (more than 500 sequences of approximately 180 species). The majority of published phylogenies of the cortinarioid fungi have, however, used the Internal Transcribed Spacer (ITS) region of the nuclear rDNA (Liu *et al.* 1997, (Høiland & Holst-Jensen 2000, Seidl 2000, Peintner *et al.* 2001, Peintner *et al.* 2002, Garnica *et al.* 2003a, Garnica *et al.* 2003b, Peintner *et al.* 2004, Frøslev *et al.* 2005, Garnica *et al.* 2005, Moreau *et al.* 2006, *c.f.* Matheny & Bougher 2006a). Bruns (2001) discusses the utility of this non-coding region, pitting its limited phylogenetic utility (relatively highly variable but often almost invariable between closely related species), against its ease of amplification and the abundance of publicly available ITS sequences which make comparison of this region an ideal first step in identifying the general taxonomic affiliations of an unknown sequence. This work aims to use the ITS to place an expanded sample of Australian sequestrate cortinarioid fungi in the context of existing cortinarioid phylogenies.

The expectation is that the cortinarioid sequestrate genera *Protoglossum*, *Quadrispora*, secotioid *Cortinarius* (ex *Thaxterogaster*) species, *Hymenogaster sensu stricto*, *Descomyces* and *Timgrovea* will group among the three clades associated with the agaricoid genera *Cortinarius*, *Hebeloma* and *Descolea*. This expectation is based on the phylogenies of Peintner *et al.* (2001), works that have supported their findings (e.g. Garnica *et al.* 2005, Moreau *et al.* 2006), affinities proposed earlier by Bougher &

Castellano (1993) based on morphology, together with morphological examinations made for the purposes of this thesis. Note that this expectation includes *Timgrovea*, a genus associated with the boletoid genus *Strobilomyces* when it was described by Bougher & Castellano (1993). The reticulate spore ornamentation that characterises this genus has also been recorded for the alveolate-spored *Descomyces giachinii* Trappe *et al.* (alveolate ornamentation defines *Timgrovea* subgenus *Timgrovea*). This and similar observations of golden, thick-walled, peridiopellis hyphae in conjunction with prominently alveolate spores suggests that species of both alveolate spored *Timgrovea* subgenus *Timgrovea* Bougher & Castellano and partially-reticulate spored *Timgrovea* subgenus *Subreticulata* Bougher & Castellano, should group among the *Descomyces*.

3.2 Materials and Methods

Two datasets of Internal Transcribed Spacer (ITS) region sequences were constructed. One included 56 sequences obtained for the purposes of this project from collections of Australian cortinarioid sequestrate fungi for which morphological data was available. The other included 142 additional sequences of cortinarioid sequestrate fungi sequenced for this project and a number of sequences obtained from GenBank for use as outgroups or that had been used in published phylogenies of cortinarioid fungi. This second dataset was split in three sub-datasets representing *Cortinarius*-like, *Descolea*-like and *Hebeloma*-like taxa.

PAUP*4.10b can calculate pair-wise distances between molecular sequences using several distance measures. The “mean distance” as calculated by PAUP*4.10b (Swofford 2001) was used as the input for cluster analysis and ordination of the molecular data. This distance metric was chosen both because it takes missing values

and weightings into account (noting that gaps were treated as missing data) and also for comparability with the morphological data set, for which the "mean" distance was the only option available in PAUP.

3.2.1 Specimens and sampling

DNA was initially sampled when specimens were sectioned for the preliminary examinations. At least some surface contamination was expected because the fruit bodies were environmental samples which had been collected, examined and dried in close proximity to other collections. The sections taken for molecular analysis consisted mostly of gleba tissue that would be relatively protected from environmental contaminants. Other possible specimen-related contaminants include apparently saprotrophic fungi colonising the specimens. It was considered unlikely, given the competitive nature of PCR and the relative proportions of target to any possible contaminant material, that there would be sufficient contaminating material to be preferentially amplified unless there was very little amplifiable DNA in the target material. An exception to this appears to be when the specimen is badly infested by presumably saprophytic fungi in which there appears to be substantial contaminant biomass and possibly less degraded DNA in that biomass.

3.2.2 Extraction

Two protocols were used in this project, a 'glass-milk' method and Qiagen *DNEasy Plant Mini Kit*. Details for the preparation of reagents for the "glass-milk" method are presented in Appendix 7. Regardless of extraction method, DNA was routinely diluted 30 or 40 fold in TE buffer (Raeder & Broda 1985) before PCR to reduce the effect of PCR inhibitors in the DNA based on the results of a preliminary dilution experiment.

3.2.2.1 Protocol 1: Glass-'milk' extraction

20 mg or less of dried herbarium material was frozen with liquid nitrogen (poured into the 1.65ml microcentrifuge tube), ground with a motorised micro-pestle with the addition of a few drops of extraction buffer (Raeder & Broda 1985) during grinding. The micro-pestle was rinsed with the remainder of the 250µl aliquot of extraction buffer to wash off any adhering fungal material. Samples were left at room temperature until all samples were ground, before proceeding to the incubation step.

The ground samples were incubated for 1-18 hours at 65 °C then centrifuged at maximum speed [either 13200 rpm (Eppendorf 5415D) or 14000 rpm (Eppendorf 5804)] for 15-20 minutes.

For each sample 200µl of supernatant was transferred to a new 1.65ml micro-centrifuge tube to which 7µl of glass-'milk' (a suspension of pH neutral, acid-washed, powdered flint glass (essentially silica) see Appendix 7 for method) and 800 µl of NaI (1mg/ml) had been added. The resulting mixtures were shaken briefly (using a vortex mixer) then incubated for 15 minutes on ice with occasional manual shaking. Two or three washing steps were then carried out, where the samples were centrifuged for 10 seconds at maximum speed to pellet the glass-milk and DNA, the supernatant discarded and then the pellet re-suspended (shaken on the vortex mixer) and centrifuged ready for the next wash step. Wash buffer was used for the first washing step and 100 % ethanol for the one or two subsequent wash steps. After discarding the supernatant from the last ethanol wash the tubes were inverted and left to dry (either on the bench-top over-night or in the laminar-flow hood) for approximately 4 hours (or until the tubes appeared dry). The DNA was re-suspended in 25µl of TE buffer and stored at -20°C.

3.2.2.2 Protocol 2: Qiagen DNEasy Plant Mini Kit

The extraction was carried out according to the manufacturer's instructions after grinding the samples in a 1.65ml tube with a micro-pestle and a small volume of liquid nitrogen.

3.2.3 Polymerase Chain Reaction (PCR)

PCR protocols were refined as the project progressed. Normal reaction volume was 20µl with the ratios of reagents as detailed in Table 7. For both protocols the first round of amplification used the primers (25µM) ITS F [Glen *et al.* (2001a): CCC T**R**T TGC TGA GAA **SY**T G**R**T C] and ITS R [Glen *et al.* (2001a): TTC CAG GAG ACT T**R**T **R**CA CGG T**Y**C]. Two micro-litres of the one fortieth dilutions of the extracted DNA were used as template for the first round of PCR. The change from *protocol 1* to *protocol 2* was an attempt to improve initial poor amplification results. This modified methodology was used to obtain the majority of the sequences used in the analysis.

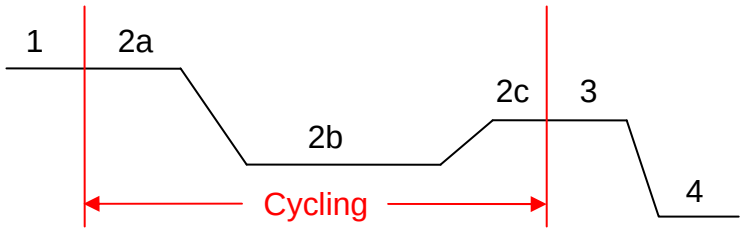
PCRs were carried out using either an Applied Biosystems Geneamp 2700 or MJ PTC 100 thermocycler. Reactions were most commonly run in individual dome-capped 0.2ml PCR tubes in an attempt to avoid cross-contamination.

For all reactions, 1/10 dilutions of the PCR product were made (in case a nested PCR was required. See section 3.2.4) before 2µl of the remaining neat PCR product was loaded onto 1% electrophoresis grade agarose gels to visualise the product using Blue/Orange Loading Dye, 6X (Promega, catalogue No. G1881) loading dye with a Lambda *Eco* R1/*Hin* dIII ladder as a guide to size.

Table 7: Concentrations of the various reagents for the two PCR protocols for the 1x reactions. Normal reaction volumes were 20µl however 50µl and 100µl reactions were used in preparation for sequencing.

	CONCENTRATIONS FOR 1X REACTION	
REAGENT	**PROTOCOL 1**	**PROTOCOL 2**
Water for injection (double deionised)		
10x buffer (Fisher Biotech)		
MgCl2 (25mM)	2.0mM	2.0mM
Bovine Serum Albumen (10mg/ml)	0.2mg/ml	0.2mg/ml
dNTP mix (10mM of each dNTP)	0.1mM for each dNTP	0.1mM for each dNTP
Forward primer (25µM)	0.25µM	0.25µM
Reverse primer (25µM)	0.25µM	0.25µM
Tth +[6] (5.5 Units/µl Fisher Biotech)	0.022 units/µl	0.05 units/µl
DNA TEMPLATE		

Table 8: Cycle profiles for the PCR protocols 1 and 2, Nested PCR and sequencing reactions.



	NUMBER OF CYCLES AND TIME FOR STEP		
STEP	**PCR AMPLIFICATION (BOTH PROTOCOLS)**	**NESTED PCR**	**SEQUENCING REACTION**
1. Initial denaturation	1 cycle x 95°C for 4 minutes	1 cycle x 94°C for 2 minutes	1 cycle x 95°C for 2 minutes
2. Cycling	**35 cycles of…**	**30 cycles of…**	**25 cycles of…**
2a. Denaturation	95°C for 30 seconds	94°C for 30 seconds	95°C for 10 seconds
2b. Annealing	56°C for 1 minute	55°C for 30 seconds	53°C for 5 seconds
2c. Extension	72°C for 2 minutes	72°C for 30 seconds	60°C for 4 minutes
3. Final extension	72°C for 8 minutes	72°C for 7 minutes	60°C for 4 minutes
4. Hold	14°C	14°C	14°C

[6] Modified *Thermus thermophilous* DNA polymerase by GeneWorks referred to as *Tth* +.

3.2.4 Nested PCR

3.2.4.1 Second round PCR

Two micro-litres of the 1/10 dilutions of first round PCR products which had not produced a visible product were used as templates for a second round of amplification. A clear band is often not obtained when neat PCR product is used for nested PCR and so the diluted product was used (Morag Glen, pers. comm.). Forward and reverse primers (25µM) for the second round of PCR were ITS 1F: CTT GGT CAT TTA GAG GAA GTA A (Gardes & Bruns 1993) and ITS 4: TCC TCC GCT TAT TGA TAT GC (White *et al.* 1990) respectively. The cycling parameters were those for nested PCR in Table 8. Again 1/10 dilutions of these PCR products were made before visualizing the PCR products on an agarose gel as detailed above.

3.2.4.2 Third round PCR

The 1/10 dilutions of second round PCR products that had not produced a band were amplified a third time using primers (25µM) ITS 5: GGA AGT AAA AGT CGT AAC AAG G and ITS 4 (White *et al.* 1990) using the nested PCR thermal cycling parameters in Table 8. These products were visualized on an agarose gel as before.

3.2.5 100µl PCRs

100µl of nested PCR product were used for sequencing template obtained by running either: two 50µl reactions in 0.2ml dome capped tubes in the AB or MJ thermocycler or as 100µl reactions in 0.5ml flat capped tubes in a Hybaid Omnigene HBTR3CM thermocycler. The concentrations of the reagents are given in Table 7. Thermal cycling parameters were the same as for the 20µl reactions (Table 8). The 1/10 dilutions of the first round PCR product were used for samples which had been successfully amplified

after one or two rounds of PCR (so even samples that had been successfully amplified dilutions of extracted DNA were nested for sequencing). If a sample had only produced a visible PCR product after three rounds of amplification, then the 1/10 dilution of the second round PCR product was used as template for the 100µl PCR.

3.2.6 PCR clean up

PCR products were cleaned using Mobio's *Ultra-Clean PCR Clean-up Kit* (either in individual tubes or in a 96-well plate format) according to the manufacturer's instructions. The cleaned PCR products were then concentrated. For individual tubes 1µl of a 5M NaCl solution and 25µl of cold 100% Ethanol was added before centrifugation for 5 minutes at 13000 rpm (≈10000 x g). The same reagents were used when concentrating PCR products cleaned in a 96-well plate excepting that the spin was for 10 minutes at 3000 x g. After centrifugation, the supernatant was decanted, the tube or plate inverted to dry and the pellet re-suspended in 20µl TE Buffer.

3.2.7 DNA Sequencing

Sequencing reactions were 10µl using 5µl template PCR product, 4µl sequencing mix (Applied Biosystems) or 2µl sequencing mix and 2µl 5x sequencing buffer (Applied Biosystems) and 1µl each of primers (3.2µM) ITS 4 or ITS 5. Sequencing reaction thermal cycling parameters are detailed in Table 8.

Once thermal cycling was finished the reaction was precipitated according to Applied Bioscience recommendations modified by Dr F. Briggs. The 10µl reaction volume was added to a 0.65ml tube containing 25µl 100% ethanol, 1µl 3M sodium acetate and 1µl 125mM EDTA (disodium salt), mixed by pipetting, then incubated at room temperature

for 20 minutes. The resulting mixture was then centrifuged for 30 minutes at 13200 to 14000rpm. As soon as the centrifuge finished, for each tube the supernatant was tipped out and a pipette used to remove as much of any remaining supernatant as possible. The pellet was washed by adding 125µl 80% ethanol before centrifugation for five minutes at 13200 to 14000rpm. The supernatant was removed as before then the samples were either dried in a SpeedyVac vacuum desiccator for 15 minutes (or in the dark at room temperature overnight) before being frozen and deposited for sequencing at the Western Australian State Agricultural Biotechnology Centre (SABC) for sequencing on either Applied Biosystems 377 or 373A DNA sequencing systems.

3.2.8 Sequence editing

Sequences were obtained in both directions (using primers ITS 5 and ITS 4) and assembled with the Staden Package. Forward and reverse chromatograms and the resulting consensus sequence were examined visually to ensure sequence quality.

3.2.9 Sequence alignment

Sequences were aligned using Clustal W (Thompson *et al.* 1994). The alignment was examined visually and adjusted where appropriate using Bioedit (Hall 1999). No region of the alignment was considered so ambiguous as to warrant removal. The 56 sequence subset of the data was aligned first. The 741bp final alignment had 430 constant characters, 51 variable but parsimony-uninformative characters and 260 parsimony-informative characters and is presented in Appendix 4.

3.2.10 Cluster analysis

Cluster analysis was performed using the program PAST - PAlaeontological STatistics, ver. 1.53 (Hammer *et al.* 2001). The 'mean' distances of the aligned molecular data as calculated by PAUP*4.10b were clustered using the Un-weighted Pair-Group Method using arithmetic Averaging (UPGMA) group-linkage method. Trees were output in nexus format and arranged for display using TREEVIEW (Page 2001). A distance cut-off was then determined subjectively for the tree with the aim of maximising the number of clusters across the whole tree that grouped more than two collections.

3.2.11 Ordination

Principal Coordinates analysis (PCO) ordination was performed on the 'mean' distances of the aligned molecular data using the 'user distance' option in PAST. Groups were determined subjectively for the ordinations based on the recognition of sets of collections that appeared to group together in the scatter plots of both Axis one against Axis two and Axis three against Axis two. The percentage variance accounted for by the first three axes was also recorded.

3.2.12 Parsimony analysis

Parsimony analysis was carried out using PAUP*4. 10b (Swofford 2001) on the aligned molecular data with the following commands:

```
Factory;
Set criterion=parsimony Maxtrees=250000 Increase=Auto
AutoInc=100 TaxLabels=full;
Hsearch start=stepwise addseq=random nreps=10 rstatus=yes;
```

The resulting trees were saved and strict and majority-rule consensus trees were calculated (Le50 was set to 'no' so that only groupings occurring in 50% or more of the trees would be included and there would be no possibility of an equally parsimonious but contradictory branch being excluded from the tree). Bootstrap support for the topology was determined by 10000 bootstrap replicates subjected to a heuristic "faststep" search where for each bootstrap replicate there is only one random-sequence-addition replicate and no branch swapping, using the following commands:

```
Bootstrap nreps=10000 brlens=yes format=nexus
treefile=filename.tre search=faststep;
```

3.2.13 Model testing

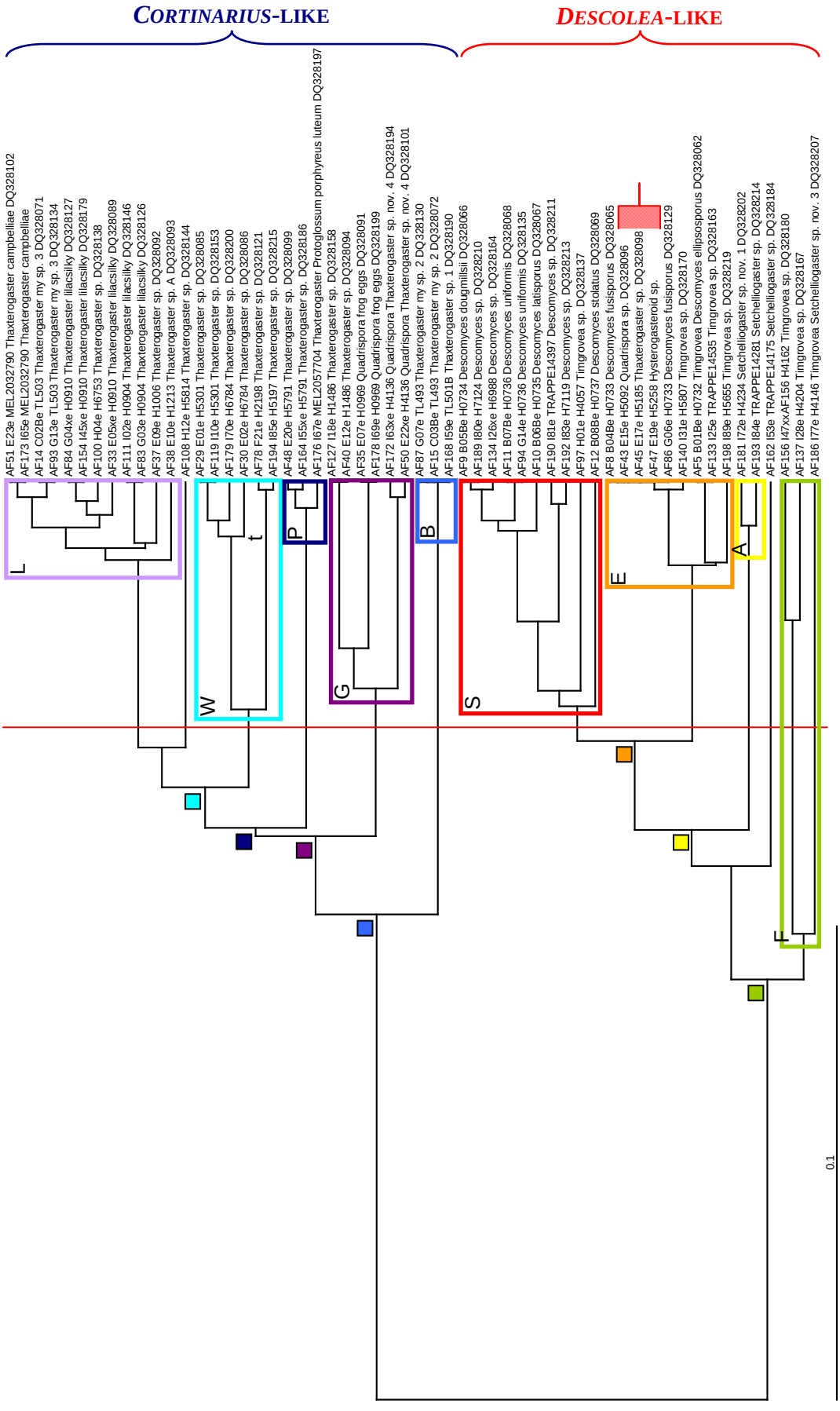
The program MRMODELTEST (Nylander 2004) was used to suggest the general form of model to be used in setting the priors for Bayesian analysis. The model suggested was also compared with the output from MODELTEST (Posada & Crandall 1998) which tests a wider range of models.

3.2.14 Bayesian analysis

Bayesian analysis was carried out using the program MRBAYES (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Except for the following commands the default settings were used:

```
Lset nst=6 rates=invgamma
Mcmcp ngen=100000 samplefreq=2000
```

The number of generations was increased by 100000 to 200000 until the standard deviation of split frequencies dropped below 0.01.


CORTINARIUS-LIKE
DESCOLEA-LIKE
AF51 E23e MEL2032790 Thaxterogaster campbelliae DQ328102
AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071
AF93 G13e TL503 Thaxterogaster my sp. 3 DQ328134
AF84 G04xe H0910 Thaxterogaster lilacsilky DQ328127
AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179
AF100 H04e H6753 Thaxterogaster sp. DQ328138
AF33 E05xe H0910 Thaxterogaster lilacsilky DQ328089
AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146
AF83 G03e H0904 Thaxterogaster lilacsilky DQ328126
AF37 E09e H1006 Thaxterogaster sp. DQ328092
AF38 E10e H1213 Thaxterogaster sp. A DQ328093
AF108 H12e H5814 Thaxterogaster sp. DQ328144
AF29 E01e H5301 Thaxterogaster sp. DQ328085
AF119 I10e H5301 Thaxterogaster sp. DQ328153
AF179 I70e H6784 Thaxterogaster sp. DQ328200
AF30 E02e H6784 Thaxterogaster sp. DQ328086
AF78 F21e H2198 Thaxterogaster sp. DQ328121
AF194 I85e H5197 Thaxterogaster sp. DQ328215
AF48 E20e H5791 Thaxterogaster sp. DQ328099
AF164 I55xe H5791 Thaxterogaster sp. DQ328186
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197
AF127 I18e H1486 Thaxterogaster sp. DQ328158
AF40 E12e H1486 Thaxterogaster sp. DQ328094
AF35 E07e H0969 Quadrispora frog eggs DQ328091
AF178 I69e H0969 Quadrispora frog eggs DQ328199
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194
AF50 E22xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328101
AF87 G07e TL493 Thaxterogaster my sp. 2 DQ328130
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
AF189 I80e H7124 Descomyces sp. DQ328210
AF134 I26xe H6988 Descomyces sp. DQ328164
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF94 G14e H0736 Descomyces uniformis DQ328135
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
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AF8 B04Be H0733 Descomyces fusisporus DQ328065
AF43 E15e H5092 Quadrispora sp. DQ328096
AF45 E17e H5185 Thaxterogaster sp. DQ328098
AF47 E19e H5258 Hysterogasteroid sp.
AF86 G06e H0733 Descomyces fusisporus DQ328129
AF140 I31e H5807 Timgrovea sp. DQ328170
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus DQ328062
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163
AF198 I89e H5655 Timgrovea sp. DQ328219
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF156 I47xxAF156 H4162 Timgrovea sp. DQ328180
AF137 I28e H4204 Timgrovea sp. DQ328167
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207
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0.1

Figure 12: Cluster analysis dendrogram for UPGMA clustering of the pair-wise 'Mean' distances of the molecular data. Coloured boxes indicate clusters discussed in the text. The red line indicates the distance cut-off for this analysis.

3.3 Results

3.3.1 Cluster analysis

The dendrogram produced by the UPGMA clustering of the 'mean' distances of the molecular data is presented in Figure 12. The most prominent feature of the tree is the division between *Cortinarius*-like and *Descolea*-like collections (0.192 distance units).

The 0.05 distance unit cut-off indicated by the red line in Figure 12 shows six major *Cortinarius*-like clusters labelled L, W, P, G and B, and the single-taxon branch joining H5814 (large spores, gelatinous peridium). This single taxon is sister to cluster L which contains smaller-spored fungi with ***L***ilac peridia (TL503, H0910, H6753, H0904, H1006, MEL2032790 and H1213). The sister cluster to that containing both cluster L and H5814 is cluster W, also comprised of smaller-spored fungi exclusively with ***W***hite peridia. The pair marked "t" is also quite distant from the remainder of the collections in this cluster. Sister to the clade containing L and W is cluster P containing '*Thaxterogaster*' collections with sub-spherical spores and gelatinous ***P***eridia. Cluster G is comprised of *Quadrispora* collection H0969, unusually large-spored '*Thaxterogaster*' H1486 and elongate-spored '*Thaxterogaster*' H4136, all characterised by ***G***elatinous peridia and relatively large non sub-spherical spores. The final cluster, B, is sister to that containing all the preceding clusters and is comprised of two collections with dry white peridia and relatively ***B***ig spores. The clusters recovered are comparable to those clusters with the same name recovered from the morphological data (especially Figure 4). It is particularly notable that the two smaller-spored clusters, L and W cluster

together as in morphological analyses (e.g. the Ward's method cluster analysis dendrogram of the Euclidian distances of the 'standardised' morphological data).

Similar to the arrangement of the six major clades of the *Cortinarius*-like cluster, the four clades (S, E, A and F) and two single taxa (TRAPPE14175 and H4146) are joined one after the other. The sister clades S and E are comprised of ***S***quat-spored (H0734, H7124, H6988, H0736, H0375) and *Descomyces albus*-like (TRAPPE14535, H7119) *Descomyces* collections and ***E***longate-spored (H0733, H5807) *Descomyces* and *Timgrovea* collections with alveolate- (H0732, TRAPPE14535 subgenus *Timgrovea*) and smaller-, partially-reticulate spores (H5655 subgenus *Subreticulata*) respectively. Three sequences, *Quadrispora* H5092, '*Thaxterogaster*' H5185 and the 'hysterogasteroid' H5258 have identical sequences to the elongate-spored *Descomyces* collections. These collections were prepared together and it is likely that these sequences were a consequence of contamination given the morphological differences between these collections and the *Descomyces* with which they grouped. The pair of collections labelled A is sister to the cluster of S and E and contains *Setchelliogaster* **a***ustraliensis*-like collections H4234 and TRAPPE14281. Broader-spored, *Setchelliogaster tenuipes*-like TRAPPE14175 is sister to the cluster containing S, E and A. The final cluster, F, is sister to that containing all the preceding *Descolea*-like clusters and is comprised of broad-, irregularly ornamented- symmetrical (H4162, H4204) and asymmetrical (H4146) spored *Timgrovea*. This last cluster is not recognised at the 0.05 distance unit cut-off but the two more symmetrically spored collections are separated from the asymmetrically-spored H4146. Three of the four *Descolea*-like clusters in Figure 12 have comparable clades in the morphological analyses. S corresponds to the pink cluster, E to the green and A to the orange. It is

also notable that two of the three collections in cluster F are also in the lime cluster in the Figure 4 dendrogram.

3.3.2 Ordination

The *P*rincipal *C*Oordinates analysis (PCO) of the pair-wise 'mean' distances of the molecular data produced the scatter plots shown in Figure 13. The percentages of the variance accounted for by each of the first three axes were: Axis one: 75.405%, Axis two: 5.001% and Axis three: 4.241%. The most prominent separation on Axes one and two is that between the *Cortinarius*-like and *Descolea*-like collections. The separation, within the *Descolea*-like group of the three broad-, irregularly ornamented-spored *Timgrovea* H4162, H4204 and H4146 (group F) and the two *Setchelliogaster australiensis*-like collections H4234 and TRAPPE14281 (group A) from the other *Descolea*-like collections is also prominent both on Axes one and two and Axes three and two.

Six groups of *Cortinarius*-like collections were discerned from the two scatter plots in Figure 13. These groups are comparable to the clusters identified in Figure 12 and are labelled accordingly. H5814 (large spores, gelatinous peridium) groups as pair Wb with H5197 '*Thaxterogaster*' from cluster W in Figure 12. The other collections from cluster W also group together in Figure 13 as group Wa. The small spored '*Thaxterogaster*' collections with lilac and white peridia group together in group L. Groups G, B and P have the same composition as the cluster-analysis-dendrogram cluster with the same name. The *Cortinarius*-like collections appear to form an irregular ring around the origin in Axes three against two. This could be seen by drawing a line joining the groups in the order L → B → P → G → Wa → Wb → L.

The *Descolea*-like collections could also be considered to form a ring on the scatter plot of Axis three against Axis two, however the separation of groups F and A from the other groups is the dominant feature of both scatter plots in Figure 13. Groups F and A have the same composition as in the cluster analysis, however the other two clusters from Figure 12, E and S, were fragmented in the PCO analysis. Group X as indicated in Figure 13 is composed of three collections not related in the cluster analysis. Broad-, irregularly reticulate-spored *Timgrovea* subgenus *Subreticulata* H4057 belonged to cluster S, small-, partially reticulate-spored *Timgrovea* subgenus *Subreticulata* H5655 belonged to cluster E and broad-spored, *Setchelliogaster tenuipes*-like TRAPPE14175 was basal to the cluster containing clusters S, E and A. Group X is closely associated with groups Ea and b and Sa and b on Axes one against two and are closer to Ea and b in the scatter plot of Axes three against two. The group labelled E in the cluster analysis dendrogram (Figure 12) was separated into two groups in Figure 13. Group Ea contains the alveolate-spored *Timgrovea* subgenus *Timgrovea* collections H0732 and TRAPPE14535 while group Eb contains the fusoid-spored *Descomyces* collections (H0733 and H5807) and the contaminated sequences of collections H5092, H5185 and H5258. The final two groups of relatively squat-spored *Descomyces* are Sb, containing the *Descomyces albus*-like collections TRAPPE14397 and H7119, and Sa containing the remaining collections from cluster S.

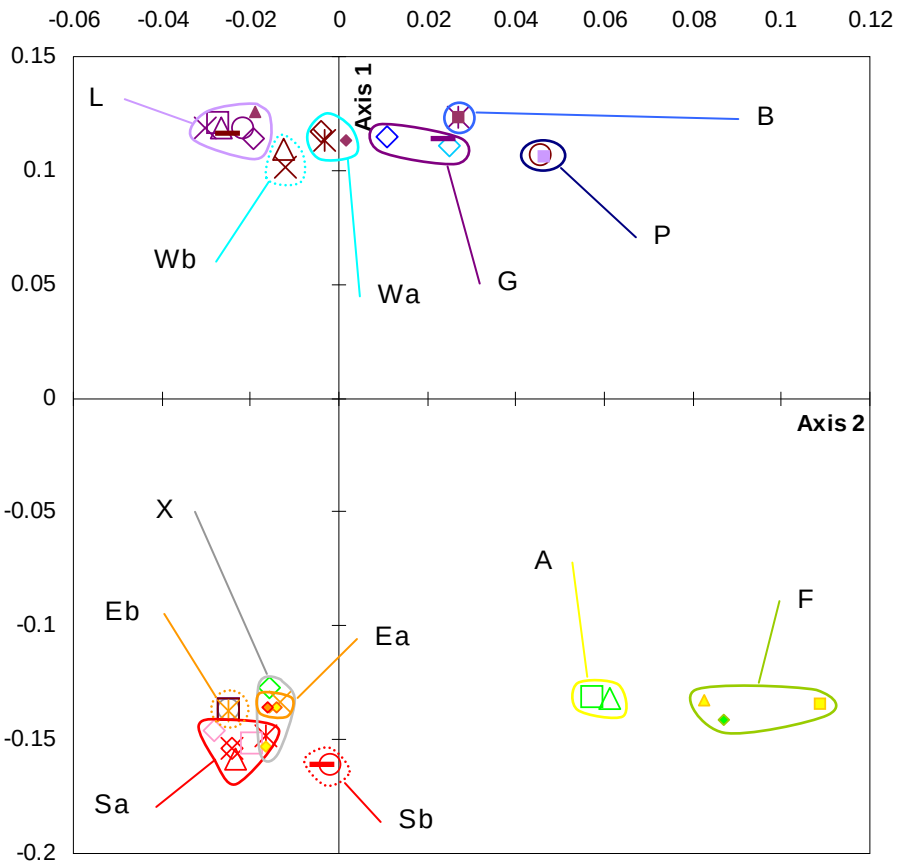
3.3.3 Parsimony analysis

Parsimony bootstrap analysis of the molecular data supported with values greater than 65%, more than 70% of branches in the 50% majority-rule consensus of the 704 equally most parsimonious trees found by the heuristic search (Figure 14). The separation of

the *Cortinarius*-like collections from the *Descolea*-like collections is supported with a bootstrap value of 100%. The topologies of these two clades however are not supported, the groups recovered and described in the cluster analysis branch from either a polytomy of *Descolea*-like clades or one *Cortinarius*-like clades.

Acknowledging the lack of support for the topology within the *Cortinarius*-like clade in Figure 14, this topology is different from that in the cluster dendrogram (Figure 12) though the same five groups were recovered. In the parsimony analysis, group G was placed closest to the *Descolea*-like clade followed by a clade containing two sister clades, one composed of collections from cluster W, and the other collections from cluster B. The position of clade B as sister to clade W is notable, as cluster B is basal to all other *Cortinarius*-like collections in the cluster analysis dendrogram. Collection H5814 which branched off between clusters W and L in Figure 12 again branches off between the clade containing the group W collections and the final *Cortinarius*-like clade, that containing the collections of group L.

The topology of the *Descolea*-like clade in Figure 14 lacks bootstrap support greater than 65% for any branch and differs from the topology of the cluster analysis dendrogram though clades representing the clusters found in that analysis are well-supported. As in Figure 12, the parsimony analysis groups clades S and E as sister clades. Group A was sister to the cluster containing these two groups in Figure 12 however the parsimony analysis groups clade A with clade F and places *Setchelliogaster tenuipes*-like collection TRAPPE14175 as a single-taxon branch between the two branches (S,E) and (A, F).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0.15 0.1 0.05 0 -0.05 -0.1 -0.15 -0.2
Axis 1
Axis 2
L
B
P
Wb
G
Wa
X
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Eb
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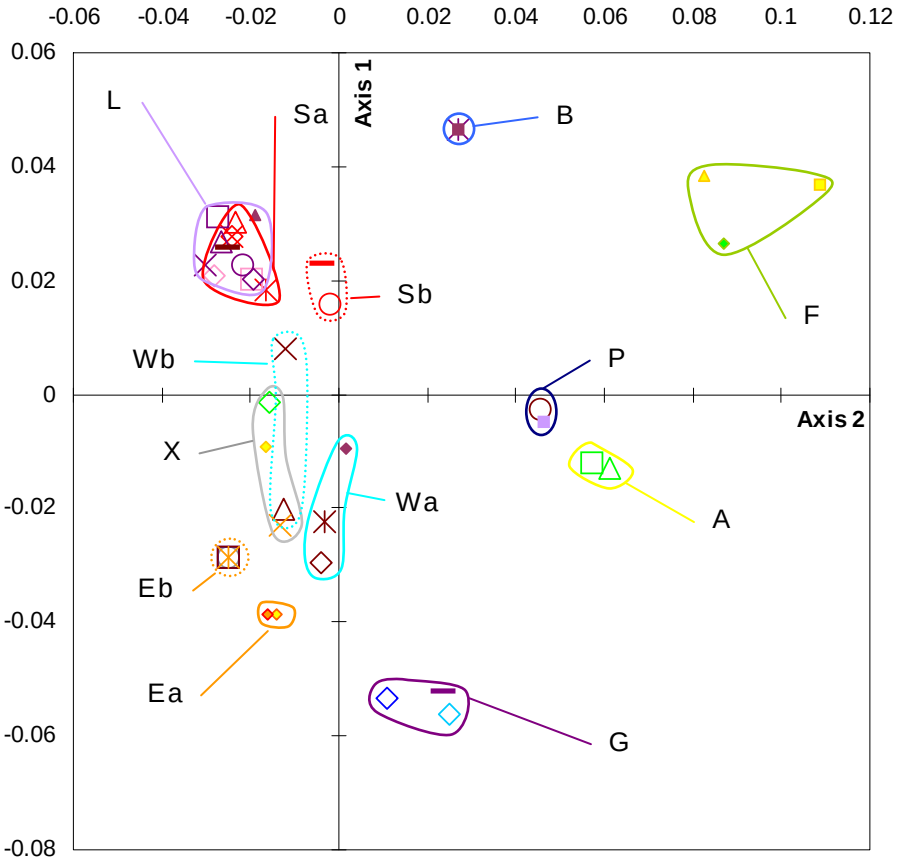
Sa
Sb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0.06 0.04 0.02 0 -0.02 -0.04 -0.06 -0.08
Axis 1
Axis 2
L
Sa
B
Sb
F
Wb
P
X
Wa
A
Eb
Ea
G

Figure 13: Principal COordinates analysis (PCO) of the pair-wise Mean distances of the molecular data set. Red lines indicate *Descolea*-like groups and blue lines *Cortinarius*-like groups. The axes are in eigenvalue units.

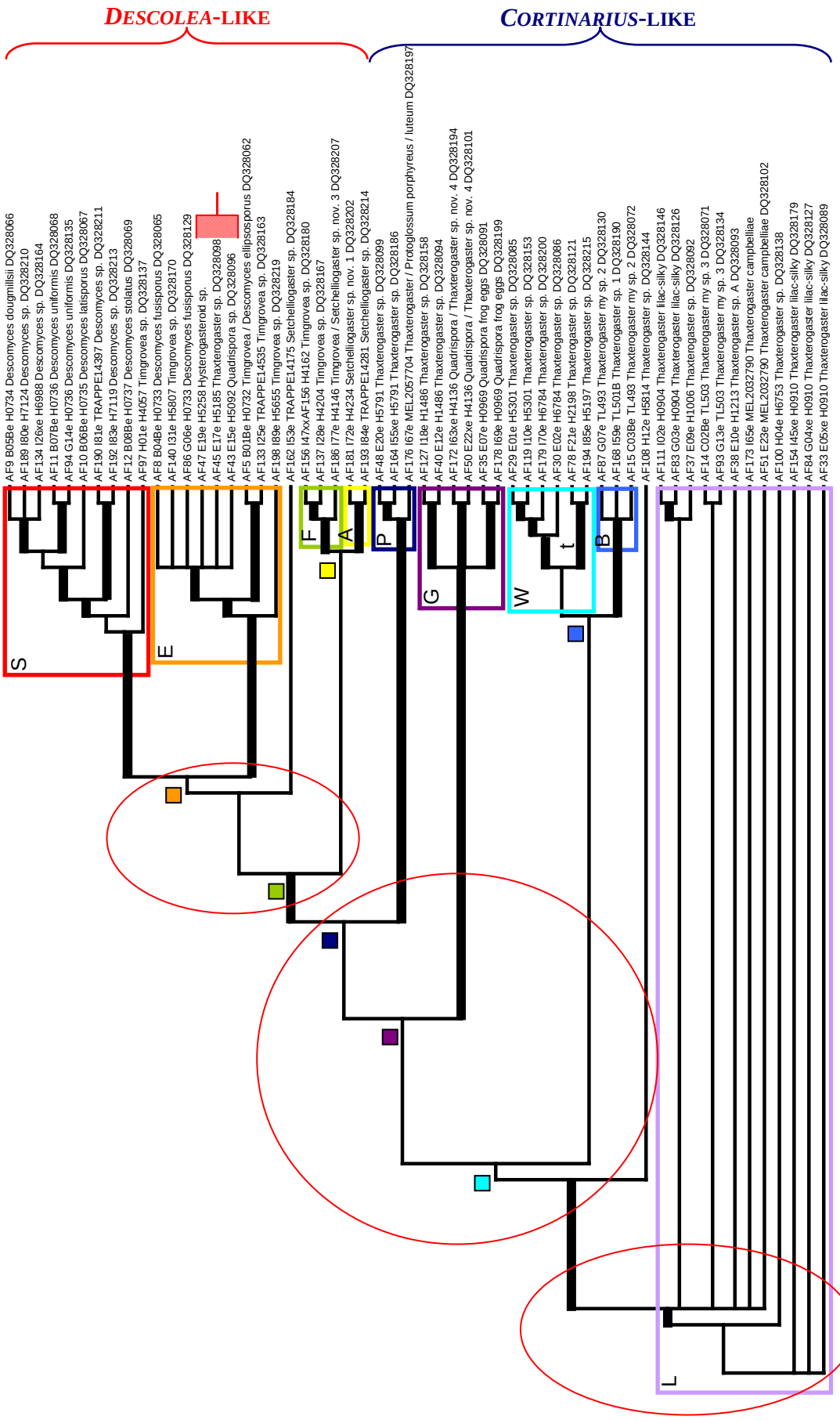
DESCOLEA-LIKE
CORTINARIUS-LIKE
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
AF189 I80e H7124 Descomyces sp. DQ328210
AF134 I26xe H6988 Descomyces sp. DQ328164
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF94 G14e H0736 Descomyces uniformis DQ328135
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
AF192 I83e H7119 Descomyces sp. DQ328213
AF12 B08Be H0737 Descomyces stolatus DQ328069
AF97 H01e H4057 Timgrovea sp. DQ328137
AF8 B04Be H0733 Descomyces fusisporus DQ328065
AF140 I31e H5807 Timgrovea sp. DQ328170
AF86 G06e H0733 Descomyces fusisporus DQ328129
AF47 E19e H5258 Hysterogasteroid sp.
AF45 E17e H5185 Thaxterogaster sp. DQ328098
AF43 E15e H5092 Quadrispora sp. DQ328096
AF5 B01Be H0732 Timgrovea / Descomyces ellipsosporus DQ328062
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163
AF198 I89e H5655 Timgrovea sp. DQ328219
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF156 I47xxAF156 H4162 Timgrovea sp. DQ328180
AF137 I28e H4204 Timgrovea sp. DQ328167
AF186 I77e H4146 Timgrovea / Setchelliogaster sp. nov. 3 DQ328207
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214
AF48 E20e H5791 Thaxterogaster sp. DQ328099
AF164 I55xe H5791 Thaxterogaster sp. DQ328186
AF176 I67e MEL2057704 Thaxterogaster / Protoglossum porphyreus / luteum DQ328197
AF127 I18e H1486 Thaxterogaster sp. DQ328158
AF40 E12e H1486 Thaxterogaster sp. DQ328094
AF172 I63xe H4136 Quadrispora / Thaxterogaster sp. nov. 4 DQ328194
AF50 E22xe H4136 Quadrispora / Thaxterogaster sp. nov. 4 DQ328101
AF35 E07e H0969 Quadrispora frog eggs DQ328091
AF178 I69e H0969 Quadrispora frog eggs DQ328199
AF29 E01e H5301 Thaxterogaster sp. DQ328085
AF119 I10e H5301 Thaxterogaster sp. DQ328153
AF179 I70e H6784 Thaxterogaster sp. DQ328200
AF30 E02e H6784 Thaxterogaster sp. DQ328086
AF78 F21e H2198 Thaxterogaster sp. DQ328121
AF194 I85e H5197 Thaxterogaster sp. DQ328215
AF87 G07e TL493 Thaxterogaster my sp. 2 DQ328130
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF108 H12e H5814 Thaxterogaster sp. DQ328144
AF111 I02e H0904 Thaxterogaster lilac-silky DQ328146
AF83 G03e H0904 Thaxterogaster lilac-silky DQ328126
AF37 E09e H1006 Thaxterogaster sp. DQ328092
AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071
AF93 G13e TL503 Thaxterogaster my sp. 3 DQ328134
AF38 E10e H1213 Thaxterogaster sp. A DQ328093
AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF51 E23e MEL2032790 Thaxterogaster campbelliae DQ328102
AF100 H04e H6753 Thaxterogaster sp. DQ328138
AF154 I45xe H0910 Thaxterogaster lilac-silky DQ328179
AF84 G04xe H0910 Thaxterogaster lilac-silky DQ328127
AF33 E05xe H0910 Thaxterogaster lilac-silky DQ328089
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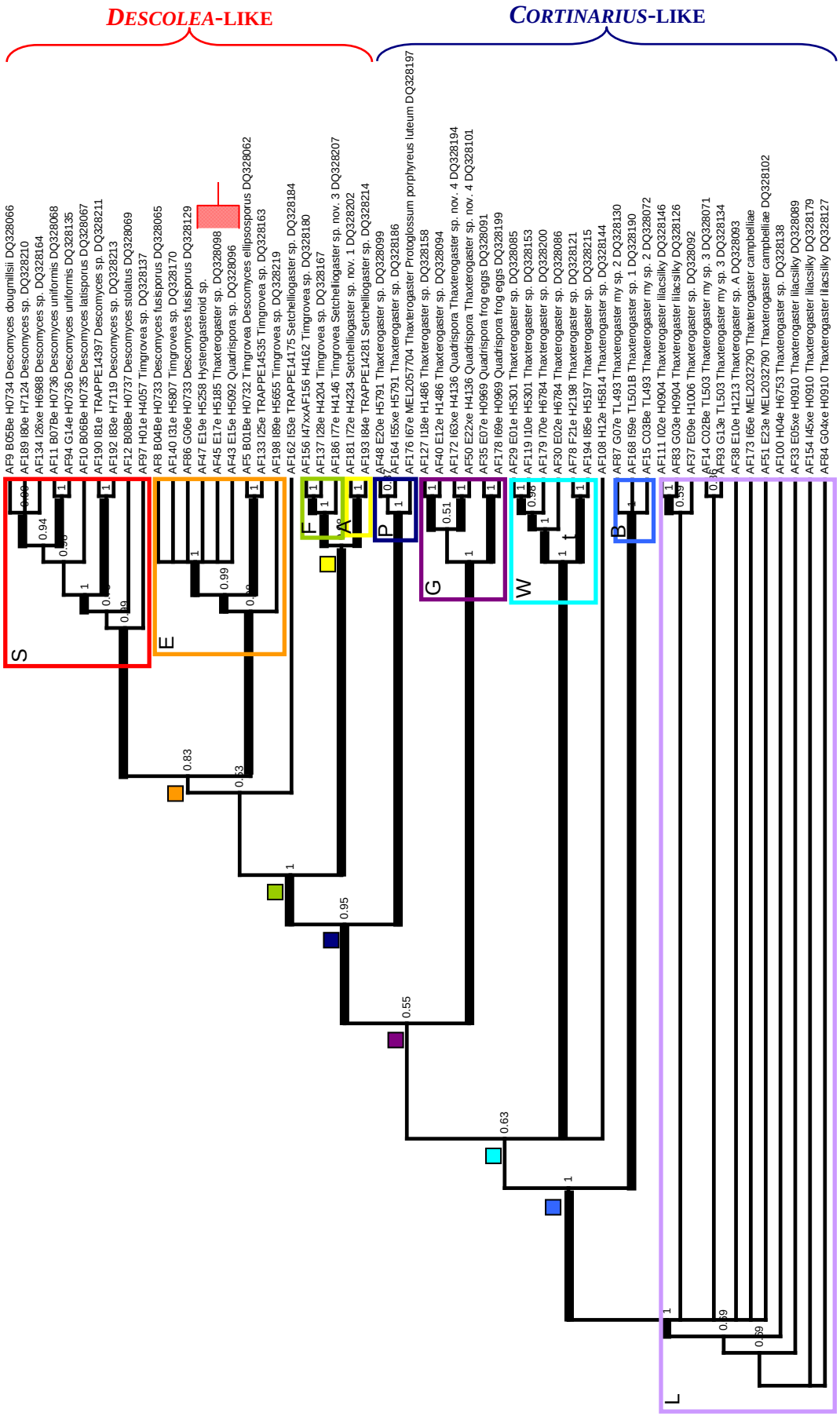
Figure 14: 50% majority rule consensus tree of the 378 trees found by a heuristic parsimony search of the molecular data. Coloured boxes indicate clusters discussed in the text, red ellipses indicate major polytomies. Bold branches have greater than 65% bootstrap support.

3.3.4 Bayesian analysis

The 50% majority-rule consensus tree resulting from Bayesian analysis molecular data indicated that more than 60% of the branches found had partition probabilities greater than 0.95 in as indicated in Figure 15. The separation of the *Cortinarius*-like collections from the *Descolea*-like collections had 100% partition probability support. The topology recovered by Bayesian analysis was similar to that recovered by parsimony analysis however particular branches within the *Cortinarius*- and *Descolea*-like clades received greater support, which reduced the degree of polytomy of these clades.

The *Cortinarius*-like collections formed clades with the same composition of collections as clades/clusters: L, W, B, G and P found in the cluster and parsimony analyses. Differences include the 95% partition probability for the branch separating clade P from all other *Cortinarius*-like clades, the placement of clade B and the placement of '*Thaxterogaster*' H5814 with its larger, coarsely ornamented spores and gelatinous peridium. Group B is basal to the *Cortinarius*-like collections in the cluster analysis dendrogram (Figure 12), sister to clade W in the parsimony analysis (Figure 14) and sister to clade L in the Bayesian analysis (Figure 15). In Figure 15 collection H5814 is part of a trichotomy with clade W and the clade containing clades B and L. This collection was sister to group L in Figure 12 and Figure 14.


DESCOLEA-LIKE
CORTINARIUS-LIKE
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
AF189 I80e H7124 Descomyces sp. DQ328210
AF134 I26xe H6988 Descomyces sp. DQ328164
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF94 G14e H0736 Descomyces uniformis DQ328135
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
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AF198 I89e H5665 Timgrovea sp. DQ328219
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF156 I47xxAF156 H4162 Timgrovea sp. DQ328180
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AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202
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AF48 E20e H5791 Thaxterogaster sp. DQ328099
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AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194
AF50 E22xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328101
AF35 E07e H0969 Quadrispora frog eggs DQ328091
AF178 I69e H0969 Quadrispora frog eggs DQ328199
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AF87 G07e TL493 Thaxterogaster my sp. 2 DQ328130
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146
AF83 G03e H0904 Thaxterogaster lilacsilky DQ328126
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AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071
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AF38 E10e H1213 Thaxterogaster sp. A DQ328093
AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF51 E23e MEL2032790 Thaxterogaster campbelliae DQ328102
AF100 H04e H6753 Thaxterogaster sp. DQ328138
AF33 E05xe H0910 Thaxterogaster lilacsilky DQ328089
AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179
AF84 G04xe H0910 Thaxterogaster lilacsilky DQ328127
S
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G
W
B
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Figure 15: 50% majority rule consensus tree of the 1502 sampled trees produced by Bayesian analysis of the molecular data. Bold branches are those with partition probabilities ≥ 0.95.

The *Descolea*-like clade has the same topology in the Bayesian analysis as it does in the parsimony analysis. As in that analysis clades F and A are sister clades however unlike that analysis, this relationship is well-supported by the Bayesian analysis with a partition probability of 0.98.

3.3.5 Expanded rDNA sequence dataset

3.3.5.1 Hebeloma *clade*

The bootstrapping of the parsimony analysis of the /Hebeloma clade showed strong separation of the outgroups (representatives of *Gymnopilus* and *Cortinarius*) from the well supported[7] /Hebeloma clade ingroup. There is, however, little bootstrap support for other structure within this ingroup.

Although there is a general lack of support for branches within the/Hebeloma clade, the three *Hymenogaster* collections sequenced for this project (highlighted in blue in Figure 16) sat in two well supported clades within this larger group. Two of these collections, AF91 (*Hymenogaster arenarius*) and AF81 (*Hymenogaster citrinus/australis c.f.*),

[7] Throughout this thesis, when referring to my own analyses and results, the phrases "well supported" or "strong support" and "poorly supported" or "weak support" in reference to branches of a phylogram indicate that the branches referred to have either greater than or less than 50% bootstrap support respectively unless otherwise stated. This is based on and for comparability with the cut-offs used by Peintner *et al.* (2001) however I acknowledge that Frøslev *et al.* (2005) suggest that only bootstrap values >65% and Bayesian Posterior Probabilities >95% indicate robust cortinarioid ITS clades.

occupied a 66% bootstrap supported clade of their own that was basal to all other well supported /Hebeloma clade branches, though this structure had less than 50% bootstrap support. The third *Hymenogaster* collection (AF191 *Hymenogaster sp.*) occupied a 65% bootstrap supported clade with *Hymenogaster subalpinus* (GenBank accession AF325640.1). This clade was itself a terminal member of another well supported (63%) clade of *Hymenogaster* species including *H. parksii* and *H. gardnerii*.

There were three main *Hymenogaster* containing clades in the /Hebeloma ingroup and a single isolated *Hymenogaster* sample. These three clades were the two mentioned above plus the third comprised of *Hymenogaster tener* and *H. alnicola*, both represented only by sequences from GenBank. The GenBank sequence of *Hymenogaster glacialis* was the isolated sample which is basal to the whole /Hebeloma clade except for the *Setchelliogaster rheophylla* collection labelled *Naucoria rheophylla*.

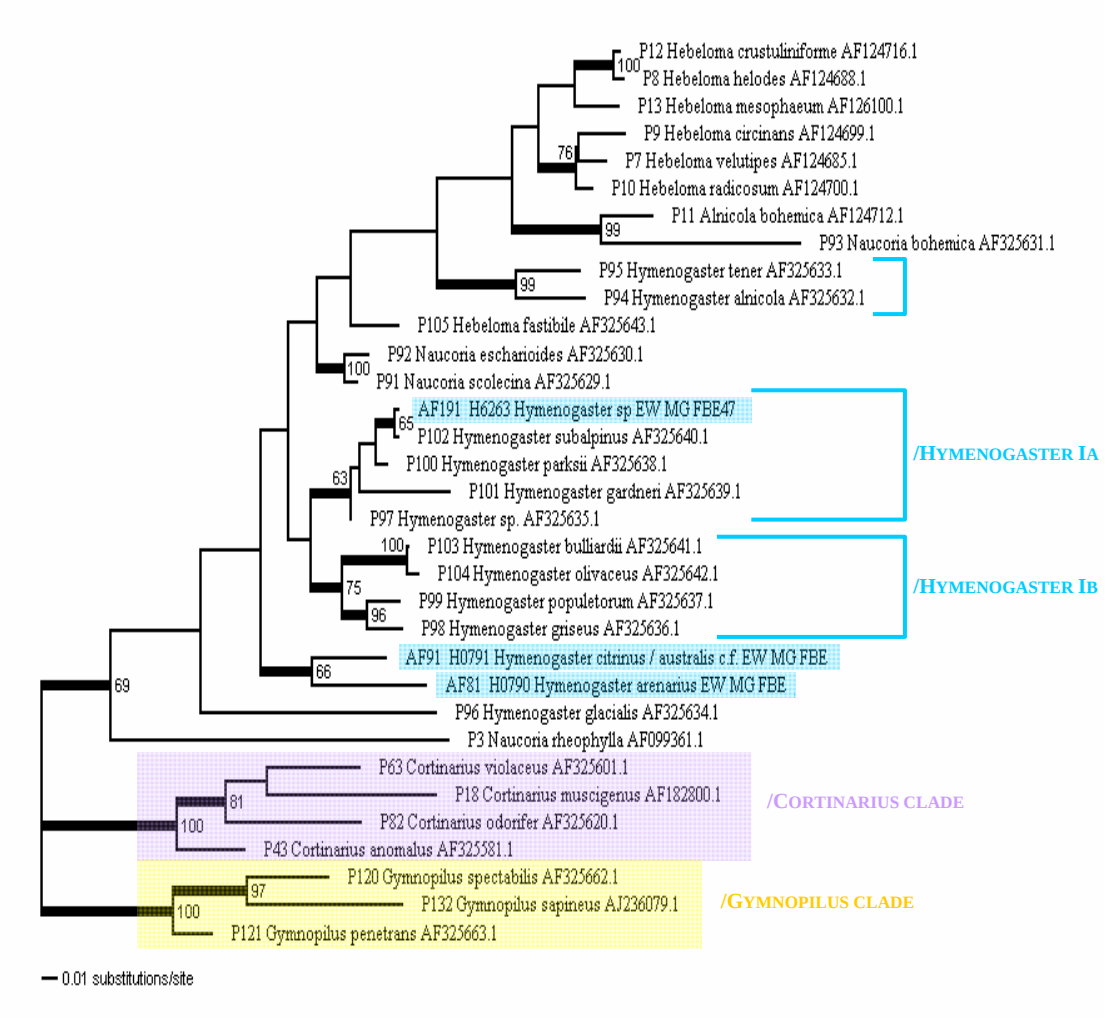


Figure 16. Phylogram produced by the maximum parsimony analysis of the /Hebeloma clade molecular dataset. The /Hymenogaster clades of Peintner *et al.* (2001) are indicated by the blue brackets. The outgroups are highlighted [/Cortinarius clade (purple), /Gymnopilus clade (yellow)]. The three *Hymenogaster* collections sequenced for this project are highlighted in blue. Bold branches have >50% bootstrap support.

3.3.5.2 Descomyces *clade*

The *Hebeloma* and *Cortinarius* sequences used as outgroups both form monophyletic groups at the base of Figure 17. The /Hebeloma clade has been placed as sister to the /Descolea clade after Peintner *et al.* (2001). The *Descomyces sp.* sample AF129 (Trappe14493) in the /Cortinarius outgroup appears to be a contaminant as does the

'hysterogasteroid' sample AF47 (H5258) situated in the /Timgrovea-Descomyces sub-clade.

Based on the working names given to the collections, seventeen clades representing putatively distinct taxa can be identified in Figure 17. It should be noted however that the deeper nodes of the tree have low bootstrap support. The two most basal of these clades form a well supported monophyletic group labelled /Setchelliogaster tenuipes – Descomyces after the dominant sequestrate forms in the clade. The *Descomyces*/*Setchelliogaster* and *Descomyces* samples (including *D. angustisporus*) of the upper sub-clade are distinct in Figure 17 from the *Setchelliogaster tenuipes, S. sp.* and *Descolea* samples in the lower sub-clade however this division has less than 50% bootstrap support. Two *Descolea phlebophora* sequences of Peintner *et al.* (2001) (GenBank accessions AF325655.1 and AF325657.1) form a well supported clade basal to the rest of the /Descomyces clade and are highlighted in orange in Figure 17.

The next clade up is labelled /Setchelliogaster australiensis – Descolea distinguishing it from the more basal, *Descomyces*-containing clade associated with *Setchelliogaster tenuipes*. This clade has five well supported sub-clades representing three published *Descolea* species, *S. australiensis* and a clade of unnamed *Setchelliogaster* collections. The *Descolea recendens, D. gunnii* and *D. maculata* sequences of Peintner *et al.* (2001) are basal to the *Setchelliogaster* containing subclades in Figure 17 however the bootstrap support for this topology is less than 50%. The two sister sub-clades of *Setchelliogaster* samples are relatively well supported, the clade of unnamed *Setchelliogaster* species has greater than 50% bootstrap however one of the four named *S. australiensis* samples (P90, AF325628.1) is not part of the well supported clade

containing the other three samples and the unnamed *Setchelliogaster* sample AF31 (H6806).

The next most apical clade is highlighted in orange in Figure 17 and contains the only named sequence of *Descomyces albellus*, two *Descolea antarctica* samples and an unnamed *Descomyces* sample [all GenBank accessioned sequences of Peintner *et al.* (2001)]. This clade has less than 50% bootstrap support and the two *Descolea antarctica* samples do not group together; in fact one (P108, AF325646.1) forms a well supported clade with the *Descomyces albellus* sample. This is the most apical clade containing samples used by Peintner *et al.* (2001) all clades above this contain only Australian collections sequenced for the purposes of this project. It should be noted though that there is less than 50% bootstrap support for the branches dividing the clades above and below this clade.

Timgrovea, as represented by seventeen sequences (marked with a ★ in Figure 17), is distributed over several clades within the large clade sister to that containing *Descolea antarctica*. Of the two 'daughter' clades of this '*Timgrovea* containing' clade, the clade towards the bottom of Figure 17 may be thought of as being *Timgrovea* 'dominated' in terms of the number of sequences it comprise while its sister clade contains more *Descomyces* than *Timgrovea* sequences though these groups have less than 50% bootstrap support.

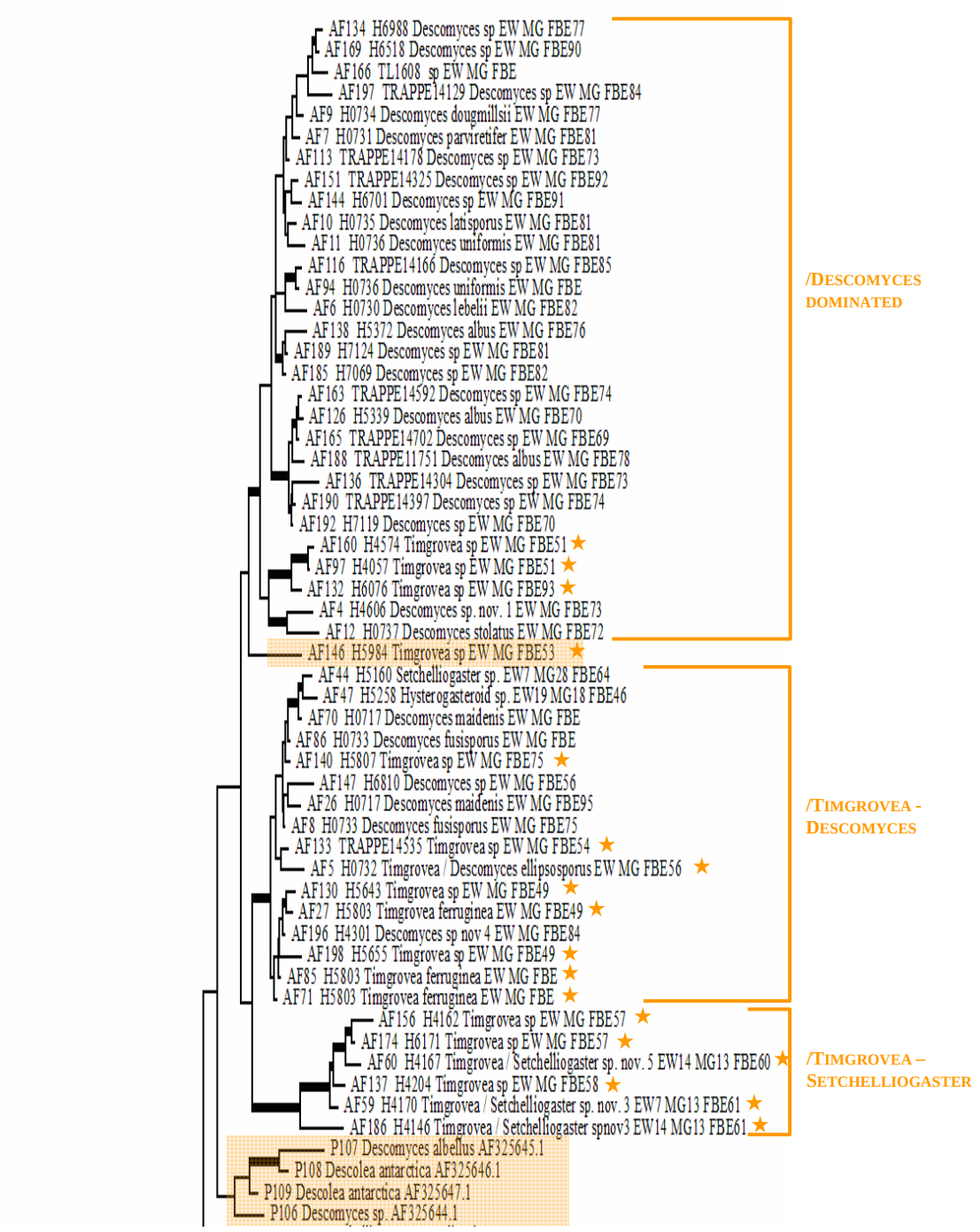


Figure 17. Phylogram produced by the maximum parsimony analysis of the /Descomyces clade molecular data and outgroups [/Cortinarius clade (purple), /Hebeloma clade (blue)]. The major sub-clades discussed in the text are bracketed while the isolated single sample *Timgrovea sp.* H5984, the poorly supported *Descolea antarctica*/*Descomyces* clade and the minor *Descolea phlebophora* clade are highlighted in orange. Bold branches have >50% bootstrap support and samples marked with a ★ were provisionally identified as *Timgrovea spp.*.

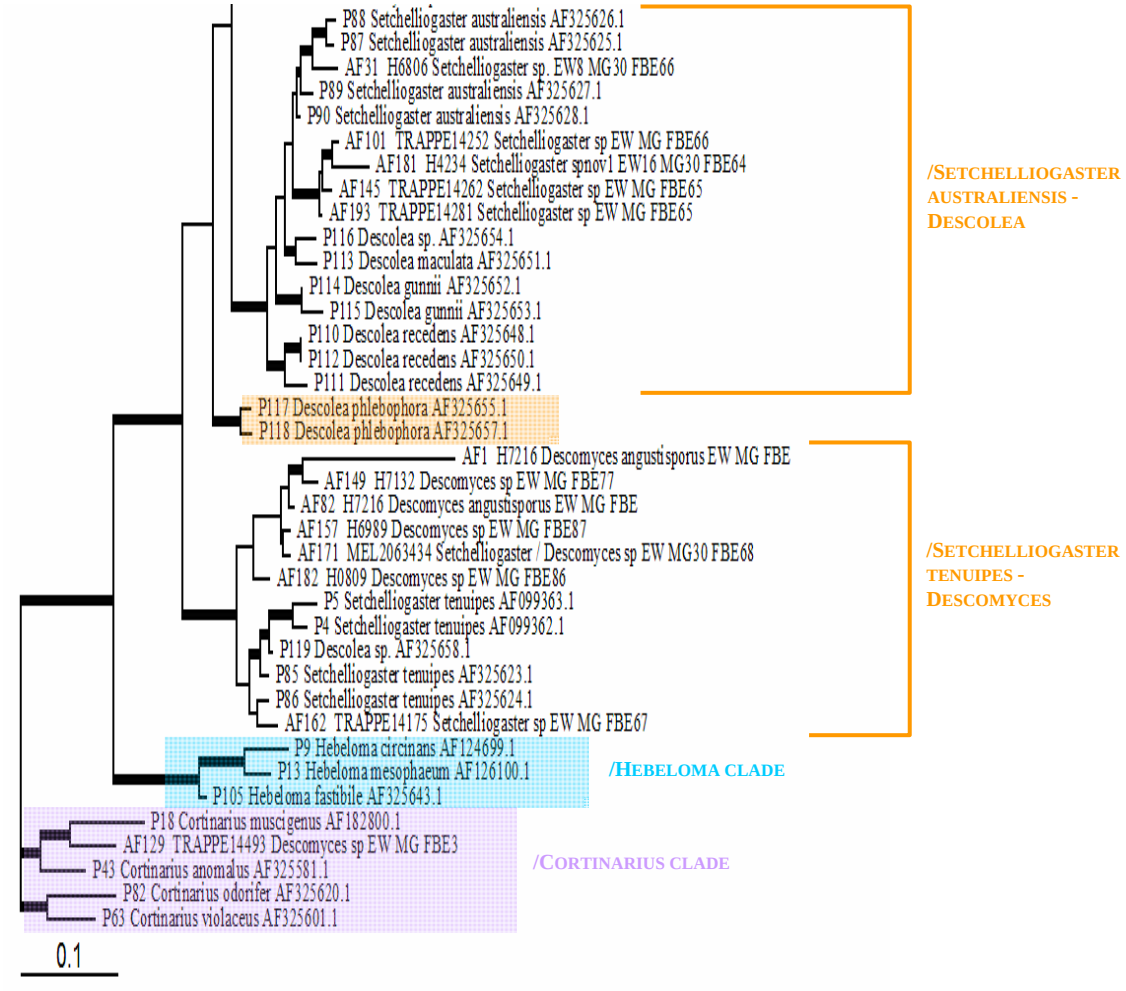


Figure 17 continued. Phylogram produced by the maximum parsimony analysis of the /Descomyces clade molecular data and outgroups [/Cortinarius clade (purple), /Hebeloma clade (blue)] The major sub-clades discussed in the text are bracketed while the isolated single sample *Timgrovea sp.* H5984, the poorly supported *Descolea antarctica*/*Descomyces* clade and the minor *Descolea phlebophora* clade are highlighted in orange. Bold branches have >50% bootstrap support and samples marked with a ★ were provisionally identified as *Timgrovea spp.*.

Though the '*Timgrovea* dominated' clade has less than 50% bootstrap support itself, it has two well supported sub-clades, one representing broad-spored, unnamed *Timgrovea/Setchelliogaster* species and the other *Timgrovea*, *Descomyces* and *Setchelliogaster* samples associated with *Timgrovea ferruginea*. A provisionally named *Descomyces* species, *D. maidenis* Trappe *nom. prov.*, *D. fusisporus* Trappe *nom. prov.* and alveolate-spored *Timgrovea*/*Descomyces ellipsosporus* Trappe *nom. prov.* form a group separate from that containing *T. ferruginea* in Figure 17 but lacking bootstrap

support greater than 50%. The topology of the /Timgrovea – Setchelliogaster sub-clade of Figure 17 has greater than 50% bootstrap support and the branch defining this sub-clade is one of the longest internal branches of the whole /Descolea clade.

A single *Timgrovea sp.* collection (AF146, H5984, highlighted orange in Figure 17) occupies a position basal to the '*Descomyces* dominated' clade. This sample represents one of three alveolate-spored *Timgrovea* subgenus *Timgrovea* collections it was possible to successfully sequence. There is however little bootstrap support for its position.

The well supported '*Descomyces* dominated' clade is the most apical of all the major well supported clades. This clade has several well supported sub-clades though there is little bootstrap support for the topology linking them. Prominent among these sub-clades is the one containing the only three *Timgrovea* samples in this clade. Sister to this *Timgrovea*-containing sub-clade is another well supported couplet of provisionally named *Descomyces stolatus* Trappe *nom. prov.* and an unnamed *Descomyces* species. Above these two clades in Figure 17 is a well supported group containing two of the three named *Descomyces albus* sequences along with several unnamed *Descomyces* samples. The third *D. albus* sample occupies a separate, though also well supported clade more apical to that containing the other collections, and is associated with two other unnamed *Descomyces* samples. This last *D. albus*-containing clade forms a poorly supported clade with two other clades, neither of which have strong bootstrap support. The one of these clades more towards the bottom of Figure 17 contains two provisionally named species *D. lebelii* Trappe *nom. prov.* and *D. uniformis* Trappe *nom. prov.* and an unnamed *Descomyces* sample. The larger, most apical clade contains a replicate sequence of *D. uniformis* Trappe *nom. prov.* along with three other

provisionally named *Descomyces* species (*D. dougmillsii* Trappe *nom. prov.*, *D. latisporus* Trappe *nom. prov.* and *D. parviretifera* Trappe *nom. prov.*) and seven unnamed *Descomyces* samples.

3.4 Discussion

Similar topologies were supported by both Parsimony bootstrap and Bayesian partition probability values for analyses of the molecular data including the separation of *Cortinarius*-like collections from *Descolea*-like collections. However neither of these methods of analysis and evaluation yielded well-resolved deeper nodes for either of these two major clades. Comparable clades/clusters of *Cortinarius*-like and *Descolea*-like collections were found in all analyses with the greatest difference in composition being seen in the groups derived from the Principal Coordinates analysis (PCO). This indicates that it was possible to consistently distinguish phylogenetically distinct groups of cortinarioid sequestrate fungi using ITS molecular data but not confidently relate them to one another. It appears that the groups are too distinct to be related perhaps because the inclusion of some longer gapped-regions (albeit only as missing data) inflated the distance between groups, or because intermediates were either not sampled, or not present the true environmental diversity. In preliminary analyses carried out on non-finalised sequences with a number of gapped regions removed and only considering parsimony informative characters, the internal topology of the *Descolea*-like clade had greater than 95% Bayesian partition probability. However the current analyses appear to present a more accurate picture of the phylogenetic structure of the data, both of the information contained in the gapped regions and the base substitution rates (estimated from the full alignment). There is the possibility that these sequences may have

diverged a very long time ago, potentially providing sufficient time for any theoretical intermediates to have been lost.

There was a high level of agreement between the major groups recovered under all analyses. The *Cortinarius*-like groups L, W, P, G and B and *Descolea*-like groups S, E, A and F are recovered with the same composition in all three dendrogram producing analyses (Figure 12, Figure 14 and Figure 15). The differences in composition in the PCO analysis relate to the interaction of relatively large within-group differences observable in the dendrograms or a more subjective grouping of the collections based on the scatter plots. Two *Descolea*-like clusters were split in the ordination isolating distinct pairs of collections Sb: TRAPPE14397 and H7119 (separated from the majority of group S collections by 0.04 distance units, as many as separate group S from group E see Figure 12) and Ea: TRAPPE14535 and H0732 (separated from group Eb by 0.033 distance units, the same distance as collection H5655 which was considered part of group X). Group X, which was not found in any other analysis, was largely a product of the interaction of the arrangement of the points on these three axes and a tendency to avoid single collections in the subjective grouping of the points on the scatter plots. These three axes accounted for nearly 85% of the variance in the data, however this still leaves a considerable percentage of the variance un-accounted for – it is likely that collections such as TRAPPE14175 and H4057 which are more divergent (long, single-taxon branches) and 'mobile' (tending to group with different sets of collections in different analyses) contribute to much of this un-accounted for variance. A tendency to avoid leaving single collections un-grouped in the ordinations (unless obviously isolated as was H1486 in the standardised morphological data PCA and PCO ordinations) was aggravated by the close spacing of the points and the lack of a rigorous method for

deciding on groups. The use of a minimum spanning tree superimposed on the ordination could aid in reducing the arbitrary element of preferential group size.

Because the higher level groupings were more consistent in the molecular analyses the formation of pairs of collections was not as important an element of patterning in the molecular data as it was for the morphological data. Pairs of collections tended to group with other related pairs and only one pair ('*Thaxterogaster*' collections H2198 and H5197) was split even by the more subjective groupings of the PCO. One or more replicate sequences were included for thirteen collections. For most collections the replicate sequences were identical, but four collections had slightly different replicate sequences – probably due to sequencing errors. In all of these four cases the replicates grouped close to one another, in two of the four cases as pairs (sequences for H5791 and H4136) and in the other two cases (H0910 and H6784), in the same clades. It is interesting to note that in preliminary analyses on non-finalised sequences with gapped-regions removed, H1213 (which groups consistently with clade/cluster L) and replicate sequences of MEL2032790 (which, when finalised, were identical) were thought to be contaminated because they grouped with unexpected collections. This suggests that the finalised sequences, including gapped regions, present a more accurate representation of the relationships between the sequences and collections.

Further work is needed to discern the phylogenetic relationships between the groups identified by ITS data within the *Cortinarius*-like and *Descolea*-like clades/clusters. The ITS data for the cortinarioid sequestrate fungi included in these analyses appears to provided phylogenetic resolution distinguishing possible subspecies (e.g. the larger- (H0904, H1006) and smaller-spored (H0910, H6753) collections of '*Thaxterogaster*' 'lilac-silky', different species (*Cortinarius campbelliae*-like collections MEL2032790

and TL503), and perhaps some groups of section or subgenus rank (group G). However the relationship of these groups to one another is not clear from the sample used in the comparative dataset. Relating the various cohesive groups within the *Cortinarius*-like and *Descolea*-like clades is likely to require sequencing of further regions. Published phylogenies of cortinarioid fungi have used the ITS, nuclear large subunit (nLSU) rDNA, nuclear and mitochondrial small subunit (nSSU or mtSSU) rDNA, and the genes encoding the two largest subunits of RNA polymerase II (RPB1 and RPB2) (Hibbett *et al.* 1997, Høiland & Holst-Jensen 2000, Seidl 2000, Peintner *et al.* 2001, Peintner *et al.* 2002, Garnica *et al.* 2003a & b, Rees *et al.* 2003, Peintner *et al.* 2004, Frøslev *et al.* 2005, Matheny & Bougher 2006a and Matheny *et al.* 2006). Other regions lodged on GenBank for *Cortinarius*, but not currently included in published papers on the subgeneric phylogeny of the genus include the translation elongation factor 1-alpha (tef1) gene, partial lac gene (laccase), mitochondrial NADH dehydrogenase subunit 5 (nad5) gene, manganese peroxidase (MnP1) gene, mitochondrial ATPase subunit 6 (ATP6) gene and the chitin synthase 1 and 2 (chs1, chs2) genes. The combined use of more conserved regions/genes with the ITS may provide good resolution within the *Cortinarius*-like and *Descolea*-like clades as seen in the improved support for internal branches gained by Frøslev *et al.* (2005) through combined analysis of ITS, RPB1 and RPB2 regions and by Matheny & Bougher (2006a) by combined analysis of RPB2 and nLSU rDNA sequences.

Chapter 4

Combined dataset

4.1 Introduction

Intuitively, if one's aim is to reconstruct the phylogeny of a suite of organisms, the more characteristics one can bring to bear on the task, the better (Farris 1986). This logic underpins the combination of different kinds of observations on the characteristics of an organism, for example molecular sequences, morphological dimensions, metabolite profiles etc., in simultaneous analyses. Various authors have suggested various reasons for combining data: that the strengths of one dataset may complement weaknesses in another (Hillis 1987), that weak but common phylogenetic signal will be additive and stand out above 'noise' (homoplasy etc.) in combined analyses (Barrett *et al.* 1991) or merely that a robust phylogeny should account for all the available data (Kluge 1989). However, Bull *et al.* (1993) points out that these advantages only hold true if the different datasets combined share a common evolutionary history. If this is not the case, the phylogenetic signal of any one dataset may be diluted by conflicting patterns in others or even converge upon an wrong topology with increasing confidence with additional data (Bull *et al.* 1993). Such conflicting patterns can arise from mechanisms such as reticulation in gene evolution and unaccounted for morphological convergences (e.g. the 'constrained evolution' of salamanders (Wake 1991), and gene versus species evolution as discussed by (Page & Charleston 1997 and Page & Charleston 1998). Thus several schools of thought have arisen regarding the combination of data on different characters including morphology.

Combined dataset

The different approaches to the combination of different datasets have been tested and reviewed by a number of authors (e.g. Tehler 1995, Nixon & Carpenter 1996, Page 1996). Broadly speaking, the three different approaches are: 'always combine', consensus tree methods and 'conditional combination' pending datasets meeting some congruence criterion. Consensus methods do not deal with all the data and conditional combination can exclude useful data that would improved the phylogenetic resolution (Lutzoni & Vilgalys 1995, Nixon & Carpenter 1996). Thus, of the three approaches to the combination of different kinds of data, the 'always combine' approach is preferred and negates the need for any congruence test.

Several tests do however, exist for assessing congruence between phylogenetic trees including those of Templeton (1983), Kishino & Hasegawa (1989), Rodrigo *et al.* (1993), the modification of the topology-dependent cladistic permutation tail probability (T-PTP) test of Faith (1991) used by Lutzoni & Vilgalys (1995) and the Incongruence-Length difference (ILD) test of Farris *et al.* (1995). These methods have been examined and several are ineffective indicators of homogeneity including the ILD (Lutzoni & Vilgalys 1995, Lutzoni & Barker 1999, Barker & Lutzoni 2002, Darlu & Lecointre 2002). Acknowledging the deficiencies of the ILD as an indicator of topological congruence, an indicator of data homogeneity and a criterion for combinability, the ILD was nevertheless carried out on the combined dataset as a readily [being incorporated into PAUP*4.1b (Swofford 2001)] and oft applied test of the reasonableness of combining the data (Barker & Lutzoni 2002).

The combination of data from various sources has been used successfully in examining fungal phylogeny. Lutzoni & Vilgalys (1995), despite only one of three tests of combinability suggesting that their molecular and morphological dataset (for lichenised

and non-lichenised Omphalinoid fungi) could be combined the parsimony analysis of the combined data agreed with that of the larger molecular dataset against which they tested it. Similarly positive results were obtained by Tehler (1995) who found that the combined analysis of their incongruent morphological and nSSU rDNA datasets produced the most parsimonious tree of all their analyses. These results suggest that combined analysis of morphology and molecular data is a potentially powerful tool for fungal phylogenetics.

4.2 Materials and Methods

On the basis of the separate analysis of the molecular data three collections identified as having contaminated sequences (H5258, H5185 and H5092 see section 3.3.1) were excluded from the combined analysis. The datasets were concatenated in two interleaved partitions with the first 741 characters being molecular data and the second 16 being morphological. For the calculation of the mean distances and parsimony analysis the data were entered as standard characters into Paup*4.1b with the following data block commands (which include the equate macros used for DNA data):

```
Begin data;
Dimensions ntax=53 nchar=757;
Format
datatype=Standard
symbols="0123456789acgtrymkswhbvdn"
equate="R = {AG} [ puRine ]
 Y = {CT} [ pYrimidine ]
 M = {AC} [ aMino ]
 K = {GT} [ Keto ]
 S = {CG} [ Strong ]
 W = {AT} [ Weak ]
```

Combined dataset

```
 H = {ACT} [ not G ]
 B = {CGT} [ not A ]
 V = {ACG} [ not T ]
 D = {AGT}[ not C ]
 N = {ACGT} [ unkNown ]
 X = ?"
interleave
gap=-
missing=?;
```

All characters were initially of equal weight and no transition/transversion bias was incorporated. For Bayesian analysis, the Nexus file was modified as the program MRBAYES is able to interpret "mixed" data types. The data block commands for the dataset to be analysed by MRBAYES were:

```
Begin data;
Dimensions ntax=53 nchar=757;
Format datatype=mixed (DNA:1-741, Standard:742-757)
interleave=yes gap=-;
```

Differential weighting was not possible using MRBAYES however three different weighting schemes were attempted for the parsimony and distance methods. The weighting schemes for each partition were the total number of characters in the other partition, the number of variable characters in the other partition or the number of parsimony informative characters in the other partition (detailed in Table 9). These weighting schemes were used to attempt to balance the contribution of the two datasets with their different number of characters.

Partition homogeneity testing (using the Incongruence-Length Difference test (Farris *et al.* 1995) as implemented by Paup*4.1b) was carried out on each weighted combined dataset.

Table 9: Weights applied to the molecular and morphological datasets under three different weighting schemes based on the features of the datasets indicated.

WEIGHTING BASED ON...	MOLECULAR	MORPHOLOGICAL
Total No. of characters	16	741
No. of variable chars	16	311
No. of parsimony informative chars	16	260

4.2.1 Cluster analysis

Cluster analysis was performed using the program PAST - PAlaeontological STatistics, ver. 1.53 (Hammer *et al.* 2001). The 'mean' distances of the aligned molecular data as calculated by PAUP*4.10b were clustered using the Un-weighted Pair-Group Method using arithmetic Averaging (UPGMA) group-linkage method. Trees were output in nexus format and arranged for display using TREEVIEW (Page 2001). A distance cut-off was then determined subjectively for the tree, with the aim of maximising the number of clusters across the whole tree grouping more than two collections.

4.2.2 Ordination

Principal Coordinates analysis (PCO) ordination was performed on the 'mean' distances of the aligned molecular data using the 'user distance' option in PAST. Groups were determined subjectively for the ordinations based on the recognition of sets of collections that appeared to group together in the scatter plots of both Axis one against

Combined dataset

Axis two and Axis three against Axis two. The percentage variance accounted for by the first three axes was also recorded.

4.2.3 Parsimony analysis

Parsimony analysis was carried out using PAUP*4. 10b (Swofford 2001) on the aligned molecular data with the following commands:

```
Set criterion=parsimony Maxtrees=250000 Increase=Auto
AutoInc=100 TaxLabels=full;
Hsearch start=stepwise addseq=random nreps=10 rstatus=yes;
```

The resulting trees were saved and strict and majority-rule consensus trees were calculated (Le50 was set to 'no' so that only groupings occurring in 50% or more of the trees would be included and there would be no possibility of an equally parsimonious but contradictory branch being excluded from the tree). Bootstrap support for the topology was determined by 10000 bootstrap replicates subjected to a heuristic "faststep" search where for each bootstrap replicate there is only one random-sequence-addition replicate and no branch swapping, using the following commands:

```
Bootstrap nreps=10000 brlens=yes format=nexus
treefile=filename.tre search=faststep;
```

4.2.4 Bayesian analysis

Bayesian analysis was carried out using the program MRBAYES (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). The General Time Reversible model with a variable gamma shaped rate distribution and a proportion of invariable sites (GTR+G+I) model suggested by the program MRMODELTEST (Nylander 2004) for the molecular dataset alone was used in the setting the priors for the molecular partition

in the Bayesian analysis of the combined data. Rates were allowed to vary between partitions. Except for the following commands the default settings were used:

```
Lset applyto=(1) nst=6 rates=invgamma
Unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all)
Prset applyto=(all) ratepr=variable
Mcmcp ngen=2000000 samplefreq=2000
```

I ran the analysis for 2000000 generations after which the standard deviation of split frequencies had dropped below 0.009.

4.3 Results

The results for the combined analysis were so similar to those of the molecular analysis that this section will focus primarily on the differences between these two sets of analyses.

4.3.1 Cluster analysis

The dendrogram produced by the UPGMA clustering of the 'mean' distances of the combined data is presented in Figure 18. The most prominent feature of the tree is the division between *Cortinarius*-like and *Descolea*-like collections (0.198 distance units).

The 0.06 distance unit cut off indicated by the red line in Figure 18 shows six major *Cortinarius*-like clusters with the same composition as those labelled L, W, P, G and B in the analysis of the molecular data. The UPGMA cluster dendrogram of the 'mean' distances of the combined data shows the same pattern of branching between the major *Cortinarius*-like clusters, as that for the molecular data. There are within-cluster differences in topology however. Within cluster L in the combined cluster analysis

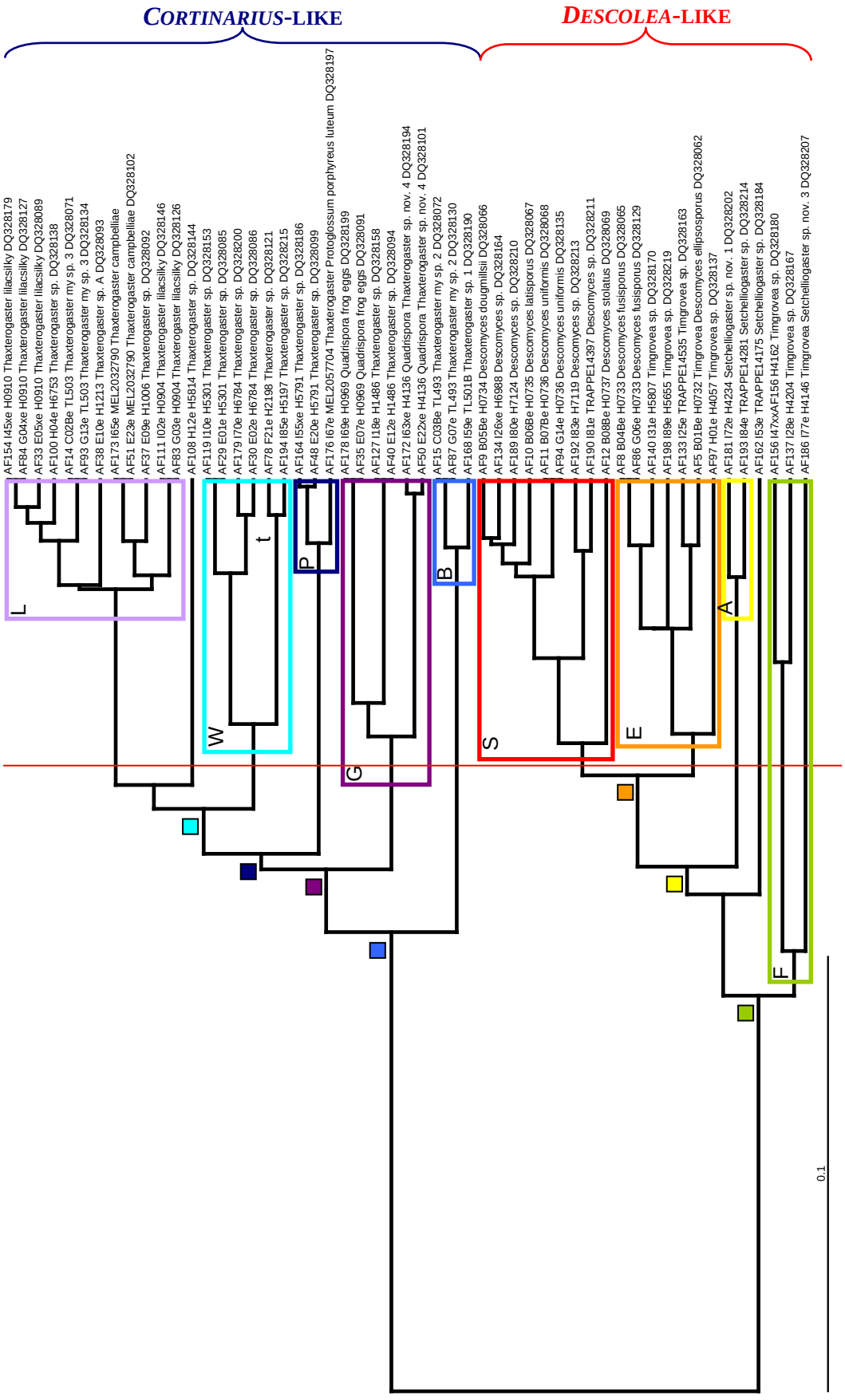
Combined dataset

(Figure 18) *Cortinarius campbelliae*-like MEL2032790 groups with the larger-spored (H0904) rather than the smaller-spored (H0910) '*Thaxterogaster*' with lilac peridia. The very small-spored '*Thaxterogaster*' with a white peridium also groups with the smaller spored collections within cluster L rather than being basal to the whole cluster. The combined cluster analysis forms a pair of the two samples of H6784 in cluster W whereas in the molecular analysis one sample subtended the clade containing the other with the two collections of H5301. Clusters P and G have the same branching pattern in the combined cluster analysis as in the molecular cluster analysis, however in cluster B, collection TL501B is distinguished from TL493.

Table 10: Listing of the clusters/clades found in analyses of the molecular and combined datasets and the two single taxon clades consistently not included in the detailed clades.

LABEL	DESCRIPTION AND COLLECTIONS
L	Smaller-spored '*Thaxterogaster*' with ***L***ilac peridia (TL503, H0910, H6753, H0904, H1006, MEL2032790 and H1213)
W	Smaller-spored '*Thaxterogaster*' with ***W***hite peridia (H5301, H6784, H5197, H2198)
P	'*Thaxterogaster*' collections with sub-spherical spores and gelatinous ***P***eridia (H5791, MEL2057704)
G	***G***elatinous peridia and relatively large non sub-spherical spores (*Quadrispora* H0969, unusually large-spored '*Thaxterogaster*' H1486 and elongate-spored '*Thaxterogaster*' H4136
B	'*Thaxterogaster*' with dry white peridia and relatively ***B***ig spores
H5814	Relatively large, coarsely ornamented spores with a gelatinous peridium with clamp connections in the outermost layer of the peridium
S	***S***quat-spored (H0734, H7124, H6988, H0736, H0375) and *Descomyces albus*-like (TRAPPE14535, H7119) *Descomyces*
E	***E***longate-spored (H0733, H5807) *Descomyces* and *Timgrovea* with alveolate- (H0732, TRAPPE14535 subgenus *Timgrovea*) and smaller-, partially reticulate-spored (H5655 subgenus *Subreticulata*)
A	*Setchelliogaster* **a***ustraliensis*-like collections H4234 and TRAPPE14281
F	Broad-, irregularly ornamented-spored *Timgrovea* H4162, H4204 and H4146
TRAPPE 14175	*Setchelliogaster tenuipes*-like

Figure 18: Cluster analysis dendrogram for UPGMA clustering of the pair-wise 'Mean' distances of the unweighted combined data. Coloured boxes indicate clusters discussed in the text. The red line indicates the distance cut-off for this analysis.


CORTINARIUS-LIKE
DESCOLEA-LIKE
AF154 I45xe H0910 Thaxterogaster iliacsilky DQ328179
AF84 G04xe H0910 Thaxterogaster iliacsilky DQ328127
AF33 E05xe H0910 Thaxterogaster iliacsilky DQ328089
AF100 H04e H6753 Thaxterogaster sp. DQ328138
AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071
AF93 G13e TL503 Thaxterogaster my sp. 3 DQ328134
AF38 E10e H1213 Thaxterogaster sp. A DQ328093
AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF51 E23e MEL2032790 Thaxterogaster campbelliae DQ328102
AF37 E09e H1006 Thaxterogaster sp. DQ328092
AF111 I02e H0904 Thaxterogaster iliacsilky DQ328146
AF83 G03e H0904 Thaxterogaster iliacsilky DQ328126
AF108 H12e H5814 Thaxterogaster sp. DQ328144
AF119 I10e H5301 Thaxterogaster sp. DQ328153
AF29 E01e H5301 Thaxterogaster sp. DQ328085
AF179 I70e H6784 Thaxterogaster sp. DQ328200
AF30 E02e H6784 Thaxterogaster sp. DQ328086
AF78 F21e H2198 Thaxterogaster sp. DQ328121
AF194 I85e H5197 Thaxterogaster sp. DQ328215
AF164 I55xe H5791 Thaxterogaster sp. DQ328186
AF48 E20e H5791 Thaxterogaster sp. DQ328099
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197
AF178 I69e H0969 Quadrispora frog eggs DQ328199
AF35 E07e H0969 Quadrispora frog eggs DQ328091
AF127 I18e H1486 Thaxterogaster sp. DQ328158
AF40 E12e H1486 Thaxterogaster sp. DQ328094
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194
AF50 E22xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328101
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF87 G07e TL493 Thaxterogaster my sp. 2 DQ328130
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
AF134 I26xe H6988 Descomyces sp. DQ328164
AF189 I80e H7124 Descomyces sp. DQ328210
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF94 G14e H0736 Descomyces uniformis DQ328135
AF192 I83e H7119 Descomyces sp. DQ328213
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
AF12 B08Be H0737 Descomyces stolatus DQ328069
AF8 B04Be H0733 Descomyces fusisporus DQ328065
AF86 G06e H0733 Descomyces fusisporus DQ328129
AF140 I31e H5807 Timgrovea sp. DQ328170
AF198 I89e H5655 Timgrovea sp. DQ328219
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus DQ328062
AF97 H01e H4057 Timgrovea sp. DQ328137
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF156 I47xxAF156 H4162 Timgrovea sp. DQ328180
AF137 I28e H4204 Timgrovea sp. DQ328167
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207
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Combined dataset

The arrangement of the four major *Descolea*-like clusters and two single taxa (TRAPPE14175 and H4146) in the UPGMA cluster dendrogram of the mean distances of the un-weighted combined data (Figure 18) is the same as that for the molecular cluster analysis though the composition of clusters S and E differ. In each molecular dendrogram *Descomyces* H0717 (with a prominent, flaring perispore) and *Timgrovea* subgenus *Subreticulata* H4057 (irregularly reticulate spore ornamentation) form single taxon branches subtending the majority of cluster/clade S collections. In Figure 18, only H0737 is associated with cluster S while H4057 is basal in cluster E. Within-cluster differences are seen in cluster S and E. The more basal position of *Descomyces* H0736, the breaking up of the identical sequences of H0734 and H7124 and the closer, less basal association of H0735 with these collections in Figure 18 differ from the molecular cluster analysis. In cluster E, aside from the absence of the three contaminated sequences, the major topological difference between Figure 18 and the molecular cluster analysis is the (albeit almost polytomous) association of H5655 with H0733 and H5807 rather than a similarly distant association with H0732 and TRAPPE14535.

4.3.2 Ordination

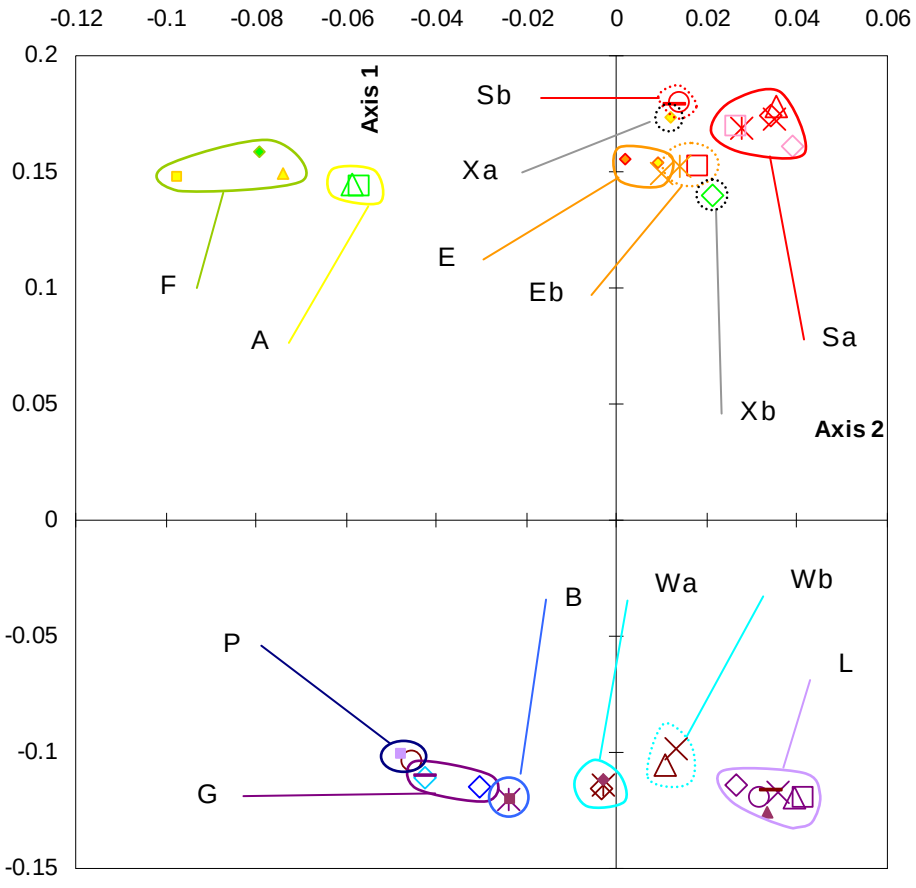
The Principal Coordinates analysis (PCO) of the pair-wise 'mean' distances of the combined data produced the scatter plots shown in Figure 19. Groups recovered from examination of the scatter plots were similar despite the changed (arbitrary) orientation of the axes. The percentages of the variance accounted for by each of the first three axes were: Axis one: 70.406%, Axis two: 5.104% and Axis three: 4.439%. In general the distribution of the groups, including the prominent *Cortinarius*-like/*Descolea*-like separation and the separation of group F from groups S, E and A were similar between

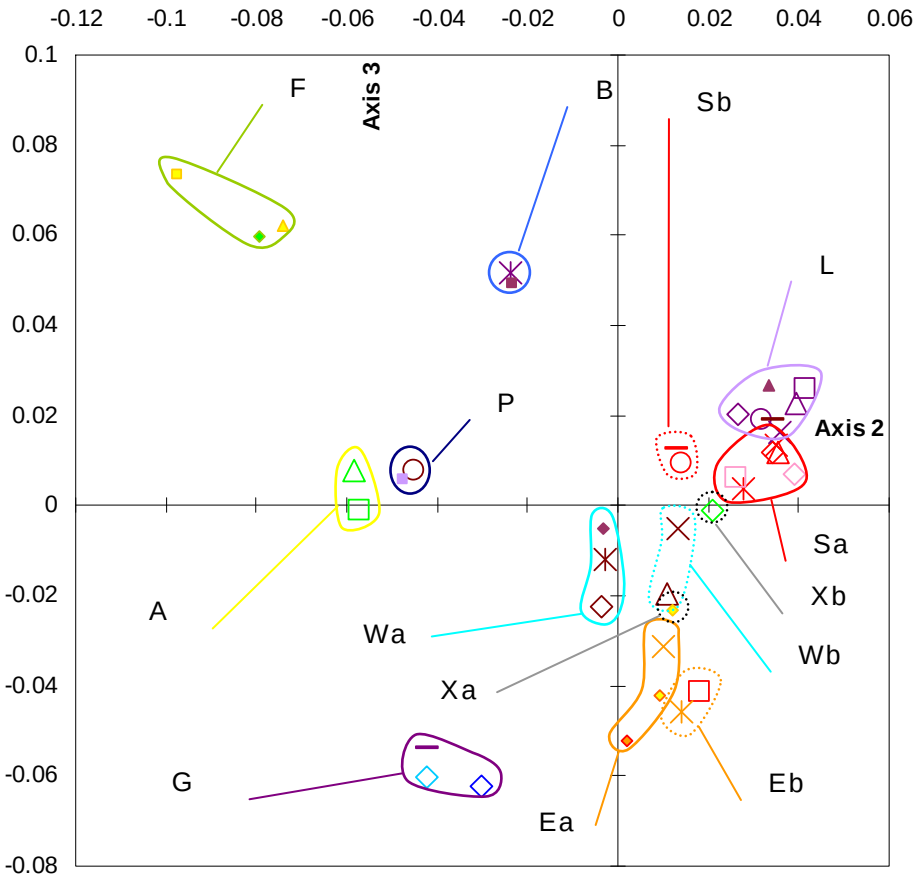
combined and molecular ordinations. There is less overlap between *Cortinarius*-like and *Descolea*-like groups on the scatter plot of Axes three and two for the combined data than there was for the molecular data alone. However, most differences occurred in the composition of the groups and the recognition of additional groups in the combined ordination.

The six groups of *Cortinarius*-like collections indicated in Figure 19 had the same composition as the groups with the same names in the molecular ordination. There is perhaps more distinction between the groups in Figure 19 especially between Wa and Wb. The groups can still be envisaged as forming a ring on Axes three and two (L → B → P → G → Wa → Wb → L), however the division of the groups in the manner (((P, B), G), (L, W)) may also be appropriate.

The ordination of the combined data (Figure 19) recovered groups of *Descolea*-like collections comparable to groups Sa, Sb, Ea, Eb, A and F. The collections grouped as group X in the molecular ordination were split between group Ea (H5655), and two single taxon groups Xa (H4057) and Xb (TRAPPE14175). Groups Ea and Eb are also much better separated from group Sa in Figure 19 than in the molecular ordination. Figure 19 also suggests associations between TRAPPE14175 (Xb) and group Sa and between H4057 and group Ea, the latter of these reflecting the position of H4057 in the cluster analysis (Figure 18). Within group Sa three subgroups were identified consisting of 1, H0737, 2, H0736 and H6988, and 3, H0734, H0735 and H7124. These groupings were also present in the molecular ordination. However these were not as apparent due to the crowding of *Descolea*-like collections on Axes one and two and the overlap between groups Sa and L on Axes three and two.

Combined dataset





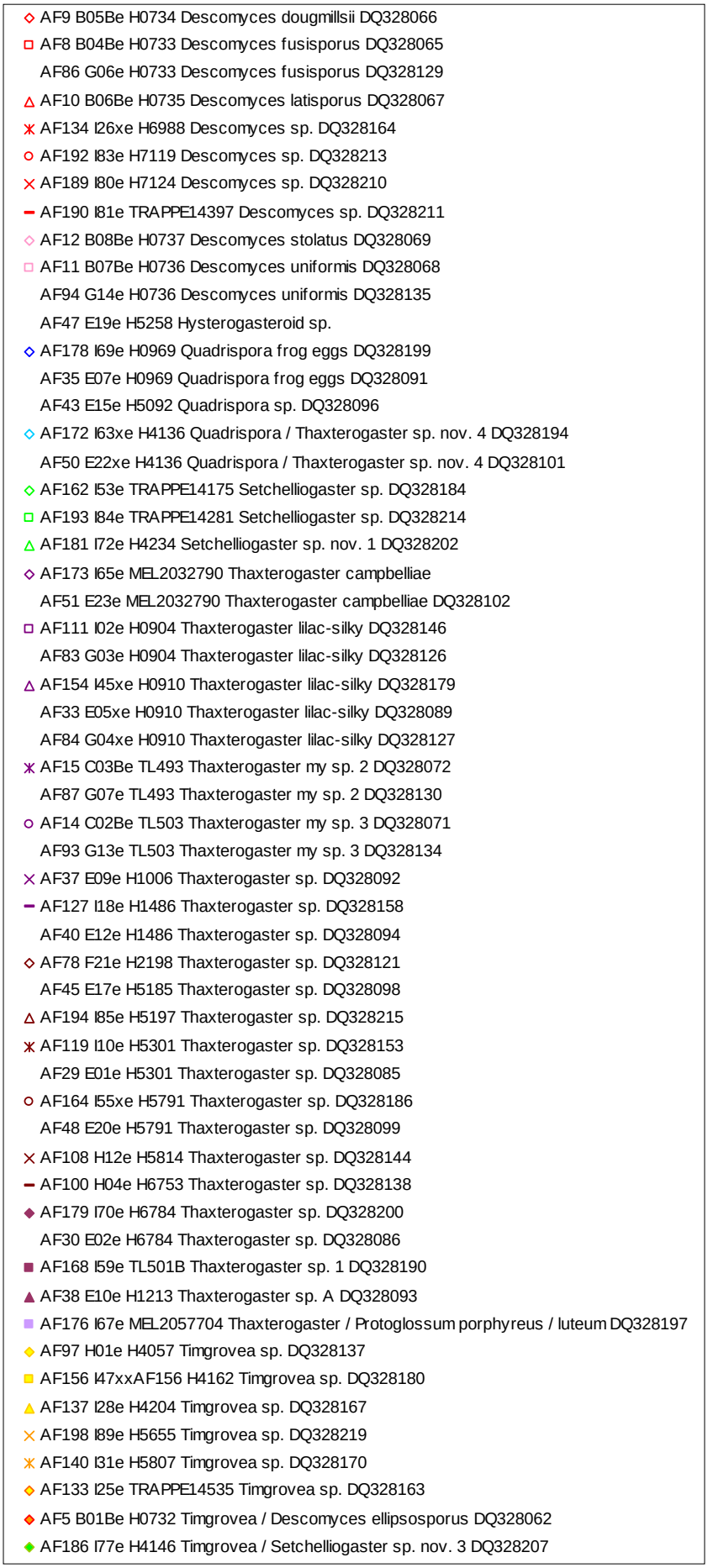


Figure 19: Principal COordinates analysis (PCO) of the pair-wise Mean distances of the unweighted combined data. The axes are in eigenvalue units.

4.3.3 Parsimony analysis

Parsimony bootstrap analysis of the combined data supported with values greater than 65%, more than 77% of branches in the 50% majority-rule consensus of the 378 equally most parsimonious trees found by the heuristic search (Figure 20). As in the molecular parsimony analysis, parsimony bootstrap analysis of the combined data supports the separation of the *Cortinarius*-like collections from the *Descolea*-like collections with a value of 100%. However, as in the molecular parsimony analysis, there is little support for the within-clade topology of either the *Cortinarius*- or *Descolea*-like clades.

Figure 20 shows that clade P is the only clade not part of the polytomy of the four major *Cortinarius*-like clades in the 50% majority-rule consensus tree from the parsimony analysis of the combined data. Though the parsimony analysis of the combined data 50% consensus tree has fewer branches relating the major *Cortinarius*-like clades than that for the molecular data, it has three more branches (18 versus 15) over all, with greater than 65% bootstrap support ('well-supported'). Ten of the eighteen 'well-supported' branches in the *Cortinarius*-like clade unite replicate sequences of single collections, five define the five major *Cortinarius*-like sub-clades, one unites H6753 with the replicate sequences of H0910 and the remaining two define two pairs of collections [(H5301, H6784) and (H2198, H5197)] in clade W. The molecular analysis lacked well-supported branches separating TL493 and TL501B, lacked support for the replicate sequence pairs for TL503 and MEL2032790, and separated replicate sequences for H6784.

Within the *Descolea*-like clade in Figure 20, sub-clades S, E, A and F join a polytomy of all four clades as in the molecular parsimony analysis. In contrast to the molecular parsimony analysis, the combined data separates the identical sequences of H0733 and H5807 but the two collections H0737 and H4057 join the polytomy directly rather than receiving bootstrap support as part of clade S. Two further differences between the combined and molecular parsimony analyses are that bootstrapping of the combined data did not support the grouping of collections H0734, H7124 and H6988 and that H0735 forms a polytomy with the clade containing those collections and H0736 in the 50% majority-rule consensus tree for the combined data parsimony analysis.

4.3.4 Bayesian analysis

The 50% majority-rule consensus tree resulting from Bayesian analysis molecular data indicated that just over 68% of the branches found had partition probabilities greater than 0.95 in as indicated in Figure 21. The separation of the *Cortinarius*-like collections from the *Descolea*-like collections had 100% partition probability support. The topology shown in Figure 21 is similar to that recovered by both the parsimony and Bayesian analysis of the molecular data.

The *Cortinarius*-like clades L, B, W and G are separated from clade P (partition probability of 0.97) as in the Bayesian analysis of the molecular data. In fact the composition and arrangement of clades in Figure 21 are the same as that in the Bayesian analysis of the molecular data with the exception of the topology of clade L. This clade has three additional internal nodes in Figure 21, none of which have partition probabilities equal to or greater than 0.95. Two branches present in clade L in the molecular Bayesian tree (but with less than 95% support) were well supported in the combined Bayesian analysis, that defining the three replicates of collection H0910 (note

that replicate AF33 rather than AF84 is sister to AF154 in Figure 21) and that defining the clade of these three collections and H1213. The pair of samples of TL503 has a partition probability value of 0.98 in Figure 21 whereas it was poorly-supported in the Bayesian analysis of the molecular data alone.

The arrangement and well-supported branches of the major *Descolea*-like sub-clades in Figure 21, is the same as in the dendrogram of the Bayesian analysis of the molecular data alone. The topology of the *Descomyces*-like clade in Figure 21 differs from that in the parsimony analysis of the combined data (Figure 20) at four points. The branch uniting the pair of sequences representing H0733 lacks support. There are additional, (though un-supported) branches separating H0735 from the clade containing H0736, H6988 etc. and separating H4057 from H0737. Finally, the branch uniting clades F and A (which was unsupported in the parsimony analysis of the combined data) has a partition probability value of 0.97 in Figure 21.

Figure 20: 50% majority rule consensus tree of the 378 trees found by a heuristic parsimony search of the unweighted combined data. Coloured boxes indicate clusters discussed in the text, red ellipses indicate polytomies. Bold branches have greater than 65% bootstrap support.


CORTINARIUS-LIKE
DESCOLEA-LIKE
AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179
AF84 G04xe H0910 Thaxterogaster lilacsilky DQ328127
AF33 E05xe H0910 Thaxterogaster lilacsilky DQ328089
AF100 H04e H6753 Thaxterogaster sp. DQ328138
AF38 E10e H1213 Thaxterogaster sp. A DQ328093
AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071
AF93 G13e TL503 Thaxterogaster my sp. 3 DQ328134
AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146
AF83 G03e H0904 Thaxterogaster lilacsilky DQ328126
AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF51 E23e MEL2032790 Thaxterogaster campbelliae DQ328102
AF37 E09e H1006 Thaxterogaster sp. DQ328092
AF108 H12e H5814 Thaxterogaster sp. DQ328144
AF119 I10e H5301 Thaxterogaster sp. DQ328153
AF29 E01e H5301 Thaxterogaster sp. DQ328085
AF179 I70e H6784 Thaxterogaster sp. DQ328200
AF30 E02e H6784 Thaxterogaster sp. DQ328086
AF78 F21e H2198 Thaxterogaster sp. DQ328121
AF194 I85e H5197 Thaxterogaster sp. DQ328215
AF178 I69e H0969 Quadrispora frog eggs DQ328199
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AF127 I18e H1486 Thaxterogaster sp. DQ328158
AF40 E12e H1486 Thaxterogaster sp. DQ328094
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194
AF50 E22xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328101
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF87 G07e TL493 Thaxterogaster my sp. 2 DQ328130
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF164 I55xe H5791 Thaxterogaster sp. DQ328186
AF48 E20e H5791 Thaxterogaster sp. DQ328099
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197
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AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
AF189 I80e H7124 Descomyces sp. DQ328210
AF134 I26xe H6988 Descomyces sp. DQ328164
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF94 G14e H0736 Descomyces uniformis DQ328135
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF192 I83e H7119 Descomyces sp. DQ328213
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
AF12 B08Be H0737 Descomyces stolatus DQ328069
AF97 H01e H4057 Timgrovea sp. DQ328137
AF198 I89e H5655 Timgrovea sp. DQ328219
AF8 B04Be H0733 Descomyces fusisporus DQ328065
AF86 G06e H0733 Descomyces fusisporus DQ328129
AF140 I31e H5807 Timgrovea sp. DQ328170
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus DQ328062
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163
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CORTINARIUS-LIKE
DESCOLEA-LIKE
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AF173 I65e MEL2032790 Thaxterogaster campbelliae
AF51 E23e MEL2032790 Thaxterogaster campbelliae DQ328102
AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072
AF87 G07e TL493 Thaxterogaster my sp. 2 DQ328130
AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190
AF119 I10e H5301 Thaxterogaster sp. DQ328153
AF29 E01e H5301 Thaxterogaster sp. DQ328085
AF179 I70e H6784 Thaxterogaster sp. DQ328200
AF30 E02e H6784 Thaxterogaster sp. DQ328086
AF78 F21e H2198 Thaxterogaster sp. DQ328121
AF194 I85e H5197 Thaxterogaster sp. DQ328215
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AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194
AF50 E22xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328101
AF178 I69e H0969 Quadrispora frog eggs DQ328199
AF35 E07e H0969 Quadrispora frog eggs DQ328091
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AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197
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AF137 I28e H4204 Timgrovea sp. DQ328167
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184
AF9 B05Be H0734 Descomyces dougmillsii DQ328066
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AF134 I26xe H6988 Descomyces sp. DQ328164
AF11 B07Be H0736 Descomyces uniformis DQ328068
AF94 G14e H0736 Descomyces uniformis DQ328135
AF10 B06Be H0735 Descomyces latisporus DQ328067
AF192 I83e H7119 Descomyces sp. DQ328213
AF190 I81e TRAPPE14397 Descomyces sp. DQ328211
AF12 B08Be H0737 Descomyces stolatus DQ328069
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AF198 I89e H5655 Timgrovea sp. DQ328219
AF8 B04Be H0733 Descomyces fusisporus DQ328065
AF86 G06e H0733 Descomyces fusisporus DQ328129
AF140 I31e H5807 Timgrovea sp. DQ328170
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus DQ328062
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163
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Figure 21: 50% majority rule consensus tree of the 1502 sampled trees produced by Bayesian analysis of the unweighted combined data. Bold branches are those with partition probabilities ≥ 0.95.

4.4 Discussion

Of the four weighting schemes attempted (un-weighted or with molecular : morphological weights: 16:741, 16:311 and 16:260) only the un-weighted partitions were considered homogeneous according to the Incongruence-Length Difference (ILD) test of Farris *et al.* (1995). This suggests that the lack of phylogenetic signal in the morphological data did not contradict the patterning in the molecular data when only worth 16 of 757 characters. Given the ratio of molecular to morphological characters it is not surprising that the patterns observed for the combined analyses are more similar to the patterns observed from analyses of the molecular data rather than the morphological data. It should be noted however that the ILD test is a relatively poor indicator of dataset combinability in simulated experiments (Barker & Lutzoni, 2002). Only combined analysis clades L, P, S and E had comparable clades in the morphological parsimony analysis (remembering that no branch in that analysis had greater than 65% bootstrap support). The manner in which this has influenced the combined analysis is questionable. For example, though group W did not appear as a clade of its own in the parsimony analysis of the morphological data, these collections were closely associated with clade L, though no relationship between these two clades was recovered in the 50% majority-rule tree for the combined data parsimony analysis. The observation, that despite the small size of the morphological dataset, it still influenced the patterning of the combined analysis was somewhat similar to the observation of Nylander *et al.* (2004) that a morphological dataset that comprised only five percent of the combined dataset still exerted considerable influence on the topology

resulting from the combined analysis. Other combined phylogenetic analyses (though not of fungi) have observed conflicting morphological and molecular datasets have 'hidden' but apparently stronger phylogenetic signal that shows strong support for certain relationships in the combined analysis that were not supported in the morphology (Gatesy & Arctander 2000). This 'hidden support' scenario did not appear to be the case, and it appears that the lack of resemblance to the morphological data is primarily due to the small size of the morphological dataset (only just over 2 percent of the characters in the combined dataset).

Combining the morphological and molecular data generated for this study appears to be of limited value, noting that the Incongruence-Length Difference test suggested the un-weighted partitions were homogenous. The lack of phylogenetic signal in the morphological data and the considerable differences in the groupings produced by separate analysis of the morphological and molecular dataset suggest that the datasets associate considerably different sets of morphological characters with phylogenetic divergence. Different separate groupings along alone do not precluded the combined analysis from being more phylogenetically informative than either separate analysis as demonstrated in several studies (e.g. in lichenised and non-lichenised Omphalinoid species (Lutzoni & Vilgalys 1995), for a weak morphology dataset combined with ITS and chloroplast Trnl-F sequences on the Asteraceae (Fernandez *et al.* 2001). The combined data tends to differentiate separate collections with identical or very similar sequences, and unite divergent replicate sequences with the same morphological characteristics (though this is merely a by-product of the duplication of the morphological data for example the three additional branches in the parsimony analysis *Cortinarius*-like clade). This method of coding sequences belonging to the same 'taxonomic unit' has been used successfully by Liu & Miyamoto (1999) where

conordinal sequences were given the same morphological traits for the purpose of estimating the phylogeny of the eutherian mammals. Though not identified as particularly important in the biplot on the morphological PCA, trends in spore size were apparent in the morphological analyses, particularly the Ward's method cluster analysis of the Euclidian distances of the 'standardised' data. Similarly, spore size was identified as a factor associated with differences in the arrangement of collections within clade L between the combined and molecular data cluster analyses. Spore size among the different groups is a potential confounding factor. It is likely that there is a tendency towards larger (and more coarsely ornamented) spores in sequestrate (especially gasteroid) fungi, perhaps as an adaptation to passing through the digestive tract of an animal vector or as a product of the redirection of resources normally used for active spore discharge (Thiers 1984). Whatever the biological cause of the convergence or tendency in spore size with gasteromycetation, the incorporation of analogous morphological characteristics in a combined dataset will weaken any molecularly homologous patterns or suggest misleading relationships where the phylogenetic signal of the molecular data is already weak.

The differences in topology between the molecular and morphological cluster analyses do not appear to resemble structural elements in the morphological cluster analysis. It is possible that the differences are due to the increased distance between collections because of the incorporation of the morphological data. The ordination of the combined data is generally slightly less crowded than that for the molecular data alone. The inclusion of the morphological data appears to have led to a more evenly distributed scatter of points on Axes one and two and a greater spread of *Descolea*-like collections along Axis three than Axis two. The characters generating similar patterns in the morphological dataset alone may have contributed similar trends in the ordination of the

combined data. The parsimony analyses of the combined and molecular data differ in having more branches (and some better supported branches) in clade L and generally more polytomy outside this clade. Bull *et al.* (1993) suggests reduced resolution in a combined analysis indicates that the phylogenies are not indicative of the same evolutionary history. The only differences between the Bayesian analyses of the molecular and combined data were in clade L and were in the form of a few different branches and better support for some of them. It seems that the differences between the molecular and combined datasets arise where the molecular data group collections that are also grouped by the morphological data, as suggested by Wiens (1998) based on computer simulations. In these cases the patterning in the morphological data can either contribute pattern where there was none or the lack of morphological patterning override poorly supported molecular branching patterns.

This attempt at combining data for the sequestrate cortinarioid fungi highlights the need for more detailed investigation and description of the characters differentiating these fungi. Successful combined analyses routinely have more than twice the non-molecular characters and employ less subjectively observed and coded characters. For example McLaughlin *et al.* (1995) used both light-microscopic characters and ultrastructure in their combined assessment of the phylogeny of the Basidiomycetes and Lutzoni & Vilgalys (1995) incorporated ecological and chemical characters alongside morphology. This thesis however, has attempted to derive phylogenetic information from selected, relatively easily-measured characters often noted in biodiversity surveys of sequestrate fungi. The characters as defined, however, appear to lack phylogenetic information and in some cases may obscure what signal does exist. The molecular data dominated the un-weighted combined analysis and thought this is not unusual, the strong contribution of the smaller morphological dataset noted by some other authors (Gatesy & Arctander

2000, Nylander *et al.* 2004) was not observed for the combined dataset presented here. Inclusion of the morphological data changed associations of a number of taxa, in all but one case, only within the major sub-clade they belonged to in the molecular analyses though this is expected when phylogenetic estimates differ (Wiens 1998). In these cases it appears that the morphological data obscures more intuitive relationships present in the separate analysis of the molecular data. The bias of the morphological data towards spore characteristics combined with a poor characterisation and coding of the diversity of peridiopellis characteristics appears to be responsible for the observed conflict between the molecular and combined analyses.

Chapter 5

Discussion and conclusions

5.1 Introduction

Did numeric analysis of the selected morphological characters recover the same pattern of groups and relationships among the cortinarioid sequestrate fungi as phylogenetic analysis of ITS data? Given the data used, the coding of that data and the analyses performed it must be concluded that no, they did not. The composition of groups recovered using the morphological data alone or as part of the combined dataset, and the relationships between those groups, differed from those recovered from the molecular data alone; though there are similarities between groups recovered from different datasets. This observation prompted three main questions; firstly, what is responsible for the disagreement between analyses, secondly, why is there such a low level of phylogenetic signal relating groups, and thirdly, what are the implications of the similarity between morphological and molecular groups given the disagreement and low phylogenetic signal. Addressing these questions should demonstrate that this thesis has not conclusively disproved the hypothesis that numeric analysis of selected morphological characters can recover the same pattern of groups and relationships as ITS data. Rather it has, firstly, reaffirmed the usefulness of morphology in distinguishing phylogenetically distinct lineages within this group, secondly, highlighted methodological limitations on the ability of the data and analyses presented here to address the hypothesis, and thirdly, provided a critique on the usefulness of the hypothesis as presented.

5.2 Disagreement on cluster/clade composition between analyses

Analyses of the morphological and combined data grouped collections differently to analyses of the molecular data alone. This indicates that the morphological data is, to some degree, in conflict with patterns of ITS variation. The roles played by analogous characters and the inadvertent overweighting of some characters (including phylogenetically unimportant characters in weak datasets) in generating these conflicting results is highlighted by the following examples.

5.2.1 Analogous characters

Analogous characters appear to be responsible for the differing placement of collections both with relation to the major clusters/clades and within them. The gelatinous peridium and larger spores of collection H5814 appear responsible for the close association of this collection with members of group P in the morphological cluster analysis. However, the ITS sequence is similar to those of the Type species of *Cortinarius*, *C. violaceus* (GenBank AY669578 Garnica *et al.* 2005, and AF325601 Peintner *et al.* 2001) which has a dry, clamped pileipellis as do clades L and W between which H5814 nests in molecular and combined analyses. It is also helpful to note that though the sequence for H5814 appears to be correct (this sequence is most similar to that of H5798 which was also placed in FBE group two in the initial morphological grouping) it was isolated morphologically and phylogenetically in the subsets of the data used in the comparative analyses. The placement of clade B relative to the other *Cortinarius*-like clades is also evidence of analogy between characteristics. Though in the comparative analyses the position of clade B is highly variable (see Table 11) in the larger molecular dataset this clade groups near a group of *Cortinarius* species with gelatinous peridia (including *C. sinapicolor* and *C. archeri*) distinct from both P and G.

A third example of analogous morphological characters leading to the rearrangement of collections in combined analyses relative to their molecular positions is among the weakly supported internal branches of clade L. Close associations between the collections MEL2032790 and H1006, and between H1213 and H6753 are responsible for the apparent 'sorting' according to spore size (in fact these pairs do share several, mostly spore-related characters). The morphological similarities between collections however could not override the sequence differences between collections H0910 and H0904. This example suggests that some sequence similarities may be analogous, exemplified by the apparently morphologically conspecific collections H0904 and H0910 grouping with distinct, different collections in the molecular analyses.

The phylogenetic significance of spore characters for the sequestrate cortinarioid fungi will require further investigation. Singer (1975) wrote "the spore, with all its characters has become, more and more, one of the most important organs on which the taxonomy of the Agaricales is based". As molecular phylogenies increasingly form the 'gold standard' against which other taxonomic arrangements are measured, the utility of spore size and shape for distinguishing sequestrate cortinarioid taxa must also be examined in this context. Regarding spore characteristics Peintner *et al.* (2001) wrote "... *Hymenogaster* was redefined and a close relationship of *Protoglossum* and *Thaxterogaster* to *Cortinarius* as well as of *Descomyces* and *Setchelliogaster* to *Descolea* were proposed based on spore morphology and other morphological characters (Bougher & Castellano 1993). Our molecular data confirm these results, thus supporting the utility of spore morphology for delimiting groups". In the same paper Peintner *et al.* (2001) suggest that a group of ex-*Thaxterogaster* species including *Cortinarius pingue* (Zeller) Peintner & M.M. Moser that formed a sister clade to species belonging to subgenus *Myxacium* section *Myxacium* shared characters including the

possession of clamp connections and "spores >10µm". Garnica *et al.* (2005) also indicate that spore shape distinguished several cortinarioid genera/subgenera including *Dermocybe*. Spore size is also quite routinely used to assist in distinguishing species and higher taxa among the sequestrate cortinarioid fungi and other cortinarioid and non cortinarioid euagaric taxa. Spore characteristics have also been successfully used in the delimitation of diverse taxa outside the euagaric clade. For example Anikster *et al.* (2005) used spore dimensions as measured by digital image analysis to distinguish species of cereal rusts (interestingly relying on the dimensions of what they call the "best fitting ellipse" of the spores, a concept that resembles the method of defining the limits of the rostrum and apiculus employed in this study). Peintner *et al.* (2003) found spore size and ornamentation to be useful in distinguishing species in the boletoid *Xerocomus* species complex and despite the observation of substantial environmental plasticity of spore characteristics observed within single isolates of some glomalean fungi Bentivenga and co-workers found that spore size and colour readily distinguished species of the glomalean fungus *Gigaspora* [*c.f.* Bentivenga *et al.* (1997) and Bentivenga & Morton (1995)]. However support for the utility of morphological characters, including spore characters is not unqualified. Peintner *et al.* (2001) suggest that when unique evolutionary solutions have arisen in response to particular ecological challenges morphological data alone may be insufficient to recover phylogenetic relationships. Indeed, in their keys to sequestrate fungi, Castellano *et al.* (1989) indicate that for several genera some sequestrate species cannot be distinguished from those of related agaricoid species on the basis of spores alone (e.g. some *Hymenogaster*, ex-*Thaxterogaster* and *Cortinarius* species). Meerts (1999) also demonstrated a positive correlation between spore size and basidiome size in *Cortinarius* (though no sequestrate species were examined). If there is, as observations made for this study suggest, a convergent tendency among cortinarioid sequestrate fungi (and probably other lineages

also) towards larger, more prominently ornamented spores as gasteromycetation proceeds, the ability for spore morphology to vary with other macromorphological characteristics indicated by Meerts (1999) strikes a cautionary note in exploiting the phylogenetic potential of spore characteristics for the cortinarioid sequestrate fungi.

5.2.2 Inadvertent character weighting

Inappropriately high effective weightings applied to some characters are also likely to contribute to disagreement between morphological and molecular estimates of phylogeny. This is seen both when presumably phylogenetically unimportant characters carry the same weight as phylogenetically important characters, and when phylogenetically important characters are inadvertently up-weighted by the inclusion of equally weighted correlated characters. Characteristics of the apiculus were included to enhance the multivariate 'description' of the spore shape, a characteristic considered phylogenetically important from preliminary examinations. However the two measurements of the apiculus were included as separate, equally weighted characters. Basidium width was also included. These characters are not generally used in phylogenetic analyses and basidium width was correlated with spore width (Correlation coefficient 0.731) as suggested by Corner (1947), Corner (1948) and Poder (1986). The degree to which these characters influenced pattern in the morphological analyses is indicated in the joint-plot of the Principal Components Analysis of the 'standardised' morphological data (Figure 7). Apiculus length and projection (AL and AP), along with rostral projection (RP) and peridium width and number of layers (PW, PL) differ between collections within group L. Weighting characteristics, such as apiculus length, as components of 'composite characters' would reduce the weight of these characters however an objective means of determining appropriate weightings is not clear. The

other issue regarding the weighting of characters is the inadvertent 'double' weighting of correlated, apparently phylogenetically important characters. Rostrum length and projection (RL and RP) were the most strongly correlated characters (correlation coefficient = 0.883). This correlation was reinforced by both characters having relatively high correlations with outer peridium type (RP:OP, 0.76, RL:OP, 0.74). While it is necessary that there be correlation between characters to discern groups, the method is compromised when these characters are not independent. The 'projection' characters were included because it was observed that some *Descomyces* spores had prominent rostra almost covered by the perispore while the entire rostrum appeared exposed in others. It may be however that the high correlation was due more to the effect of considering the rostral projection of collections without rostra to be negative.

The influence of the morphological data on the within-cluster/within-clade associations of collections is seen in the topology of group S compared using different analyses. The morphological cluster analysis (Figure 4) groups collections initially classified in FBE group 81 (broad 'shouldered' spore) separately from H0734 and H6988 (both belonging to FBE group 77 with narrower spores) with the later two collections placed in distinct clusters. However, in the molecular analyses they are united with the group 81 collections in well supported clades (*c.f.* Figure 15). The combined cluster analysis grouped the two FBE77 collections together, indicating that the morphological differences that separated them from each other and the FBE81 collections, when combined with the molecular characters, distinguish these collections from FBE81 collection H7124 which appears to have an identical molecular sequence. Thus the morphological data, particularly the spore morphology, is providing a different picture of the associations between these fungi. Consequently the analysis may have benefited from the use of different characters, such as the structure of underlying layers of the

peridiopellis or geographic or host association. The utility of spore characters is, however, attested to by the association (in most analyses) of the two collections H7119 and TRAPPE14397 both, after these analyses, considered to be *Descomyces albus*-like. Despite similar spore size and shape these collections were initially placed in separate FBE groups (74 and 70 respectively) because a polycystoderm was not observed in TRAPPE14379. While the consistent grouping of these collections is not conclusive evidence of their conspecificity (*c.f.* Bruns 2001) it does suggest that the use of spore characteristics can unite collections that might otherwise be separated on the basis of characters subject to artefacts of observation or development, such as one's ability to observe a polycystoderm.

5.3 Between-cluster/clade topology and estimation of the phylogeny

5.3.1 Between cluster/clade topology

There are several topological differences between the various dendrograms recovered by the different analyses. However, despite these differences in the various 50% majority rule consensus trees and cluster analyses, in the phylogenetic analyses all *Cortinarius*-like clades but P, and *Descolea*-like clades but that joining F and A, consistently stemmed from either *Cortinarius*- or *Descolea*-like polytomies respectively. The placement of the collections H5814 and TRAPPE 14175 also differ between analyses, particularly between those for the morphological data alone and those including the molecular data. In the Ward's method clustering of the Euclidian distances of the 'standardised' morphological data (Figure 4) H5814 is a member of the clade comparable to group P, whereas in the analyses containing molecular data this

collection groups with clades/clusters L and W. Similarly TRAPPE14175 is a member of a cluster with representatives of clade cluster F in Figure 4 but is sister to that group containing clades/clusters S and E in analyses incorporating molecular data. In addition to these topological differences, the relative placements of clades B, G and P are quite variable and the associations among the *Descolea*-like clades differs between the phonetic and phylogenetic methods. Clade/cluster B is closely associated with cluster P in Figure 4, basal to the *Cortinarius*-like cluster in the UPGMA clustering of the 'mean' distances of the molecular and combined data (Figure 12 and Figure 18), sister to clade W in the molecular parsimony analysis, part of a polytomy with clades W and G in the parsimony analysis of the combined data, and sister to clade L in the molecular and combined Bayesian analyses. These differences had less than 65% bootstrap support, or 95% partition probability support for those analyses for which support could be calculated, except for the placement of clade P as basal in those analyses incorporating molecular data. The between clade topology of the *Descolea*-like clades differed between the cluster analyses of the morphological and molecular data, the Bayesian analysis of the molecular and combined data and the parsimony analysis of the combined data. In these last three analyses TRAPPE14175 subtended sister clades S and E and was in turn subtended by a branch bearing sister clades A and F. The UPGMA clustering of the mean distances of the molecular data did not pair clusters A and F but linked cluster A to the pair of S and E and subtended these three by TRAPPE14175 and placed F basal to the whole clade. The Ward's method clustering of the Euclidian distances of the 'standardised' morphological data clustered A and F but not S and E and incorporated TRAPPE14175 among group F (recalling that groups bear the same name if 50% or more of their collections are in common between analyses). The two collections H5814 and TRAPPE14175 are the only representatives of their taxa (an unnamed secotioid *Cortinarius* and *Setchelliogaster tenuipes*

respectively). Thus these collections are 'isolated' in terms of both sequence and morphological data. This 'isolation' means that there are no other collections that group with these collections in the differing analyses – giving the impression that these collections 'move' more than others. There is a relatively large degree of rearrangement occurring between the different sub-clades of both major clades. The link between clades B and P appears to be the size and ornamentation of the spores. These characters appear to override differences in outer peridium type in the morphological analyses however the dry peridium appears to be more indicative of patterns of ITS variation. At a fundamental level this is because there is very little signal determining the relative placements of the major *Cortinarius*- and *Descolea*-like sub-clades in any of the datasets considered.

Table 11: Simplified between clade/cluster topologies of the dendrograms for selected analyses in New Hampshire format. Letters represent clades/clusters and between clade/cluster topological features discussed in the text are underlined. "H" or a bold clade letter indicates the position of H5814 and "*T*" or an *italicised* clade letter indicates the position of TRAPPE14175.

ANALYSIS	TREE STRUCTURE
Euclidian distance Ward's clustering method 'standardised' morphological data (Figure 4)	((L, W), ((P, B), G)), (((A, *F*), S), E)
'Mean' distance UPGMA clustering molecular and combined data (Figure 12 and Figure 18)	(((((L,H), W), P), G), B), (((S, E), A), *T*), F)
Molecular data parsimony analysis (Figure 14)	((((L, H), (B, W))), G), P), ((S, E), *T*) (A, F))
Combined data parsimony analysis (Figure 20)	(((L, H), B, W, G), P), ((S, E), *T*), (A, F))
Molecular and combined data Bayesian analysis (Figure 15 and Figure 21)	(((((L, B), H), W), G), P), ((S, E), *T*), (A, F))

Few relationships between the various *Cortinarius*- and *Descolea*-like sub-clades were confidently recovered in the analyses. The separation of the basal *Cortinarius*-like clade P from the other *Cortinarius*-like sub-clades has greater than 95% partition probability in the Bayesian analyses of the molecular and combined datasets but that is the only well supported relationship between any of these sub-clades. Similarly, among the *Descolea*-like sub-clades, only the branch joining clades A and F in the Bayesian

analysis of the molecular and combined datasets is 'well-supported' (≥ 95% partition probability). It appears that both the morphological and the molecular datasets lack phylogenetic resolving power above the level of the sub-clades indicated here. Several factors are suspected of compromising the phylogenetic utility of the morphological dataset. Firstly there were only sixteen characters for forty to fifty-six operational taxonomic units. This small taxonomic sample size limits both the number of OTUs exhibiting a particular trait (and hence the ability of the various methods to group collections based on that character) and the low number of characters means that the support that any branch is also low. This is evidenced by the relatively low proportions of variance accounted for by the first three axes of the morphological principal coordinates analysis (PCO) compared to that of the molecular PCO. Some continuous characters that appear to confer phylogenetic signal to the cluster analysis of the 'standardised' data are compromised by being broken up into discrete characters for parsimony and Bayesian analysis. Such characters include spore size and the degree of spore reticulation. The reticulation of the ornamentation especially appears to be phylogenetically informative as H0732, TRAPPE14535 (alveolate spored *Timgrovea* subgenus *Timgrovea*) and H5655 (small-, partially-reticulate spored *Timgrovea* subgenus *Subreticulata*) group closely in all analyses incorporating molecular data (clade/cluster E) but not in analyses of the morphological data alone. Some characters that appear to be useful in separating taxa were not included in the morphological dataset for analysis. Characters such as peridium colour (as a very approximate surrogate for pigment content), the presence of clamps in the various tissues of the basidiome, and the structure of underlying layers of the peridiopellis were not included in this analysis. These characters were considered either unreliably observed or difficult to quantify meaningfully. For example, the determination of basidiome colour is subjective even using colour charts, was not uniformly recorded for all herbarium

collections and is affected by the apparent colour of multiple-layered peridia such as that of *Descomyces* that may appear more white or more golden brown depending on the development and separation of the outer fibrillose layer. Finally the high number of states (most quantitative characters were divided into ten equal 'bins' and all non-empty bins included as separate states) relative to the number of characters and putative taxa may have compromised how informative any given character was in terms of conferring similarity of one group to another. For example, if there were so many states in a particular character that only two groups shared any two states and these patterns were compromised by other patterns, then the phylogenetic usefulness of those characters would be reduced.

5.3.2 Phylogenetic resolution

There is very little phylogenetic signal in the ITS data with which to compare patterns derived from the morphological data. Only one branch in the *Cortinarius*-like clade and one in the *Descolea*-like clade in the Bayesian analyses of the molecular and combined datasets had greater than a 95% partition probability. Examination of the clades recovered in the context of the larger molecular dataset suggests that the clades obtained represent monophyletic assemblages – at least to the degree that can be determined by the ITS. Collections that form the five *Cortinarius*-like clades (L, B, W, G, P) and the four *Descomyces*-like clades (S, E, A, F) along with the two single collections H5814 and TRAPPE14175 group with collections from the same or comparable FBE groups in the larger molecular dataset. Also, when sequences from GenBank are incorporated into the larger dataset, the clades identified remain cohesive and are associated with similar taxa. Clade W appears to belong to the /Telamonia 1 clade (/Obtusi) of Peintner *et al.* (2001) while clade L is associated with the /Phlegmacium 1 clade of Peintner *et al.*

(2001) [/Purpurascentes, Garnica *et al.* (2005)]. H5814 groups with another collection from the same FBE group (group 2, H5798) with sequences of *Cortinarius minoscaurus*. Clade P forms a cohesive group with sequences of *Cortinarius fulvoochrascens* and ex *Cuphocybe* species *Cortinarius dulciolens* with which the sequences of *Protoglossum luteum* grouped in Peintner *et al.* (2001). Sister to this clade in the larger analysis was part of the /Myxacium 1 clade of Peintner *et al.* (2001) identified as section *Myxacium* by Seidl (2000). A clade representing *Myxacium* section *Defibulati* (*c.f.* Peintner *et al.* 2001 and Seidl 2000) includes the clade G collections with *Quadrispora* and '*Thaxterogaster*' sequences from GenBank (*c.f.* Peintner *et al.* 2001). It is useful to note here that the lack of clamps on the gelatinous hyphae of these collections was a character that distinguished them from the large-spored collection H5814, the gelatinised peridiopellis hyphae of which bore clamps. The final *Cortinarius*-clade, B, appears to be relatively isolated but groups with other Australian/Southern Hemisphere *Cortinarius* species including *C. archeri* and *C. sinapicolor* and the Western Australian *Cortinarius sebosus* named as an outcome of this project (Francis & Bougher 2004). Likewise the *Descomyces*-like sub-clades all appear to be part of monophyletic groups when incorporated into a larger molecular dataset. Clades S and E each group with similar collections as separate clades associated with *Descolea antarctica*. The two *Descomyces* collections included by Peintner *et al.* (2001) grouped with *Descolea antarctica*. Suspected *Setchelliogaster tenuipes* collection TRAPPE14175 groups with other collections of this species and, surprisingly, the elongate spored *Descomyces angustisporus* named as part of this project (Francis & Bougher 2004). The separation of *D. angustisporus* is surprising because of the remarkable macro-morphological homogeneity of the genus *Descomyces*. All published species are gasteroid with thin whitish peridia, and with overlying yellow fibrillose patches formed by thick-walled golden hyphae. The association of *D.*

angustisporus with *S. tenuipes* represents a novel lineage of *Descomyces*-like fungi demonstrating again multiple origins of highly similar sequestrate forms. Another new lineage of *Descolea*-like fungi is represented by collections of group F that, with related collections, form a well supported clade labelled /Timgrovea-Setchelliogaster in Figure 17. It is significant to note that the *Timgrovea* collections of both subgenera nest within the various *Descolea*-like clades. Though various alveolate collections group together closely (e.g. H0732 and TRAPPE14535 *Timgrovea* subgenus *Timgrovea*), several other collections that also fit the circumscription of *Timgrovea* occupy positions in different sub-clades.

This ability of the ITS to distinguish groups but not provide well resolved relationships between them was observed in all five published works examining the molecular basis for subgeneric division of *Cortinarius*. Seidl (2000) and Peintner *et al.* (2001) used the ITS while Garnica *et al.* (2003a & b), Peintner *et al.* (2004) and Garnica *et al.* (2005) have used both the ITS and the nuclear large subunit rDNA. None of these papers has obtained a well resolved tree where relationships between major clades of *Cortinarius* have received high support (Bayesian partition probabilities, bootstrap values or both) though, as in the work presented here, the clades themselves have been well supported. Similar patterns of support can be made for the *Descolea*-like and *Hebeloma*-like clades as represented in Peintner *et al.* (2001) and Moreau *et al.* (2006). Frøslev *et al.* (2005) recovered high support for the sister relationship of the /Fulvi sub-clade of the /Calochroi clade to that clade containing the /Calochroi and /Rufoolivacei sub-clades but not for the /Rufoolivacei clade itself. Such results indicate that even the combination of two coding regions RPB1 and RPB2 with the ITS cannot fully, confidently resolve the relationships between the sub-clades of *Cortinarius*. Bruns (2001) puts the case forward that though the ITS is at once too variable to distinguish

distantly related fungi, and often not variable enough to resolve relationships between closely associated species, it is useful for identifying species groups and for identification of related taxa through the public databases. Matheny & Bougher (2006a), using RPB2 and nLSU sequences to estimate the phylogeny of *Auritella* were able to obtain high bootstrap support for the separation of *Auritella* from *Inocybe* and for intrageneric separations corresponding to geographic (Africa/Australia) and putative host plant association (Fabaceae/Myrtaceae). This indicates that multiple gene phylogenies can recover high bootstrap support for subgeneric divisions in cortinarioid fungi. The applicability of this finding to the subgeneric phylogeny of *Cortinarius* is qualified however because fewer collections were examined in Matheny & Bougher (2006a) than in Frøslev *et al.* (2005), and the suggestion of Matheny *et al.* (2006) that the Inocybaceae are more closely related to the Strophariaceae than to *Cortinarius*. This suggests that the aspect of the hypothesis seeking to recover similar between-group relationships by analyses of molecular and morphological data erred in presuming that the ITS molecular data would produce reliable relationships. Indeed this was suspected. However, because of the abundance of publicly available cortinarioid ITS sequences, this region was chosen to integrate previously unincorporated taxa and collections into largely ITS based phylogenies of the cortinarioid sequestrate fungi (Peintner *et al.* 2001, Moreau *et al.* 2006). Although, ideally, other regions would have been incorporated, this work has never-the-less provided associations for Australian collections within the major sub-clades of *Cortinarius*-, *Descolea*- and *Hebeloma*-like clades that may facilitate the incorporation of these species/collections into later analyses. The discovery of novel lineages of *Descolea*-like sequestrate fungi and of new species augmenting apparently Australian/Southern Hemisphere endemic clades should also assist in informing the taxonomic scope of further work.

5.4 Agreement between molecular and morphological group composition

Certain collections group consistently together in analyses of morphological, molecular and combined data. These consistent groupings of collections formed the 'core' of the groups recognised in the different analyses. Groups were identified in each analysis but those from analyses incorporating molecular data were better supported and more consistent. 'Core' collections were defined as those that grouped together in at least four molecular (or combined) and three morphological analyses. Table 12 lists the collections examined in the comparative analyses against the group into which they were placed in each analysis. 'Core' collections are indicated in the colour of the group for which they are a core part. Some groups in resolved/supported analyses have more than twice the number of collections than there are core collections. This is because these analyses showed a considerable degree of chaining (e.g. the parsimony analysis of the morphological data) or fewer discernable groups (the PCA of the Euclidian distances of the 'standardised' morphological data). In those cases the whole group bears the name and colour (e.g. P and B respectively in the above examples) of the apical clade or prominent group. With the mentioned limits for recognition of 'core' collections, only *Cortinarius*-like groups L, W, B, and P and *Descolea*-like groups S, E and A are recovered in both molecular and morphological analyses.

The absence of core collections for group F appears to be because of morphological similarities between H4146 and group A collections which group with F in all analyses incorporating molecular data. H4146 is included with group A collections in the UPGMA clustering and principal coordinates analysis (PCO) of the 'mean' distances of the morphological data, associations that appear largely based on spore width, rostral

projection, basidium width and to a lesser degree ornamentation type. It is likely that the conversion of the morphological data to multi-state categorical variables is responsible for the association of H4146 with clade A, because this collection is associated with clade F collection H4204 in analyses using the 'standardised' morphological data. Of the morphological analyses, clusters from the Ward's method and UPGMA cluster analyses of the 'standardised' morphological data Euclidian distances show the greatest similarity to groupings based on analyses incorporating the molecular data. The information loss inherent in converting the quantitative variables (such as spore width and rostral projection), to categorical variables by the use of a set, arbitrary number of equal sized bins (ten or as many as were not empty to a maximum of ten). In seeking to test for an association between basidiome size and spore size (Meerts 1999) used a threefold difference in cap diameter to distinguish large and small basidiomes. Using such subjective methods may ensure that collections related by variable traits are not separated by the arbitrary choice of bins. This has implications for analysis because, regardless of how close the raw values for a particular character are for two collections, if, because of the size classes chosen, the values are placed in different bins, those collections immediately have maximum distance for that character, as long as characters are unordered with equal substitution rates.

The core collections of *Cortinarius*-like groups P and B are replicate samples putative taxa while *Descolea*-like groups S and E each grouped pairs of collections representing two putative taxa in each clade. *Cortinarius*-like groups W and L also group pairs of collections that appear closely related though perhaps not conspecific. In both of these clades there are morphological differences that coincide with molecular separation. The larger and smaller-spored collections of '*Thaxterogaster* lilac-silky' are separated molecularly, and there are differences in the hyphae of the subtending peridiopellis

layers between molecularly distinct members of group W. Further work will be necessary to determine the implications of the ITS variation in these taxa. Do the collections sampled here represent different members of a cryptic species complex, or is this an indication that for certain lineages of *Cortinarius* [respectively /Phlegmacium 1 and /Telamonia 1(/Obtusi)] the ITS is still more variable than usually encountered, providing resolution to the subspecies level or lower? The association of what initially appear to be morphologically distinct *Descomyces* within single groups was unexpected given the morphological similarity of members of the *Cortinarius*-like groups. The squat-spored *Descomyces* (H0736 and H0735) group with the more tapered spores of *Descomyces albus*-like collections H7119 and TRAPPE14397. Similarly, the elongate-spored *Descomyces* collections H0733 and H5807 group with the apparently broad spored, alveolate-ornamented *Timgrovea* subgenus *Timgrovea* collections TRAPPE14535 and H0732. In the multi-state morphological dataset only four characters (PW, PL, QS and QB) have no state in common between the four core collections of group S whereas the core collections of group E have seven characters where there is no shared state between the two putative species (AL, RL, PW, OT, PD, QS and QB). This could explain the observation that the core collections of group E were grouped with other collections more often than the core collections of group S (see Table 12). Why has morphology given similar results to molecular data when at first glance the morphology of the pairs making up the core collections of clades E and S appears divergent within these clades? Spore width (SW), number of spores (SN), ornamentation type (OT), outer peridium code (OP) are some of the presumably phylogenetically important characters that have the same value for various core collections in groups S and E. Some, presumably less phylogenetically important, characters including apiculus length, and basidium width, none-the-less have a common

state for all or most collections of groups S and E and reinforce the cohesion of these groups across various analyses and datasets.

5.5 Implications for the systematic position of the genera of cortinarioid sequestrate fungi

The findings of this thesis impact upon the taxonomy of the sequestrate cortinarioid fungi, particularly among the *Descolea*-like forms. This thesis has produced the first *Timgrovea* ITS sequences to be made publicly available via GenBank (accessions given in Table 13, Appendix 1). The incorporation of collections from *Timgrovea* subgenus *Timgrovea* and subgenus *Subreticulata* has enabled this thesis to test the theory (as raised in section 1.2 of the introduction) that both these subgenera belong to the *Descolea*-like clade. In all analyses performed for this study, putative *Timgrovea* collections, of both subgenera, have nested among the *Descolea*-like fungi. As can be seen from Table 12, putative *Timgrovea* subgenus *Timgrovea* and subgenus *Subreticulata* collections are 'core collections' of two groups, E and F, recovered in the majority of analyses of both molecular and morphological data. Indeed, even when incorporated into the extended molecular dataset with other euagaric outgroups all putative *Timgrovea* collections were aligned among the *Descolea*-like clade, both in the parsimony analysis of the extended molecular dataset (among *Descomyces* collections Figure 17) and by pair-wise multiple alignment using Clustal W [Appendix 5 and Thompson *et al.* (1994)]. In no analysis, however, did all putative *Timgrovea* collections form a single monophyletic lineage.

The polyphyly of the genus *Timgrovea* among *Descomyces* that such observations imply is qualified, however, by two observations. Firstly, the generally low level of

bootstrap support in Figure 17 questions the reliability of the topology as illustrated suggesting it may well change if more data were included. Ideally such additional data would involve the inclusion of other molecular regions (coding regions such as RPB1 and 2 to attempt to resolve the deeper nodes as the more apical branches were relatively well supported by the ITS) and a larger taxonomic sample, particularly of subgenus *Timgrovea* that was represented in this study by only three sequences.

The second qualification arises from the observation that the degree and appearance of the reticulate ornamentation varies among *Timgrovea* collections, especially those of subgenus *Subreticulata*. This raises the possibility that some collections responsible for the apparent polyphyly exhibit a form of partially-reticulate ornamentation merely analogous to that of *Timgrovea ferruginea* (Cribb) Bougher & Castellano, the Type of subgenus *Subreticulata* - for example the broad, irregularly-reticulate spored collections of molecular clade F. Clade F, which is comprised of two putative, secotioid *Setchelliogaster*/*Timgrovea* species with partially-reticulate spores, appears to represent a distinct lineage within the *Descolea*-like fungi (Figure 17). This cautionary note regarding the dependence of the polyphyly of *Timgrovea* upon interpretation of spore ornamentation is supported by the observation that alveolate-spored collections TRAPPE14535 and H0732 (subgenus *Timgrovea*) and *Timgrovea ferruginea* collections (e.g. H5655, H5803, subgenus *Subreticulata*) form a paraphyletic assemblage with *Descomyces* and *Setchelliogaster* collections (including *D. fusisporus* Trappe *nom. prov.* H0733, H5807) in Figure 17, separate from other partially reticulate forms. There is also diversity in spore ornamentation among molecularly distant alveolate-spored *Timgrovea* subgenus *Timgrovea*. For example the molecularly isolated alveolate-spored H5984 (Figure 17) has much smaller, less polygonal alveoli than the *Timgrovea* subgenus *Timgrovea* collections in clade E (TRAPPE14535, H0732). This

diversity of reticulate ornamentation types among the *Descolea*-like fungi begs more detailed ultrastructural and possibly developmental study to investigate this apparently homoplasic character.

Ultrastructural examination of the different wall elements comprising the various ornamentation types found in the /Descolea clade could test hypotheses as to potential transitions between these ornamentation types. The phylogenetic association of *Timgrovea* with *Descolea* and *Setchelliogaster* is a promising factor to encourage such studies as it raises the possibility that the methods used to successfully generate *Descolea* and *Setchelliogaster* fruit bodies in mycorrhizal pot-cultures may be applied to *Timgrovea* (Bougher 1987, Lago *et al.* 2001). Pot-cultures may be able to supply ample material of *Timgrovea* and *Descomyces* fruit bodies for developmental studies, and for assessment of morphological plasticity. A high degree of plasticity has been observed in other members of the /Descolea clade (Martin & Rocabruna 1999, (Martin & Moreno 2001, Lago *et al.* 2001). Peintner *et al.* (2001) suggest this capacity for basidiome plasticity is ancestral for this clade. Testing could also determine if such plasticity also extends to the degree of reticulation of the spore ornamentation.

Developmental studies may also serve to clarify the diversity of peridiopellis structures observed in *Timgrovea* species. An examination of the origin and structure of the golden peridial hyphae of *Timgrovea* species (including thick-walled hyphae resembling those of *Descomyces* observed for all *Timgrovea* collections in the comparative analyses Table 15, Appendix 3) and in the description of *Timgrovea reticulata* in Bougher & Castellano (1993) could provide insight into the relationship of *Timgrovea* to *Descomyces*. The observation by Cribb (1956) and Bougher & Castellano (1993) of a glabrous blue-green peridium in *Timgrovea subtropica* (Cribb) Bougher & Castellano is

at odds with peridia known from species within the *Descolea* clade. No collections of this species were examined in the current study. Collections of this morphologically atypical *Timgrovea* species should be included if possible in future examinations of the genus to confirm its taxonomic position.

Thus it seems probable, given the utility of the ITS for discerning closely related sequences (Bruns 2001), that not only *Timgrovea* subgenus *Subreticulata* [as suggested by Francis & Bougher (2003)] but also subgenus *Timgrovea* belong to the /Descolea clade of Peintner *et al.* (2001). However the taxonomic position of the genus *within* this clade is currently unconfirmed and should be reassessed with an expanded taxonomic sample size and expanded molecular and morphological datasets.

This thesis has shown the *Descolea*-like genus *Descomyces* Bougher & Castellano to be more phylogenetically diverse than previously known. *Descomyces* has been considered to represent the most reduced form in the evolutionary series linking *Descolea*, *Setchelliogaster* and *Descomyces* (Bougher & Castellano 1993). Peintner *et al.* (2001), recovered the two *Descomyces* collections included in their analysis as a monophyletic group associated with *Descolea antarctica* and noted that *Descomyces* lacks the morphological plasticity exhibited by other genera in their /Descolea clade. The analyses presented in this thesis not only show a greater diversity of phylogenetically and morphologically distinct species of *Descomyces* (note the consistent distinctiveness of group S and group E *Descomyces* across the majority of analyses Table 12) but has also brought to light the affinity of *Descomyces angustisporus* with *Setchelliogaster australiensis*. This represents a separate origin for the highly consistent *Descomyces* morphology as discussed in section 5.3.2. Indeed nothing in the morphology of *Descomyces angustisporus* as examined suggested that it

would be so phylogenetically distant from putative collections of the Type of the genus, *Descomyces albus* including the morphologically and molecularly supported pair of group S collections TRAPPE14397 and H7119 (Figure 17). Further investigation of this second lineage of *Descomyces*-like fungi, employing a greater sample of authenticated collections of *D. angustisporus*, could seek to explore what factors underpin the morphological similarities of the two groups of *Descomyces* species. Observations made for this thesis suggest that there may be allometric tendencies or perhaps constraints on *Descomyces* spores. For example, *D. angustisporus* has elongate, narrowly fusiform spores with the widest point of the spore roughly mid-way between the apex and hilum. Other *Descomyces* collections grouping among the main group of *Descomyces* exhibit a variety of spore shapes. These range from broadly citriform spores, again with a 'central' widest point, to almost pyriform spores with a very distended rostrum and bulbous proximal end such that the widest point of the spore is much closer to the hilar appendix. Investigation of such allometric characteristics in a more sophisticated manner than that carried out in this thesis could make use of digital image analysis and computer generated shape descriptors such as those used for *Puccinia* spores (Anikster *et al.* 2005) or fruit types in *Lithocarpus* (Fagaceae) (Cannon & Manos 2001) to provide more, less-subjective characters for phylogenetic or multivariate analyses. Such investigations would however need to distinguish between phylogenetic and developmental, physiological or environmental influences on spore shape for this group.

This thesis has supported the findings of Peintner *et al.* (2001) regarding the division of the sequestrate cortinarioid fungi among the *Cortinarius*-, *Descolea*- and *Alnicola/Hebeloma*-like lineages. Though bootstrap values are not as high for some of the deeper nodes, Figure 16 and Figure 17, as in figures six and seven of Peintner *et al.*

(2001) show the association of *Hymenogaster sensu stricto* with *Hebeloma* [and ex-*Naucoria Alnicola* collections (Moreau *et al.* 2006)] and the association between *Descomyces* (including *Timgrovea*), *Setchelliogaster* and *Descolea*. Using Clustal W to align the expanded set of *Cortinarius, Protoglossum* and *Quadrispora* sequences obtained for this study with a large set of published *Cortinarius* sequences (Appendix 5) also showed a similar grouping of sequestrate *Cortinarius, Protoglossum* and *Quadrispora* forms in a range of subgeneric clades of *Cortinarius* [e.g. those of Peintner *et al.* (2001), Peintner *et al.* (2004), Garnica *et al.* (2005)].

5.6 Has the hypothesis been conclusively disproved...

and if not how might retesting be improved?

No, the hypothesis that numeric analysis of selected morphological characters can recover the same pattern of groups and relationships among the cortinarioid sequestrate fungi as phylogenetic analysis of ITS data has not been conclusively disproved. The work presented here, and even the hypothesis itself, are too limited to have satisfactorily dealt with the hypothesis, yet this work indicates that it may well be possible to achieve congruent phylogenies using morphological and molecular data. Limitations on the work presented here relate to the data used, the preparation of this data for analysis and the analyses used. The possibility of finding agreement between morphological and molecular data is supported by the agreement observed between the morphological and molecular analyses presented in this study, even given the limitations outlined in the following sections.

Table 12: Groupings from cluster analysis (CA) and ordination (PCA/PCO), and parsimony and Bayesian analyses for morphological, molecular (ITS) and combined datasets. 'Core' members (those grouping together in molecular, combined and at least three morphological analyses) are indicated by the colour of the group of which they are a core part.

		MORPHOLOGICAL							ITS				COMB.			
CODE	NAME	**CA** Std. Euc. Wards. (Figure 4)	**CA** Std. Euc. UPGMA (Figure 5)	**CA** Mul. Mean UPGMA (Figure 6)	**PCA** Std. Euc. (Figure 7)	**PCO** Mul. Mean (Figure 9)	**PARSIMONY** (Figure 10)	**BAYESIAN** (Figure 11)	**CA** Mean UPGMA (Figure 12)	**PCO** Mean (Figure 13)	**PARSIMONY** (Figure 14)	**BAYESIAN** (Figure 15)	**CA** Mean UPGMA (Figure 18)	**PCO** (Figure 19)	**PARSIMONY** (Figure 20)	**BAYESIAN** (Figure 21)
MEL2032790	*Thaxterogaster campbelliae*	L	W	P	L		P		L	L	L	L	L	L	L	L
H1006	*Thaxterogaster sp.*	L	L	P	B		P		L	L	L	L	L	L	L	L
H0904	*Thaxterogaster* lilac-silky	L	L	W	L	L	P		L	L	L	L	L	L	L	L
TL503	*Thaxterogaster* sp. 3	L	L	L	L	W	L		L	L	L	L	L	L	L	L
H0910	*Thaxterogaster* lilac-silky	L	L	L	L	W	L		L	L	L	L	L	L	L	L
H6753	*Thaxterogaster sp.*	W	W	L		L	L		L	L	L	L	L	L	L	L
H1213	*Thaxterogaster sp.* A	W	W	L		L	L		L	L	L	L	L	L	L	L
H5814	*Thaxterogaster sp.*	P	P	P	B	P	P		L	W	L		L	W	L	
H5301	*Thaxterogaster sp.*	W	W	W	W	L	L		W	W	W	W	W	W	W	W
H6784	*Thaxterogaster sp.*	W	W	W	W	W	L	W	W	W	W	W	W	W	W	W
H2198	*Thaxterogaster sp.*	W	W	W	W	L	L	W	W	W	W	W	W	W	W	W
H5197	*Thaxterogaster sp.*	W	W	W	W	W	P		W	W	W	W	W	W	W	W
H5791	*Thaxterogaster sp.*	P	P	P	B	P	P		P	P	P	P	P	P	P	P
MEL2057704	*Thaxterogaster/Protoglossum porphyreus/luteum*	P	P	P	B	P	P		P	P	P	P	P	P	P	P
H1486	*Thaxterogaster sp.*				B	P	P		G	G	G	G	G	G	G	G
H0969	*Quadrispora* frog eggs			P	G	P	P		G	G	G	G	G	G	G	G
H4136	*Quadrispora/Thaxterogaster sp. nov. 4*	P	P	P	G		P		G	G	G	G	G	G	G	G
TL493	*Thaxterogaster* sp. 2	B	B	P	B	B	P		B	B	B	B	B	B	B	B
TL501B	*Thaxterogaster sp. 1*	B	B	P	B	B	P		B	B	B	B	B	B	B	B
H0734	*Descomyces dougmillsii*		S			S	S		S	S	S	S	S	S	S	S

Table 12 continued: Groupings from cluster analysis (CA) and ordination (PCA/PCO), and parsimony and Bayesian analyses for morphological, molecular (ITS) and combined datasets. 'Core' members (those grouping together in molecular, combined and at least three morphological analyses) are indicated by the colour of the group of which they are a core part.

		MORPHOLOGICAL							ITS				COMB.			
CODE	NAME	CA Std. Euc. Wards. (Figure 4)	CA Std. Euc. UPGMA (Figure 5)	CA Mul. Mean UPGMA (Figure 6)	PCA Std. Euc. (Figure 7)	PCO Mul. Mean (Figure 9)	PARSIMONY (Figure 10)	BAYESIAN (Figure 11)	CA Mean UPGMA (Figure 12)	PCO Mean (Figure 13)	PARSIMONY (Figure 14)	BAYESIAN (Figure 15)	CA Mean UPGMA (Figure 18)	PCO (Figure 19)	PARSIMONY (Figure 20)	BAYESIAN (Figure 21)
H7124	*Descomyces sp.*	S	S	S	S	E	S		S	S	S	S	S	S	S	S
H6988	*Descomyces sp.*	S		S		S	S		S	S	S	S	S	S	S	S
H0736	*Descomyces uniformis*	S	S	S	S	S	S		S	S	S	S	S	S	S	S
H0735	*Descomyces latisporus*	S	S	S	S	S	S		S	S	S	S	S	S	S	S
TRAPPE14397	*Descomyces sp.*	S	S	S	S	S	S		S	S	S	S	S	S	S	S
H7119	*Descomyces sp.*	S	S	S	S	S	S		S	S	S	S	S	S	S	S
H0737	*Descomyces stolatus*	S		E	S	S	E	S	S	S	S	S	S	S	S	S
H4057	*Timgrovea sp.* (subg. *Subreticulata*)	E	E		E	E	E	E	S	E	S	S	E	E	S	S
H0733	*Descomyces fusisporus*	E	E	E	E		E	S	E	E	E	E	E	E	E	E
H5807	*Descolea sp.* (elongate spored)	E	E	E	E		E		E	E	E	E	E	E	E	E
H0732	*Timgrovea/Descomyces ellipsosporus* (subg. *Timgrovea*)	E	E		E	E	E	E	E	E	E	E	E	E	E	E
TRAPPE14535	*Timgrovea sp.* (subg. *Timgrovea*)	E	E	S	E	E	S		E	E	E	E	E	E	E	E
H5655	*Timgrovea sp.* (subg. *Subreticulata*)	E				E	S		E	E	E	E	E	E	E	E
H4234	*Setchelliogaster sp. nov. 1*	A	A	A	A	A			A	A	A	A	A	A	A	A
TRAPPE14281	*Setchelliogaster sp.*	A	A	A	A	A	P		A	A	A	A	A	A	A	A
TRAPPE14175	*Setchelliogaster sp.*		F			E	E			E				S		
H4162	*Timgrovea sp.* (subg. *Subreticulata*)		S				S		F	F	F	F	F	F	F	F
H4204	*Timgrovea sp.* (subg. *Subreticulata*)	F	F			E	E	E	F	F	F	F	F	F	F	F
H4146	*Timgrovea/Setchelliogaster sp. nov. 3* (~ subg. *Subreticulata*)	F	F	A		A	S		F	F	F	F	F	F	F	F

5.6.1 Limitations of the analyses

The phylogenetic analysis methods employed in this study necessitated the conversion of quantitative morphological data into categorical variables. This caused the loss of information and introduced artificial and maximal divisions between collections in the analyses because these characters were unordered characters with equal substitution rates. Possible means of incorporating phylogenetic signal from quantitative morphological traits include, developing/applying phylogenetic methods that can incorporate quantitative data without recoding, using phenetic, distance-based methods as an approximation to phylogenetic methods, or finding a better means of coding the data.

5.6.2 Limitations of the data

The data used in this study to address the hypothesis were limited. The limitations included: the relatively small number of taxa incorporated into the comparative analyses, the choice and utility of both those characters that were included and those that were not and finally the limiting effects of the way the morphological data was coded for analysis.

5.6.2.1 Taxon sampling

Sample size effects have been noted as influencing the placement and apparent isolation of collections such as H5814 and TRAPPE14175 which belong to phylogenetically distinct lineages in the larger molecular dataset, but their distinctiveness is obscured by being the only representatives of their clades in the comparative analyses. The impact of single isolated collections on the patterns of similarity in the various datasets also warrants further investigation. Peintner *et al.* (2001) suggests the use of multiple

replicate sequences for a single putative taxon. No agaricoid taxa were included in the comparative analyses on the presumption that analogous gasteromycetation-related characters may have obscured phylogenetic patterns given the limitations of the morphological dataset. Further work is needed to determine the validity of this presumption and, even if it holds true, there is the possibility of including a comparable subset of characters as indicated in the hypothesis by the use of the phrase "selected morphological characters". Sample size also interacts with the coding of characters in that when there are few putative taxa of the rank that can be resolved by the data relative to the number of states in each character some characters can become effectively uninformative at the rank of resolution. For example if a character may have one of ten states and six of eight major clades exhibit one state each while two exhibit two states each, but no clade exhibits states from another clade then that character is essentially autapomorphic for each clade and confers no signal as to between group relationships.

5.6.2.2 Character selection

Extending the comparative analyses presented here should incorporate an expanded suite of both molecular and morphological characters. The utility of the ITS for identification of comparable lineages, and its limited usefulness for elucidating between group relationships has been reinforced by the work presented here. Bruns (2001) lists several coding regions used in the fungal phylogenetics including EF1-alpha, Beta tubulin, the RNA polymerases and Chitinase subunits. However to date only the ITS, nLSU (including divergent domains D1 and D2) and RPB1 and RPB2 have been utilised in publications examining the sub-familial phylogeny of the Cortinariaceae. These studies attempting to find molecular and morphological bases for generic and

sub-generic classification of, and within, *Cortinarius* and related genera show similarly poorly resolved between-clade relationships (*c.f.* Seidl 2000, Peintner *et al.* 2001, Garnica *et al.* 2003a & b, Peintner *et al.* 2004, Frøslev *et al.* 2005, Garnica *et al.* 2005 and Moreau *et al.* 2006). The lack of resolution shown in these phylogenies may represent diverged lineages which lack intermediate fungi and sequences that might provide support for branching patterns between groups. Regardless of the cause of the lack of resolution of between clade relationships, it is clear, especially from the combined ITS, RPB1 and RPB2 phylogeny of Frøslev *et al.* (2005) that, if the phylogenetic signal linking these clades exists, many more regions must be incorporated to recover it. The work of Rokas *et al.* (2003) suggesting that the concatenation of eight to twenty genes may be necessary to confidently recover species trees appears to be a pertinent challenge for researchers seeking to estimate the phylogeny of the cortinarioid sequestrate fungi.

Many more morphological characters exist for the cortinarioid sequestrate fungi than have been included here. These include the appearance of the fresh basidiome, including colour, environmental and geographic data, and host plant associations, chemical phenotypic characters including pigments and other secondary metabolites and developmental, cytological and ultrastructural characteristics. The morphological dataset presented here was intentionally limited to characters considered comparable to identification methods routinely used in biodiversity surveys of sequestrate fungi. It is expected that more detailed and in-depth examination and definition of the characteristics of the fungi in question will lead to more accurate delimitations of analogous and homologous characters. This is supported by observations that characteristics not incorporated into the morphological dataset (such as the presence of

clamps in the peridium or observations on development) may be useful in distinguishing the different forms of gelatinised peridiopellis exhibited by H5814 and G group fungi.

Further work linking sequence data with peridial layer development and the mature phenotype could also aid in tracing the multiple origins of characters, such as gelatinous peridia, suggested by authors including Peintner *et al.* (2001). Ontogeny and developmental variation can be a source of both taxonomically useful characters and of potentially taxonomically confounding variation. Kluge & Strauss (1985) and Reijnders (1991) review the application of studies of ontogeny to taxonomic problems and emphasise the usefulness of such studies for discerning homologous tissues in morphologically dissimilar fungi. Examinations of fruit body development have revealed associations between sequestrate and agaricoid taxa that have subsequently been supported by molecular studies. The homology of the primary angiocarpy of *Battarrea stevenii* with agaricoid taxa is a particularly striking example. This homology was revealed in the patterns of differentiation of the of the stipe, gleba and layers volva in the former (Jacobson *et al.* 1999, Reijnders 2000) and is supported by the placement of *Battarrea* and *Tulostoma* among the Agaricales in the MOR phylogeny (Hibbett *et al.* (2005) http://mor.clarku.edu). Examination of morphological characters at various stages of development is particularly important when these characters are phenotypically plastic or phylogenetically analogous. Based on observations of developmental and phenotypic plasticity among *Laccaria*, *Podohydnangium* and *Hydnangium*, Bougher *et al.* (1993) stress the importance of adequately sampling the developmental and phenotypic variation of putative taxa, particularly when these are distinguished on the basis of gross, mature basidiome morphology. Similarly taxonomically important patterns of morphological variability have been demonstrated between species of *Descolea* and *Setchelliogaster* (Martin & Rocabruna 1999, Lago *et*

al. 2001). Authors such as Bentivenga & Morton (1995) have used developmental studies to elucidate the biology underpinning genetically and environmentally variable, but taxonomically informative, characteristics such as those of spore size and colouration (Bentivenga *et al.* 1997). For the cortinarioid sequestrate fungi this approach is especially relevant given the substantial influence spore characteristics imposed on the relationships recovered from the morphological analyses of this thesis. Soehner (1962) used spore developmental characteristics in delimiting sections and subsections of *Hymenogaster* but stressed the difficulty in accurately discerning some of the distinctions in ornamentation structure and developmental stage. While spore characteristics were used by Bougher & Castellano (1993) in their delimitation of *Hymenogaster* [supported by the molecular phylogeny of Peintner *et al.* (2001)], distinctive spore morphology in the cortinarioid genus *Inocybe* has been shown to be homoplasic (Kropp & Matheny 2004). Large changes in development may be brought about by relatively simple genetic changes (Bruns *et al.* 1989, Baura *et al.* 1992, Hibbett *et al.* 1994). It is possible that several such mechanisms exist and have been independently responsible for the multiple origins of sequestrate cortinarioid forms. This may also explain the observation of Peintner *et al.* (2001) that "certain sequestrate forms seem to be more frequent in certain groups" and the observation that the sequestrate habit is arrived at by apparently different pathways e.g. non-expansion of the pileus (more common in /myxacium I) or persistence of the partial veil (more common in /phlegmacium clades) (Table 1). Examination of the development of morphological characters could provide information preventing the incorporation of characters analogous by either plasticity or homoplasy in phylogenetic analyses.

This project was also limited because it was based mainly upon herbarium collections and because some characters, especially fresh appearance, were not uniformly recorded

for much of that material, these characters could not be coded and included in the analyses presented here. May (1991) used a standard method of observing, recording and coding colour of basidiomes of *Laccaria* species that generated taxa supported by molecular phylogeny (G. Mueller pers. com. 2006). However such a method would need to be modified to deal with a range of peridium types as broad as that dealt with here, where multi-layered peridial surfaces complicate the discernment of 'peridium colour'. Finding methods of accurately coding differing peridial structures is also a challenge for future work. Developmental studies may provide a means of incorporating an ontological, and perhaps more phylogenetically informative, aspect into the assessment and coding of homologous peridial layers and structural elements.

5.6.2.3 Character coding

The necessary coding of the morphological data was a substantial limiting factor in the incorporation of morphological data into this assessment of the phylogeny of the sequestrate cortinarioid fungi. As with all morphometric analyses complex, continuous and 'circular' characters present challenges in attempting to code them as discrete characters suitable for phylogenetic analysis. Continuous characters provide a challenge in determining how best to break the character into discrete states. In the analysis presented here equal sized bins were employed for this purpose. Future work should revise this approach. As suggested previously, a method that sets a more subjective but less arbitrary size-class division may ensure that meaningful patterns are preserved. It is possible to break 'circular' characters with informative results as mentioned above (e.g. May 1991), though this requires a reliably standardised record of fresh colour. Particularly pertinent to the patterns observed in the analyses presented here are the limitations due to sub-optimal coding of complex characters such as

peridiopellis structure and composition. There is a trade-off between the inclusion of the diversity of information present in complex 'organs' and the inadvertent up-weighting of correlated characters from the same organ. In the analyses presented here the general appearance of the outer layer of the peridiopellis was used as a surrogate for the complex characteristics of peridiopellis structure and hyphal composition. When so defined, this 'outer peridiopellis' character was seen to be homoplasic for H5814 and collections in clade/cluster G. Future work would need to address the oversimplification inherent in the coding of peridiopellis structure used here. This observation, that some fungal 'organs' convey diverse but correlated information necessitates consideration of character weighting and the recognition of composite characters. It has been suggested that complex or strongly correlated characters should be weighted proportionally to the number of correlated/associated characters (Swofford & Begle 1993). In the analyses presented here some characteristics intrinsically related to others were not included to avoid overweighting (e.g. spore and basidium length when both the width and the ratio of length to width of these organs were included). Future work should carefully investigate and define correlations and associations between characters as a means of more objectively deciding those characters that form 'complex characters' and appropriate weightings. However, it might be argued that all characters do not convey the same phylogenetic signal, in terms of either magnitude or direction. This work has questioned the influence certain characters, such as apiculus length and basidium width, have had on the analyses. It should be noted that the 'importance' of a character or a fungal organ to phylogenetic investigations depends on the manner in which it is used. For example, based on developmental studies on sequestrate fungi, Reijnders (2000) suggested that the apiculus was more phylogenetically important than often acknowledged, because of its relation to spore release. Equal weighting of weaker characters or even proportional weighting for

'complex characters' may not be ideal if one's aim is to discern morphological characters that reveal molecular phylogenetic patterns.

5.6.3 Potential for future work

This thesis holds out the potential for future numeric analysis of a larger, more detailed, more effectively-coded, selective morphological dataset to recover the same groups and pattern of relationships as a well resolved multi-region molecular phylogeny for the cortinarioid sequestrate fungi. Addressing the issues outlined above should resolve some or all of the limitations of the current work and further the integration of the phenotype into the understanding of the phylogeny of the cortinarioid sequestrate fungi.

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Appendix 1

Collections

Table 13. Collections examined for this study, those included in the comparative analyses and expanded molecular dataset are listed first against the analysis codes used throughout the text. Accession numbers are included for those collections for which ITS sequences were submitted to GenBank. FBE group refers to the subjective grouping carried out on the basis of the preliminary morphological examinations.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GenBank ACCESSION	FBE GROUP
E5512	*Russula*	*clelandii*	AF73		
			AF95	DQ328136	
H0717	*Descomyces*	*maidenis* Trappe *nom. prov.*	AF26	DQ328082	95
			AF70	DQ328115	
H0719	*Hymenogaster*	*sp.*	AF112		42
H0720	*Mackintoshia*	*persica*	AF153		47
H0726	*Thaxterogaster*	*redactus*	AF143	DQ328172	8
H0727	*Thaxterogaster*	*campbelliae*	AF175	DQ328196	30
H0728	*Thaxterogaster*	*pyriformis*	AF103	DQ328141	16
H0730	*Descomyces*	*lebelii* Trappe *nom. prov.*	AF6	DQ328063	82
H0731	*Descomyces*	*parviretifer*	AF7	DQ328064	81
H0732	*Timgrovea/Descomyces*	*ellipsosporus* Trappe *nom. prov.*	AF5	DQ328062	56
H0733	*Descomyces*	*fusisporus* Trappe *nom. prov.*	AF8	DQ328065	75
			AF86	DQ328129	
H0734	*Descomyces*	*dougmillsii* Trappe *nom. prov.*	AF9	DQ328066	77
H0735	*Descomyces*	*latisporus* Trappe *nom. prov.*	AF10	DQ328067	81
H0736	*Descomyces*	*uniformis* Trappe *nom. prov.*	AF11	DQ328068	81
			AF94	DQ328135	
H0737	*Descomyces*	*stolatus* Trappe *nom. prov.*	AF12	DQ328069	72
H0790	*Hymenogaster*	*arenarius*	AF81	DQ328124	
H0791	*Hymenogaster*	*citrinus/australis c.f.*	AF91	DQ328132	
H0809	*Descomyces*	*sp.*	AF182	DQ328203	86
H0904	*Thaxterogaster*	*lilac silky*	AF83	DQ328126	19
			AF111	DQ328146	

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GenBank ACCESSION	FBE GROUP
H0910	*Thaxterogaster*	*lilac silky*	AF33	DQ328089	19
			AF84	DQ328127	
			AF154	DQ328179	
H0920	*Thaxterogaster*	*lilac silky*	AF34	DQ328090	19
H0969	*Quadrispora*	*frog eggs*	AF35	DQ328091	45
			AF178	DQ328199	
H1006	*Thaxterogaster*	*sp.*	AF37	DQ328092	34
H1013	*Thaxterogaster*	*sp.*	AF109	DQ328145	27
H1120	*Thaxterogaster*	*sp. a*	AF79	DQ328122	40
H1134	*Thaxterogaster*	*sp. a*	AF141	DQ328171	40
H1194	*Thaxterogaster*	*sp. a*	AF28	DQ328084	34
			AF72	DQ328117	
H1202	*Thaxterogaster*	*sp.*	AF184	DQ328205	24
H1213	*Thaxterogaster*	*sp. a*	AF38	DQ328093	36
H1364	*Quadrispora*	*sp.*	AF180	DQ328201	45
H1446	*Thaxterogaster*	*sp.*	AF195	DQ328216	34
H1486	*Thaxterogaster*	*sp.*	AF40	DQ328094	5
			AF127	DQ328158	
H2129	*Timgrovea*	*sp.*	AF123		56
H2192	*Thaxterogaster*	*sp.*	AF77	DQ328120	41
			AF128	DQ328159	
H2193	*Thaxterogaster*	*sp.*	AF39		3
H2195	*Thaxterogaster*	*sp.*	AF80	DQ328123	36
			AF183	DQ328204	
H2198	*Thaxterogaster*	*sp.*	AF78	DQ328121	38
H3000	*Hymenogaster*	*sensu stricto*	AF90		47
H3059	*Thaxterogaster*	*sp.*	AF41	DQ328095	
H4002	*Mycoamaranthus*	*auriorbis*	AF152		
H4032	*Mycoamaranthus*	*auriorbis*	AF155		
H4057	*Timgrovea*	*sp.*	AF97	DQ328137	51
H4136	*Quadrispora/Thaxterogaster*	*sp. nov. 4*	AF50	DQ328101	14
			AF172	DQ328194	
H4146	*Timgrovea/Setchelliogaster*	*sp. nov. 3*	AF186	DQ328207	61
H4162	*Timgrovea*	*sp.*	AF156	DQ328180	57
H4167	*Timgrovea/Setchelliogaster*	*sp. nov. 5*	AF60	DQ328109	60
H4170	*Timgrovea/Setchelliogaster*	*sp. nov. 3*	AF59	DQ328108	61
H4204	*Timgrovea*	*sp.*	AF137	DQ328167	58
H4221	*Aroramyces*	*gelatinosporus*	AF99		

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GenBank ACCESSION	FBE GROUP
H4234	*Setchelliogaster*	*sp. nov. 1*	AF181	DQ328202	64
H4301	*Descomyces*	*sp. nov. 4*	AF196	DQ328217	84
H4323	*Thaxterogaster*	*sp.*	AF167	DQ328189	2
H4424	*Cribbea*	*sp.*	AF122	DQ328156	
H4574	*Timgrovea*	*sp.*	AF160	DQ328182	51
H4606	*Descomyces*	*sp. nov. 1*	AF4	DQ328061	73
H4770	*Thaxterogaster*	*sp.*	AF117	DQ328151	35
H4798	*Thaxterogaster*	*sp.*	AF139	DQ328169	25
			AF42		
H4850	*Thaxterogaster*	*sp.*	AF161	DQ328183	13
H5008	*Thaxterogaster*	*sp.*	AF177	DQ328198	25
H5092	*Quadrispora*	*sp. (oblongispora?)*	AF43	DQ328096	45
H5160	*Setchelliogaster*	*sp. (australiensis?)*	AF44	DQ328097	64
H5183	*Hymenogaster*	*aureus?*	AF89		
H5185	*Thaxterogaster*	*sp.*	AF45	DQ328098	44
H5197	*Thaxterogaster*	*sp.*	AF46		38
			AF194	DQ328215	
H5234	*Timgrovea*	*sp. (nov. 2)*	AF98		51
H5255	*Thaxterogaster*	*sp.*	AF135	DQ328165	37
H5258	*Hysterogasteroid*	*sp.*	AF47		46
H5286	*Thaxterogaster*	*sp.*	AF19	DQ328076	41
			AF110		
H5301	*Thaxterogaster*	*sp.*	AF18	DQ328075	43
			AF29	DQ328085	
			AF119	DQ328153	
H5328	*Hysterogasteroid*	*sp.*	AF75		46
H5330	*Thaxterogaster*	*sp.*	AF76	DQ328119	41
H5339	*Descomyces*	*albus*	AF126	DQ328157	70
H5362	*Thaxterogaster*	*sp.*	AF20	DQ328077	37
H5368	*Russuloid*	*sp.*	AF64		47
H5372	*Descomyces*	*albus*	AF138	DQ328168	76
H5643	*Timgrovea*	*sp.*	AF130	DQ328161	49
H5655	*Timgrovea*	*sp.*	AF198	DQ328219	49
H5791	*Thaxterogaster*	*sp.*	AF48	DQ328099	1
			AF164	DQ328186	
H5798	*Thaxterogaster*	*sp.*	AF62	DQ328110	2
H5803	*Timgrovea*	*ferruginea*	AF27	DQ328083	49
			AF36		
			AF71	DQ328116	
			AF85	DQ328128	
H5807	*Descolea*	*sp.* (elongate spores)	AF140	DQ328170	75

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
H5814	*Thaxterogaster*	*sp.*	AF108	DQ328144	2
H5984	*Timgrovea*	*sp.*	AF146	DQ328175	53
H6076	*Timgrovea*	*sp.*	AF132	DQ328162	93
H6171	*Timgrovea*	*sp. nov. 4*	AF174	DQ328195	57
H6263	*Hymenogaster*	*sp.*	AF191	DQ328212	47
H6358	*Protoglossum*	*violaceum*	AF25	DQ328081	
			AF69	DQ328114	
H6406	*Thaxterogaster*	*sp.*	AF49	DQ328100	14
H6446	*Gymnopaxillus*	*sp.*	AF61		47
H6472	*Hysterogasteroid*	*sp.*	AF63		46
H6518	*Descomyces*	*sp.*	AF169	DQ328191	90
H6558	*Thaxterogaster*	*sp.*	AF21	DQ328078	33
H6558			AF115	DQ328149	
H6564	*Thaxterogaster*	*sp.*	AF22	DQ328079	41
H6585	*Thaxterogaster*	*sp.*	AF23	DQ328080	39
H6585			AF170	DQ328192	
H6591	*Zelleromyces*	*sp.*	AF131		
H6646	*Cortinarius*	*walpolei*	AF88	DQ328131	
H6701	*Descomyces*	*sp.*	AF144	DQ328173	91
H6728	*Thaxterogaster*	*sp.*	AF74	DQ328118	39
H6732	*Thaxterogaster*	*sp.*	AF104	DQ328142	
H6739	*Thaxterogaster*	*sp.*	AF17	DQ328074	39
H6753	*Thaxterogaster*	*sp.*	AF100	DQ328138	39
H6784	*Thaxterogaster*	*sp.*	AF30	DQ328086	43
			AF179	DQ328200	
H6806	*Setchelliogaster*	*sp.*	AF31	DQ328087	66
H6810	*Descomyces*	*sp.*	AF147	DQ328176	56
H6878	*Hysterogasteroid*	*sp.*	AF150		46
H6915	*Thaxterogaster*	*sp.*	AF106	DQ328143	38
H6946	*Thaxterogaster*	*sp.*	AF120	DQ328154	44
H6957	*Genus?*	*sp.*	AF125		46
H6975	*Hysterogasteroid*	*sp.*	AF58		46
H6988	*Descomyces*	*sp.*	AF134	DQ328164	77
H6989	*Descomyces*	*sp.*	AF157	DQ328181	87
H7069	*Descomyces*	*sp.*	AF185	DQ328206	82
H7119	*Descomyces*	*sp.*	AF192	DQ328213	70
H7124	*Descomyces*	*sp.*	AF189	DQ328210	81
H7127	*Thaxterogaster*	*sp.*	AF121	DQ328155	22
H7132	*Descomyces*	*sp.*	AF149	DQ328177	77
H7216	*Descomyces*	*angustisporus*	AF82	DQ328125	
H7265	*Cortinarius*	*sebosus*	AF92	DQ328133	

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
HL456	*Thaxterogaster*	*sp.*	AF32	DQ328088	41
MEL 2032790	*Thaxterogaster*	*campbelliae*	AF51	DQ328102	35
			AF173		
MEL 2049699	*Hymenogaster*	*sp.*	AF159		2
MEL 2056701	*Thaxterogaster*	*levisporus*	AF124		32
MEL 2056847	*Thaxterogaster*	*sp.*	AF142		27
MEL 2057505	*Thaxterogaster*	*levisporus*	AF187	DQ328208	16
MEL 2057536	*Thaxterogaster*	*levisporus*	AF114	DQ328148	42
MEL 2057547	*Thaxterogaster*	*levisporus*	AF55	DQ328105	42
MEL 2057558	*Thaxterogaster*	*leucocephalus*	AF52	DQ328103	42
MEL 2057565	*Thaxterogaster*	*levisporus*	AF53		42
MEL 2057704	*Thaxterogaster/protoglossum*	*porphyreus/luteum*	AF176	DQ328197	1
MEL 2057999	*Thaxterogaster/Cortinarius*	*sp.*	AF102	DQ328140	23
MEL 2059043B	*Thaxterogaster*	*levisporus*	AF65	DQ328111	42
MEL 2059057	*Thaxterogaster*	*sp.*	AF57	DQ328107	26
MEL 2063434	*Setchelliogaster/Descomyces*	*sp.*	AF171	DQ328193	68
MEL 2063437	*Thaxterogaster*	*sp.*	AF54	DQ328104	12
			AF107		
MEL 2063439	*Thaxterogaster*	*sp.*	AF118	DQ328152	17
MEL 2063445	*Thaxterogaster*	*sp.*	AF158		41
MEL 2079347	*Thaxterogaster*	*pyriformis*	AF56	DQ328106	9
MEL 2136538	*Thaxterogaster*	*pingue*	AF66	DQ328112	11
NEGATIVE 1	*Negative*	*1 pg 44 bk 1*	AF96		

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
PERTH 00960403	*Quadrispora*	*tubercularis*	AF24		
			AF67	DQ328113	
			AF68		
PERTH 06234615	*Descomyces*	*angustisporus*	AF1	DQ328058	
PERTH 06234623	*Cortinarius*	*walpolei*	AF2	DQ328059	
PERTH 06234631	*Cortinarius*	*sebosus*	AF3	DQ328060	
TL1608	*Genus?*	*sp.*	AF166	DQ328188	
TL493	*Thaxterogaster*	*my sp. 2*	AF15	DQ328072	4
			AF87	DQ328130	
TL501A	*Thaxterogaster*	*my sp. 1*	AF16	DQ328073	41
TL501B	*Thaxterogaster*	*sp. 1*	AF168	DQ328190	4
TL502A	*Thaxterogaster*	*my sp. 3*	AF13	DQ328070	41
TL503	*Thaxterogaster*	*my sp. 3*	AF14	DQ328071	37
			AF93	DQ328134	
TRAPPE 11751	*Descomyces*	*albus*	AF188	DQ328209	78
TRAPPE 14129	*Descomyces*	*sp.*	AF197	DQ328218	84
TRAPPE 14166	*Descomyces*	*sp.*	AF116	DQ328150	85
TRAPPE 14175	*Setchelliogaster*	*sp.*	AF162	DQ328184	67
TRAPPE 14178	*Descomyces*	*sp.*	AF113	DQ328147	73
TRAPPE 14201	*Descomyces*	*sp.*	AF148		72
TRAPPE 14252	*Setchelliogaster*	*sp.*	AF101	DQ328139	66
TRAPPE 14262	*Setchelliogaster*	*sp.*	AF145	DQ328174	65
TRAPPE 14281	*Setchelliogaster*	*sp.*	AF193	DQ328214	65
TRAPPE 14293	*Setchelliogaster*	*sp.*	AF105		66
TRAPPE 14304	*Descomyces*	*sp.*	AF136	DQ328166	73
TRAPPE 14325	*Descomyces*	*sp.*	AF151	DQ328178	92
TRAPPE 14397	*Descomyces*	*sp.*	AF190	DQ328211	74

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
TRAPPE 14493	*Descomyces*	*sp.*	AF129	DQ328160	3
TRAPPE 14535	*Timgrovea*	*sp.*	AF133	DQ328163	54
TRAPPE 14592	*Descomyces*	*sp.*	AF163	DQ328185	74
TRAPPE 14702	*Descomyces*	*sp.*	AF165	DQ328187	69
AWCLARI DGE 2115	*Descomyces*	*giachinii*			
AWCLARI DGE 2616	*Timgrovea*	*reticulata*			
AWCLARI DGE 2669	*Timgrovea*	*reticulata*			
AWCLARI DGE 2994	*Descomyces*	*giachinii*			
BEATON EO229A	*Hymenogaster*	*reticulatus*			
BRIP1768	*Descomyces*	*sp. nov. 5*			
CANB 624350.1	*Timgrovea*	*sp.*			
CANB 628210.1	*Setchelliogaster*	*australiensis*			
CANB 628667.1	*Descomyces*	*albellus*			
CANB 628686.1	*Setchelliogaster*	*australiensis*			
CANB 628689.1	*Setchelliogaster*	*australiensis*			
CANB 628840.1	*Descomyces*	*albellus*			
CBG 3405749	*Thaxterogaster*	*sp.*			
CBG 9405741	*Protoglossum*	*sp.*			
CBG 9405742	*Protoglossum*	*sp.*			
CBG 9405743	*Hymenogaster*	*sp.*			
CBG 9405744	*Hymenogaster*	*sp.*			
CBG 9405745	*Protoglossum*	*sp.*			
CBG 9405758	*Thaxterogaster*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
CBG 9405759	*Setchelliogaster*	*sp.*			
CBG 9405760	*Descomyces*	*albus?*			
CBG 9405770	*Protoglossum*	*sp.*			
CBG 9405774	*Thaxterogaster*	*aff. levisporus*			
CBG 9405781	*Thaxterogaster*	*sp.*			
CBG 9405783	*Setchelliogaster?*	*sp.*			
CBG 9405785	*Descomyces*	*albus?*			
CBG 9405786	*Gautieria*	*monosporus*			
CBG 9405788	*Descomyces*	*albus?*			
CBG 9405852	*Thaxterogaster*	*sp.*			
CBG 9405855	*Protoglossum*	*sp.*			
CBG 9405857	*Descomyces*	*albus?*			
CBG 9405860	*Hymenogaster*	*sp.*			
CBG 9405861	*Protoglossum*	*sp.*			
CBG 9415752	*Protoglossum*	*sp.*			
CBG 9511828	*Genus?*	*sp.*			
CBG 9901106	*Descomyces*	*albus*			
CBG 9901112	*Descomyces*	*albus*			
DR1	*Quadrispora*	*oblongispora?*			
DR10	*Protoglossum*	*sp.*			
DR11	*Protoglossum*	*sp.*			
DR12	*Setchelliogaster*	*sp.*			
DR13	*Protoglossum*	*sp.*			
DR14	*Descomyces*	*sp.*			
DR2	*Austrogautieria*	*sp.*			
DR3	*Protoglossum*	*sp.*			
DR5	*Thaxterogaster?*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
DR6	*Protoglossum*	*sp.*			
DR7	*Thaxterogaster/Protoglossum/Quadrispora*	*sp.*			
DR8	*Thaxterogaster?*	*sp.*			
DR9	*Thaxterogaster/Potoglossum*	*sp.*			
E3801	*Pholiotina*	*sp.*			
E3802	*Cuphocybe*	*phaeomyxa?*			
E3803	*Cortinarius*	*rotundisporus*			
E3804	*Tubaria*	*rufofulva*			
E3812	*Dermocybe*	*sp.*			
E3814	*Cuphocybe*	*sp.*			
E3815	*Cortinarioid*	*sp.*			
E3816	*Pholiotina*	*sp.*			
E3817	*Inocybe*	*australiensis?*			
E3818	*Galerina*	*patagonia?*			
E3819	*Gymnopilus*	*sp.*			
E3820	*Conocybe*	*sp.*			
E3821	*Dermocybe*	*sp.*			
E3822	*Cortinarius*	*sp.*			
E5707	*Cortinarius*	*sp.*			
G.BEATON 39	*Setchelliogaster*	*australiensis*			
H0051	*Descomyces*	*sp.*			
H0121	*Cortinarius*	*sp.*			
H0145	*Descomyces*	*albellus*			
H0150	*Descomyces*	*nov. sp. 2 or 3*			
H0175	*Protoglossum*	*luteum*			
H0177	*Cortinarius*	*sp.*			
H0180	*Protoglossum*	*sp.*			
H0201	*Cortinarius*	*sp.*			
H0213	*Descomyces*	*albellus*			
H0218	*Descomyces*	*albellus*			
H0278	*Descomyces*	*sp.*			
H0280	*Descomyces*	*sp.*			
H0284	*Descomyces*	*albellus*			
H0354	*Cortinarius*	*sp.*			
H0358	*Cortinarius*	*sp.*			
H0359	*Dermocybe*	*globuliformis*			
H0376	*Descomyces*	*albellus*			
H0395	*Dermocybe*	*globuliformis*			
H0410	*Descomyces*	*nov. sp. 2*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
H0417	*Descomyces*	*sp.*			
H0424	*Descomyces*	*sp.*			
H0450	*Descomyces*	*sp.*			
H0458	*Protoglossum*	*sp.*			
H0461	*Descomyces*	*albellus*			
H0482	*Descomyces*	*sp.*			
H0535	*Descomyces*	*sp.*			
H0539	*Protoglossum*	*sp.*			
H0542	*Descomyces*	*nov. sp 2*			
H0553	*Descomyces*	*sp.*			
H0566	*Descomyces*	*albellus*			
H0574	*Descomyces*	*albus*			
H0584	*Descomyces*	*albellus*			
H0596	*Descomyces*	*albus*			
H0608	*Descomyces*	*sp.*			
H0653	*Descomyces*	*albus*			
H0654	*Cortinarius*	*sp.*			
H0665	*Descomyces*	*sp.*			
H0716	*Descomyces*	*sp.*			
H0725	*Thaxterogaster*	*sp.*			
H0808	*Descomyces*	*sp.*			
H0812	*Descomyces*	*sp.*			
H0830	*Descomyces*	*sp.*			
H0855	*Descomyces*	*sp.*			
H0891	*Thaxterogaster*	*sp.*			
H0892	*Thaxterogaster*	*sp.*			
H0905	*Thaxterogaster*	*lilac silky*			
H0906	*Octaviania*	*tasmanica?*			
H0907	*Podohydnangium*	*sp.*			
H0911	*Descomyces*	*sp.*			
H0912	*Thaxterogaster*	*lilac silky*			
H0913	*Hysterogaster?*	*tasmanicus?*			
H0914	*Thaxterogaster*	*lilac silky*			
H0915	*Thaxterogaster*	*lilac silky*			
H0918	*Hysterogaster?*	*tasmanicus?*			
H0919	*Hysterogaster?*	*tasmanicus?*			
H0921	*Thaxterogaster*	*yellowish orange dry*			
H0922	*Hysterogaster?*	*tasmanicus?*			
H0923	*Hysterogaster?*	*tasmanicus?*			
H0924	*Setchelliogaster*	*sp. australiensis?*			
H0926	*Podohydnangium*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
H0929	*Thaxterogaster*	*lilac silky*			
H0930	*Thaxterogaster*	*lilac silky*			
H0933	*Protoglossum*	*sp.*			
H0934	*Protoglossum*	*sp.*			
H0936	*Dermocybe*	*globuliformis*			
H0941	*Descomyces*	*sp.*			
H0945	*Cortinarius*	*sp.*			
H0953	*Descomyces*	*sp.*			
H0960	*Genus?*	*sp.*			
H0962	*Protoglossum*	*orange lumpy*			
H0964	*Thaxterogaster*	*lilac*			
H0965	*Thaxterogaster*	*lilac*			
H0968	*Protoglossum*	*viscid lilac*			
H0970	*Protoglossum*	*luteum?*			
H0971	*Dermocybe*	*globuliformis*			
H0973	*Descomyces*	*sp.*			
H1018	*Thaxterogaster*	*sp.*			
H1021	*Thaxterogaster*	*sp.*			
H1023	*Setchelliogaster*	*australiensis*			
H1041	*Descomyces*	*albus*			
H1047	*Descomyces*	*longibasidia*			
H1049	*Descomyces*	*sp.*			
H1070	*Thaxterogaster*	*sp.*			
H1111	*Descomyces*	*sp. nov. 1*			
H1183	*Descomyces*	*sp.*			
H1188	*Descomyces*	*sp.*			
H1204	*Thaxterogaster*	*sp.*			
H1218	*Thaxterogaster*	*sp.*			
H1231	*Thaxterogaster*	*sp.*			
H1260	*Thaxterogaster*	*sp.*			
H1274	*Thaxterogaster*	*sp.*			
H1308	*Thaxterogaster*	*sp.*			
H1326	*Hysterogaster?*	*sp.*			
H1341	*Thaxterogaster*	*sp.*			
H1388	*Setchelliogaster*	*sp.*			
H1425	*Descomyces*	*albellus*			
H1447	*Descomyces*	*albus*			
H1452	*Thaxterogaster*	*sp.*			
H1490	*Descomyces*	*albus*			
H1498	*Descomyces*	*sp.*			
H1585	*Descomyces*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
H2021	*Descomyces*	*sp.*			
H2027	*Descomyces*	*sp. nov. 2 or 3 near oblongisporus (angustisporus)*			
H2030	*Descomyces*	*sp. nov. 2 or 3 near oblongisporus*			
H2085	*Descomyces*	*sp.*			
H2086	*Thaxterogaster*	*sp.*			
H2114	*Descomyces*	*sp. nov. 7*			
H2142	*Descomyces*	*longibasidia*			
H2194	*Thaxterogaster*	*sp.*			
H4051	*Timgrovea*	*kirramaensis*			
H4116	*Descomyces*	*sp. nov. 1*			
H4158	*Descomyces*	*sp. nov. 1*			
H4162	*Timgrovea*	*sp. nov. 4*			
H4192	*Descomyces*	*sp. nov. 1*			
H4200	*Timgrovea*	*sp. nov. 5*			
H4204	*Timgrovea*	*sp. nov. 5*			
H4220	*Thaxterogaster*	*sp.*			
H4247	*Descomyces*	*sp. nov. 1*			
H4250	*Descomyces*	*sp.*			
H4259	*Timgrovea*	*montgloriosus*			
H4260	*Descomyces*	*sp.*			
H4315	*Cortinarius*	*walpolei*			
H4392	*Descomyces*	*sp.*			
H4573	*Descomyces*	*sp. nov. 1*			
H4574	*Timgrovea*	*kirramaensis*			
H4597	*Descomyces*	*sp.*			
H4607	*Descomyces*	*sp.*			
H4725	*Descomyces*	*sp. nov. 9*			
H4769	*Descomyces*	*sp.*			
H4847	*Descomyces*	*sp.*			
H4866	*Protoglossum*	*sp.*			
H4903	*Descomyces*	*sp.*			
H4904	*Descomyces*	*sp.*			
H4905	*Descomyces*	*sp.*			
H4906	*Descomyces*	*sp.*			
H5024	*Timgrovea*	*sp. nov. 2*			
H5036	*Thaxterogaster*	*sp.*			
H5052	*Thaxterogaster*	*sp.*			
H5061	*Thaxterogaster*	*sp.*			
H5129	*Quadrispora?*	*oblongispora?*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
H5141	*Setchelliogaster*	*sp.*			
H5147	*Descomyces*	*sp.*			
H5149	*Thaxterogaster*	*sp.*			
H5155	*Quadrispora?*	*sp.*			
H5164A	*Setchelliogaster*	*australiensis?*			
H5164B	*Setchelliogaster*	*australiensis?*			
H5177	*Setchelliogaster*	*australiensis?*			
H5179	*Thaxterogaster*	*sp.*			
H5189	*Descomyces*	*albus*			
H5196	*Descomyces*	*albellus*			
H5235	*Thaxterogaster*	*sp.*			
H5243	*Descomyces*	*sp.*			
H5249	*Descomyces*	*albellus*			
H5252	*Setchelliogaster*	*australiensis?*			
H5312	*Hysterogaster?*	*sp.*			
H5318	*Hysterogaster?*	*sp.*			
H5363	*Descomyces*	*albus*			
H5369	*Thaxterogaster*	*sp.*			
H5382	*Thaxterogaster*	*sp.*			
H5388	*Thaxterogaster*	*sp.*			
H5396	*Thaxterogaster*	*sp.*			
H5540	*Descomyces*	*sp. nov. 6*			
H5825	*Descomyces*	*sp.*			
H5833	*Descomyces*	*sp. nov. 1*			
H5851	*Descomyces*	*sp. nov. 1*			
H6070	*Descomyces*	*sp. nov. 1*			
H6128	*Descomyces*	*sp.*			
H6179	*Descomyces*	*albus*			
H6204	*Protoglossum*	*sp.*			
H6212	*Descomyces*	*nov. sp 2*			
H6235	*Thaxterogaster*	*basipurpurea*			
H6236	*Thaxterogaster*	*basipurpurea*			
H6248	*Cortinarius*	*sebosus*			
H6255	*Thaxterogaster*	*basipurpurea*			
H6355	*Descomyces*	*sp.*			
H6357	*Thaxterogaster*	*luteirufescens*			
H6363	*Descomyces*	*sp.*			
H6371	*Cortinarius*	*sp.*			
H6388	*Quadrispora*	*tubercularis*			
H6415	*Thaxterogaster*	*sp.*			
H6454	*Thaxterogaster*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
H6579	*Thaxterogaster*	*sp.*			
H6640	*Cortinarius*	*sp.*			
H6642	*Descomyces*	*sp.*			
H6665	*Thaxterogaster*	*sp wht sml sprs*			
H6670	*Thaxterogaster*	*sp.*			
H6671	*Quadrispora*	*oblongispora*			
H6672	*Thaxterogaster*	*sp.*			
H6677	*Cortinarius*	*sp.*			
H6684	*Cortinarius*	*basipurpureus*			
H6688	*Thaxterogaster/Protoglossum*	*purpureum/violaceum*			
H6777	*Thaxterogaster*	*sp.*			
H6779	*Hysterogaster?*	*sp.*			
H6782	*Thaxterogaster*	*sp.*			
H6802	*Descomyces*	*sp.*			
H6832	*Thaxterogaster*	*sp.*			
H6837	*Descomyces*	*sp.*			
H6868	*Descomyces*	*sp.*			
H6882	*Descomyces*	*sp.*			
H6925	*Thaxterogaster*	*sp.*			
H6956	*Thaxterogaster*	*sp.*			
H6966	*Thaxterogaster*	*sp.*			
H6980	*Descomyces*	*sp.*			
H7002	*Cortinarius*	*sp.*			
H7003	*Thaxterogaster*	*sp.*			
H7004	*Thaxterogaster*	*sp.*			
H7005	*Thaxterogaster*	*sp wht sml sprs*			
H7021	*Descomyces*	*albellus*			
H7032	*Descomyces*	*sp.*			
H7059	*Descomyces*	*sp.*			
H7064	*Descomyces*	*albellus*			
H7076	*Descomyces*	*sp.*			
H7087	*Descomyces*	*sp.*			
H7121	*Descomyces*	*sp.*			
H7138	*Descomyces*	*sp.*			
H7196	*Descomyces*	*sp.*			
H7205	*Descomyces*	*sp.*			
H7246	*Thaxterogaster*	*sp gpf lrg sprs*			
H7250	*Thaxterogaster*	*sp gpf lrg sprs*			
H7252	*Descomyces*	*sp.*			
H7259	*Protoglossum*	*luteum*			
H7260	*Dermocybe*	*globuliformis*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
H7263	*Descomyces*	*sp.*			
H7272	*Descomyces*	*angustisporus*			
H7280	*Descomyces*	*sp.*			
H7282	*Descomyces*	*sp.*			
H7283	*Descomyces*	*sp.*			
H7287	*Thaxterogaster*	*sp gpf lrg sprs*			
H7290	*Descomyces*	*sp.*			
H7298	*Descomyces*	*sp.*			
H7299	*Descomyces*	*sp.*			
H7300	*Descomyces*	*sp.*			
H7312	*Descomyces*	*sp.*			
H7317	*Setchelliogaster*	*australiensis*			
H7322	*Descomyces*	*sp.*			
H7325	*Descomyces*	*sp.*			
H7327	*Cortinarius*	*sp.*			
H7328	*Descomyces*	*sp.*			
H7337	*Cortinarius*	*sp.*			
H7339	*Descomyces*	*sp.*			
H7344	*Auritella*	*geoaustralis*			
H7345	*Descomyces*	*longibasidia sp. nov*			
H7350	*Descomyces*	*angustisporus*			
H7357	*Cortinarius*	*luteirufescens*			
H7561	*Cortinarius*	*sp.*			
H7570	*Descomyces*	*sp.*			
H7642	*Descomyces*	*sp.*			
H7660	*Protoglossum*	*luteum*			
H7717	*Descomyces*	*sp.*			
H7732	*Descomyces*	*sp.*			
HL1367	*Protoglossum*	*sp.*			
HL1671	*Hymenogaster*	*sp.*			
HL3573	*Setchelliogaster*	*australiensis*			
HL3664	*Protoglossum*	*sp.*			
HL3718	*Protoglossum*	*sp.*			
HL445	*Thaxterogaster*	*sp.*			
HL539	*Hymenogaster*	*sp.*			
HL592	*Setchelliogaster*	*sp.*			
HL810	*Thaxterogaster*	*sp.*			
HL855	*Thaxterogaster*	*sp.*			
HO 100542	*Descomyces (Hymenogaster)*	*albellus (albidus)*			
HO 100544	*Timgrovea (Hymenogaster)*	*macrosporus*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
HO 100573	*Hymenogaster*	*maideni*			
HO 100580	*Descomyces (Hymenogaster)*	*albellus*			
HO 100651	*Arcangeliella (Hymenogaster)*	*nanus*			
HO 100654	*Gymnoglossum (Dendrogaster, Hymenogaster)*	*fulvus*			
HO 100659	*Arcangeliella (Hymenogaster)*	*nanus*			
HO 100665	*Hymenogaster*	*sp.*			
HO 100666	*Thaxterogaster*	*levisporus*			
HO 100668	*Gymnoglossum (Dendrogaster, Hymenogaster)*	*fulvus*			
HO 100734	*Arcangeliella (Hymenogaster)*	*nanus*			
HO 100736	*Arcangeliella (Hymenogaster)*	*nanus*			
HO 89538	*Hymenogaster (Hysterangium)*	*atratum*			
HO 89552	*Protoglossum (Hymenogaster, Hysterangium)*	*viscidum*			
HO 89558	*Hysterogaster (Hymenogaster, Hysterangium)*	*fusisporum*			
HORAK 73/275	*Descomyces*	*sp. nov. 6*			
KA5995	*Descomyces*	*sp.*			
KB7995	*Descomyces*	*sp.*			
MEL 1053377	*Thaxterogaster*	*sp.*			
MEL 2024698	*Thaxterogaster? Setchelliogaster?*	*sp.*			
MEL 2024715	*Thaxterogaster*	*sp.*			
MEL 2031839	*Descomyces/Setchellio gaster*	*sp.*			
MEL 2040397	*Thaxterogaster*	*sp.*			
MEL 2056660	*Thaxterogaster*	*sp.*			
MEL 2056692	*Timgrovea/Descomyces*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
MEL 2056839	*Thaxterogaster*	*sp.*			
MEL 2057437	*Thaxterogaster*	*sp.*			
MEL 2057510	*Thaxterogaster*	*sp.*			
MEL 2057535	*Thaxterogasater*	*sp.*			
MEL 2057548	*Thaxterogaster*	*sp.*			
MEL 2057554	*Thaxterogaster*	*sp.*			
MEL 2057555	*Thaxterogaster*	*sp.*			
MEL 2057560	*Thaxterogaster*	*sp.*			
MEL 2057561	*Thaxterogaster*	*sp.*			
MEL 2057564	*Thaxterogaster*	*sp.*			
MEL 2057572	*Thaxterogaster*	*sp.*			
MEL 2058001	*Thaxterogaster*	*sp.*			
MEL 2058007	*Thaxterogaster*	*sp.*			
MEL 2058426	*Thaxterogaster*	*sp.*			
MEL 2058457	*Thaxterogaster*	*sp.*			
MEL 2058484	*Thaxterogaster*	*sp.*			
MEL 2058523	*Thaxterogaster? Setchelliogaster?*	*sp.*			
MEL 2058533	*Thaxterogaster*	*sp.*			
MEL 2058670	*Thaxterogaster*	*sp.*			
MEL 2058674	*Descomyces*	*sp.*			
MEL 2058676	*Thaxterogaster*	*sp.*			
MEL 2058688	*Thaxterogaster? Setchelliogaster?*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
MEL 2058693	*Descomyces*	*sp.*			
MEL 2058700	*Setchelliogaster*	*sp.*			
MEL 2058758	*Setchelliogaster*	*sp.*			
MEL 2058768	*Thaxterogaster*	*sp.*			
MEL 2058991	*Thaxterogaster*	*sp.*			
MEL 2059085	*Thaxterogaster*	*sp.*			
MEL 2059195	*Thaxterogaster*	*sp.*			
MEL 2059207	*Thaxterogaster*	*sp.*			
MEL 2059208	*Thaxterogaster*	*sp.*			
MEL 2059211	*Thaxterogaster/Protoglossum?*	*sp.*			
MEL 2059879	*Thaxterogaster*	*sp.*			
MEL 2059906	*Descomyces/Setchelliogaster*	*sp.*			
MEL 2059919	*Thaxterogaster*	*sp.*			
MEL 2059964	*Descomyces*	*sp.*			
MEL 2059986	*Thaxterogaster*	*sp.*			
MEL 2061024	*Thaxterogaster*	*sp.*			
MEL 2063155	*Thaxterogaster*	*sp.*			
MEL 2063331	*Descomyces*	*sp.*			
MEL 2063428	*Hysterogaster?*	*sp.*			
MEL 2079349	*Thaxterogaster*	*sp.*			
MEL 2136537	*Thaxterogaster?*	*sp.*			
PDD 11590	*Descomyces*	*sp. nov. 4*			
PERTH 00964115	*Setchelliogaster/Timgrovea*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
PERTH 05306809	*Thaxterogaster*	*sp.*			
TL1047	*Descomyces*	*lebelii* Trappe *nom. prov.*			
TL1104	*Descomyces*	*lebelii* Trappe *nom. prov.*			
TL1107	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1113	*Thaxterogaster*	*sp. 1*			
TL1116	*Unknown*	*orangy brown gleba spores smooth, thick walled*			
TL1117	*Thaxterogaster*	*sp. 2*			
TL1133	*Protoglossum*	*sp. nov.*			
TL1134	*Thaxterogaster*	*sp. 1 (young)*			
TL1135	*Thaxterogaster*	*sp. 1*			
TL1159	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1174	*Thaxterogaster*	*sp. 1 or protoglossum sp?*			
TL1212	*Descomyces*	*lebelii* Trappe *nom. prov.*			
TL1214	*Mixed collection A: Thaxterogaster sp. 1; B: Same/similar to TL1234*	*sp.*			
TL1214A	*Thaxterogaster*	*sp. 1*			
TL1214B	*Unknown*	*spores thick walled non-dextrinoid inamyloid*			
TL1226	*Thaxterogaster*	*sp. 1*			
TL1232	*Descomyces*	*albellus*			
TL1234	*Unknown*	*spores thick walled non-dextrinoid inamyloid*			
TL1248	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1249	*Descomyces*	*lebelii* Trappe *nom. prov.*			
TL1263	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1264	*Thaxterogaster*	*sp. 2*			
TL1265	*Thaxterogaster*	*sp. 1*			
TL1280	*Descomyces*	*albellus*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
TL1295	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1309	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1327	*Descomyces*	*albus*			
TL1337	*Unknown*	*smooth spores slightly citriform may be same as tl1448*			
TL1347	*Descomyces*	*albellus*			
TL1356	*Royoungia*	*boletoides (-like, spores slightly broader?)*			
TL1357	*Thaxterogaster*	*sp. 1*			
TL1382	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1388	*Thaxterogaster*	*sp. 1 or 3*			
TL1389	*Thaxterogaster*	*sp. 2*			
TL1395	*Descomyces*	*albus*			
TL1406	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1414	*Thaxterogaster*	*sp. 1*			
TL1419	*Thaxterogaster*	*sp. 1*			
TL1422	*Thaxterogaster*	*sp. 3?*			
TL1435	*Thaxterogaster*	*sp. 1*			
TL1437	*Thaxterogaster*	*sp. 1*			
TL1441	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1448	*Setchelliogaster*	*sp (more or less smooth spored)*			
TL1454	*Thaxterogaster*	*sp. 2*			
TL1478	*Thaxterogaster*	*sp. 1*			
TL1480	*Octaviania*	*sp.*			
TL1482	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1484	*Thaxterogaster*	*sp. 1*			
TL1485	*Thaxterogaster*	*sp. 1*			
TL1489	*Thaxterogaster*	*sp. 1*			
TL1499	*Quadrispora*	*oblongispora*			
TL1506	*Thaxterogaster*	*sp. 1 (sterile?)*			
TL1511	*Thaxterogaster*	*sp. 1*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
TL1513	*Thaxterogaster*	*sp. 1 (? spores seem almost if not smooth and are often strongly/acutely obovate*			
TL1517	*Thaxterogaster*	*sp. 1*			
TL1525	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL1569	*Descomyces*	*sp.*			
TL1590	*Descomyces*	*sp.*			
TL273	*Cortinarius*	*strongly velar cort 1*			
TL276	*Descomyces*	*albellus/hymenogaster s.s. sp. 1*			
TL288	*Cortinarius*	*strongly velar cort 1*			
TL292	*Descomyces*	*albus/lebelii* Trappe *nom. prov.*			
TL300	*Descomyces*	*lebelii* Trappe *nom. prov.*			
TL304	*Cortinarius*	*strongly velar cort 2 "white bloom"*			
TL308	*Cortinarius*	*strongly velar cort 1*			
TL319	*Protoglossum*	*sp. 2 (sp. "silver")*			
TL326	*Cortinarius*	*strongly velar cort 1*			
TL353	*Protoglossum*	*sp. 1 ("collar & orange basal context")*			
TL375	*Thaxterogaster*	*sp. 1*			
TL376	*Thaxterogaster*	*sp. 1*			
TL390	*Thaxterogaster*	*sp. 1*			
TL407	*Thaxterogaster*	*sp. 1*			
TL419	*Thaxterogaster*	*sp. 1*			
TL421	*Protoglossum*	*sp. 4 (not luteum)*			
TL425	*Thaxterogaster*	*sp. 1*			
TL430	*Thaxterogaster*	*sp. 3*			
TL448	*Thaxterogaster*	*sp. 3*			
TL455	*Thaxterogaster*	*sp. 1*			
TL459	*Thaxterogaster*	*sp. 1*			
TL462	*Thaxterogaster*	*sp. 2*			
TL470	*Protoglossum*	*sp. 3*			
TL471	*Thaxterogaster*	*sp. 2*			
TL474	*Protoglossum*	*luteum*			
TL478	*Thaxterogaster*	*sp. 1*			
TL496	*Thaxterogaster*	*sp. 2*			
TL499	*Thaxterogaster*	*sp. 2*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
TL501	*Mixed collection A: Thaxterogaster sp. 1; B: Thaxterogaster sp. 2*	*sp.*			
TL502	*Mixed collection A: Thaxterogaster sp. 3; B: Thaxterogaster sp. 1*	*sp.*			
TL502B	*Thaxterogaster*	*sp. 1*			
TL506	*Thaxterogaster*	*sp. 1*			
TL508	*Thaxterogaster*	*sp. 3*			
TL518	*Thaxterogaster*	*sp. 1*			
TL521	*Thaxterogaster*	*sp. 1*			
TL524	*Unknown*	*secotioid; long, thick-walled, non-dextrinoid spores*			
TL534	*Quadrispora*	*oblongispora ? (perid diff colour to tl1499 and description in bougher & castellano 1993)*			
TL536	*Thaxterogaster*	*sp. 1*			
TL548	*Thaxterogaster*	*sp. 1*			
TL549	*Protoglossum*	*sp. 5*			
TL553	*Thaxterogaster*	*sp. 3 or 1*			
TL554	*Thaxterogaster*	*sp. 1*			
TL569	*Descomyces*	*lebelii* Trappe *nom. prov.*			
TL578	*Thaxterogaster*	*sp. 1*			
TL581	*Thaxterogaster*	*sp. 1*			
TL584	*Quadrispora*	*oblongispora*			
TL588	*Thaxterogaster*	*sp. 1*			
TL597	*Thaxterogaster*	*sp. 1*			
TL602	*Protoglossum*	*sp. 1*			
TL607	*Protoglossum*	*sp. 1*			
TL608	*Descomyces*	*sp.*			
TL609	*Thaxterogaster*	*sp. 3*			
TL621	*Thaxterogaster*	*sp. 1 or 3*			
TL625	*Thaxterogaster*	*sp. 1*			
TL638	*Thaxterogaster*	*sp. 1*			
TL640	*Protoglossum*	*sp. 6*			
TL653	*Thaxterogaster*	*sp. 1*			
TL661	*Thaxterogaster*	*sp. 3 or 1*			
TL663	*Thaxterogaster*	*sp. 1*			
TL670	*Thaxterogaster*	*sp. 1*			
TL671	*Thaxterogaster*	*sp. 1*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
TL673	*Descomyces*	*lebelii* Trappe *nom. prov. (spores similar to latisporus but inflated cells in perid)*			
TL674	*Descomyces*	*lebelii* Trappe *nom. prov. (same as 695?)*			
TL677	*Thaxterogaster*	*sp. 1*			
TL678	*Thaxterogaster*	*sp. 1*			
TL679	*Mixed collection A: Thaxterogaster 1, B: Zelleromyces sp or spp (mixed collection?)*	*sp.*			
TL679A	*Thaxterogaster*	*sp. 1*			
TL695	*Descomyces*	*lebelii* Trappe *nom. prov.*			
TL841	*Thaxterogaster*	*sp. 1*			
TL852	*Thaxterogaster*	*sp. 1*			
TL892	*Thaxterogaster*	*sp. 1*			
TL897	*Thaxterogaster*	*sp. 1*			
TL900	*Thaxterogaster*	*sp. 1*			
TRAPPE 14061	*Descomyces*	*sp.*			
TRAPPE 14065	*Descomyces*	*sp.*			
TRAPPE 14076	*Descomyces*	*sp.*			
TRAPPE 14082	*Descomyces*	*sp.*			
TRAPPE 14092	*Descomyces*	*sp.*			
TRAPPE 14099	*Descomyces*	*sp.*			
TRAPPE 14498	*Descomyces*	*malajczukii*			
TRAPPE 14535	*Timgrovea*	*reticulata*			
TRAPPE 14637	*Cortinarius*	*sp.*			
TRAPPE 14643	*Cortinarius*	*sp.*			
TRAPPE 14651	*Descomyces*	*sp.*			
TRAPPE 14660	*Cortinarius*	*sp.*			
TRAPPE 14662	*Descomyces*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
TRAPPE 14664	*Descomyces*	*sp.*			
TRAPPE 14667	*Descomyces*	*sp.*			
TRAPPE 14672	*Descomyces*	*sp.*			
TRAPPE 14679	*Descomyces*	*sp.*			
TRAPPE 14705	*Descomyces*	*sp.*			
TRAPPE 14711	*Descomyces*	*sp.*			
TRAPPE 14730	*Descomyces*	*sp.*			
TRAPPE 14731	*Descomyces*	*sp.*			
TRAPPE 14732	*Descomyces*	*sp.*			
TRAPPE 14736	*Descomyces*	*sp.*			
TRAPPE 14738	*Descomyces*	*sp.*			
TRAPPE 14742	*Descomyces*	*sp.*			
TRAPPE 14763	*Descomyces*	*sp.*			
TRAPPE 14814	*Cortinarius*	*sp.*			
TRAPPE 14843	*Protoglossum*	*sp.*			
TRAPPE 14877	*Descomyces*	*sp.*			
TRAPPE 14905	*Descomyces*	*sp.*			
TRAPPE 14920	*Descomyces*	*nov. sp 2*			
TRAPPE 14925	*Descomyces*	*sp.*			
TRAPPE 14940	*Descomyces*	*sp.*			
TRAPPE 14943	*Cortinarius*	*sp.*			
TRAPPE 14951	*Descomyces*	*sp.*			
TRAPPE 14955	*Cortinarius*	*sp.*			

Table 13 continued.

PRIMARY ACCESSION	WORKING GENUS	WORKING SPECIES	ANALYSIS CODE	GENBANK ACCESSION	FBE GROUP
TRAPPE 16825	*Dermocybe*	*globuliformis*			
TRAPPE 19806	*Descomyces*	*giachinii*			
TRAPPE 19811	*Descomyces*	*giachinii*			
TRAPPE 4025	*Mycolevis*	*siccigleba*			
TRAPPE 6874	*Descomyces*	*malajczukii*			
TRAPPE 6985	*Descomyces*	*nov. sp 2 (angustisporus)*			
TRAPPE 712	*Hymenogaster*	*alnicola*			
TRAPPE 8408	*Setchelliogaster*	*tenuipes*			
TWMB 237	*Hymenogaster?*	*violaceus?*			
TWMB 255	*Thaxterogaster*	*sp.*			
TWMB 373	*Thaxterogaster*	*campbelliae*			
TWMB 374	*Hymenogaster*	*macrosporus*			
TWMB 431	*Descomyces*	*sp.*			
TWMB 471	*Thaxterogaster*	*levisporus*			
TWMB 597	*Thaxterogaster*	*sp.*			
TWMB 599	*Thaxterogaster*	*sp.*			
TWMB 611	*Thaxterogaster*	*sp.*			
TWMB 611A	*Thaxterogaster*	*sp.*			
TWMB 611B	*Thaxterogaster*	*sp.*			
TWMM 160	*Setchelliogaster*	*tenuipes*			
TWMM 73	*Thaxterogaster*	*sp.*			
TWMM 8	*Thaxterogaster*	*sp.*			

Appendix 2

'FBE' groups resulting from the preliminary morphological examination

Table 14: FBE ('final by-eye') groups resulting from the preliminary morphological examination. Some groups have been collapsed into neighbouring groups and putative taxon names have been applied in some cases. Brief descriptions of selected microscopic characters are provided. Abbreviations: SP.**=spores,** O.**=ornamentation** P.**=peridipellis,** B.**=basidia.**

GROUP	PUTATIVE TAXON	EXAMPLE COLLECTIONS	DESCRIPTION
1	*Protoglossum luteum*	H5791, MEL2057704	**SP:** sub-spherical, **O:** bacculate to 1µm tall. **P:** a myxocutis of unclamped to rarely clamped hyphae in a hyaline matrix. **B:** 2-spored.
2	*Thaxterogaster sp.*	MEL2049699, H4323, H5798, H5814	**SP:** broad-elliptical, **O:** tuberculate to 1.5µm. **P:** a loosely woven myxocutis of clamped hyphae. **B:** 4-spored.
3	*Thaxterogaster sp.*	H2193	**SP:** elliptical, **O:** tuberculate to 1µm. **P:** an ixomyxocutis of unclamped hyphae in a hyaline matrix. **B:** 4-spored.
4	*Thaxterogaster* sp. 2	TL493, TL501B	**SP:** broad-elliptical, **O:** warts and tubercles to 1.5µm tall. **P:** a cutis of thin clamped hyphae overlaying a thicker hypocutis of broader clamped hyphae. **B:** 4-spored.
5	*Thaxterogaster sp.*	H1486	**SP:** unusually large, broad-turbinate, **O:** warts and short ridges to 1µm tall. **P:** an ixocutis of clamped hyphae overlaying a pseudoparehchymatous layer. **B:** 4-spored.
8	*Thaxterogaster redactus*	H0726	**SP:** elliptical, **O:** warts to 1µm tall. **P:** a loosely-woven cutis of thin clamped hyphae overlaying a sub-cellular layer. **B:** 2-spored.
9	*Thaxterogaster porphyreus*	MEL2079347	**SP:** elliptical, **O:** warts to 1µm tall. **P:** an ixomyxocutis of thin clamped hyphae overlaying a thicker hypocutis of broader clamped hyphae. **B:** 4-spored.
11	*Thaxterogaster pingue*	MEL2136538	**SP:** elliptical to sligthly adaxially flattened, **O:** very fine warts to 0.5µm tall. **P:** a repent ixocutis of clamped hyphae overlaying a hypocutis of inflated clamped hyphae. **B:** 4-spored.
12	*Thaxterogaster sp.*	MEL2063437	**SP:** broad-elliptical, **O:** warts and tubercles to 1.5µm tall. **P:** a cutis of thin clamped hyphae overlaying a thicker hypocutis of broader clamped hyphae. **B:** (2-) 4-spored. (may be the same as group 2)

Table 14 continued: Abbreviations: SP.=**spores,** O.=**ornamentation** P.=**peridipellis,** B.=**basidia.**

GROUP	PUTATIVE TAXON	EXAMPLE COLLECTIONS	DESCRIPTION
13	*Thaxterogaster sp.*	H4850	**SP:** elliptical, **O:** fine warts to 1µm tall. **P:** a myxocutis of much-branched unclamped hyphae overlaying a pseudoparenchymatous layer. **B:** 4-spored.
14	*Thaxterogaster sp. nov. 4*	H4136, H6406	**SP:** elliptical, **O:** warts to 0.5µm tall. **P:** an ixomyxocutis of thin unclamped hyphae overlaying a hypocutis of broader clamped hyphae. **B:** 2-spored.
16	*Thaxterogaster* pyriformis	MEL2057505, H0728	**SP:** elliptical, **O:** warts <0.5µm tall. **P:** an ixomyxocutis of thin clamped hyphae overlaying a pigmented hypocutis of clamped hyphae. **B:** 4-spored.
17	*Thaxterogaster sp.*	MEL2063439	**SP:** elliptical, **O:** warts to 0.5µm tall. **P:** an ixo-cutis of thin clamped hyphae overlaying a pigmented layer hyphae. **B:** 4-spored.
19	*Thaxterogaster campbelliae*	H0904, H0910, H0920	**SP:** elliptical slightly asymmetrical, **O:** warts to 0.5µm tall. **P:** three layered, a cutis over a subcellular layer over a hypocutis all layers with clamps. **B:** 4-spored.
22	*Thaxterogaster sp.*	H7127	**SP:** elliptical and asymmetrical, **O:** warts to 0.5µm tall. **P:** an ixocutis of thin, clamped hyphae overlaying a hypocutis of thicker-walled clamped hyphae. **B:** 4-spored.
23	*Thaxterogaster sp.*	MEL2057999	**SP:** broad-elliptical, **O:** warts 0.5µm tall. **P:** a myxocutis of thin clamped hyphae overlaying a thicker hypocutis of broader clamped hyphae. **B:** 4-spored. Red reaction in KOH.
24	*Thaxterogaster sp.*	H1202	**SP:** small, elliptical, **O:** fine and sparese warts < 0.5µm tall. **P:** a trichoderm of very thin clamped hyphae overlaying a hypocutis of clamped hyphae. **B:** 4-spored.
25	*Thaxterogaster sp.*	H4798, H5008	**SP:** elliptical, **O:** fine and sparse warts < 0.5µm tall. **P:** an ixomyxocutis of thin clamped hyphae in a gelatinous matrix overlaying a pseudoparenchymatous layer. **B:** 4-spored.
26	*Thaxterogaster sp.*	MEL2059057	**SP:** elliptical and asymmetrical, **O:** warts to 0.5µm tall. **P:** a cutis of unclamped hyphae. **B:** 4-spored.
27	*Thaxterogaster sp.*	H1013, MEL2056847	**SP:** elliptical, **O:** warts to tubercles to 1.5µm tall. **P:** a cutis of yellowish clamped hyphae. **B:** 4-spored.
30	*Thaxterogaster campbelliae*	H0727	**SP:** elliptical, **O:** warts to 1µm tall. **P:** a cutis of (in some cases) inflated clamped hyphae. **B:** 4-spored.
32	*Thaxterogaster levisporus*	MEL2056701	**SP:** small, elliptical, **O:** warts <0.5µm tall. **P:** a cutis of thin, unclamped hyphae. **B:** 4-spored.

Table 14 continued: Abbreviations: SP.**=spores,** O**.=ornamentation** P**.=peridipellis,** B.**=basidia.**

GROUP	PUTATIVE TAXON	EXAMPLE COLLECTIONS	DESCRIPTION
33	*Thaxterogaster sp*.	H6558	**SP:** small, elliptical, **O:** warts <0.5µm tall. **P:** a cutis of thin, unclamped hyphae. **B:** 4-spored. (may be the same as group 32)
34	*Thaxterogaster sp. A*	H1006, H1446, H1194	**SP:** elliptical, **O:** warts to 0.5µm tall. **P:** a cutis of clamped hyphae. **B:** 4-spored.
35	*Thaxterogaster campbelliae*	MEL2032790, H4770	**SP:**, **O:** warts to 0.5µm tall. **P:** three layered, a cutis over a subcellular layer over a hypocutis all layers with clamps **B:** 4-spored.
36	*Thaxterogaster sp. A*	H2195, H1213	**SP:** elliptical, **O:** warts to 0.5µm tall. **P:** outer layer with intermixed inflated hyphae over a hypocutis. **B:** 4-spored.
37	*Thaxterogaster* sp. 3	TL503, H5255, H5362	**SP:** elliptical, **O:** warts to 0.5µm tall. **P:** three layered, a cutis over a subcellular layer over a hypocutis all layers with clamps **B:** 4-spored.
38	*Thaxterogaster sp.*	H2198, H5197, H6915	**SP:** broad-elliptical, **O:** verruculose, <0.5µm tall. **P:** a cutis of thin- to thick-walled, clamped hyphae. **B:** 4-spored.
39	*Thaxterogaster sp.*	H6585, H6728, H6739, H6753	**SP:** elliptical, **O:** warts to 0.5µm tall. **P:** a cutis of (in some cases) inflated clamped hyphae. **B:** 4-spored.
40	*Thaxterogaster sp. A*	H1120, H1134	**SP:** elliptical, **O:** warts and short ridges to 0.5µm tall. **P:** an interwoven cutis of clamped hyphae. **B:** 4-spored.
41	*Thaxterogaster* sp. 1	TL501A, TL502A, H2192, H5286, H5330, H6564, HL456, MEL2063445	**SP:** elliptical, **O:** verruculose, <0.5µm tall. **P:** a cutis of clamped and (in some cases) inflated hyphae. **B:** 4-spored.
42	*Thaxterogaster levisporus*	H0719, MEL2057558, MEL2057536, MEL2057547, MEL2057565, MEL2059043B	**SP:** elliptical, **O:** verruculose <0.5µm tall. **P:** three layered, a cutis over a layer of inflated, thick walled hyphae, over a hypocutis; all layers with clamps. **B:** 4-spored.
43	*Thaxterogaster sp.*	H5301, H6784	**SP:** elliptical, **O:** verruculose <0.5µm tall. **P:** three layered, a trichoderm over a subcellular layer over a hypocutis; all layers with clamps. **B:** 4-spored.
44	*Thaxterogaster sp.*	H5185, H6946	**SP:** elliptical, **O:** warts <0.5µm tall. **P:** a cutis of clamped hyphae. **B:** 2-spored.
45	*Quadrispora oblongispora*	H0969, H1364, H5092	**SP:** elliptical, in tetrads, **O:** warts and tubercles to 1µm tall. **P:** an ixomyxocutis of thin clamped hyphae in a hyaline matrix overlaying a hypocutis of clamped hyphae. **B:** 4-spored.

Table 14 continued: Abbreviations: SP.=**spores,** O.=**ornamentation** P.=**peridipellis,** B.=**basidia.**

GROUP	PUTATIVE TAXON	EXAMPLE COLLECTIONS	DESCRIPTION
46	Hysterogasteroid sp.	H6957, H5258, H5328, H6472, H6878, H6975	**SP:** elliptical, **O:** none, spores smooth. **P:** a cutis of thin clamped hyphae (a subset of collections had clamped hyphae). **B:** 4-spored.
47	Miscellany including *Gymnopaxillus sp.*, *Hymenogaster sensu stricto*, *Mackintoshia persica*, russuloid sp.	H6446, H3000, H6263, H0720, H5368	
49	*Timgrovea ferruginea*	H5803, H5643, H5655	**SP:** citriform, **O:** irregular reticulum to 1µm tall. **P:** degraded golden-brown hyphae over a hypocutis all layers clamped. **B:** 4-spored.
51	*Timgrovea sp. (nov. 2)*	H4057, H4574, H5234	**SP:** citriform, **O:** irregular reticulum to 1µm tall. **P:** degraded golden-brown hyphae over a hypocutis all layers clamped. **B:** 2-spored.
53	*Timgrovea sp.*	H5984	**SP:** pyriform, **O:** reticulate to 1µm tall. **P:** degraded golden-brown hyphae over a hypocutis all layers clamped. **B:** 2-spored.
54	*Timgrovea sp.*	TRAPPE14535	**SP:** broadly fusiform, **O:** alveolate to 2.5µm tall. **P:** degraded golden-brown hyphae over a hypocutis all layers clamped. **B:** 2-spored.
56	*Timgrovea ellipsosporus* Trappe *nom. prov.*	H6810, H2129, H0732	**SP:** broadly fusiform, **O:** alveolate to reticulate, to 2.5µm tall. **P:** golden-brown hyphae over a hypocutis all layers clamped. **B:** 2-spored.
57	*Timgrovea, sp.*	H4162, H6171	**SP:** broadly sub-citriform, **O:** irregular reticulum to 1µm tall. **P:** degraded golden-brown hyphae over a hypocutis all layers clamped. **B:** 2-spored.
58	*Timgrovea sp.*	H4204	**SP:** broadly sub-citriform, **O:** irregular reticulum to 0.5µm tall. **P:** degraded golden hyphae over a pseudoparenchymatous layer, all layers clamped. **B:** 4-spored.
60	*Timgrovea/Setchelliogaster sp. nov. 5*	H4167	**SP:** broadly fusiform to amygdalyform, **O:** irregular reticulum to 1.5µm tall. **P:** degraded golden-brown hyphae over a hypocutis all layers clamped. **B:** 2-spored.
61	*Timgrovea/Setchelliogaster sp. nov. 3*	H4146, H4170	**SP:** broadly sub-citriform, **O:** isolated warts to an irregular reticulum to 1µm tall. **P:** a hypocutis grading into thick-walled golden hyphae, all layers clamped. **B:** (2)4-spored.
64	*Setchelliogaster australiensis*	H5160, H4234	**SP:** amygdaliform, **O:** isolated fine warts <0.5µm tall. **P:** degraded golden-brown hyphae over a polycystoderm all layers clamped. **B:** 4-spored.
65	*Setchelliogaster sp.*	TRAPPE14262 TRAPPE14281	**SP:** amygdaliform, **O:** isolated fine warts <0.5µm tall. **P:** degraded golden-brown hyphae over a layer with some inflated cells all layers clamped. **B:** 4-spored.

Table 14 continued: Abbreviations: SP.=**spores,** O.=**ornamentation** P.=**peridipellis,** B.=**basidia.**

GROUP	PUTATIVE TAXON	EXAMPLE COLLECTIONS	DESCRIPTION
66	*Setchelliogaster sp.*	H6806, TRAPPE14252, TRAPPE14293	**SP:** fusiform to amygdaliform **O:** isolated fine warts <0.5µm tall. **P:** degraded golden-brown hyphae over a hypocutis, all layers clamped. **B:** 4-spored.
67	*Setchelliogaster sp.*	TRAPPE14175	**SP:** citriform, **O:** isolated very fine warts to 1µm tall. **P:** degraded golden hyphae over a hypocutis all layers clamped. **B:** 2-spored.
68	*Setchelliogaster*/*Descomyces sp.*	MEL2063434	**SP:** essentially ellipsoid but with a broad, short rostrum, **O:** isolated very fine warts to 3µm tall, perispore flaring to cover/surround the apiculus. **P:** a hyaline cutis of clamped hyphae. **B:** 2-spored.
69	*Descomyces sp.*	TRAPPE14702	**SP:** lacrymoid (narrowed towards prominent rostrum) **O:** isolated warts and short ridges to 2µm tall. **P:** degraded golden hyphae over a myxocutis all layers clamped. **B:** 2-spored.
70	*Descomyces albus*	H5339, H7119	**SP:** lacrymoid, **O:** isolated warts and short ridges to 2µm tall, perispore attached at hilum. **P:** degraded golden hyphae over a polycystoderm all layers clamped. **B:** 2-spored.
72	*Descomyces stolatus* Trappe *nom. prov.*	TRAPPE14201, H0737	**SP:** broadly citriform to broadly pyriform, **O:** isolated warts and short ridges to 2.5µm tall, perispore flaring in 'corners' of the spore. **P:** golden hyphae over a hypocutis all layers clamped. **B:** 2-spored.
73	*Descomyces sp. nov. 1*	TRAPPE14178, TRAPPE14304, H4606	**SP:** lacrymoid to narrowly pyriform, **O:** isolated warts and short ridges, perispore flaring to 3.5µm tall and surrounding the apiculus. **P:** degraded golden hyphae over a myxocutis all layers clamped. **B:** 2-spored.
74	*Descomyces sp.*	TRAPPE14397, TRAPPE14592	**SP:** fusoid to lacrymoid, **O:** isolated warts and short ridges to 1.5µm tall, perispore attached at hilum. **P:** degraded golden hyphae over a myxocutis all layers clamped. **B:** 2-spored.
75	*Descomyces fusisporus* Trappe *nom. prov.*	H0733, H5807	**SP:** lacrymoid, **O:** isolated warts to 0.5µm tall, apiculus exposed. **P:** abundant golden hyphae over a hypocutis all layers clamped. **B:** 2-spored.
76	*Descomyces sp.*	H5372	**SP:** fusoid, **O:** isolated warts to 1µm tall. **P:** a cutis of clamped hyphae. **B:** 2-spored.
77	*Descomyces dougmillsii* Trappe *nom. prov.*	H0734, H6988, H7132	**SP:** lacrymoid, **O:** isolated warts and short ridges to 2µm tall, perispore flaring at 'corners' of spores but rostrum prominent. **P:** golden hyphae over a polycystoderm all layers clamped. **B:** 2-spored.

Table 14 continued: Abbreviations: SP.=**spores,** O.=**ornamentation** P.=**peridipellis,** B.=**basidia.**

GROUP	PUTATIVE TAXON	EXAMPLE COLLECTIONS	DESCRIPTION
78	*Descomyces albus*	TRAPPE11751	**SP:** pyriform, **O:** isolated warts to 1µm tall, distended-rostrum prominent, perispore closely adhering. **P:** golden hyphae over a pseudoparenchymatous layer, all layers clamped. **B:** 2-spored.
81	*Descomyces latisporus* Trappe *nom. prov.*	H0735, H0731, H7124, H0736	Also included *D.* parviretifer Trappe *nom. prov.* and *D. uniformis* Trappe *nom. prov.* **SP:** broadly citriform to squat-pyriform, **O:** isolated warts and short ridges to 2µm tall, apiculus covered and rostrum almost covered by perispore. **P:** abundant golden hyphae over a hypocutis all layers clamped. **B:** 2-spored.
82	*Descomyces* lebelii Trappe *nom. prov.*	H0730, H7069	**SP:** ellipsoidal but slightly constricted at apex, **O:** isolated warts and short ridges to 1µm tall. **P:** golden hyphae over a hypocutis, all layers clamped. **B:** 2-spored.
84	*Descomyces sp. nov. 4*	TRAPPE14129, H4301	**SP:** lacrymoid to fusoid, **O:** isolated warts and short ridges to 0.5µm tall. **P:** a hyaline cutis of clamped hyphae. **B:** 4-spored.
85	*Descomyces sp.*	TRAPPE14166	**SP:** broadly lacrymoid, **O:** warts and short ridges to 0.5µm tall. **P:** degraded cutis clamped. **B:** 4-spored.
86	*Descomyces sp.*	H0809	**SP:** fusoid, **O:** short ridges aranged perpendicular to the long axis of the spore to 1µm tall, apiculus and rostrum exposed. **P:** degraded golden hyphae over a hypocutis, all layers clamped. **B:** 4-spored.
87	*Descomyces sp.*	H6989	**SP:** lacrymoid to sub-ellipsoid (narrowed at apex) **O:** warts and short ridges to 1.5µm tall, apiculus and rostrum covered by perispore. **P:** myxoocutis, clamped. **B:** 4-spored.
90	*Descomyces sp.*	H6518	**SP:** narrowly fusoid, **O:** warts and scattered short ridges (similar to those of FBE86) to 1µm tall. **P:** not seen. **B:** 4-spored.
91	*Descomyces sp.*	H6701	**SP:** broadly lacrymoid, **O:** isolated warts and short ridges to 1.5µm tall, apiculus and rostrum usually covered by perispore. **P:** degraded golden hyphae over a mixed layer of hyphae and inflated end-cells, all layers clamped. **B:** 2-spored.
92	*Descomyces sp.*	TRAPPE14325	**SP:** lacrymoid to pyriform, **O:** isolated warts and short ridges to 2µm tall, rostrum exposed. **P:** a mixed layer of hyphae and inflated end-cells, all layers clamped. **B:** 2-spored.
93	*Timgrovea sp.*	H6076	**SP:** pyriform to broadly lacrymoid, **O:** isolated and aggregated warts <0.5µm tall, apiculus and rostrum exposed. **P:** a hyaline cutis, clamped. **B:** 2-spored.

Table 14 continued: Abbreviations: SP.**=spores,** O**.=ornamentation** P**.=peridipellis,** B.**=basidia.**

GROUP	PUTATIVE TAXON	EXAMPLE COLLECTIONS	DESCRIPTION
95	Miscellany including *Descomyces maidenis* Trappe *nom. prov.*	H0717	

Appendix 3

Morphological taxon by character matrix

Table 15: Matrix of taxa examined in detail against morphological characters. Character codes (see Table 4) followed by percentage signs indicate values 'standardised' as a percentage of the maximum value while character codes without percentage signs indicate recoded '10-bin' multi-state values

COLLECTION	OP	OP%	MP%	MP	BW%	BW	AP%	AP	AL%	AL
AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179	2	60	15.24	1	47.63	2	26.00	4	22.31	0
AF100 H04e H6753 Thaxterogaster sp. DQ328138	2	60	13.33	1	51.28	2	27.30	4	24.86	0
AF14 C02Be TL503 Thaxterogaster sp. 3 DQ328071	2	60	20.32	2	47.93	2	25.33	4	25.26	0
AF38 E10e H1213 Thaxterogaster sp. A DQ328093	2	60	11.11	1	40.83	1	16.80	4	16.57	0
AF173 I65e MEL2032790 Thaxterogaster campbelliae	2	60	27.11	2	55.62	3	41.30	5	38.67	2
AF37 E09e H1006 Thaxterogaster sp. DQ328092	2	60	32.11	3	53.25	3	42.45	5	40.63	2
AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146	2	60	19.62	1	52.72	3	30.64	5	32.73	1
AF108 H12e H5814 Thaxterogaster sp. DQ328144	0	20	40.89	3	58.68	3	44.80	5	43.09	2
AF119 I10e H5301 Thaxterogaster sp. DQ328153	2	60	4.00	0	42.90	1	43.40	5	34.25	1
AF179 I70e H6784 Thaxterogaster sp. DQ328200	2	60	1.33	0	41.12	1	49.00	6	40.33	2
AF78 F21e H2198 Thaxterogaster sp. DQ328121	2	60	8.00	0	42.60	1	36.40	5	28.73	1
AF194 I85e H5197 Thaxterogaster sp. DQ328215	2	60	5.33	0	58.58	3	39.90	5	31.49	1
AF164 I55xe H5791 Thaxterogaster sp. DQ328186	0	20	24.89	2	56.21	3	34.30	5	42.54	2
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197	0	20	30.22	2	52.07	2	32.20	5	40.33	2
AF178 I69e H0969 Quadrispora frog eggs DQ328199	0	20	32.59	3	49.11	2	78.81	7	67.94	5
AF127 I18e H1486 Thaxterogaster sp. DQ328158	1	40	100.00	9	100.00	9	2.80	3	37.57	2
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194	0	20	19.56	1	63.91	4	50.40	6	39.78	2
AF15 COSBe TL493 Thaxterogaster sp. 2 DQ328072	2	60	43.64	4	89.35	8	60.45	6	62.03	4
AF168 !59eTL501B Thaxterogaster sp. 1 DQ328190	2	60	40.89	3	72.78	5	64.40	7	76.80	6
AF9 BOSBe H0734 Descomyces dougmillsii Trappe *nom. prov.* DQ328066	3	80	46.50	4	49.11	2	5.92	3	52.27	3
AF134 I26xe H6988 Descomyces sp. DQ328164	4	100	48.00	4	55.03	3	4.20	3	50.28	3
AF189 I80e H7124 Descomyces sp. DQ328210	4	100	39.11	3	53.25	3	2.80	3	36.46	1
AF10 B06Be H0735 Descomyces latisporus Trappe *nom. prov.* DQ328067	4	100	53.93	5	53.25	3	8.40	3	66.30	5
AF11 B07Be H0736 Descomyces uniformis Trappe *nom. prov.* DQ328068	3	80	61.75	5	59.17	3	-11.05	2	45.94	2
AF192 I83e H7119 Descomyces sp. DQ328213	4	100	76.27	7	53.55	3	-6.32	2	62.38	4
AF190 !81eTRAPPE14397 Descomyces sp. DQ328211	4	100	61.78	5	55.62	3	0.00	3	70.17	5
AF12 BOSBe H0737 Descomyces stolatus Trappe *nom. prov.* DQ328069	4	100	93.78	8	59.17	3	-51.80	0	51.38	3
AF8 B04Be H0733 Descomyces fusisporus Trappe *nom. prov.* DQ328065	4	100	29.33	2	57.40	3	91.00	8	87.85	7
AF140 I31e H5807 Descomyces sp. DQ328170	3	80	40.00	3	51.53	2	65.33	7	84.71	7
AF198 I89e H5655 Timgrovea sp. DQ328219	4	100	42.67	4	56.21	3	26.60	4	53.59	3
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163	4	100	74.67	6	53.25	3	42.70	5	98.34	8
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus Trappe *nom. prov.* DQ328062	4	100	41.33	3	62.72	4	76.30	7	100.00	8
AF97 H01e H4057 Timgrovea sp. DQ328137	4	100	41.06	3	59.76	4	100.00	9	84.71	7
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202	2	60	8.04	0	68.05	5	33.33	5	26.84	0
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214	4	100	15.56	1	67.46	5	45.50	5	36.46	1
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184	2	60	33.78	3	55.62	3	35.70	5	40.33	2
AF156 !47xxAF156 H4162 Timgrovea sp. DQ328180	2	60	50.22	4	51.78	2	11.20	3	40.88	2
AF137 I28e H4204 Timgrovea sp. DQ328167	2	60	34.67	3	76.33	6	35.70	5	39.23	2
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207	2	60	30.67	2	69.03	5	2.80	3	30.39	1
AF45 E17e H5185 Thaxterogaster sp. DQ328098	2	60	15.35	1	36.69	0	52.18	6	41.19	2
AF47 E19e H5258 Hysterogasteroid sp.	2	60	0.00	0	56.21	3	34.36	5	27.12	0
AF43 E15e H5092 Quadrispora sp. DQ328096	0	20	27.11	2	53.85	3	43.40	5	43.09	2

Table 15 continued: Matrix of taxa examined in detail against morphological characters. Character codes (see Table 4) followed by percentage signs indicate values 'standardised' as a percentage of the maximum value while character codes without percentage signs indicate recoded '10-bin' multi-state values

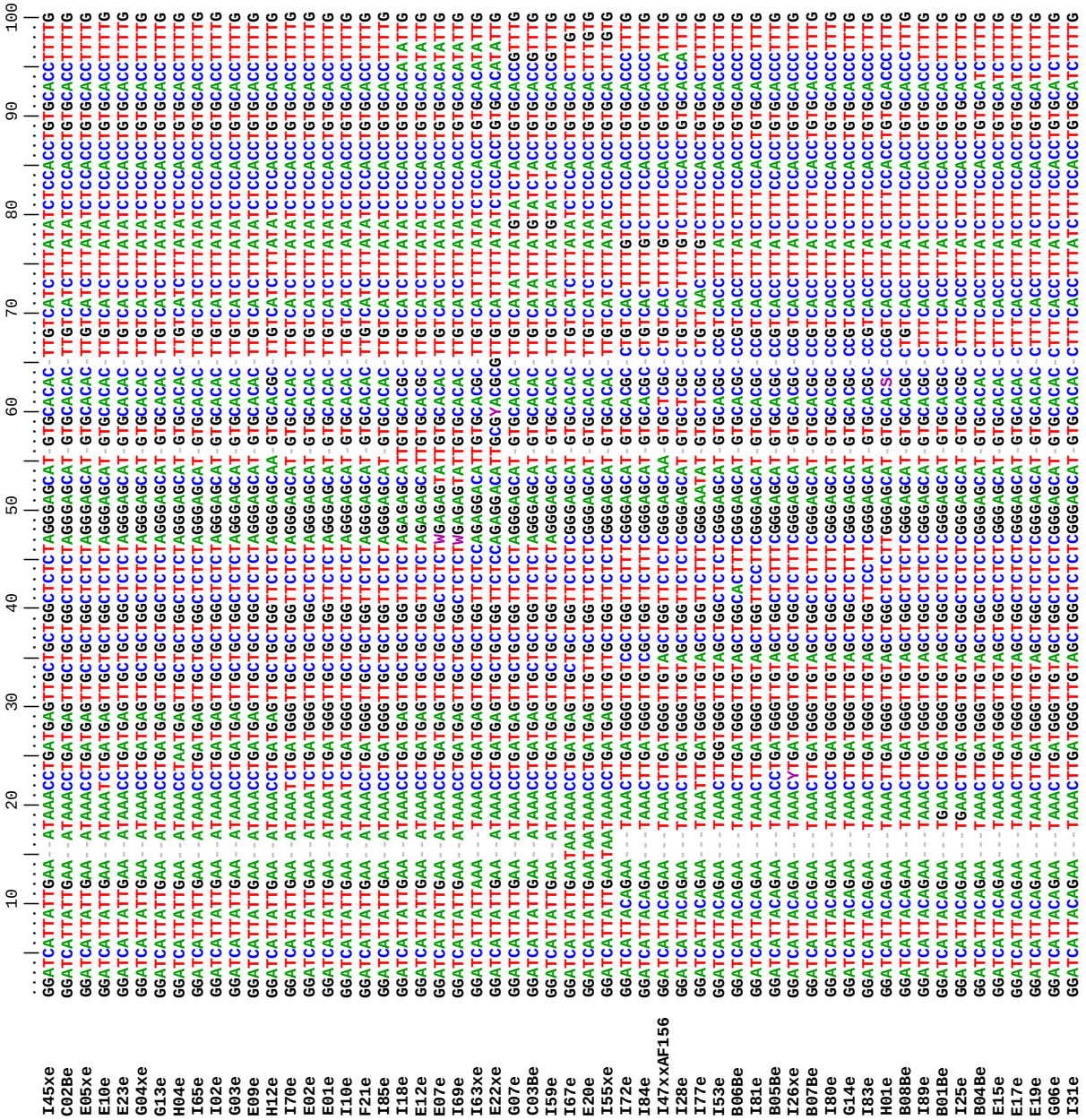
COLLECTION	QS%	QS	QB%	QB	PW%	PW	PL%	PL	PD%	PD	OT%	OT
AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179	75.59	4	69.07	5	27.86	3	81.82	4	75	3	40	1
AF100 H04e H6753 Thaxterogaster sp. DQ328138	75.22	4	58.05	3	24.14	2	54.55	2	75	3	40	1
AF14 C02Be TL503 Thaxterogaster sp. 3 DQ328071	72.78	4	77.50	6	25.05	2	81.82	4	75	3	40	1
AF38 E10e H1213 Thaxterogaster sp. A DQ328093	73.04	4	61.80	3	11.02	1	27.27	0	75	3	40	1
AF173 I65e MEL2032790 Thaxterogaster campbelliae	67.04	2	64.04	4	30.24	3	45.45	1	75	3	40	1
AF37 E09e H1006 Thaxterogaster sp. DQ328092	65.38	2	76.88	6	31.43	3	81.82	4	75	3	40	1
AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146	75.00	4	74.94	6	33.05	3	90.91	4	50	2	40	1
AF108 H12e H5814 Thaxterogaster sp. DQ328144	61.68	1	71.25	5	39.74	4	72.73	3	75	3	40	1
AF119 I10e H5301 Thaxterogaster sp. DQ328153	73.93	4	75.56	6	26.24	2	68.18	3	75	3	40	1
AF179 I70e H6784 Thaxterogaster sp. DQ328200	70.31	3	65.15	4	12.96	1	36.36	0	75	3	40	1
AF78 F21e H2198 Thaxterogaster sp. DQ328121	69.96	3	67.21	4	16.63	1	27.27	0	75	3	40	1
AF194 I85e H5197 Thaxterogaster sp. DQ328215	64.00	2	69.57	5	16.52	1	54.55	2	75	3	40	1
AF164 I55xe H5791 Thaxterogaster sp. DQ328186	61.62	1	82.28	7	61.34	6	81.82	4	75	3	40	1
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197	57.49	0	79.55	6	23.33	2	100.00	5	75	3	40	1
AF178 I69e H0969 Quadrispora frog eggs DQ328199	87.71	7	61.56	3	100.00	9	54.55	2	75	3	40	1
AF127 I18e H1486 Thaxterogaster sp. DQ328158	60.96	1	56.92	3	63.50	6	54.55	2	75	3	40	1
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194	82.40	6	94.69	9	87.47	8	81.82	4	75	3	40	1
AF15 COSBe TL493 Thaxterogaster sp. 2 DQ328072	63.25	2	67.66	4	26.89	2	54.55	2	75	3	40	1
AF168 !59eTL501B Thaxterogaster sp. 1 DQ328190	62.52	1	77.24	6	40.82	4	81.82	4	75	3	40	1
AF9 BOSBe H0734 Descomyces dougmillsii Trappe *nom. prov.* DQ328066	80.18	5	91.82	8	8.75	1	68.18	3	100	4	40	1
AF134 I26xe H6988 Descomyces sp. DQ328164	86.46	7	69.41	5	13.61	1	40.91	1	25	1	40	1
AF189 I80e H7124 Descomyces sp. DQ328210	71.01	3	55.18	2	26.57	2	81.82	4	25	1	40	1
AF10 B06Be H0735 Descomyces latisporus Trappe *nom. prov.* DQ328067	69.71	3	73.46	5	12.31	1	54.55	2	25	1	40	1
AF11 B07Be H0736 Descomyces uniformis Trappe *nom. prov.* DQ328068	66.21	2	77.98	6	11.66	1	54.55	2	25	1	40	1
AF192 I83e H7119 Descomyces sp. DQ328213	91.93	8	81.83	7	30.67	3	81.82	4	25	1	40	1
AF190 !81eTRAPPE14397 Descomyces sp. DQ328211	90.35	7	67.10	4	20.09	2	81.82	4	25	1	40	1
AF12 BOSBe H0737 Descomyces stolatus Trappe *nom. prov.* DQ328069	72.83	4	63.24	4	10.15	1	54.55	2	75	3	40	1
AF8 B04Be H0733 Descomyces fusisporus Trappe *nom. prov.* DQ328065	100.00	9	93.61	8	11.66	1	54.55	2	75	3	40	1
AF140 I31e H5807 Descomyces sp. DQ328170	97.90	9	100.00	9	6.16	0	54.55	2	75	3	40	1
AF198 I89e H5655 Timgrovea sp. DQ328219	68.62	3	53.58	2	12.53	1	54.55	2	75	3	80	3
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163	78.83	5	80.23	6	26.57	2	81.82	4	25	1	100	4
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus Trappe *nom. prov.* DQ328062	82.46	6	54.71	2	27.00	2	54.55	2	50	2	80	3
AF97 H01e H4057 Timgrovea sp. DQ328137	82.00	6	80.70	6	8.10	1	54.55	2	50	2	60	2
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202	77.22	5	43.26	0	7.13	1	54.55	2	50	2	40	1
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214	84.31	6	58.36	3	21.38	2	72.73	3	50	2	40	1
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184	73.07	4	71.94	5	15.55	1	54.55	2	50	2	40	1
AF156 !47xxAF156 H4162 Timgrovea sp. DQ328180	63.36	2	82.11	7	27.54	3	68.18	3	75	3	60	2
AF137 I28e H4204 Timgrovea sp. DQ328167	68.76	3	52.67	2	9.07	1	54.55	2	50	2	60	2
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207	70.44	3	54.67	2	23.76	2	54.55	2	25	1	60	2
AF45 E17e H5185 Thaxterogaster sp. DQ328098	73.48	4	69.34	5	20.73	2	54.55	2	75	3	40	1
AF47 E19e H5258 Hysterogasteroid sp.	76.79	4	56.79	3	23.97	2	27.27	0	0	0	20	0
AF43 E15e H5092 Quadrispora sp. DQ328096	85.55	6	66.62	4	33.48	3	54.55	2	75	3	40	1

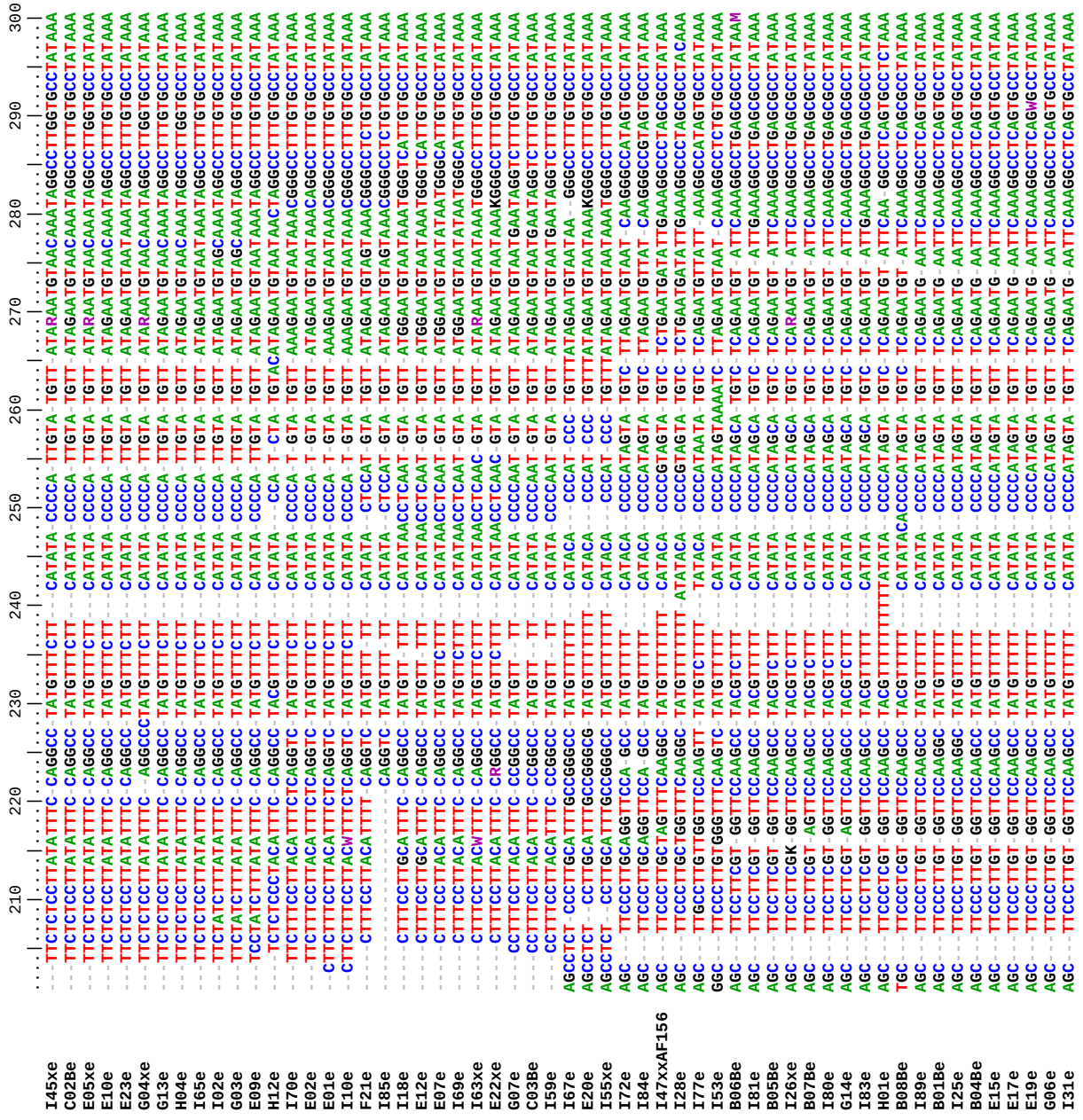
Table 15 continued: Matrix of taxa examined in detail against morphological characters. Character codes (see Table 4) followed by percentage signs indicate values 'standardised' as a percentage of the maximum value while character codes without percentage signs indicate recoded '10-bin' multi-state values

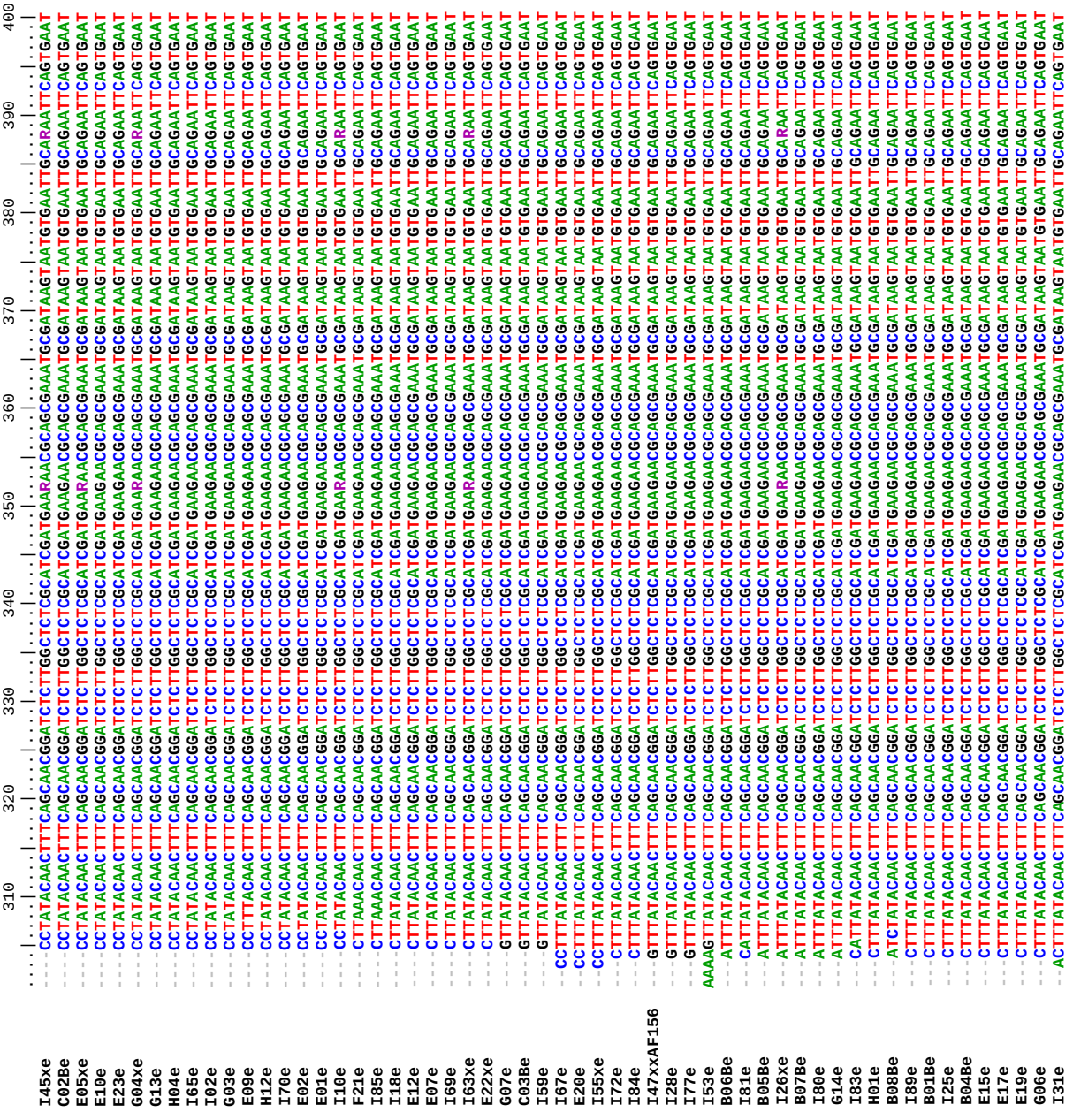
COLLECTION	SW%	SW	SN%	SN	SF%	SF	RP%	RP	RL%	RL
AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179	33.99	1	100.00	2	50	0	-17.90	4	0.00	0
AF100 H04e H6753 Thaxterogaster sp. DQ328138	34.18	1	100.00	2	50	0	-14.77	4	0.00	0
AF14 C02Be TL503 Thaxterogaster sp. 3 DQ328071	34.15	1	100.00	2	50	0	-25.57	4	0.00	0
AF38 E10e H1213 Thaxterogaster sp. A DQ328093	29.71	0	100.00	2	50	0	-12.75	4	0.00	0
AF173 I65e MEL2032790 Thaxterogaster campbelliae	42.51	2	100.00	2	50	0	-34.23	3	0.00	0
AF37 E09e H1006 Thaxterogaster sp. DQ328092	47.14	3	100.00	2	50	0	-40.27	3	0.00	0
AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146	40.56	2	100.00	2	50	0	-21.27	4	0.00	0
AF108 H12e H5814 Thaxterogaster sp. DQ328144	64.81	5	100.00	2	50	0	-46.31	3	0.00	0
AF119 I10e H5301 Thaxterogaster sp. DQ328153	33.08	1	100.00	2	50	0	-9.40	4	0.00	0
AF179 I70e H6784 Thaxterogaster sp. DQ328200	35.27	1	100.00	2	50	0	-2.01	5	0.00	0
AF78 F21e H2198 Thaxterogaster sp. DQ328121	39.56	2	100.00	2	50	0	-12.08	4	0.00	0
AF194 I85e H5197 Thaxterogaster sp. DQ328215	40.15	2	100.00	2	50	0	-8.05	4	0.00	0
AF164 I55xe H5791 Thaxterogaster sp. DQ328186	55.56	4	100.00	2	50	0	-33.56	3	0.00	0
AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197	60.02	4	50.00	0	50	0	-37.58	3	0.00	0
AF178 I69e H0969 Quadrispora frog eggs DQ328199	53.68	3	100.00	2	100	1	-41.26	3	0.00	0
AF127 I18e H1486 Thaxterogaster sp. DQ328158	100.00	9	50.00	0	50	0	-114.77	0	0.00	0
AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194	47.14	3	100.00	2	50	0	-28.86	4	0.00	0
AF15 COSBe TL493 Thaxterogaster sp. 2 DQ328072	72.08	6	100.00	2	50	0	-46.37	3	0.00	0
AF168 !59eTL501B Thaxterogaster sp. 1 DQ328190	69.53	5	100.00	2	50	0	-55.03	2	0.00	0
AF9 BOSBe H0734 Descomyces dougmillsii Trappe *nom. prov.* DQ328066	58.08	4	50.00	0	50	0	40.78	6	41.42	3
AF134 I26xe H6988 Descomyces sp. DQ328164	60.69	4	50.00	0	50	0	100.00	9	90.53	8
AF189 I80e H7124 Descomyces sp. DQ328210	59.51	4	50.00	0	50	0	51.68	7	31.95	2
AF10 B06Be H0735 Descomyces latisporus Trappe *nom. prov.* DQ328067	59.15	4	50.00	0	50	0	34.90	6	38.66	3
AF11 B07Be H0736 Descomyces uniformis Trappe *nom. prov.* DQ328068	60.96	4	50.00	0	50	0	16.96	5	47.34	4
AF192 I83e H7119 Descomyces sp. DQ328213	58.76	4	50.00	0	50	0	43.73	7	63.37	5
AF190 !81eTRAPPE14397 Descomyces sp. DQ328211	60.35	4	50.00	0	50	0	37.58	6	45.56	4
AF12 BOSBe H0737 Descomyces stolatus Trappe *nom. prov.* DQ328069	66.92	5	50.00	0	50	0	61.74	7	74.56	6
AF8 B04Be H0733 Descomyces fusisporus Trappe *nom. prov.* DQ328065	63.13	5	50.00	0	50	0	95.97	9	82.25	7
AF140 I31e H5807 Descomyces sp. DQ328170	60.27	4	50.00	0	50	0	65.41	7	62.33	5
AF198 I89e H5655 Timgrovea sp. DQ328219	52.10	3	100.00	2	50	0	36.91	6	54.44	4
AF133 I25e TRAPPE14535 Timgrovea sp. DQ328163	70.03	5	50.00	0	50	0	61.07	7	100.00	8
AF5 B01Be H0732 Timgrovea Descomyces ellipsosporus Trappe *nom. prov.* DQ328062	69.11	5	50.00	0	50	0	69.80	8	71.60	6
AF97 H01e H4057 Timgrovea sp. DQ328137	62.37	4	50.00	0	50	0	75.42	8	52.97	4
AF181 I72e H4234 Setchelliogaster sp. nov. 1 DQ328202	57.36	4	100.00	2	50	0	62.64	7	52.58	4
AF193 I84e TRAPPE14281 Setchelliogaster sp. DQ328214	52.10	3	100.00	2	50	0	53.69	7	36.09	3
AF162 I53e TRAPPE14175 Setchelliogaster sp. DQ328184	66.41	5	50.00	0	50	0	35.57	6	32.54	2
AF156 !47xxAF156 H4162 Timgrovea sp. DQ328180	70.29	5	50.00	0	50	0	32.21	6	52.07	4
AF137 I28e H4204 Timgrovea sp. DQ328167	70.29	5	50.00	0	50	0	68.46	8	48.52	4
AF186 I77e H4146 Timgrovea Setchelliogaster sp. nov. 3 DQ328207	62.29	4	79.17	1	50	0	53.69	7	52.66	4
AF45 E17e H5185 Thaxterogaster sp. DQ328098	37.50	1	50.00	0	50	0	-18.30	4	0.00	0
AF47 E19e H5258 Hysterogasteroid sp.	41.55	2	100.00	2	50	0	0.00	5	0.00	0
AF43 E15e H5092 Quadrispora sp. DQ328096	50.93	3	100.00	2	100	1	-34.90	3	0.00	0

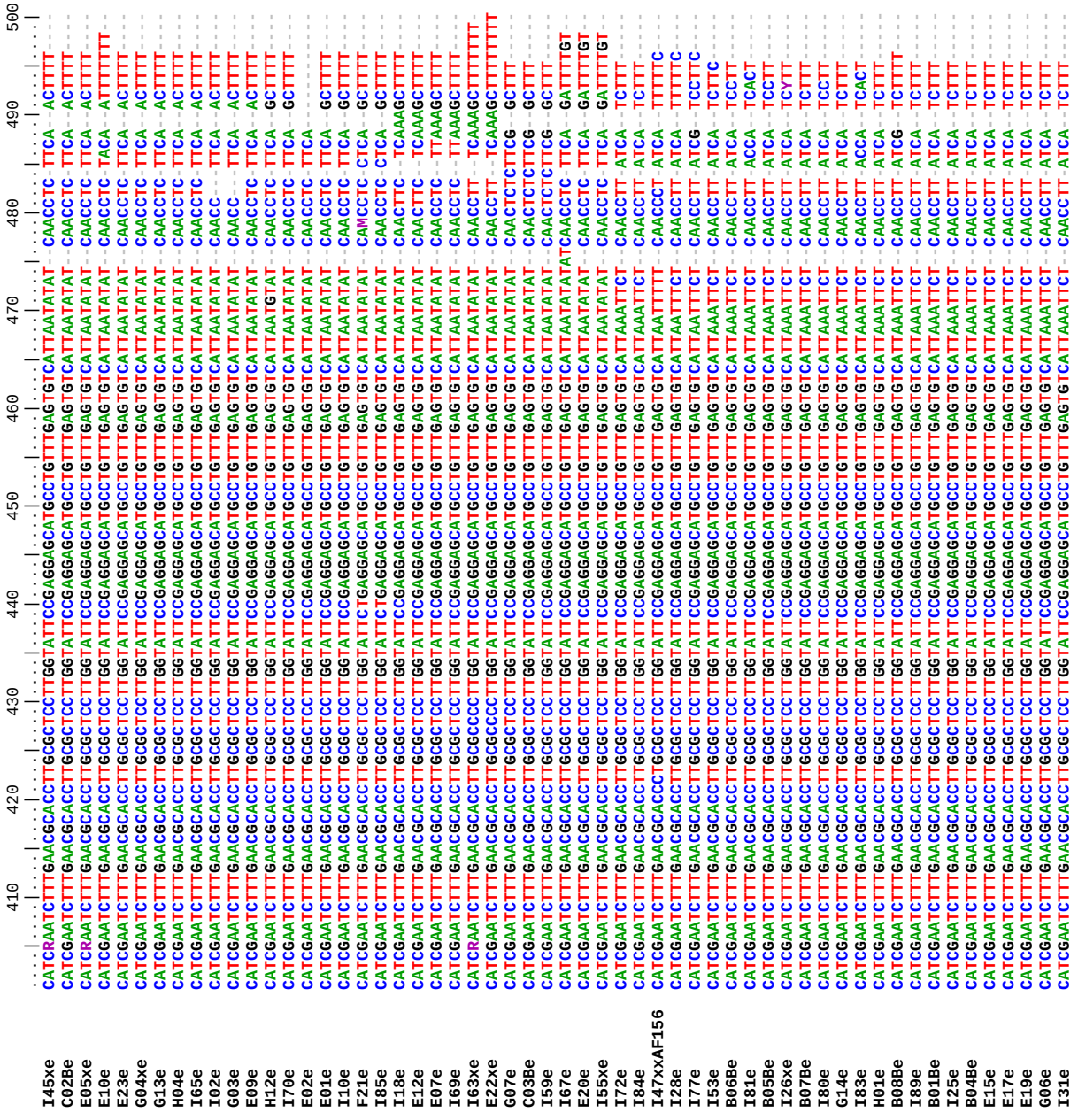
Appendix 4

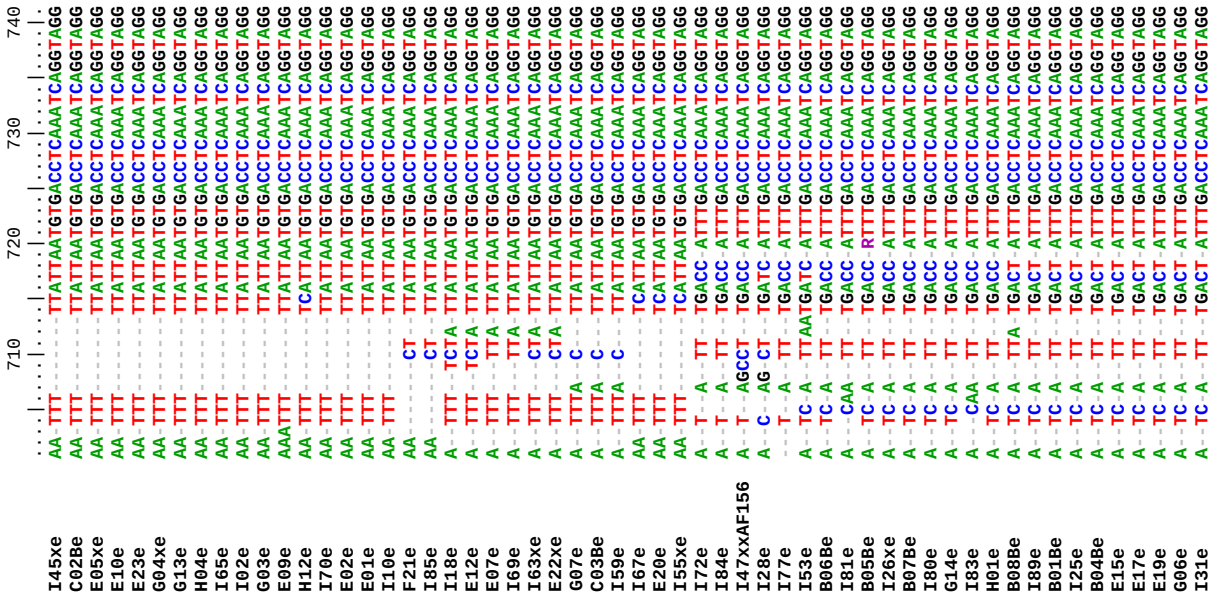
Multiple sequence alignment











Appendix 5

Clustal W alignment of *Cortinarius*-like ITS sequences

5.1 Subgeneric clades associated with Cortinarius*-like fungi sequenced for the current study*

All 94 *Cortinarius*-like sequences obtained for this study and the 420 *Cortinarius* and *Dermocybe* sequences publicly available on GenBank were not constructed into an unambiguous alignment. Rather the following table was produced from the examination of the Clustal W (Thompson *et al.* 1994) distance 'guide tree' without any further modification of the machine alignment. It is presented as a prliminary indication of the affinity of the *Cortinarius*-like sequences represented in Australian herbaria examined for the purposes of the current study to published subgeneric clades of *Cortinarius*.

Table 16: Published subgeneric clades of *Cortinarius* and the GenBank accessions from these clades with which *Cortinarius*-like sequestrate fungi sequenced for this study were aligned by Clustal W. Unshaded sequences are those generated by the current study. Sequences shaded in grey are those representing the published clade in the Clustal W alignment.

PUBLISHED CLADE	SEQUENCES ALIGNED BY CLUSTAL W
(Peintner *et al.* 2004) /OCHROLEUCI	AF184 I75e H1202 Thaxterogaster sp. DQ328205 FBE24
(Garnica *et al.* 2005) Included the clade but it was the unnamed sister clade to AMARESCENTES	Cor0431 Cortinarius croceocaeruleus gbAF389143.1
	Cor0432 Cortinarius croceocaeruleus gbAY669590.1
	Cor0599 Cortinarius pluvius gbAF389142.1
(Garnica *et al.* 2005) PSEUDOTRIUMPHANTES	AF21 C09Be H6558 Thaxterogaster sp. DQ328078 FBE33
	AF115 I06e H6558 Thaxterogaster sp. DQ328149 FBE33
	AF65 F08xe MEL2059043B Thaxterogaster levisporus DQ328111 FBE42
	AF112 I03e H0719 Hymenogaster sp. FBE42
	AF19 C07xe H5286 Thaxterogaster sp. DQ328076 FBE41
	Cor0508 Cortinarius iringa gbAY669624.1

Table 16 continued: Published subgeneric clades of *Cortinarius* and the GenBank accessions from these clades with which *Cortinarius*-like sequestrate fungi sequenced for this study were aligned by Clustal W. Unshaded sequences are those generated by the current study. Sequences shaded in grey are those representing the published clade in the Clustal W alignment.

PUBLISHED CLADE	SEQUENCES ALIGNED BY CLUSTAL W
(Peintner *et al.* 2001) **PHLEGMACIUM I** (Peintner *et al.* 2004) **/PURPURASCENTES**	AF14 C02Be TL503 Thaxterogaster my sp. 3 DQ328071 FBE37
	AF93 G13e TL503 Thaxterogaster my sp. 3 DQ328134 FBE37
	AF175 I66e H0727 Thaxterogaster campbelliae DQ328196 FBE30
	AF23 C11B2xe H6585 Thaxterogaster sp. DQ328080 FBE39
	AF170 I61xe H6585 Thaxterogaster sp. DQ328192 FBE39
	AF51 E23e MEL2032790 Thaxterogaster campbelliae DQ328102 FBE35
	AF173 I65e MEL2032790 Thaxterogaster campbelliae FBE35
	AF74 F17e H6728 Thaxterogaster sp. DQ328118 FBE39
	AF20 C08Be H5362 Thaxterogaster sp. DQ328077 FBE37
	AF17 C05Be H6739 Thaxterogaster sp. DQ328074 FBE39
	AF100 H04e H6753 Thaxterogaster sp. DQ328138 FBE39
	AF33 E05xe H0910 Thaxterogaster lilacsilky DQ328089 FBE19
	AF84 G04xe H0910 Thaxterogaster lilacsilky DQ328127 FBE19
	AF154 I45xe H0910 Thaxterogaster lilacsilky DQ328179 FBE19
	AF79 F22e H1120 Thaxterogaster sp. A DQ328122 FBE40
	AF135 I24e H5255 Thaxterogaster sp. DQ328165 FBE37
	AF109 H13e H1013 Thaxterogaster sp. DQ328145 FBE27
	AF141 I32e H1134 Thaxterogaster sp. A DQ328171 FBE40
	AF28 D05e H1194 Thaxterogaster sp. A DQ328084 FBE34
	AF72 F15e H1194 Thaxterogaster sp. A DQ328117 FBE34
	AF37 E09e H1006 Thaxterogaster sp. DQ328092 FBE34
	AF117 I08xe H4770 Thaxterogaster sp. DQ328151 FBE35
	Tha1726 Thaxterogaster campbellae gbAF325558.1
(Peintner *et al.* 2001) **PHLEGMACIUM I** (Peintner *et al.* 2004) **/PURPURASCENTES** (Garnica *et al.* 2005) **PURPURASCENTES**	AF13 C01Be TL502A Thaxterogaster my sp. 3 DQ328070 FBE41
	AF52 E24e MEL2057558 Thaxterogaster leucocephalus DQ328103 FBE42
	AF34 E06e H0920 Thaxterogaster lilacsilky DQ328090 FBE19
	AF83 G03e H0904 Thaxterogaster lilacsilky DQ328126 FBE19
	AF111 I02e H0904 Thaxterogaster lilacsilky DQ328146 FBE19
	AF56 E28xxAF56 MEL2079347 Thaxterogaster pyriformis DQ328106 FBE9
	AF121 I12e H7127 Thaxterogaster sp. DQ328155 FBE22
	AF38 E10e H1213 Thaxterogaster sp. A DQ328093 FBE36
	AF57 E29xe MEL2059057 Thaxterogaster sp. DQ328107 FBE26
	Cor0693 Cortinarius submagellanicus gbAY669614.1
	Tha1727 Thaxterogaster fragile gbAF325559.1

Table 16 continued: Published subgeneric clades of *Cortinarius* and the GenBank accessions from these clades with which *Cortinarius*-like sequestrate fungi sequenced for this study were aligned by Clustal W. Unshaded sequences are those generated by the current study. Sequences shaded in grey are those representing the published clade in the Clustal W alignment.

PUBLISHED CLADE	SEQUENCES ALIGNED BY CLUSTAL W
(Garnica *et al.* 2005) **PURPURASCENTES**	AF104 H08e H6732 Thaxterogaster sp. DQ328142
	AF195 I86e H1446 Thaxterogaster sp. DQ328216 FBE34
	Cor0408 Cortinarius chalybaeus gbAY669613.1
(Peintner *et al.* 2001) **PHLEGMACIUM I** (Peintner *et al.* 2004) **/PURPURASCENTES** (Garnica *et al.* 2005) **PURPURASCENTES**	Cor0602 Cortinarius porphyropus gbAF325560.1
	Cor0603 Cortinarius porphyropus gbAY174854.1
	Cor0619 Cortinarius purpurascens gbAY174858.1
	Cor0620 Cortinarius purpurascens gbAY669538.1
(Seidl 2000) **MYXACIUM SECTION MYXACIUM** (Peintner *et al.* 2001) **MYXACIUM I** (Peintner *et al.* 2004) **/MYXACIUM SENSU STRICTO** (Garnica *et al.* 2005) **MYXACIUM**	AF66 F09xe MEL2136538 Thaxterogaster pingue DQ328112 FBE11
	Cor0421 Cortinarius collinitus gbAY083181.1
	Cor0422 Cortinarius collinitus gbAY033096.1
	Cor0552 Cortinarius muscigenus gbAY083185.1
	Cor0551 Cortinarius muscigenus gbAF182800.1
	Tha1729 Thaxterogaster pingue gbAF325570.1
	Tha1730 Thaxterogaster pingue gbAF325571.1
	Cor0545 Cortinarius mucosus gbAF182801.1
	Cor0546 Cortinarius mucosus gbAY669591.1
	Cor0547 Cortinarius mucosus gbAF325574.1

Table 16 continued: Published subgeneric clades of *Cortinarius* and the GenBank accessions from these clades with which *Cortinarius*-like sequestrate fungi sequenced for this study were aligned by Clustal W. Unshaded sequences are those generated by the current study. Sequences shaded in grey are those representing the published clade in the Clustal W alignment.

PUBLISHED CLADE	SEQUENCES ALIGNED BY CLUSTAL W
(Peintner *et al.* 2001) **MYXACIUM I** (Peintner *et al.* 2004) **/MYXACIUM SENSU LATO** (Garnica *et al.* 2005) included the clade but did not name it	AF35 E07e H0969 Quadrispora frog eggs DQ328091 FBE45
	AF178 I69e H0969 Quadrispora frog eggs DQ328199 FBE45
	AF180 I71e H1364 Quadrispora sp. DQ328201 FBE45
	AF40 E12e H1486 Thaxterogaster sp. DQ328094 FBE5
	AF127 I18e H1486 Thaxterogaster sp. DQ328158 FBE5
	AF143 I34e H0726 Thaxterogaster redactus DQ328172 FBE8
	AF50 E22xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328101 FBE14
	AF172 I63xe H4136 Quadrispora Thaxterogaster sp. nov. 4 DQ328194 FBE14
	AF103 H07xe H0728 Thaxterogaster pyriformis DQ328141 FBE16
	AF118 I09e MEL2063439 Thaxterogaster sp. DQ328152 FBE17
	AF187 I78xe MEL2057505 Thaxterogaster levisporus DQ328208 FBE16
	Qua1615 Quadrispora oblongispora gbAF325566.1
	Tha1732 Thaxterogaster redactus gbAF325568.1
	Cor0435 Cortinarius cycneus gbAF389123.1
	Cor0530 Cortinarius magellanicus gbAF389124.1
(Seidl 2000) **MYXACIUM SECTION DEFIBULATI** (Peintner *et al.* 2001) **MYXACIUM I** (Peintner *et al.* 2004) **/MYXACIUM SENSU LATO**	AF25 D02e H6358 Protoglossum violaceum DQ328081 FBE
	AF69 F12e H6358 Protoglossum violaceum DQ328114 FBE
	AF41 E13xxAF41 H3059 Thaxterogaster sp. DQ328095 FBE
	AF49 E21e H6406 Thaxterogaster sp. DQ328100 FBE14
	AF159 I50e MEL2049699 Hymenogaster sp. FBE2
	AF161 I52e H4850 Thaxterogaster sp. DQ328183 FBE13
	AF167 I58e H4323 Thaxterogaster sp. DQ328189 FBE2
	Cor0525 Cortinarius lividoochraceus embAM113951.1
	Cor0526 Cortinarius lividoochrascens gbAF325565.1
	Cor0544 Cortinarius mucifluus gbAF182795.1
	Cor0720 Cortinarius vanduzerensis gbAF182793.1
	Cor0615 Cortinarius pseudosalor gbAF182792.1
	Cor0721 Cortinarius vanduzerensis gbAF182794.1
	Tha1728 Thaxterogaster paveleckii gbAF325564.1

Table 16 continued: Published subgeneric clades of *Cortinarius* and the GenBank accessions from these clades with which *Cortinarius*-like sequestrate fungi sequenced for this study were aligned by Clustal W. Unshaded sequences are those generated by the current study. Sequences shaded in grey are those representing the published clade in the Clustal W alignment.

PUBLISHED CLADE	SEQUENCES ALIGNED BY CLUSTAL W
(Peintner *et al.* 2001) **THAXTEROGASTER II (SISTER TO MYXACIUM I)** (Peintner *et al.* 2004) **SISTER TO /MYXACIUM SENSU LATO**	Tha1731 Thaxterogaster porphyreum gbAF325577.1
(Peintner *et al.* 2001) **CUPHOCYBE** (Peintner *et al.* 2004) **/CORRUGATUS** (Garnica *et al.* 2005) as "C. luteum" near C. minoscaurus	AF176 I67e MEL2057704 Thaxterogaster Protoglossum porphyreus luteum DQ328197 FBE1
	AF48 E20e H5791 Thaxterogaster sp. DQ328099 FBE1
	AF164 I55xe H5791 Thaxterogaster sp. DQ328186 FBE1
	Cor0446 Cortinarius dulciolens gbAF325610.1
	Pro1592 Protoglossum luteum gbAF325612.1
	Cor0475 Cortinarius fulvoochrascens gbAF389139.1
(Peintner *et al.* 2004) **/DERMOCYBE SENSU LATO** (Garnica *et al.* 2005) **SPLENDIDI**	AF102 H06e MEL2057999 Thaxterogaster Cortinarius sp. DQ328140 FBE23
	Cor0482 Cortinarius globuliformis gbAF325582.1
	Cor0483 Cortinarius globuliformis gbAY669602.1
	Cor0510 Cortinarius kula gbAY669643.1
	Cor0684 Cortinarius splendidus gbAY669598.1
	Der0833 Dermocybe splendida gbAF325583.1
(Peintner *et al.* 2004) **/DERMOCYBE SENSU LATO**	AF139 I30e H4798 Thaxterogaster sp. DQ328169 FBE25
	AF177 I68xxAF177 H5008 Thaxterogaster sp. DQ328198 FBE25
	Cor0463 Cortinarius firmus gbAF389163.1
(Garnica *et al.* 2005) include the clade but do not name it	AF3 A03e PERTH06234631 Cortinarius sebosus DQ328060
	AF92 G12e H7265 Cortinarius sebosus DQ328133
	AF15 C03Be TL493 Thaxterogaster my sp. 2 DQ328072 FBE4
	AF87 G07e TL493 Thaxterogaster my sp. 2 DQ328130 FBE4
	AF168 I59e TL501B Thaxterogaster sp. 1 DQ328190 FBE4
	Cortinarius archeri gbAF112142.1, gbAY669610
	Cortinarius austrovaginatus gbAY669635.1
	Cortinarius sinapicolor gbAF112145.1, gbAY669604

Table 16 continued: Published subgeneric clades of *Cortinarius* and the GenBank accessions from these clades with which *Cortinarius*-like sequestrate fungi sequenced for this study were aligned by Clustal W. Unshaded sequences are those generated by the current study. Sequences shaded in grey are those representing the published clade in the Clustal W alignment.

PUBLISHED CLADE	SEQUENCES ALIGNED BY CLUSTAL W
(Peintner *et al.* 2001) **TELAMONIA I** (Peintner *et al.* 2004) **/ACUTUS** (Garnica *et al.* 2005) **OBTUSI**	AF16 C04Be TL501A Thaxterogaster my sp. 1 DQ328073 FBE41
	AF78 F21e H2198 Thaxterogaster sp. DQ328121 FBE38
	AF194 I85e H5197 Thaxterogaster sp. DQ328215 FBE38
	AF32 E04e HL456 Thaxterogaster sp. DQ328088 FBE41
	AF54 E26xxAF54 MEL2063437 Thaxterogaster sp. DQ328104 FBE12
	AF106 H10xe H6915 Thaxterogaster sp. DQ328143 FBE38
	AF120 I11xxAF120 H6946 Thaxterogaster sp. DQ328154 FBE44
	AF2 A02e PERTH06234623 Cortinarius walpolei DQ328059 FBE
	AF88 G08e H6646 Cortinarius walpolei DQ328131 FBE
	AF179 I70e H6784 Thaxterogaster sp. DQ328200 FBE43
	AF29 E01e H5301 Thaxterogaster sp. DQ328085 FBE43
	AF119 I10e H5301 Thaxterogaster sp. DQ328153 FBE43
	AF53 E25e MEL2057565 Thaxterogaster levisporus FBE42
	AF55 E27e MEL2057547 Thaxterogaster levisporus DQ328105 FBE42
	AF114 I05e MEL2057536 Thaxterogaster levisporus DQ328148 FBE42
	AF80 F23xe H2195 Thaxterogaster sp. DQ328123 FBE36
	AF183 I74xe H2195 Thaxterogaster sp. DQ328204 FBE36
	AF77 F20e H2192 Thaxterogaster sp. DQ328120 FBE41
	AF128 I19e H2192 Thaxterogaster sp. DQ328159 FBE41
	AF158 I49e MEL2063445 Thaxterogaster sp. FBE41
	AF30 E02e H6784 Thaxterogaster sp. DQ328086 FBE43
	AF76 F19e H5330 Thaxterogaster sp. DQ328119 FBE41
	Cor0294 Cortinarius acutovelatus gbAY083175.1
	Cor0295 Cortinarius acutovelatus gbAY669655.1
	Cor0296 Cortinarius acutus gbAF325578.1
	Cor0436 Cortinarius cystidiocatenatus gbAY669651.1
	Cor0512 Cortinarius laetus gbAF389170.1
	COB0239 Cortinarius obtusus embAJ438981.2
(Garnica *et al.* 2005) include the clade but do not name it	AF62 F05e H5798 Thaxterogaster sp. DQ328110 FBE2
	AF108 H12e H5814 Thaxterogaster sp. DQ328144 FBE2
	Cor0539 Cortinarius minoscaurus gbAY669628.1

5.2 Accessioned sequences included in the Clustal W alignment but not used to define subgeneric clade associations

The following is a list of those accessions also included in the Clustal W alignment of the expanded *Cortinarius*-like molecular dataset. These sequences were not aligned to sequences generated for the current study as closely as those listed in Table 16 that were used to indicate subgeneric clade associations.

Cortinarius absarokensis gbAF182797.1
Cortinarius achrous gbAY033105.1
Cortinarius alboroseus gbAY033097.1
Cortinarius alboroseus gbAY033098.1
Cortinarius alboserrulatus gbAY669620.1
Cortinarius alboviolaceus gbAF325596.1
Cortinarius alboviolaceus gbAF325597.1
Cortinarius alboviolaceus gbAY669657.1
Cortinarius alcalinophilus gbDQ083770.1
Cortinarius aleuriosmus gbAY669537.1
Cortinarius allutus gbAF325585.1
Cortinarius allutus gbAY669531.1
Cortinarius alnetorum gbAY083176.1
Cortinarius alnetorum gbAY083177.1
Cortinarius alnetorum gbAY669695.1
Cortinarius amoenus gbAF389160.1
Cortinarius anisatus gbDQ117931.1
Cortinarius anisatus gbDQ120753.1
Cortinarius anisatus gbDQ120756.1
Cortinarius anomalus embAJ236071.1
Cortinarius anomalus gbAF325581.1
Cortinarius anserinus gbAY174805.1
Cortinarius anserinus gbAY174806.1
Cortinarius anserinus gbAY174807.1
Cortinarius aprinus embAJ889942.1
Cortinarius aprinus gbAY669663.1
Cortinarius arcuatorum gbAF503552.1
Cortinarius arcuatorum gbAY033120.1
Cortinarius arcuatorum gbAY174824.1
Cortinarius argutus gbAY669535.1
Cortinarius armeniacus gbAF325595.1
Cortinarius armeniacus gbDQ117925.1
Cortinarius armillatus embAJ236075.1
Cortinarius armillatus gbAF037223.1
Cortinarius armillatus gbDQ114744.1
Cortinarius atrocoerulaeus gbAY083178.1
Cortinarius atrovirens gbAY174848.1
Cortinarius aureocalceolatus gbAY669569.1
Cortinarius aureomarginat gbDQ102660.1
Cortinarius australis gbAY669615.1
Cortinarius austrocyanites gbAY669626.1
Cortinarius austrosaginus gbAY669619.1
Cortinarius badiovinaceus gbAF389152.1
Cortinarius balaustinus gbAY669693.1
Cortinarius balteatoalbus gbAY669517.1
Cortinarius balteatoalbus gbAY669533.1
Cortinarius balteatocumatilis gbAY174801.1
Cortinarius balteatus gbAY669526.1
Cortinarius barbarorum gbDQ083773.1
Cortinarius barbarorum gbDQ323959.1
Cortinarius belleri gbAY669685.1
Cortinarius betuletorum gbAY040712.1
Cortinarius biformis gbAY669688.1
Cortinarius bigelowii gbAF325617.1
Cortinarius bivelus gbAY669682.1
Cortinarius bolaris gbAF389169.1
Cortinarius boudieri gbAY174860.1
Cortinarius boudieri gbAY174861.1

Cortinarius bovinus embAJ889943.1
Cortinarius bovinus gbDQ139983.1
Cortinarius brunneus embAJ236076.1
Cortinarius brunneus gbAF325590.1
Cortinarius brunneus gbAF430287.1
Cortinarius bulliardi gbAY669659.1
Cortinarius bulliardii gbAF389154.1
Cortinarius caeruleoburneus gbAY669634.1
Cortinarius caerulescens embAJ889944.1
Cortinarius caerulescens gbAY174862.1
Cortinarius caerulescens gbAY669515.1
Cortinarius caesiocanescens gbAY669546.1
Cortinarius caesiocortinatus gbAY174809.1
Cortinarius caesiostramineus gbAY669519.1
Cortinarius cagei gbAY669676.1
Cortinarius caligatus gbAY669553.1
Cortinarius callisteus gbAY040713.1
Cortinarius callisteus gbAY669594.1
Cortinarius calochrous gbAY174842.1
Cortinarius calochrous gbDQ083766.1
Cortinarius calochrous gbDQ323960.1
Cortinarius camptoros gbAY669540.1
Cortinarius caninus gbAY669646.1
Cortinarius cannarius gbAY669630.1
Cortinarius caperatus gbAY669575.1
Cortinarius caperatus gbDQ367911.1
Cortinarius casimiri embAJ889945.1
Cortinarius castoreus gbAY033117.1
Cortinarius catharinae gbAY669560.1
Cortinarius cedretorum gbAY669564.1
Cortinarius cedriolens gbAY083179.1
Cortinarius cephalixus gbAY174783.1
Cortinarius cephalixus gbAY174784.1
Cortinarius cephalixus gbAY174786.1
Cortinarius cereifolius gbAY174847.1
Cortinarius cf. submeleagris gbAY669638.1
Cortinarius chrysomallus gbDQ102670.1
Cortinarius cinnabarinus gbAY669662.1
Cortinarius citrinolilacinus gbAY174830.1
Cortinarius citrinus gbAY174820.1
Cortinarius citrinus gbAY174821.1
Cortinarius citrinus gbAY174825.1
Cortinarius citriolens gbAF325607.1
Cortinarius claricolor gbAY669522.1
Cortinarius coalescens gbAY669552.1
Cortinarius coerulescens gbAF389134.1
Cortinarius collariatus gbAY033114.1
Cortinarius collariatus gbAY033115.1
Cortinarius collinitus gbDQ367896.1
Cortinarius corrosus gbAY669562.1
Cortinarius corrosus gbDQ323964.1
Cortinarius cotoneus gbAY669597.1
Cortinarius crassus gbAY669544.1
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Cortinarius emodensis gbAY669576.1
Cortinarius erythraeus gbAY669605.1
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Cortinarius flexipes gbAY669683.1
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Cortinarius fraudulosus gbAY669551.1
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Cortinarius vulpinus gbAY174811.1
Cortinarius xanthophyllus gbAY174827.1
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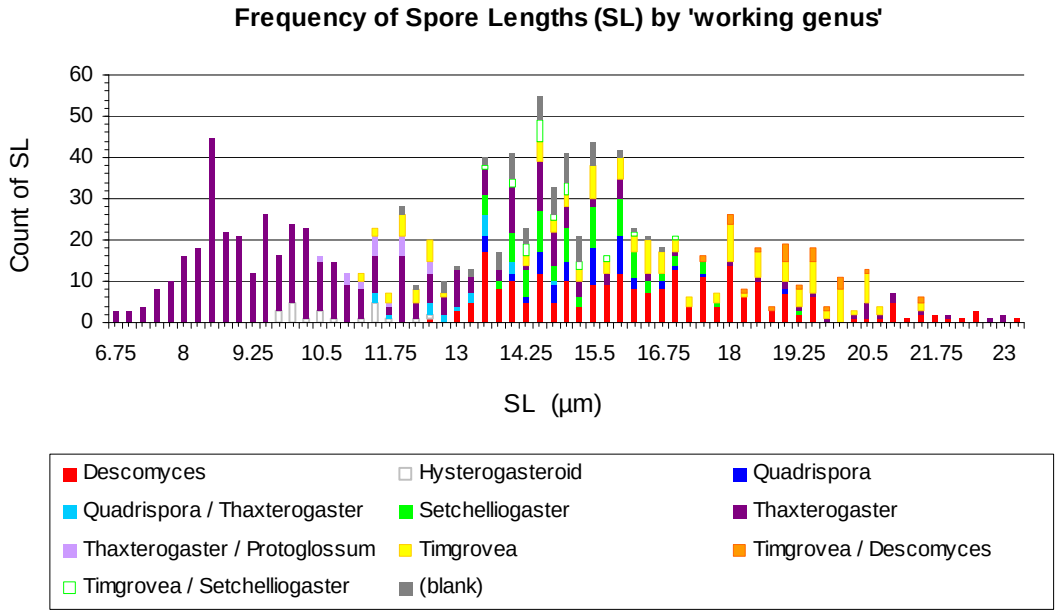
Appendix 6

Examples of graphs used for morphological data exploration and coding

a.



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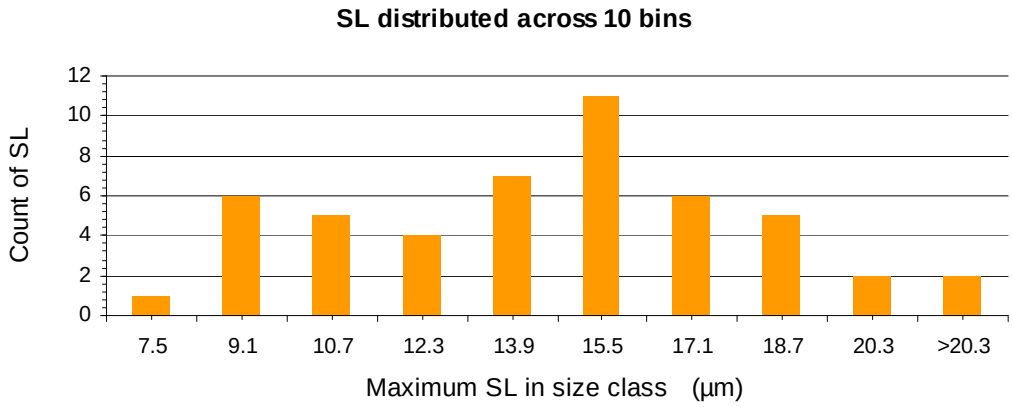
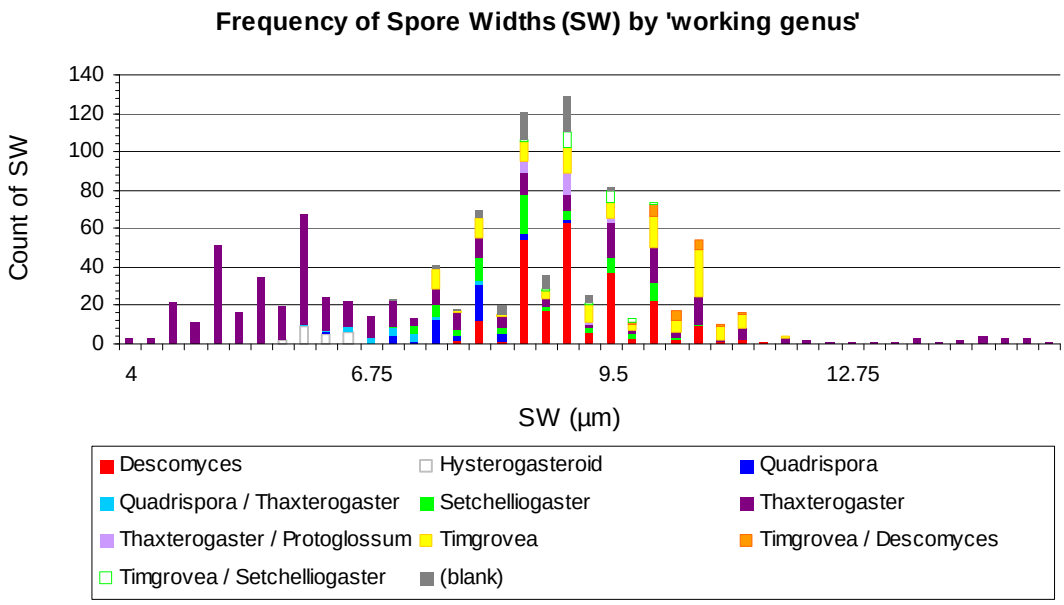


Figure 22: Histograms of Spore Length (SL) data for: a. 74 bins coloured by 'working genus' (bin size based on the smallest interval between any two length measurements), and b. 10 bins.

a.



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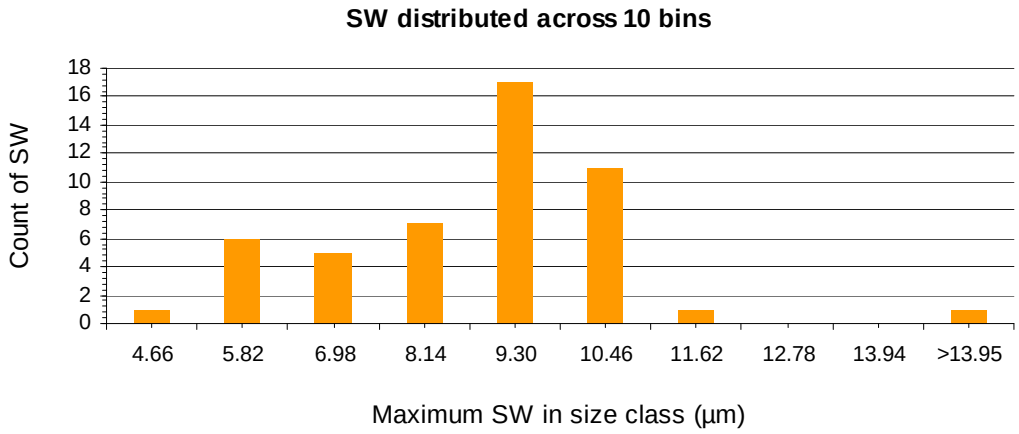

Figure 23: Histograms of Spore Width (SW) data for: a. 66 bins coloured by working genus (bin size based on the smallest interval between any two width measurements), and b. 10 bins.

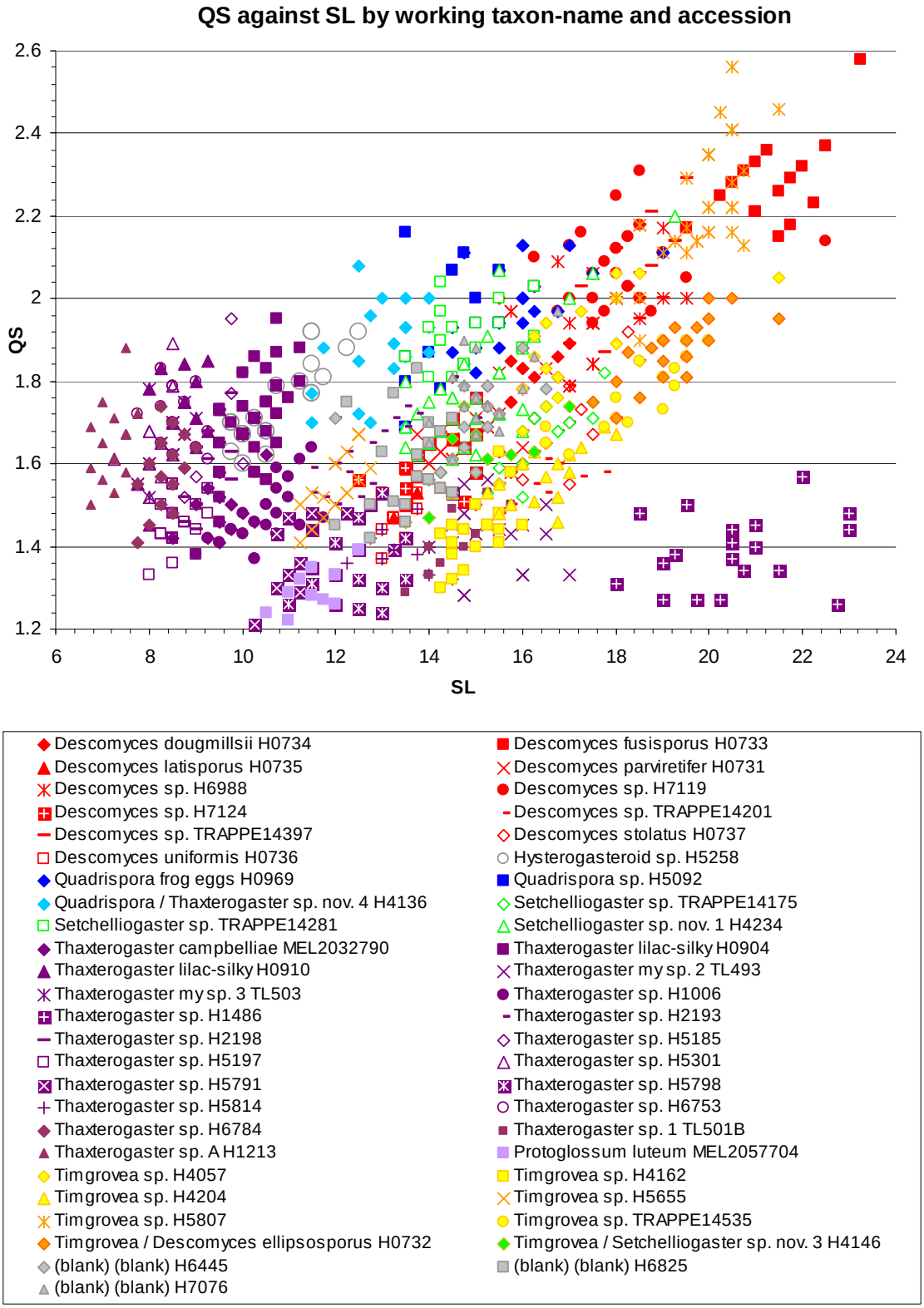

Figure 24: Scatter plot of the ratio of spore length to width (QS) against spore lenth (SL) for the collections examined in the comparative analysis of the morphological data.

Appendix 7

Preparation of reagents

7.1 Preparation of Glass-'milk'

A suspension of powdered silica. Use powdered silica 325 mesh or finer (from a ceramic shop) or powdered (not fumed) silica from a commercial chemical supplier.

1. Resuspend 400 g of glass powder in 800 ml of double deionized H_2O in a 2l flask.
2. Stir for 60 minutes.
3. Allow to settle for 90 minutes.
4. Take the supernatant (which contains the 'fines' of interest) and pellet by centrifuging for 10 minutes at 6000 rpm to (using a GSA rotor).
5. Discard supernatant and resuspend pellet in 300 ml ddH_2O add nitric acid (H_2NO_3) to 50 %.
6. Bring close to the boil in fume hood.
7. Pellet glass as before.
8. Wash pellet 4-6 times with ddH_2O (check that pH has returned to neutral)
9. Store the final pellet as 50 % slurry in ddH_2O store at -70 °C, working aliquot at 4 °C.

7.2 Preparation of extraction buffer

From Raeder & Broda (1985)

To make 500 ml stock solution add:

1. 100 ml of 200 mM Tris HCl pH 8.5.
2. 25 ml of 250 mM NaCl.
3. 25 ml 25 mM EDTA (disodium salt).
4. 25 ml 0.5 % SDS.
5. 325 ml UV filtered/Baxter water, and store at 4 °C.

7.3 Preparation of sodium iodide (NaI) solution

1. Dissolve 100 g sodium iodide (NaI) in 100 ml UV filtered/Baxter water
2. Add 1.5 g sodium sulphite ($NaSO_3$).
3. Filter through Whatman No. 1 filter paper and store at 4 °C in an opaque bottle. If the solution starts to turn yellow, add a little more $NaSO_3$.

7.4 Preparation of wash buffer

To make 500 ml 1 M stock solution add

1. 10 ml of 10 mM Tris pH 7.5.
2. 1 ml of 1 mM EDTA (disodium salt).
3. 10 ml of 100 mM NaCl.
4. Make up volume to 500 ml with 50 % ethanol and store at 4 °C.

Appendix 8

Reprints

HISTORICAL AND CURRENT PERSPECTIVES IN THE SYSTEMATICS OF AUSTRALIAN CORTINARIOID SEQUESTRATE (TRUFFLE-LIKE) FUNGI

Anthony A. Francis [1, 2] & Neale L. Bougher [1]

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Abstract

Historically, sequestrate macrofungi with purported affinities to *Cortinarius* and related agarics, have been classified among taxa representing a broad range of basidiome forms and phylogenetic affinities. In recent decades increasing recognition of the ecological function and importance of sequestrate macrofungi has provided an impetus for research into their ecology and taxonomy. The classification of the cortinarioid sequestrate fungi remains contentious despite an intensifying research effort. Related cortinarioid genera spanning agaricoid to gastroid basidiome forms share characters including spore structure, pigmentation and ornamentation; basidiome pigmentation and development; and similarities in molecular sequences. This paper provides an account of historical and current information on the systematic position of cortinarioid sequestrate fungi, based on published classical and molecular studies. The authors provisionally accept as 'cortinarioid', 16 genera of sequestrate fungi having purported affinities to *Cortinarius* and related agaricoid fungi. Thirty-nine published species in 11 of these genera are currently reported from Australia. The history of collecting and classifying cortinarioid sequestrate fungi, as outlined in this paper, illustrates well the effect of increased scientific effort in uncovering previously unrecognised relationships among components of Australia's unique and diverse fungal flora.

A.A. Francis & N.L. Bougher (2002) [2003]. Historical and current perspectives in the systematics of Australian cortinarioid sequestrate (truffle-like) fungi. *Australasian Mycologist* 21 (3): 81–93.

Introduction

A wide variety of sequestrate (truffle-like) macrofungi have been aligned with *Cortinarius* and related agaricoid (mushroom-like) fungi. These phylogenetic affinities have not always been recognised and, in a number of cases, remain uncertain. Early fungal taxonomists placed the sequestrate fungi together under polyphyletic groupings such as the Gasteromycetes, based on the common trait of enclosed hymenia. As a result, the cortinarioid sequestrate fungi largely share the same early history of study as the sequestrate fungi in general. For Australian sequestrate fungi this shared history of classification and collection is detailed in Bougher & Lebel (2001), Castellano & Bougher (1994), Lebel & Castellano (1999), May (2001) and May & Wood (1997). As different morphological, chemical and developmental characters were considered, sequestrate fungi were progressively separated into new families and orders, including the Cortinariales, that more closely reflected their relationships with other fungi. Research employing molecular technology has affirmed the polyphyletic nature of many traditional cortinarioid taxa, and provided additional support for a number of postulated links between sequestrate and non-sequestrate forms.

Sequestrate fungi are those in which the spores, commonly statismospores (not forcibly discharged), mature inside an enclosed, underground, semi-underground or less often emergent fruitbody, remaining there until the fruitbody decomposes or is eaten. The cortinarioid sequestrate fungi exhibit a variety of basidiome forms, differing in the degree of gasteromycetation (pileus, stipe, veil and hymenophore development) found independently or together with characteristics such as statismospory (Table 1). Particular sequestrate genera have been affiliated with *Cortinarius* on the basis of spore structure, pigmentation and ornamentation; basidiome pigmentation and development; and similarity in molecular sequence data (*e.g.* Bougher & Castellano 1993, Moncalvo *et al.* 2002, Peintner *et al.* 2001, Singer 1951). However, opinions have, and continue to differ on the significance of the various characters used to determine the phylogenetic relationships of the cortinarioid fungi, and thus which taxa should be included.

This paper presents a history of the classification and collection of sequestrate fungi currently considered related to *Cortinarius* and closely allied agarics, with a particular focus on Australia. The Australian assemblage of cortinarioid sequestrate fungi is particularly diverse and represents a range of sequestrate forms, ecological associations and interactions (Bougher & Lebel 2001). However, the Australian assemblage of these fungi is only partially known.

Table 1. Some broad groupings of morphological forms found among the cortinarioid sequestrate fungi (adapted from Peintner *et al.* 2001).

Character	Sequestrate Cortinarii with persistent veils	Secotioid	Gastroid
Pileate	Yes	Yes or no	No
Hymenophore structure	Straight to more or less anastomosed lamellae	Anastomosed lamellae or a loculate gleba	Anastomosed lamellae, a loculate gleba or lining the single chamber of the basidiome
Stipe/columella	Generally possessing a well-developed stipe/columella	With a stipitate to very short columella traversing the gleba	With or without a columella or internal sterile tissue
Habit	Epigeous to hypogeous	Epigeous to hypogeous	Hypogeous
Spore release	Ballistosporic or statismosporic	Commonly statismosporic	Statismosporic
References and examples	Described as hypogeous *Cortinarii* by Thiers & Smith (1969)	*Thaxterogaster* (Singer 1951) synonymised with *Cortinarius* (Peintner *et al.* 2002)	*Protoglossum* Massee (1891)

The concept of Cortinarioid sequestrate fungi

Despite the relatively large body of information regarding the cortinarioid sequestrate fungi, their taxonomy is currently in a state of flux. For this reason we have decided to deal with the informal grouping 'cortinarioid sequestrate fungi', acknowledging that different authors have placed these fungi in a variety of genera and suprageneric taxa. For the purposes of this paper then, we consider cortinarioid sequestrate genera to be those that at some time, have been placed in either the Cortinariaceae or the Hymenogasteraceae and, as yet, have not been shown to have stronger affinities with taxa outside these families. Under this definition we accept 16 genera as cortinarioid sequestrate fungi (Table 2).

For many taxa inclusion or exclusion from the cortinarioid sequestrate fungi is, however, inconclusive. For example, Bougher & Castellano (1993) suggest *Timgrovea* is related to *Strobilomyces* (Boletales). However, as the spores of *Timgrovea* subgenus *Subreticulata* are quite similar to those of *Descomyces*, we include it among the cortinarioid sequestrate fungi (Francis & Bougher unpublished data). As a second example, Smith (1965) first described the 'amyloid'-spored *Mycolevis*, tentatively as a second genus in the Cribbeaceae. Smith (1965) also demonstrated, regarding mounts made in Melzer's solution, that 'not all violet or blue-black material (especially granules) are necessarily 'truly amyloid'. In the case of *Mycolevis*, Smith appears to have considered the structure of the spore ornamentation (*e.g.* the presence of a conspicuous perisporium), at least possibly, more phylogenetically informative than the amyloid Melzer's reaction. No published study has rigorously examined this possibility, and yet this genus has been aligned with the cortinarioid fungi by the reduction of the Cribbeaceae to synonymy with the Cortinariaceae (*e.g.* Kirk *et al.* 2001). We consider it unlikely that the *Mycolevis* amyloid reaction is significantly different from that observed in the Russulaceae, and believe that molecular data aligns *Mycolevis* with this family (Prof. J. Trappe pers. comm.). We therefore exclude *Mycolevis* from our concept of the cortinarioid sequestrate fungi. Finally, the genera *Aroramyces*, *Cribbea*, *Destuntzia*, *Kjeldsenia*, *Mackintoshia* and *Mycoamaranthus* are included within our concept of the cortinarioid sequestrate fungi. In most cases inclusion is based on the discussion included with the original descriptions of the genera without supporting molecular evidence for such a phylogenetic affinity (Figure 1). These genera should be considered as tentatively included, pending further study.

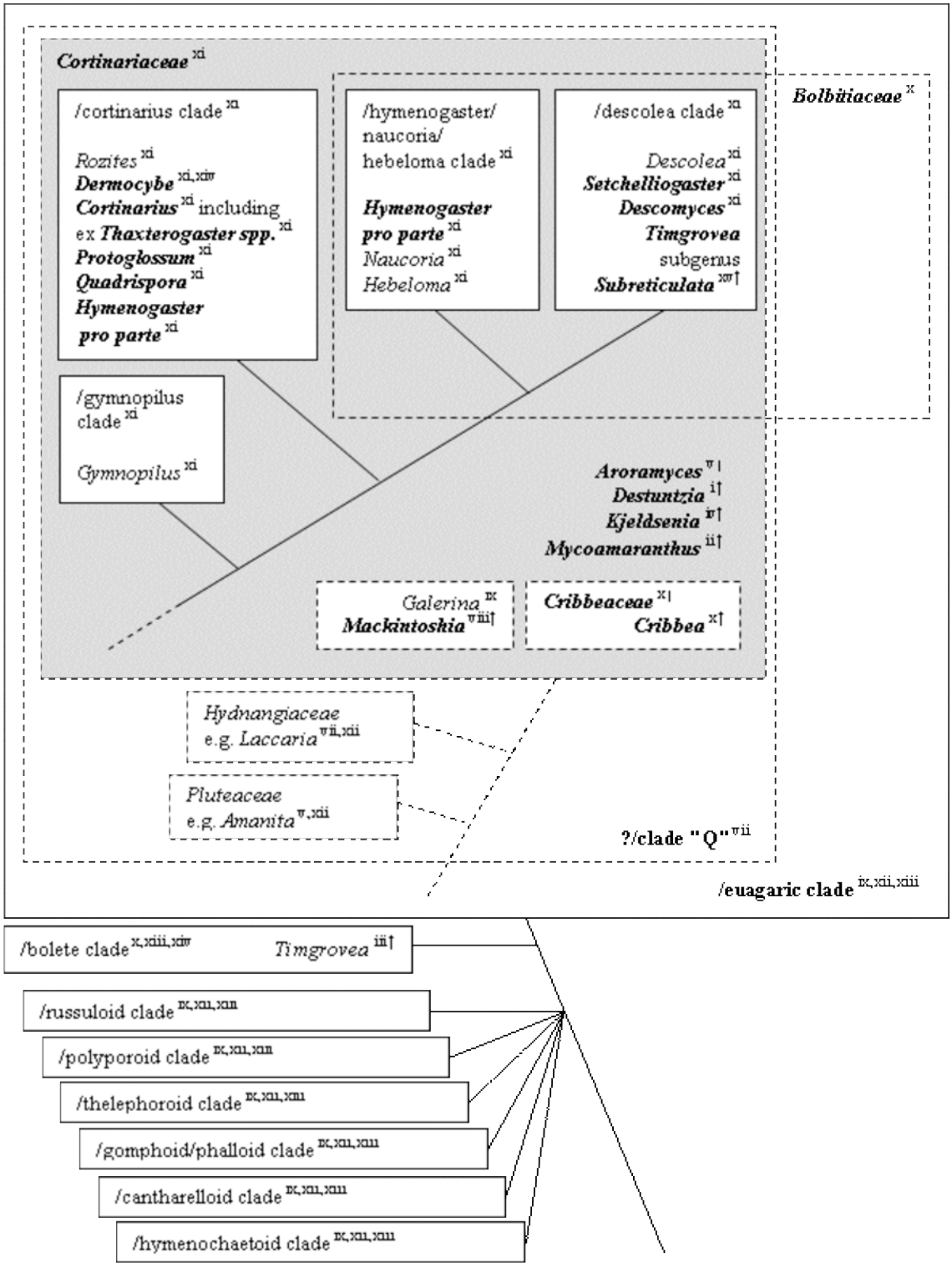

Figure 1: Diagrammatic representation of the postulated taxonomic position of sequestrate and selected agaricoid cortinarioid fungi among the Cortinariaceae, and selected homobasidiomycete groups. † indicates taxa not included in published molecular phylogenies at the time of publication. Taxa including known cortinarioid sequestrate forms are indicated in bold. Dotted lines indicate uncertainty in group membership, or in phylogenetic links to other fungi inside or outside the groups indicated. Taxa grouped according to: [i] Fogel & Trappe (1985), [ii] Castellano *et al.* (1992), [iii] Bougher & Castellano (1993), [iv] Colgan *et al.* (1995), [v] Hibbett *et al.* (1997), [vi] Castellano *et al.* (2000), [vii] Moncalvo *et al.* (2000), [viii] Pacioni & Sharp (2000), [ix] Hibbett & Thorn (2001), [x] Kirk *et al.* (2001), [xi] Peintner *et al.* (2001), [xii] Binder & Hibbett (2002), [xiii] Moncalvo *et al.* (2002), [xiv] Bougher & Trappe (2002), [xv] Francis & Bougher (unpublished).

Table 2. Genera and number of published species considered cortinarioid sequestrate fungi by the current authors, based on references as provided. World numbers from CABI Bioscience Databases (http://194.131.255.4), Australian numbers from May & Wood (1997), and Bougher (1997). Selected additional references with particular importance to distribution and Australian taxa are provided. Peintner *et al.* (2002) synonymised *Thaxterogaster* with *Cortinarius*; however, we have maintained the genus name for the purpose of this table.

Genus	Number of known species		Comments/References
	World	Australia	
Aroramyces Castellano *et* Verbeken	2	1	Australian and African, Castellano *et al.* (2000).
Cortinarius (Pers.) Gray (Sequestrate forms)	6	0	Sequestrate forms from North America, Kashmir, and several un-named species in Australia (Francis & Bougher unpublished data). Coined as 'hypogeous *cortinarii*' (Thiers & Smith 1969), Watling (1980), Bougher & Malajczuk (1986).
Cribbea A.H. Smith *et* D.A. Reid	4	3	Australian and Argentinean, Smith & Reid (1962).
Dermocybe (Fr.) Wünsche	1	1	The only known sequestrate member of this genus is Australian, Bougher & Trappe (2002).
Descomyces Bougher *et* Castellano	4	2	Australasian, Bougher & Castellano (1993).
Destuntzia Fogel *et* Trappe	5	0	One unnamed species occurs in Australia. Castellano & Bougher (1994), Fogel & Trappe (1985).
Geoinocybe	-	-	*Gen. ined.* Trappe & Claridge (pers. comm. J. Trappe, '*Genus B*' *in* Bougher & Lebel 2001). Also cited by Matheny *et al.* (2002).
Hymenogaster Vittad.	100	6	Worldwide; many of the Australian species have been recombined (Bougher & Castellano 1993)
Kjeldsenia Colgan *et al.*	1	0	North American, Colgan *et al.* (1995).
Mackintoshia Pacioni *et* C. Sharp	1	0	African, Pacioni & Sharp (2000).
Mycoamaranthus Castellano *et al.*	3	1	Australasian and African, Castellano *et al.* (1992), Castellano *et al.* (2000).
Protoglossum Massee	6	5	Worldwide, formerly *Cortinomyces* (see May 1995), Bougher & Castellano (1993).
Quadrispora Bougher *et* Castellano	2	2	Australian endemic, Bougher & Castellano (1993).
Setchelliogaster Pouzar	7	2	Worldwide. In association with *Eucalyptus* (Bougher & Lebel 2001), Beaton *et al.* (1985b), Pouzar (1958).
Thaxterogaster Singer	60	12	Worldwide, synonymised with *Cortinarius* by Peintner *et al.* (2002).
Timgrovea Bougher *et* Castellano	5	4	Australian and Chinese, Bougher & Castellano (1993).
TOTAL	**207**	**39**	

Classification of Cortinarioid sequestrate fungi

Early developments

Early intuitive classifications grouped the sequestrate fungi now known to have affinities with genera among the Cortinariales, along with a variety of other sequestrate fungi in large polyphyletic groups (for many years also including ascomycetes). Vittadini (1831) proposed the first dedicated classification for sequestrate fungi, including the original description of the genus *Hymenogaster* (Lebel & Castellano 1999). Vittadini (1831) classified the Tuberaceae of Fries' *Systema Mycologicum* into two subfamilies, the Hymenogastereae and the

Tubereae. The Hymenogastereae had three sections: Prototypi, Summi and Centrales which accommodated *Hymenogaster* (Vittadini 1831).

Berkeley (1840) pointed out that many of the Gasteromycetes had basidia, not asci as had been generally supposed to that time. This observation, along with monographic works by Tulasne & Tulasne on the Nidulariaceae (1844) and the Hymenogastrales (1851) contributed to the exclusion of the sequestrate Ascomycetes, such as *Tuber*, from the Gasteromycetes (Cunningham 1944). Tulasne & Tulasne (1851) also proposed the existence of evolutionary links between hypogeous and epigeous ascomycetes (*i.e.* Tuberales and Pezizales). Parallel links among the Basidiomycota were not proposed until considerably later and the Gasteromycetes, minus the ascomycetes, continued to be classified as a distinct basidiomycete lineage. In his *Outlines of British Fungology*, Berkeley (1860) produced a system of classification in accordance with this understanding of the Gasteromycetes, including them at the rank of family. Within the Gasteromycetes, Berkeley included six genera besides *Hymenogaster* in the Hypogaei.

In the later part of the 19th Century, continuing examination of the structure and development of fungi elucidated more characteristics uniting basidiomycete taxa than had been recognised previously. Microscopic features were also gradually incorporated into classifications of the basidiomycetes (*e.g.* Fischer's (1900) inclusion of basidial and hymenial characteristics in his system of classification). By the early 20th century, mycology and the study of sequestrate fungi had advanced to a point where some authors began to acknowledge the artificial nature of contemporary gasteromycete classifications and anticipate major revisions in the taxonomy of the Hymenogasteraceae *s. lat.* (*e.g.* Rodway 1912a).

Bridging the gap between agaricoid and gasteroid fungi

During the 20th century micro-morphology, chemotaxonomy and developmental studies engendered new and increasingly natural fungal classifications, identifying sequestrate fungi with affinities to various agaricoid taxa, including *Cortinarius*. Sequestrate and agaricoid taxa united by obvious and consistent characters, such as the amyloid spore ornamentation and peridial sphaerocysts of the 'astrogastraceous series' (*e.g.* Heim 1934, Malençon 1931, Singer & Smith 1960), were first to be linked in basidiomycete 'evolutionary series'. Such theories strengthened the expectation that other sequestrate and agaricoid taxa would be related in a similar fashion. However, Cunningham's monographic work of 1944, *The Gasteromycetes of Australia and New Zealand*, illustrates the continuing tendency around this time, despite such theories, to treat the Gasteromycetes as a single taxonomic unit. According to this work the order Hymenogastrales was comprised of two families, the Hymenogasteraceae and the Secotiaceae. The Hymenogasteraceae had two subfamilies and two named tribes including, alongside *Hymenogaster*, eight genera since found to be non-cortinarioid.

Recognition of the links between sequestrate fungi and agaricoid forms intensified in the mid to late 20th century. For example, Singer (1951) incorporated a discussion on the similarities between *Cortinarius* and the new secotioid genus *Thaxterogaster*. Singer & Smith (1959) suggested a possible relationship between the secotioid *Setchelliogaster* and the agaricoid *Conocybe* (this connection to the Bolbitiaceae is noteworthy as Kirk *et al.* 2001 also classify *Descolea* in this family see Figure 1). Smith & Singer (1959) also detailed a series related to *Boletus* and *Suillus*, including *Rhizopogon* and *Chamonixia*, two genera formerly aligned with *Hymenogaster* (*e.g.* Cunningham 1944). The discovery of sequestrate *Cortinarius* species (*e.g.* Thiers & Smith 1969) contributed to the understanding of the diversity of basidiome forms among the cortinarioid sequestrate fungi. A major factor contributing to the retention of sequestrate forms in artificial taxa, such as the gasteromycetes, were theories suggesting that agaricoid forms had developed from gastroid ancestors (*e.g.* Singer 1986). These theories were challenged by discoveries concerning morphological plasticity of sequestrate basidiomes. In the late 1960s culturing techniques lead to the observation of mating intercompatibility between secotioid and agaricoid forms (*e.g.* Rosinski & Robinson 1968). Similarly, evidences of morphological plasticity have been found among the cortinarioid sequestrate fungi. For example, Lago *et al.* (2001) demonstrated a high degree of phenotypic plasticity within single collections and axenic cultures of *Setchelliogaster* and *Descolea* species. Such observations blurred traditional taxonomic boundaries based on basidiome morphology. Basidiome development is thought to be under relatively simple genetic control and hence potentially frequently mutated (Bruns *et al.* 1989). This means that some sequestrate basidiome forms, previously used to distinguish taxa (*e.g.* secotioid *Thaxterogaster* from agaricoid *Cortinarius* species) may have arisen more than once, making such taxa polyphyletic (Peintner *et al.* 2001, 2002). Such research into the sequestrate fungi continued to raise questions about the boundaries between agaricoid and sequestrate genera and, more broadly, the grounds for maintaining the Gasteromycetes as a distinct taxonomic entity.

Significant changes to the way in which sequestrate fungi are classified were set in motion by discoveries of the 1970s. Increasing recognition of the multiple origins of the sequestrate fungi provided an impetus for the reassessment of polyphyletic groupings such as the Gasteromycetes. Extensive collection of fungi in the Southern Hemisphere begun during this period also highlighted the richness and uniqueness of the regions sequestrate fungal flora (Lebel & Castellano 1999).

The 1980s was a period of seminal discussion on the links between agaricoid and sequestrate genera, including summations of the then known (or suspected) evolutionary series (*e.g.* Singer 1986, Thiers 1984). Thiers (1984) named *Hymenogaster* as the closest gastroid form to the Cortinariaceae, as did Singer (1986) building on works by Singer, Smith and others (*e.g.* Singer 1958, Smith 1973). Jülich (1981) formulated a system of classification that, acknowledging the links between non-sequestrate and sequestrate forms, raised a number of groups to ordinal level, including the Cortinariales. Since this work, the cortinarioid sequestrate fungi have been placed either in the Cortinariales or retained in the order Hymenogastrales. For example, Beaton *et al.* (1985a, b) dealt with the sequestrate Cortinariales of Victoria, including *Setchelliogaster* and *Thaxterogaster* in the Cortinariaceae and *Hymenogaster* in the Hymenogasteraceae.

In 1993 *Hymenogaster s. str.* was delimited by excluding four of the eight species originally included by Vittadini (1831) and proposing four new genera, *Cortinomyces*, *Descomyces*, *Quadrispora* and *Timgrovea* (Bougher & Castellano 1993). *Hymenogaster s. lat.* had encompassed a very mixed bag of species lumped together on the basis of having brown, ornamented and/or perisporial spores (Pegler & Young 1987). Bougher & Castellano (1993) reassessed the previously dominant view that the genus *Hymenogaster s. lat.* represented the most reduced form of the evolutionary series related to *Cortinarius*. Their paper demonstrated the polyphyletic nature of *Hymenogaster s. lat.* indicating that only a subset of species formerly included in this group (designated *Cortinomyces*) were likely to be closely aligned with *Cortinarius*. *Cortinomyces* was later deemed invalid as the type species chosen for it, *Protoglossum luteum*, had been used to typify *Protoglossum* by Massee in 1891 (May 1995).

The Australian Biological Resources Study (ABRS) commenced the *Fungi of Australia* series in 1996. This project has provided a major impetus to fungal taxonomy in Australia including Walker's classification of 1996. Walker (1996) is unusual among modern classifications in that, rather than attempting to develop the most natural classification possible, Walker maintains the artificial Euholobasidiomycete 'Group 1' (the Gasteromycetes). Grgurinovic's *Larger Fungi of South Australia*, published in 1997, used the concept of the Cortinariales *sensu* Jülich (1981). Grgurinovic (1997) included the genera *Setchelliogaster* and *Thaxterogaster* (Cortinariaceae) in the Cortinariales but, in keeping with Cleland (1934) and Jülich (1981), the Hymenogastrales was reserved for gastroid taxa.

Modern classifications of the higher fungi have treated *Cortinarius* and related genera in various ways. Higher taxa containing *Cortinarius* have been variously designated at the levels of order (Cortinariales Jülich), family (Cortinariaceae R. Heim ex Pouzar) and tribe (Cortinarieae *e.g.* Singer 1986). The degree to which these classifications integrate sequestrate forms into predominantly agaricoid higher taxa also varies. Some classifications maintain the sequestrate genera independently of their agaricoid relations but note their affinities (*e.g.* Singer 1986, Walker 1996). Following the lead of Jülich (1981) other classifications have further highlighted relationships between sequestrate and agaricoid cortinarioid taxa by including orders containing, for example, both *Hymenogaster* and *Cortinarius* in the Cortinariales (*e.g.* Pegler *et al.* 1993). Thus, largely based on morphological methods, traditional taxonomy has identified some sequestrate taxa that appear to be more closely related to agaricoid Cortinariales than to other sequestrate or agaricoid genera.

The molecular age

Techniques for analysing the structures of nucleic acids (DNA and RNA) have made additional characters available to systematic mycology. Nucleic acid technologies have provided new insights into the evolutionary relationships of, and the diversity among, the cortinarioid sequestrate fungi. Molecular work on basidiomycete phylogeny has generally either focussed on elucidating the broad picture of evolutionary relatedness among the higher taxa, or the relationships among smaller components thereof. For example Hibbett & Thorn (2001) present a classification of the Homobasidiomycetes, synthesising the results of published molecular studies with eight major clades (monophyletic groups) derived from a previous study by Hibbett *et al.* (1997). The eight clades are the euagaric, bolete, russuloid, polyporoid, thelephoroid, gomphoid-phalloid, cantharelloid and hymenochaetoid clades. This work provided further support for the theory that a number of basidiome forms formerly used to distinguish taxa (*e.g.* gilled, pored or toothed hymenophores) had arisen more than once. Binder

& Hibbett (2002) supported the groups resolved in Hibbett & Thorn (2001) demonstrating that the bolete clade is the sister taxon of the euagaric clade. Works such as these use only a relatively few 'representative' taxa (sometimes only one) to represent higher taxonomic groupings. For example Binder & Hibbett (2002) use only *Cortinarius iodes* Berk. & M.A. Curtis to represent all the cortinarioid fungi, agaricoid or otherwise. Moncalvo *et al.* (2002) deals with the euagaric clade identified in Binder & Hibbett (2002) and Hibbett & Thorn (2001), citing the work of Peintner *et al.* (2001) and others with regard to the position of sequestrate taxa nested within the euagaric clade. The '/cortinarioid clade' is poorly supported in Moncalvo *et al.* (2002), as are many other clades along the 'spine' of their tree. This means that their analysis could not confidently resolve the position of the /cortinarioid clade relative to any of the other euagaric clades, including those in the primary ingroup of Peintner *et al.* (2001), and other cortinarioid agaric taxa. The size of the data matrix used (number of characters multiplied by the number of taxa) is given as the primary reason for this. Nevertheless, these works support the contention that relationships exist between sequestrate and agaricoid fungi as suggested by morphology, and propose some links that have not been previously suspected (*e.g.* the nesting of the Lycoperdales within the Agaricaceae).

These 'larger scale' studies place cortinarioid taxa in the euagaric clade, often close to clades containing representatives of the Hydnangiaceae and Pluteaceae (Binder & Hibbett 2002, Hibbett *et al.* 1997, Moncalvo *et al.* 2000, Figure 1). Other molecular studies, focussed on more specific target groups, indicate that sequestrate forms, including cortinarioid fungi, have been derived a number of times from within predominantly agaricoid clades, rather than representing an ancestral state (*e.g.* Peintner *et al.* 2001 cf. Singer 1986). Studies examining specific, purportedly cortinarioid taxa, have also effected changes in the taxonomy of the cortinarioid sequestrate fungi. For example, molecular technology has shown the Gautieriaceae to have affinities to the Gomphales and Phallales, leading to the separation of this family from the Hymenogasteraceae (Humpert *et al.* 2001).

Arguments for the inclusion of the *Descolea-Setchelliogaster-Descomyces* complex among the cortinarioid fungi have also been strengthened by molecular studies (Martin & Moreno 2001, Peintner *et al.* 2001 cf. the lack of connection between the /cortinarioid and /bolbitioid clades in Moncalvo *et al.* 2002). Peintner *et al.* (2001) provide a molecular phylogeny of the cortinarioid sequestrate fungi as defined by Bougher & Castellano (1993), Singer (1951), Singer & Smith (1963), Thiers (1984) and Thiers & Smith (1969), based on nuclear rDNA sequences from the Internal Transcribed Spacer (ITS) region. The Peintner *et al.* (2001) phylogeny supports the division of *Hymenogaster s. lat.* The phylogeny indicates that all the genera described in Bougher & Castellano (1993), except *Timgrovea* (which was not examined), were nested within their target group of cortinarioid taxa. This demonstrates the benefits of molecular analyses in indicating phylogenetic associations when morphological techniques have been inconclusive, for example, *Quadrispora* (cf. Bougher & Castellano 1993). Peintner *et al.* (2001) found *Hymenogaster s. str.* to be most closely related to species of *Hebeloma* and *Naucoria*. This supports statements by Bougher & Castellano (1993) indicating that *Hymenogaster* was not the most reduced form of the *Cortinarius-Thaxterogaster* complex. Peintner *et al.* (2001) also support the contention that *Cortinarius* itself is paraphyletic, and indicate that distinct lineages within *Cortinarius* (sometimes given the rank of subgenera *e.g. Myxacium, etc.*), and related genera, have given rise to different sequestrate forms, as suggested by Bougher & Castellano (1993). Peintner *et al.* (2002) collapsed *Thaxterogaster* into *Cortinarius* citing the ITS phylogenies of Peintner *et al.* (2001) that supported earlier theories that *Thaxterogaster* is polyphyletic (*e.g.* Horak & Moser 1965, Moser 1964). It now seems likely that a range of sequestrate taxa will be aligned to subgenera of *Cortinarius* and other cortinarioid agaricoid taxa. Indeed this process has already begun with the recombination of *Cortinarius globuliformis* as *Dermocybe globuliformis* (Bougher & Malajczuk 1986, Bougher & Trappe 2002). Integrating studies examining smaller numbers of taxa, with each other and with larger studies such as Moncalvo *et al.* (2002), should provide better resolution of interrelationships between diverse taxa, including the cortinarioid sequestrate fungi.

Various classical and molecular classifications have suggested that a number of lineages of sequestrate fungi arose from among the agaricoid allies of *Cortinarius* and from within this genus (Figure 1). Similarly, different authors have considered these same sequestrate fungi to be related to quite distinct agaricoid or boletoid fungi. The current edition of the *Dictionary of the Fungi* (Kirk *et al.* 2001) acknowledges the discoveries in molecular technology yet makes few linkages between cortinarioid agarics and related sequestrate genera. For example, the *Dictionary* includes *Protoglossum* in the Hymenogasteraceae (Boletales) and places *Descolea* and *Setchelliogaster* in different families of the Agaricales—Cortinariaceae and Bolbitiaceae respectively (Kirk *et al.* 2001 cf. Bougher & Castellano 1993, Lago *et al.* 2001, Peintner *et al.* 2001). Indeed Singer & Smith (1959) note similarities between *Setchelliogaster* and some genera of the Bolbitiaceae such as *Conocybe*. Neither Peintner *et al.* (2001) nor Moncalvo *et al.* (2002) attempt to thoroughly integrate their molecular phylogeny with current

taxonomy and nomenclature. Instead, clades comprised exclusively of currently named taxa are generally recognised under the accepted name for that group, while groups containing combinations of currently named taxa are given informal names indicated by specified differences in how they are written (for example the '/' sign is used in Moncalvo *et al.* 2002). In this paper, we too have chosen to delay comprehensive reassessment of the systematics and nomenclature of the cortinarioid sequestrate fungi, pending more conclusive integrated morphological and molecular examinations.

Collection and study of Cortinarioid sequestrate fungi in Australia

Early collection and study of Australian Cortinarioid sequestrate fungi

The infrequent collection of Australian sequestrate fungi in the early 19th century began to change with increasing involvement of resident collectors, mycologists and Australian government departments and universities. The private collector J. Drummond, working in Western Australia from 1828 to 1863, collected at least one fragment of a *Hymenogaster* species, sent to Berkeley and now lodged at K. However, owing to poor preservation it cannot be identified (Hilton 1983). Working in the Victorian Department of Agriculture, McAlpine (1895) published a comprehensive bibliography and systematically arranged checklist of the known Australian fungi incorporating six orders of Gasteromycetes, including the Hymenogasteraceae. The Hymenogasteraceae (*sensu* McAlpine 1895) included three species *Hymenogaster* and four other genera. Collection and classification of Australian fungi were also advanced by the work of R. Rodway in Tasmania. Rodway (1912a, b) compiled all known Australian species considered as Hymenogasteraceae *s. lat.* including four other genera (13 spp.) alongside *Hymenogaster s. lat.* (six spp.). Rodway (1912a) refuted Cooke's statement (Cooke 1892) that Australia had few hypogeous sequestrate fungi and concluded that Australia indeed had a rich hypogeous fungal flora. In subsequent publications Rodway added a further seven *Hymenogaster* species to this list (Rodway 1918, 1919). The increase in the number of species found is illustrated by comparing the number of species of Tasmanian Hymenogasteraceae in the work of 1912 with the species list from Rodway (1923). In this later work there were eight *Hymenogaster* and 28 other sequestrate species in five other genera, not including four suppressed names.

The 20th century to 1970

During the 20th Century interest in fungi as pathogens, symbionts, and as of importance to medicine, overtook colonialist exploration and biodiversity inventory as the primary rationale behind mycological research in Australia (May 2001). J.B. Cleland, a resident collector and taxonomist, contributed significantly to the knowledge of sequestrate fungi in Australia with the publication of his *Toadstools and Mushrooms and other Larger Fungi of South Australia* in 1934. The section on Gasteromycetes was based largely on the work of Cunningham and the author's own extensive writings (in particular the 'Australian Fungi' series *e.g.* Cleland & Cheel 1919, 1923). Cleland incorporated in the order Hymenogastrales, seven other genera (30 spp.) alongside *Hymenogaster* (10 spp.) and *Dendrogaster* (three spp.). In 1944, Cunningham published *The Gasteromycetes of Australia and New Zealand*. In this work, two families, the Hymenogasteraceae and the Secotiaceae, made up the Hymenogastrales, one of the five orders of the class Gasteromycetes. *Hymenogaster* (11 species) along with two other genera (14 spp.) made up the Hymenogastereae of the Hymenogastroideae. Cribb, working at the University of Queensland, wrote a series of papers on various genera considered to belong to the Hymenogastrales in which she included six *Hymenogaster* species (Cribb 1956).

Collection and study of Cortinarioid sequestrate fungi since 1970

Recognition of the role of fungi in ecosystems increased during the 1970s and 1980s. This provided a further impetus for research into the distribution, ecology, biology and systematics of fungi, including cortinarioid sequestrate forms. As awareness of the need to collect specimens of Australian fungi developed, and collections were made, the magnitude of Australia's sequestrate fungal flora became increasingly evident. Activity in collecting and defining sequestrate fungal taxa increased markedly during the 1980s. Systematic mycology and the collection of sequestrate macrofungi in Australia benefited from visits by such overseas mycologists as E. Horak, R. Petersen, G. Samuels, J. Trappe and co-workers, and R. Watling around this period (May 2001).

This effort has lead to the acquisition of high quality herbarium collections including those held at the CSIRO Mycology Herbarium, Floreat, Perth Western Australia, CANB, DAR, MEL, and OSU. Increased research also resulted in an expansion of the literature on cortinarioid sequestrate fungi. Both the 'Gasteroid Basidiomycota of Victoria state' and 'Australasian truffle-like fungi' series included papers on cortinarioid sequestrate fungi (Beaton *et al.* 1985a, Castellano & Trappe 1990, 1992). In 1982, an extensive program of collecting sequestrate

fungi was initiated by Malajczuk and Trappe in south-western Australia as a preliminary study of the diversity of ectomycorrhizal fungi with possible applications to mycorrhizal inoculation of *Eucalyptus* species in plantations and mine-spoil reclamation (Lebel & Castellano 1999). The team of scientists responsible for the study was later expanded to include Castellano, Reddell and Bougher for collecting throughout Australia including the Northern Territory, Queensland, Tasmania and Western Australia (Bougher pers. comm., Lebel & Castellano 1999). The CSIRO Forestry and Forest Products Mycology Herbarium, Perth, Western Australia established as a result of this work and parallel work on epigeous fungi, currently has approximately 5000 collections of sequestrate fungi. Of these approximately 20 per cent are cortinarioid sequestrate forms, a proportion which may approximate the abundance of these fungi in Australian ecosystems relative to other sequestrate fungi. Beaton *et al.* (1985a, b) dealt with the sequestrate Cortinariales of Victoria (Australia) including two *Setchelliogaster* and seven *Thaxterogaster* species in the Cortinariaceae and 10 *Hymenogaster* species in the Hymenogasteraceae. Growing interest in sequestrate fungi, and an increasing number of collections and publications produced on the subject, provided the impetus for the reassessment of *Hymenogaster s. lat.* by Bougher & Castellano (1993). Castellano & Trappe (1990) and Trappe *et al.* (1996) published a nomenclatural bibliography of Australian sequestrate fungi. In 1990 they included one sequestrate *Cortinarius*, four *Cribbea*, 37 *Hymenogaster*, one *Protoglossum*, one *Rozites* (originally described as a *Thaxterogaster*), five *Setchelliogaster* and 48 *Thaxterogaster* species. In 1996 they added a further four *Timgrovea*, three *Descomyces*, six *Cortinomyces*, and two *Quadrispora* species (including synonyms) (Castellano & Trappe 1990, Trappe *et al.* 1996).

In recent years a focus on mycophagy among Australia's native marsupials has also provided significant stimulus to mycology, especially the study of sequestrate fungi, and raised the profile of these fungi among ecologists and forest managers (*e.g.* Claridge *et al.* 1996). Seven cortinarioid sequestrate genera were listed by Claridge *et al.* (1996) as providing food for animal mycophagists. Australian sequestrate fungi have featured in several other major works including Grgurinovic's *Larger Fungi of South Australia* which was published in 1997 based on a re-examination of Cleland's specimens. This work included one *Setchelliogaster* species and two *Thaxterogaster* species (Cortinariaceae) and one species now considered to be non-cortinarioid, in the Hymenogastrales. Bougher & Syme (1998) produced *Fungi of Southern Australia*, featuring 125 species of macrofungi and emphasising the relationship of sequestrate fungi to agaricoid forms. This work featured one *Cortinarius* (now *Dermocybe*), one *Descomyces*, and two *Thaxterogaster* species.

Concurrently with rising awareness of the roles of fungi in ecosystems, molecular technology has emerged as a promising tool for the investigation of fungal taxonomy and ecology. Linking identified fungi to molecular sequences from mycelium in the soil or on roots is potentially a major tool for integrating molecular distribution/association data into our understanding of ecosystem functioning. This technology is already being applied for fungi of Australian ecosystems (*e.g.* Glen *et al.* 2001). The ability to identify fungi from vegetative mycelium is an especially promising development for the study of sequestrate fungi because of the difficulty of finding their often hypogeous fruitbodies. Of the 16 sequestrate genera considered cortinarioid by the current authors (Table 2), 11 occur in Australia, and three are Australasian endemics. There are currently 207 species of cortinarioid sequestrate fungi (as designated in Table 2) listed for the world (CABI Bioscience Databases) of which 39 (approximately 20 per cent) have been reported from Australia (Table 3). The current authors and many colleagues believe that Australia possesses numerous undiscovered species of cortinarioid sequestrate fungi.

Conclusions

Recognition of sequestrate members of the Cortinariaceae and Cortinariales has been a relatively recent development. For much of the time between the naming of *Cortinarius* (Persoon 1801) and the naming of *Thaxterogaster* (Singer 1951), it was thought that the gasteromycetes were a separate lineage from the hymenomycetes, rather than a polyphyletic assemblage arising more than once from among hymenomycete ancestors. This perspective continues to blur taxonomic concepts classically based on basidiome morphology, the genetic control of which appears relatively simple and potentially subject to frequent mutation (Bruns *et al.* 1989, Peintner *et al.* 2001). Reassessments of morphological and molecular characteristics uniting sequestrate taxa to *Cortinarius* and allied agarics have supported the view that both the Cortinariaceae and the Hymenogasteraceae are polyphyletic (Bougher & Castellano 1993, Peintner *et al.* 2001). However, the conceptual framework and historical taxonomic legacy continues to influence the classification of cortinarioid sequestrate genera (cf. Bougher & Castellano 1993, Kirk *et al.* 2001).

Table 3. Published Australian cortinarioid sequestrate fungi (taken from May & Wood 1997 unless otherwise indicated). [i] Bougher (1997), [ii] Castellano *et al.* (2000), [iii] Bougher & Trappe (2002). Peintner *et al.* (2002) synonymised *Thaxterogaster* with *Cortinarius*; however, we have retained the genus name for the purposes of this table. The specific epithets of the feminine generic names *Hymenogaster* and *Thaxterogaster* (Peintner *et al.* 2002) have been corrected in accordance with the current International Code of Botanical Nomenclature (Art. 23.5, or 32.5, or 60.1, Greuter *et al.* 2000). May & Wood (1997) also list un-identified specimens of the genera: *Destuntzia*, *Hymenogaster*, *Protoglossum*, *Setchelliogaster* and *Thaxterogaster*, that are not listed below.

Name and Reference
Aroramyces gelatinosporus (Cribb) Castellano, *Kartsenia* 40: 13 (2000)[I]
Cribbea gloriosa (D.A. Reid) A.H. Sm. & D.A. Reid, *Mycologia* 54: 99 (1962)
C. lamellata (J.W. Cribb) A.H. Sm. & D.A. Reid, *Mycologia* 54: 101 (1962)
C. reticulatum (J.W. Cribb) A.H. Sm. & D.A. Reid, *Mycologia* 54: 101 (1962)
Dermocybe globuliformis (Bougher) Bougher & Trappe, *Australasian Mycologist* 21 (1): 1–3 (2002)[ii]
Descomyces albellus (Massee & Rodway) Bougher & Castellano, *Mycologia* 85: 282 (1993)
D. albus (Klotzsch) Bougher & Castellano, *Mycologia* 85: 280 (1993)
Hymenogaster aurea Rodway, *Pap. & Proc. Roy. Soc. Tasmania* 1923: 152 (1924)
H. fuliginea G. Cunn., *New Zealand J. Sci. Technol., ser. B,* 22: 299 (1941)
H. lycoperdinea Vittad., *Monogr. Tuberac.* 22 (1831)
H. monospora E.L. Stewart & Trappe, *Trans. Brit. Mycol. Soc.* 65: 331 (1975)
H. nana Massee & Rodway *in* Massee, *Bull. Misc. Inform. Kew* 1899: 180 (1899)
H. nana Massee & Rodway f. ***gilva*** Rodway, *nom. inval.*, Art. 32.1
H. nana Massee & Rodway f. ***globosa*** Rodway, *nom. inval.* Art. 32.1
H. tasmanica (G. Cunn.) G.W. Beaton, Pegler & T.W.K. Young, *Kew Bull.* 40: 590 (1985)
Mycoamaranthus auriobis Castellano, Trappe & Malajczuk, *Austral. Syst. Bot.* 5: 613 (1992)
Protoglossum cribbiae (A.H. Sm) T.W. May, *Muelleria* 8: 287 (1995)
P. luteum Massee, *Grevillia* 19: 97 (1891)
P. purpureum (J.W. Cribb) T.W. May, *Muelleria* 8: 288 (1995)
P. violaceum (Massee & Rodway) T.W. May, *Muelleria* 8: 288 (1995)
P. viscidum (Massee & Rodway) T.W. May, *Muelleria* 8: 288 (1995)
Quadrispora musispora Bougher & Castellano, *Mycologia* 85: 286 (1993)
Q. oblongispora (G.W. Beaton, Pegler & T.W.K. Young) Bougher & Castellano, *Mycologia* 85: 286 (1993)
Setchelliogaster australiensis G.W. Beaton, Pegler & Young, *Kew Bull.* 40: 169 (1985)
S. tenuipes (Setch.) Pouzar, *_eská Mykol.* 12: 34 (1958)
Thaxterogaster brunnea G.W. Beaton, Pegler & T.W.K. Young, *Kew Bull.* 40: 174 (1985)
T. basipurpurea Bougher, *Mycotaxon* 63: 43 (1997)[I]
T. campbelliae (Berk. & Broome ex Zeller & C.W. Dodge) G.W. Beaton, Pegler & T.W.K. Young, *Kew Bull.* 40: 173 (1985)
T. cunninghamii E. Horak, *Beih. Nova Hedwigia* 43: 92 (1973)
T. leucocephala (Massee) Singer & A.H. Sm., *Brittonia* 10: 210 (1958)
T. levispora (Massee & Rodway) G.W. Beaton, Pegler & T.W.K. Young, *Kew Bull.* 40: 172 (1985)
T. luteirufescens Bougher, *Mycotaxon* 63: 44 (1997)[I]
T. piriformis (Cleland & G.Cunn.) M.M.Moser *in* Horak & Moser, *Nova Hedwigia* 10: 223 (1956)
T. porphyrea (G. Cunn.) Singer, *Lilloa* 26: 105 (1953)
T. redacta G.W. Beaton, Pegler & T.W.K. Young, *Kew Bull.* 40: 177 (1985)
T. scabrosa (Cooke & Massee) A.H. Sm. & Singer *in* Singer & Smith, *Madroño* 17: 23 (1963)
Timgrovea ferruginea (J.W. Cribb) Bougher & Castellano, *Mycologia* 85: 290 (1993)
T. macrospora (G. Cunn) Bougher & Castellano, *Mycologia* 85: 289 (1993)
T. reticulata (G. Cunn) Bougher & Castellano, *Mycologia* 85: 289 (1993)
T. subtropica (J.W. Cribb) Bougher & Castellano, *Mycologia* 85: 290 (1993)

The incorporation of classical taxonomic methods with molecular technology has the potential to elucidate natural relationships between taxa while also yielding readily applicable information for studying the biology of the cortinarioid sequestrate fungi. However, the placement of these fungi within the euagaric clade is currently unresolved. A number of purportedly cortinarioid taxa have not yet been included in any published molecular phylogeny. For the cortinarioid sequestrate fungi, the integration of molecular phylogenetics with classical nomenclature is just beginning. Integrating molecular and morphological, chemical, and ecological information in combined analyses has the potential to advance understanding of the currently unresolved systematics of the cortinarioid sequestrate fungi.

The history of collection for the cortinarioid sequestrate fungi has been influenced by the nature of these organisms and the changing focus of human investigations. The collection and study of these fungi in Australia has been sporadic, and remains far from complete. It is probable that there are many more than the current 39 recorded species in Australia. They may be substantial components of important ecological guilds within many Australian ecosystems as mycorrhizal associates and food sources for animal mycophagists in addition to providing other environmental services such as decomposition, nutrient capture and cycling. Further research is needed to fill the large gaps in our knowledge concerning the interrelationships of sequestrate and agaricoid cortinarioid genera, their distribution, associations and function in order to adequately assess their role in Australian ecosystems.

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CORTINARIOID SEQUESTRATE (TRUFFLE-LIKE) FUNGI OF WESTERN AUSTRALIA

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Abstract

Revised descriptions are presented of nine previously published cortinarioid sequestrate species, and four new species, occurring in Western Australia. A key to the Western Australian species is also included. The new species, *Descomyces angustisporus*, *Quadrispora tubercularis*, *Cortinarius walpolensis* and *Cortinarius sebosus* are not known to occur outside Western Australia. The Western Australian species represent five of the 11 genera, and nine of the 39 cortinarioid sequestrate species previously known to occur in Australia. In view of the broad distribution of some cortinarioid sequestrate species in Australia, and large areas of the continent poorly explored for these fungi, the current authors believe that the species presented here represent only a portion of the cortinarioid sequestrate fungi present in Western Australia. It is likely that many other species are yet to be collected and/or named.

A.A. Francis & N.L. Bougher (2004). Cortinarioid sequestrate (truffle-like) fungi of Western Australia. *Australasian Mycologist* 23 (1): 1–26.

Introduction

Sixteen genera constitute the informal grouping 'cortinarioid sequestrate fungi' defined as including taxa of sequestrate fungi that at some time, have been placed in either the Cortinariaceae or the Hymenogasteraceae and, as yet, have not been shown to have stronger affinities with taxa outside these families (Francis & Bougher 2003). Sequestrate fungi are those in which the spores mature inside an enclosed fruitbody, remaining there until the fruitbody decomposes or is eaten (Bougher & Lebel 2001). The term sequestrate fungi therefore incorporates fruitbody forms historically described as secotioid or gasteroid as well as agaricoid forms in which the persistent partial veil encloses the hymenophore. A number of sequestrate fungal taxa have been allied with the genus *Cortinarius* and its mushroom-like allies on the basis of various combinations of characteristics including: spore structure, pigmentation and ornamentation; basidiome structure, pigmentation and development; and, similarity in molecular sequence data (*e.g.* Bougher & Castellano 1993, Moncalvo *et al.* 2002, Peintner *et al.* 2001, Singer 1951). Australia is currently known to have 11 genera of these fungi. Six of the genera are now known to occur in Western Australia.

As discussed in a previous paper (Francis & Bougher 2003) the taxonomy of the sequestrate cortinarioid fungi is in a state of flux with a number of authorities placing taxa included under our definition in other more distantly related families within the Agaricales. We will discuss, for the genera dealt with in this paper, the reasons for their inclusion as sequestrate cortinarioid fungi. Also, the acceptance of some of the genera of cortinarioid sequestrate fungi is contentious. For example, rDNA Internal Transcribed Spacer (ITS) sequences (Peintner *et al.* 2001) support the hypothesis that *Thaxterogaster* is polyphyletic. Accordingly, Peintner *et al.* (2002b) synonymised *Thaxterogaster* under *Cortinarius*. Molecular evidence from other studies also indicates that *Cortinarius* itself is paraphyletic (*e.g.* Peintner *et al.* 2002a). The *Cortinarius* species detailed in this paper are all secotioid (*sensu* Francis & Bougher 2003), that is with the peridium *not* pileate/expanded but more or less globose enclosing the hymenophore.

The history of collection of sequestrate fungi in Western Australia includes some collections of 19th century Australian naturalists such as James Drummond and a large number of more recent collections held in herbaria at the CSIRO Mycology Forestry and Forest Products Herbarium Perth, OSU, MEL and PERTH. These collections have arisen as a result of more general and often Australia-wide research into the sequestrate fungi. This research

has been summarised by Lebel & Castellano (1999), May (2001) and, with particular focus on the sequestrate cortinarioid fungi, by Francis & Bougher (2003) and the reader is referred to those references for a more in-depth treatment of the history surrounding the collection of sequestrate fungi in Australia. Works detailing the Western Australian sequestrate cortinarioid fungi include Bougher & Syme (1998) which includes colour illustrations of the cortinarioid sequestrate fungi *Dermocybe globuliformis*, *Descomyces albus*, *Cortinarius luteirufescens* and *C. basipurpureus* (the latter two as *Thaxterogaster*). Large areas of Western Australia are poorly explored for sequestrate fungi, and it is likely that many more species of cortinarioid sequestrate fungi remain to be found and/or named. The collections available to the current authors have mostly come from forest regions in the south west of Western Australia and from areas extending inland into lower rainfall woodland regions. In this paper we present revised descriptions of species of cortinarioid sequestrate fungi known to occur in Western Australia, including nine previously published species and four new species.

Methods

Macroscopic characters were described for fresh specimens in the field, which were subsequently air dried as herbarium vouchers. Microscopic examination was carried out with both fresh (when available) and herbarium material. We examined collections lodged mainly at PERTH, MEL, OSU, the Mycology Herbarium, CSIRO FFP, Perth, Australia (coded as 'H' or 'E' numbers) and the working collection of Prof. J. Trappe (coded as 'Trappe' numbers). Collections coded as 'Trappe' numbers are all eventually to be lodged in public herbaria. These have been indicated in brackets after the number. Colour codes for macroscopic features are from Kornerup & Wanscher (1978). Line drawings and measurements of spores in 3% KOH were made with the aid of an Olympus BH2–DA drawing attachment. Congo red was applied to hyaline structures revived in 3% KOH (*e.g.* basidia). Spore measurements include the hilar appendix but not the ornamentation or perisporium. Shapes are according to Kirk *et al.* (2001). Spores have been drawn at 2000× magnification and other elements at 1000× magnification; figures illustrating both spores and other elements have separate bars indicating 10 µm at the relevant scales.

Results

More than 160 collections of cortinarioid sequestrate fungi from Western Australia were at hand for this work representing a range of morphologically defined taxa. Thirteen species are treated in this paper. Nine previously published species are listed here as occurring either naturally or in plantations/plantings within Western Australia. Of these nine, two (*Cortinarius luteirufescens* and *C. basipurpureus*) are currently known only from Western Australia as are the four new species described in the paper. Additionally, a number of other species (potentially four to eight) are represented in existing collections of Western Australian fungi. However, owing to the poor condition or limited number of collections and absence of macromorphological data, these have not been treated in this paper. Definition of the additional species awaits adequate supporting specimens and data.

As one might expect, different species of sequestrate cortinarioid fungi appear to exhibit different distributions. Sampling for sequestrate fungi has not been exhaustive and large areas of the State remain unexplored for these fungi (and fungi in general). However, based on the data currently available to us, we relate some tentative patterns. In Western Australia most collections of sequestrate cortinarioid species appear to have been centralised on the southern coast, with two of the new species described in this paper coming from further north around the Kellerberrin area—Western Australia wheatbelt. It must be noted that these locations coincide with major focal areas of collecting effort by the CSIRO Forestry and Forest Products mycology group and colleagues. Despite this obvious weakness in the sampling design, it appears that *Descomyces angustisporus* Francis & Bougher *sp. nov.* does to some extent replace *D. albus* and *D. albellus* towards the wheatbelt region. *Cortinarius sebosus* represents the only secotioid *Cortinarius* (*Thaxterogaster*) to be found so far in this drier, more northerly wheatbelt region. Also, the area from Walpole to Two Peoples Bay also appears to be a centre of diversity for sequestrate cortinarioid fungi in Western Australia. Further extensive, well-planned sampling is required if a more accurate picture of the biological resources inherent in our fungal biodiversity is to be better understood.

Key to genera of cortinarioid sequestrate fungi currently recorded from Western Australia. Note: Numbers next to genus names in the key refer to the order in which the genera are treated in this paper.

1 Spores with a smooth, rostrate apex.
  2 Basidiomes secotioid; spores prominently asymmetrical. **6. *Setchelliogaster*** (only species currently described from Western Australia, *S. australiensis*)
  2: Basidiomes gastroid; spores more or less symmetrical. **3. *Descomyces***
1: Spores with a rounded, ornamented apex.
  3 Spores retained in tetrads after release from the basidium. **5. *Quadrispora***
  3: Spores not retained in tetrads after release from the basidium.
    4 Basidiomes pileate; pileus expanded; hymenophore covered by persistent partial veil; pileus, stipe and surrounding conspicuous mycelium bright yellow. **2. *Dermocybe*** (only species currently described from Australia, *D. globuliformis*)
    4: Basidiomes not as above.
      5 Basidiomes with a truncate to percurrent (may be dendroid) stipe/columella. **1. *Cortinarius***
      5: Basidiomes with columella lacking to truncate, not percurrent. **4. *Protoglossum***

The six genera included in this work as sequestrate cortinarioid fungi are *Setchelliogaster* Pouzar, *Descomyces* Bougher & Castellano, *Quadrispora* Bougher & Castellano, *Cortinarius* (Pers.) Gray, *Dermocybe* (Fr.) Wünsche and *Protoglossum* Massee. All of these genera fall within the Cortinariaceae as accepted by Peintner *et al.* (2001). In addition, the genera *Setchelliogaster* and *Descomyces*, like *Descolea*, lack the true germ pore characteristic of the Bolbitiaceae in which they were placed by Kirk *et al.* (2001) (Bougher 1987, Bougher & Castellano 1993) and their position among the cortinarioid fungi has been supported by other molecular studies (Martin & Moreno 2001). *Thaxterogaster* has recently been reduced to synonymy under *Cortinarius* by Peintner *et al.* (2002b). Similarities between the two genera had been noted since the first description of *Thaxterogaster* (Singer 1951) and the polyphyly of *Thaxterogaster* had also been suspected (*e.g.* Horak & Moser 1965, Moser 1964). Bougher & Castellano (1993) discuss the links between *Protoglossum* (*Cortinomyces*) and *Cortinarius*. No conclusions were given in that paper as to the placement of *Quadrispora*, however, the current authors consider basidiome form and spore structure (in all but symmetry) to be sufficiently similar to that of *Cortinarius* (*Thaxterogaster*) and *Protoglossum* to warrant including *Quadrispora* as a sequestrate cortinarioid fungus. *Dermocybe* was initially described as a subgenus of *Cortinarius* (chiefly distinguished by pigment characteristics) and forms a monophyletic group of fungi nested within *Cortinarius* (Chambers *et al.* 1999, Peintner *et al.* 2001). Further discussion on membership within our concept of cortinarioid sequestrate fungi is provided in Francis & Bougher (2002). Thus the main characters uniting the sequestrate cortinarioid fungi are spore characteristics (most commonly brown, ornamented spores which lack a true germ pore) coupled with basidiome characteristics (including pigmentation and peridiopellis structure) the interpretation of which are supported by the analysis of molecular characteristics.

1. *Cortinarius*

Key to species of secotioid *Cortinarius* currently recorded from Western Australia.

1 Mature peridium white to off-white. **4. *Cortinarius walpolensis***
1: Mature peridium not white to off-white.
  2 Spores 14–21 × 9–18 µm. **3. *C. sebosus***
  2: Spores smaller.
    3 Peridium yellow with slight orange tint, with orange-red stains; spores ovoid to ellipsoidal to oblong-ellipsoidal, 12–15 × 7.5–9 µm. **2. *C. luteirufescens***
    3: Peridium initially cream to pale tan becoming grey-brown or reddish/purplish brown or sometimes grey-violet; spores broad-ovoid to broadly pyriform 10–13 × 7.5–9 µm. **1. *C. basipurpureus***

1. *Cortinarius basipurpureus* (Bougher) Peintner & M.M. Moser, *Mycotaxon* 81: 178 (2002b) (Figure 1, Plate 2F)

= *Thaxterogaster basipurpureum* Bougher, *Mycotaxon* 63: 43 (1997).

Basidiomes hypogeous, fruiting singly or in groups, under litter, gastroid, 5–15 × 8–25 mm, globose, ellipsoidal to pyriform, often pleated around the stipe. *Peridium* initially cream to pale tan (4B4–5B4) becoming grey-brown or reddish/purplish brown (6F7–7F7 or 10E5) or sometimes grey–violet (duller than 17B3), with a layer of thick non-glutinous slime overlying the peridial surface that is covered in small lumps, not bruising, often with adhering debris, thick (approx. 1 mm), of two layers, outer layer broader and gelatinised, inner layer greyish in section and not gelatinised. *Gleba* loculate, cream when young maturing to dark brown (6E8–7F8), dry, not rapidly disintegrating after maturity, locules to 1 mm broad, empty, may be distinctly radially arranged. *Columella/sterile tissue* a percurrent to truncate stipe, columella to 6 mm broad, stipe to 3 mm long by 5 mm wide, central, terete, tapering, whitish to cream, dry, but with a viscous gelatinous purple collar at junction of peridium and stipe, minutely pubescent, of one layer, solid, base tapering; basal mycelium whitish, inconspicuous. *Macrochemical tests* 15% KOH very dark brown on gleba, $FeSO_4$ no reaction to dull brown on gleba. *Odour* similar to camphor or mothballs, *taste* not distinctive.

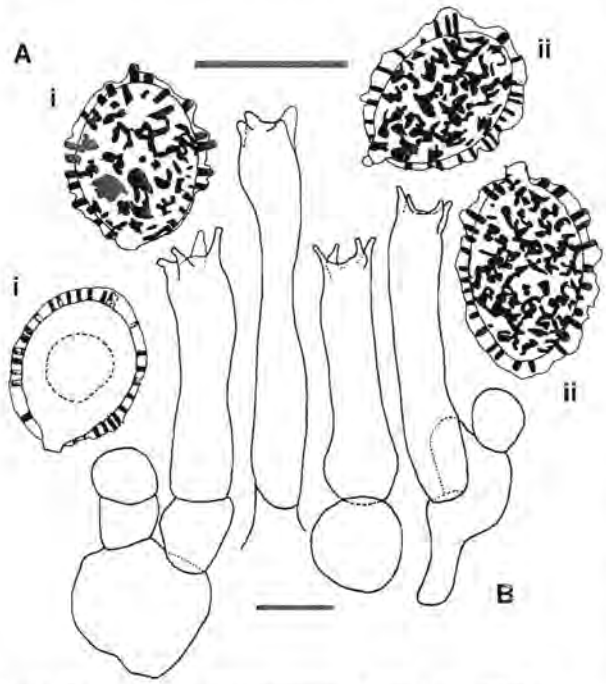


Figure 1. *Cortinarius basipurpureus*. **A** Spores i H6684. ii H62236. Scale bar = 10 µm. **B** Basidia, hymenial and subhymenial elements H6236. Scale bar = 10 µm.

Spores bright brown (KOH), axially symmetrical, more or less orthotropic, broad-ovoid to broad-pyriform, (10–) 10.5–12.5 (–13) × 7.5–9 µm mean of 30 spores 11.0 × 8.5 µm, Q = 1.39-1.82, 1.62 ± 0.1 (KOH), ornamented with rods and short ridges, ornaments to 2 × 2 µm in profile appearing irregular and quite densely arranged in face view; perisporium yellowish (KOH), obvious, adhering closely to the ornamentation; spores not aggregating; hilar appendix to 2 µm broad, truncate, entire; spores thin-walled, inamyloid, non-dextrinoid; the apex rounded and ornamented. *Basidia* hyaline, cylindrical to cylindro-clavate, 4-spored, 33–65 × 7–11 µm. *Cystidia* absent. *Hymenium* palisade, with hyaline, non-gelatinised, clavate (although sometimes becoming globose or pyriform after the basidia collapse), thin-walled, elements to 25 × 35 µm. *Subhymenium* approximately 10 µm broad, largely undifferentiated, a layer of hyaline, non-gelatinised, more or less cylindrical, thin-walled, approximately 5–8 µm broad hyphae. *Hymenophoral trama* parallel, hyaline or yellow-encrusted, non-gelatinised, more or less cylindrical, thin-walled, 3–15 µm broad hyphae. *Peridiopellis* (in longitudinal section) of two layers, outer broader layer: loosely interwoven in a hyaline matrix, hyaline, gelatinised and intact, cylindrical, generally thin-walled (although some hyphae with faint annular wall thickenings, walls to 0.5 µm thick or small (< 0.5 µm tall), rounded, undulating interior wall projections), 2–7 µm broad hyphae; inner layer: interwoven to subparallel, of hyphae encrusted with concentrated clusters of a bright golden yellow (KOH) pigment, non-gelatinised, inflated (sometimes appearing as polygonal cells), thick-walled, 4–10 µm broad. *Clamp connections* absent.

Habitat and distribution: Abundant throughout coastal south Western Australia in a variety of soils under *Eucalyptus* woodlands and *Gastrolobium* thickets in association with mycorrhizal plants including *Gastrolobium* sp., *Eucalyptus jacksonii*, *Corymbia* [*Eucalyptus*] *calophylla*, *Agonis flexuosa* and *Allocasuarina decussata*. Fruiting June–August.

Collections examined: **W.A.**: Two Peoples Bay Nature Reserve, Hakea Gully, in thicket dominated by *Gastrolobium* sp., 31 July 1995, coll. *A. Danks s.n.* holotype PERTH 0425629, isotype H7302. Upper Hakea,

Two Peoples Bay Nature Reserve, under *Gastrolobium*, 31 July 1995, coll. *Alan Danks s.n.* H0891. Lower Hakea, Two Peoples Bay Nature Reserve, under *Gastrolobium*, 2 Aug. 1995, coll. *Alan Danks s.n.* H0892. Walpole-Nornalup National Park, The Knoll Drive, parking area, Walpole, under *Corymbia* [*Eucalyptus*] *calophylla*, *Agonis flexuosa*, 6 June 1992, coll. *K. Syme & N. Bougher s.n.* H6235. Walpole-Nornalup National Park, Shedley Drive, under *Eucalyptus jacksonii* and *Allocasuarina decussata*, 6 June 1992, coll. *N. Bougher & K. Syme s.n.* H6236. Firebreak Gully, Two Peoples Bay, under *Agonis flexuosa*, 21 Aug. 1992, coll. *K. Syme s.n.* H6255. Cemetery Road, Walpole-Nornalup National Park, under *Eucalyptus jacksonii*, 13 July 1994, coll. *T. Lebel & D. Brown s.n.* H6672. Corner of Thomson and Rate Roads, Walpole-Nornalup National Park, under *Eucalyptus jacksonii*, 14 July 1994, coll. *D. Brown, T. Lebel & N. Bougher s.n.* H6684. Hilltop Road Walpole-Nornalup National Park, 15 July 1994, coll. *N. Bougher s.n.* H7003. Two Peoples Nature Reserve, Hakea Gully, in thicket dominated by *Gastrolobium* sp., 31 July 1995, coll. *A. Danks s.n.* H7303.

Etymology: In reference to the purple colour of the flesh at the base of the stipe.

Discussion: Macroscopically, the characteristic purple flesh at the base of the stipe readily distinguishes *Cortinarius basipurpureus* from *Protoglossum violaceum* (see discussion for that species). The peridium of *C. basipurpureus* is also usually darker, duller and with more brown tones than that of *P. violaceum*.

2. *Cortinarius luteirufescens* (Bougher) Peintner & M.M. Moser, *Mycotaxon* 81: 180 (2002b)
(Figure 2, Plate 2A)

= *Thaxterogaster luteirufescens* Bougher, *Mycotaxon* 63: 44 (1997).

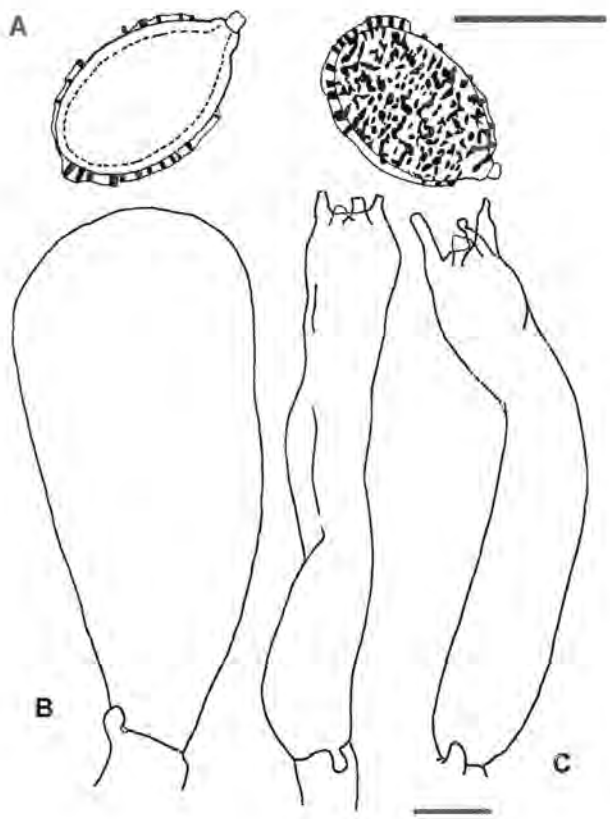


Figure 2. *Cortinarius luteirufescens* H6357. **A** Spores, scale bar = 10 µm. **B** Inflated hymenial element. **C** Basidia, scale bar = 10 µm.

Basidiomes hypogeous to subhypogeous, found growing singly, buried in soil under litter, gastroid, up to 20 mm diam., globose or broadly ellipsoidal, often pleated around the stipe. *Peridium* yellow with slight orange tint (4A6–4B7) with orange-red (6B8 or 7B8) stains in small patches, covered in thick non-glutinous slime, smooth, not bruising, few adhering debris, thick (approx. 1 mm), of two layers, outer layer broader and gelatinised, inner layer whitish in section and not gelatinised. *Gleba* loculate, bright rich brown (7E8–8F7), dry, not rapidly disintegrating after maturity, large irregular locules to 0.5 mm broad, empty, not noticeably radially arranged. *Columella/sterile tissue* a truncate or percurrent stipe-columella, if truncate extending a third to half of the way to the peridium, if percurrent up to 2–3 mm broad within the gleba, stipe very short (up to 1 mm long), white in section, central, terete, tapering, pale dull yellow, dry, but with a viscous gelatinous red collar at junction of peridium and stipe, matted-fibrillose, no obvious layering, solid, base tapering or bulbous; basal mycelium not conspicuous. *Macrochemical tests* 15% KOH on gleba instantly brown darkening further within a few minutes, $FeSO_4$ not recorded. *Odour* and *taste* not distinctive.

Spores bright brown (KOH), sometimes slightly axially asymmetrical, orthotropic, ovoid to elliptical to oblong-elliptical in both face view and profile excepting some slightly

adaxially flattened spores, 12–15 × 7.5–9 µm mean of 20 spores 13.5 ± 1.0 × 8.5 ± 0.5 µm, Q = 1.44–1.81, 1.64 ± 0.1 (KOH), ornamented with rods and very short ridges, ornaments to 1 µm tall in profile appearing irregular and densely crowded in face view; perisporium pale yellowish (KOH), conspicuous, adhering closely to the ornamentation; spores not aggregating; hilar appendix to 2 µm, conspicuous, tapering to truncate, entire; spores thin-walled, inamyloid non-dextrinoid (orange-brown in Melzer's solution); apex rounded and ornamented. *Basidia* hyaline, cylindro-clavate, 4-spored, 50–83 × 8–16 µm. *Cystidia* absent. *Hymenium* palisade, of yellowish (KOH), non-gelatinised, clavate, ellipsoidal or apically attenuated, thin-walled, 25–70 × 5–38 µm hymenial elements. *Subhymenium* to 58 µm broad, pseudoparenchymatous, of hyaline, non-gelatinised, broad, thin-walled, to 15 µm broad hyphae and end-cells. *Hymenophoral trama* to 40 µm thick, parallel, of hyaline and brown-encrusted, non-gelatinised, cylindrical, thin-walled, 3–11 µm broad hyphae, with some oleiferous hyphae also present. *Peridiopellis* (in longitudinal section) of three layers, the outermost layer to 14 µm thick, interwoven to subparallel, of hyaline, gelatinised and non-gelatinised, narrow cylindrical, thin-walled (though sometimes with minutely peg-like hyaline wall ornamentation), 3–5 µm broad hyphae overlying a middle layer, to 14.5 µm thick, of parallel to subparallel hyphae heavily encrusted with a golden brown (in KOH) pigment, non-gelatinised, inflated (sometimes appearing as polygonal cells), thick-walled, to 22 µm broad (polygonal cells to 30 µm broad), overlaying the innermost layer which is broad (to 613 µm), of interwoven to subparallel, hyaline, non-gelatinised, more or less cylindrical, thin-walled, to 15 µm broad hyphae. *Clamp connections* present in hymenium, rare in peridium.

Habitat and distribution: Known only from two collections from southern Western Australia from among litter under *Corymbia* [*Eucalyptus*] *ficifolia* and *Corymbia* [*Eucalyptus*] *calophylla*. Found fruiting from June–August.

Collections examined: **W.A.**: Walpole-Nornalup National Park, Nut Road, under *Eucalyptus ficifolia*, 10 June 1993, coll. *N. Bougher s.n.* holotype PERTH 04259599, isotype H6357. Two Peoples Bay Nature Reserve, Firebreak Gully, under *Corymbia* [*Eucalyptus*] *calophylla*, 13 Aug. 1991, coll. *K. Syme KS331/91* H6254.

Etymology: Referring to the yellow, red-stained peridium of this species.

Discussion: *Cortinarius luteirufescens* is known from only two collections. This species may sometimes be confused with *Protoglossum luteum* because of variability in peridium colour and stipe-columella structure. These two, sometimes co-occurring, species are distinguished by the presence, in *Cortinarius luteirufescens*, of orange-red stains on the peridium and a collar of red slime around the stipe in combination with longer, narrower (ellipsoidal to oblong-ellipsoidal) spores, the ornamentation of which is more pronounced towards the apex of the spore. *Protoglossum luteum* by contrast lacks a glutinous collar and stains on its peridium (which may be much darker brown that that of *Cortinarius luteirufescens*), and has strongly globose to broadly ellipsoidal spores with relatively uniform spore ornamentation.

3. ***Cortinarius sebosus*** A.A. Francis & N.L. Bougher *sp. nov.*
(Figure 3, Plate 2B)

Inter *Cortinarios* (*sensu* Peintner *et al.* 2002b) hymenophoro incluso saltem usque ad fructificationis maturitatem sic pertans combinatione sporarum relative magnarum grosse ornatarum cum peridio eburneo luteo-griseo obscure purpureove leniter viscido et bulbo basali non concolori distinguenda.
Typus hic designatus PERTH 06234631.

Basidiomes hypogeous, fruiting in small groups within the first 15 cm of soil, secotioid, 12–25 × 10–14 mm, irregularly globose to subpyriform, margin inrolled and somewhat pleated around the base. *Peridium* cream (paler than 5B4), yellow- or greenish grey, or dull purple (near 17D3 to 18E4), slightly viscid when collected, smooth although initially covered with easily removable fibrils that make the peridium feel 'greasy' to the touch radially arranged from the base, not bruising, without much adhering debris, to 1 mm thick, of one layer, grey in longitudinal section. *Gleba* sublamellate of convoluted lamellae, initially white in button stage, then pale pinkish tan (near 6C3 to 6C4) maturing to bright brown (near 7E8), dry, not rapidly disintegrating after maturity, edges of tramal plates smooth and usually entire, lamellae rather loosely packed and radially arranged. *Columella/sterile tissue* a percurrent stipe-columella, to 7 mm broad inside the gleba, in longitudinal section white with a yellow tinge after being cut, central, terete, surface of base on mature specimens usually yellow (near 4A5) but may also be white, cream or orange, dry, smooth (though in some specimens conspicuously covered with white fibrils that extend onto the peridium and appear continuous with the partial veil), not forming

distinct layers, solid, base angular-bulbous, to 8 mm wide, protruding up to 3 mm below the peridium; partial veil inconspicuous but present between inrolled margin and stipe-columella, cortinoid, concolourous with overlying fibrils; basal mycelium inconspicuous. *Macrochemical tests* 15% KOH and $FeSO_4$ no reaction on peridium, flesh or the gleba. *Odour* is not distinctive and *taste* is slightly sour.

Spores yellow-brown (KOH), pale singly, symmetrical, orthotropic, ellipsoidal to subglobose or obovoid, 14–21 × 9–18 µm, mean of 62 spores 17.5 ± 1.5 × 12 ± 1.5 µm, Q = 0.99–1.63, 1.46 ±0.11 (KOH), ornamented with warts, rods and small ridges, ornaments to 1 µm tall in profile, ornaments irregular and quite isolated in face view; perisporium yellowish (KOH), conspicuous, usually adhering closely but may flare irregularly (perhaps as a consequence of non-uniform ornament height); mature spores not aggregating; hilar appendix to 1.5 µm, conspicuous, slightly tapering truncate, entire; spores thin-walled, inamyloid, non-dextrinoid (bright yellow brown in Melzer's solution); apex rounded or slightly flattened, ornamented. *Basidia* hyaline to slightly yellowish, clavate but may appear cylindro-clavate to broadly obspathulate if distorted by pressure/slide preparation, 2-spored, 37–55 × 8–13.5 µm. *Cystidia* absent. *Hymenium* a palisade of hyaline, non-gelatinised, clavate to ellipsoidal, thin-walled, to 19 × 11 µm broad clamped elements. *Subhymenium* 5–10 µm broad, undifferentiated, of yellowish, non-gelatinised, more or less cylindrical, often branching, thin-walled, 2.5–4.5 µm broad hyphae. *Hymenophoral trama* to 8.5 µm thick, subparallel to parallel, of hyaline, non-gelatinised, more or less cylindrical, thin-walled, 4–11 µm broad hyphae. *Peridiopellis* (in longitudinal section) of two layers, outer layer 75–200 µm thick, quite loosely interwoven, of hyaline, gelatinised and intact, cylindrical, thin-walled, very thin (approximately 1–2.5 µm broad) hyphae, inner layer 370–450 µm, more tightly interwoven, of yellow-pigmented, non-gelatinised, broad, thin-walled, 8–21.5 µm broad hyphae. *Clamp connections* present in the peridium and hymenophoral trama.

Habitat and distribution: Collected from Jarrahdale (average rainfall >1000 mm/yr) under *Eucalyptus marginata* and *Corymbia* [*Eucalyptus*] *calophylla* to Kellerberrin (average rainfall <400 mm/yr) under *Allocasuarina campestris*. Fruiting June and July.

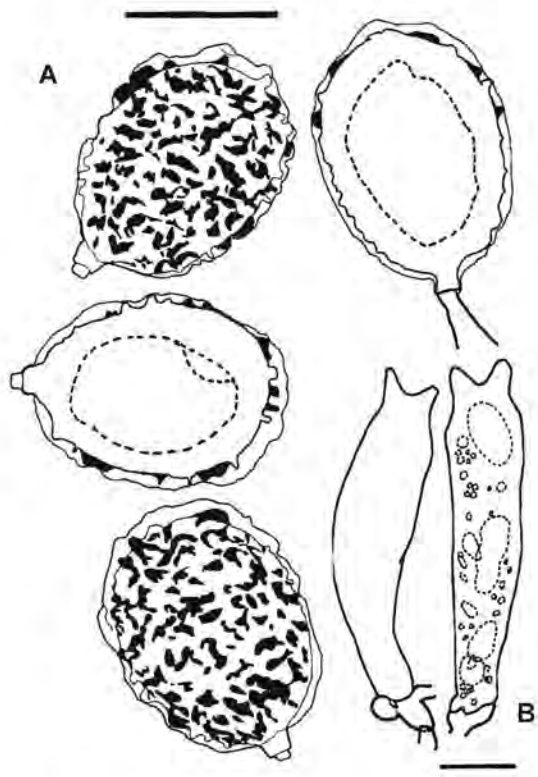


Figure 3: *Cortinarius sebosus* sp. nov. PERTH 06234631. **A** Spores, scale bar = 10 µm. **B** Basidia, scale bar = 10 µm.

Collections examined: **W.A.**: Hilltop between Site 17A and B, Higginson Road, 16 km along Bencubbin-Kellerberrin Rd, north of Kellerberrin, under *Allocasuarina campestris*, 5 July 1995, coll. *W. Dunstan s.n.* holotype here designated as PERTH 06234631, isotype H7265. Ashendon Road, next to a siding road, under *Eucalyptus marginata*, *Corymbia* [*Eucalyptus*] *calophylla*, 13 June 1992, coll. *N. Bougher, D. Xu, R. Hilton, K. Syme & R. Shultz s.n.* H6248. Hilltop site between Site 17A and B, Higginson Rd, 16 km along Bencubbin-Kellerberrin Rd, Kellerberrin, under *Allocasuarina campestris*, 29 June 1995, coll. *N. Bougher s.n.* H7250.

Etymology: In reference to the greasy fibrils initially covering the peridium of this species.

Discussion: *Cortinarius sebosus* is characterised by the combination of the large, coarsely ornamented spores, and short, white to orange basal 'bulb' that contrasts with the peridium. No other described species of secotioid *Cortinarius* has this combination of characters. *Cortinarius peraurantiacus* Peintner & M.M. Moser and *Cortinarius cartilagineus* (G. Cunn.) Peintner & M.M. Moser also possess small button-like stipe portions. However, these species have smaller spores than *Cortinarius sebosus* (13–15 × 7–9 µm and 14–15 × 8.5–10 µm respectively) and their stipes are concolourous with their peridia. The infrageneric relationships of *Cortinarius sebosus* are currently undetermined; however, a study is being undertaken by

the current authors to assess the phylogenetics of the Australian sequestrate cortinarioid fungi including this species. It should be noted that the term 'greasy' in reference to the peridial fibrils (hence '*sebosus*'), does not refer to any production of lipids on the peridial surface, but is merely used to describe its particularly greasy texture.

4. ***Cortinarius walpolensis*** A.A. Francis & N.L. Bougher *sp. nov.*
(Figure 4, Plate 1G)

Cortinarius levisporus primo adspectu maxime simile, sed peridio multo latiore (360–1000 µm crasso), peridiopellis hyphis latioribus luteis non gelatinosis crassiparietis non incrustatis, et basidiis longioribus (36.5–39.5 × 6.5–9 µm).
Typus hic designatus PERTH 06234623.

Basidiomes hypogeous, fruiting in small groups in deep litter, gastroid, 6–19 × 4–19 mm, globose, subglobose or turbinate with a flattened apex, often with a short stipe-like basal protrusion. *Peridium* white to off-white sometimes with cinnamon fibrils, dry, fibrillose with a satin sheen, bruising and discolouration of fibrils (cinnamon to brown) inconsistent, without much adhering debris, thin (0.5 mm), of a single layer, white in longitudinal section. *Gleba* loculate, pale-tan brown maturing to ochre-brown, rusty and finally brown (near 7E7 to 7D7), dry, not rapidly disintegrating after maturity, locules to 1 mm long, empty, labyrinthoid with no obvious radial arrangement. *Columella/sterile tissue* a truncate to percurrent columella tapering from the bulbous base towards the apex, 9–12 × 1–2 mm, white to translucent in section, central, more or less terete, white, dry, silky, of a single layer, solid and fibrous, base bulbous protruding up to 3 mm below peridium; basal mycelium of conspicuous white rhizomorphs. *Macrochemical tests* not recorded. *Odour* faintly fungoid or slightly of iodine, *taste* not recorded.

Spores golden brown (KOH), slightly asymmetrical, heterotropic, elliptical in profile, in face view elliptical but very slightly adaxially flattened, 7–10 × 3–5.5 µm, mean of 80 spores 8.0 ± 0.5 × 5.0 ± 0.5 µm, Q = 1.45–2.67, 1.72 ± 0.16 (KOH), ornamented with small warts or rods, ornaments to 0.5 µm in profile, slightly irregular in face view; perisporium absent or inconspicuous and closely adhering; mature spores not aggregating; hilar appendix around 0.5 µm long, conspicuous, equal, truncate, entire; thin-walled, inamyloid, non- to weakly dextrinoid (dark orange-brown); apex rounded and ornamented. *Basidia* yellowish, cylindro-clavate to cylindrical, 4-spored, 36.5–39.5 × 6.5–9 µm. *Cystidia* absent. *Hymenium* palisade, of brown-yellow, non-gelatinised, inflated, thin-walled, to 25 × 8 µm clamped elements. *Subhymenium* 5–10 µm wide, pseudoparenchymatous, of brown-yellow, non-gelatinised, short-branched, thin-walled, 3.5–9 µm broad elements. *Hymenophoral trama* 12.5–72.5 µm, subparallel to interwoven, of brown-yellow to hyaline, non-gelatinised, cylindrical, thin-walled, 4.5–17.5 µm broad hyphae. *Peridiopellis* (in longitudinal section) structure varies in a gradient 360–1000 µm thick from the outside towards the hymenium but does not form distinct layers, towards the outermost peridiopellis the hyphae are interwoven (sometimes so tightly as to appear pseudoparenchymatous) and undulating, hyaline, non-gelatinised (nor are those closer to the hymenium), cylindrical to somewhat inflated, thin-walled and 2–11.5 µm broad, closer to the hymenium the hyphae quickly become subparallel, yellow, more uniformly cylindrical, thick-walled (to 2 µm thick), 6–10 µm broad and grade in to the hymenophoral trama. *Clamp connections* present in all tissues but obscured in thick-walled hyphae.

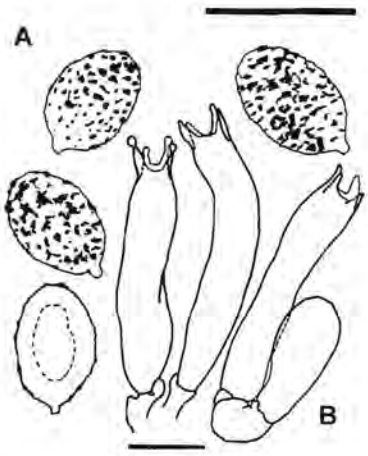


Figure 4: *Cortinarius walpolensis sp. nov.* PERTH 06234623.
A Spores. Note the small size of the spores compared with those of *Descomyces* and *Quadrispora* species, scale bar = 10 µm.
B. Basidia, scale bar = 10 µm.

Habitat and distribution: In Western Australia known from four collections found in deep litter among *Eucalyptus marginata*, *Corymbia* [*E.*] *marginata* and *Allocasuarina campestris* in the Walpole region. Fruiting June and July.

Collections examined: **W.A.**: Hull Road, Dawson Plantation, near Walpole, under *Eucalyptus marginata* and *Corymbia* [*E.*] *calophylla*, 13 July 1994, coll. *T. Lebel s.n.* holotype here designated as PERTH

06234623, isotype H6646. Yarra's property, North Walpole Road, under *Allocasuarina campestris*, 12 June 1988, coll. *Y. Korczynskyi s.n.* H4315. Cemetery Road Walpole-Nornalup National Park, under *Allocasuarina campestris*, 13 July 1994, coll. *N. Bougher, M. Brundrett, D. Brown & T. Lebel s.n.* H6665. Hilltop Road Walpole-Nornalup National Park, 15 July 1994, coll. *T. Lebel s.n.* H7005.

Etymology: In reference to the area from which this fungus has been collected.

Discussion: *Cortinarius walpolensis* appears to be closely related to *C. levisporus* (Massee & Rodway) Peintner & M.M. Moser with the two species chiefly distinguished by differences in peridiopellis structure. In *Cortinarius levisporus* (isotype HO100666 Rodway 653 examined), the peridium is a very thin (15–30 µm wide) repent cutis of thin (1–3 µm), slightly gelatinised, hyaline hyphae, with a hyaline encrustation on the outer surface, whereas the peridium of *C. walpolensis* is very broad (360–1000 µm), non-gelatinised and yellow-pigmented, and the broader (2–11.5 µm), thick-walled hyphae have no visible encrustations in KOH. The basidia of *Cortinarius walpolensis* are also larger than those of *C. levisporus* (25–34 × 4–6 µm Beaton *et al.* 1985). Spore size would have suggested placement of both of these species in *Thaxterogaster* section *Microsporogaster* Singer & Smith (1963), however, we await further molecular data as to the phylogenetic status of that section. *Cortinarius walpolensis* is, so far, only known from the south coast region of Western Australia, whereas *C. levisporus* occurs in Victoria and Tasmania (Beaton *et al.* 1985).

2. *Dermocybe*

Dermocybe globuliformis (Bougher) Bougher & Trappe, *Australasian Mycologist* 20: 2 (2002)
(Figure 5, Plate 2C, H)

= *Cortinarius globuliformis* Bougher *in* Bougher & Malajczuk, *Transactions of the British Mycological Society* 86: 301 (1986).

Basidiomes subhypogeous to hypogeous, fruiting in small groups among litter and soil, pileate, short, squat, pileus 5–30 mm broad, broadly convex, flattening to become plane, finally slightly upturned with or without a depressed centre, margin initially incurved and entire becoming plane and split in older specimens. *Peridium* bright yellow (2A6–2B6) becoming dark grey-brown when exposed to atmosphere for long periods, dry, smooth and shiny to fibrillose, not bruising, without much adhering debris, thin, one-layered. *Gleba* lamellate, gills initially bright yellow maturing to rust brown (6E8), dry, rather fragile, eroded in mature basidiomes, crowded, radially arranged, attachment adnate or subdecurrent. *Columella/sterile tissue* stipe very short in relation to pileus diameter, 2–7 mm long, 2–4 mm broad, central, terete, equal, initially bright yellow becoming dull, of two layers, solid, base rounded but not bulbous; partial veil membranous sometimes remaining entire covering hymenium otherwise splitting radially to absent shiny yellow to dull yellow; basal mycelium conspicuous and matt like, bright yellow. *Macrochemical tests* 15% KOH dull red on peridium and pileus context, bright red on stipe context, dark red-brown to black on lamellae, $FeSO_4$ no reaction to dull yellow on pileal peridium. *Odour* and *taste* not recorded.

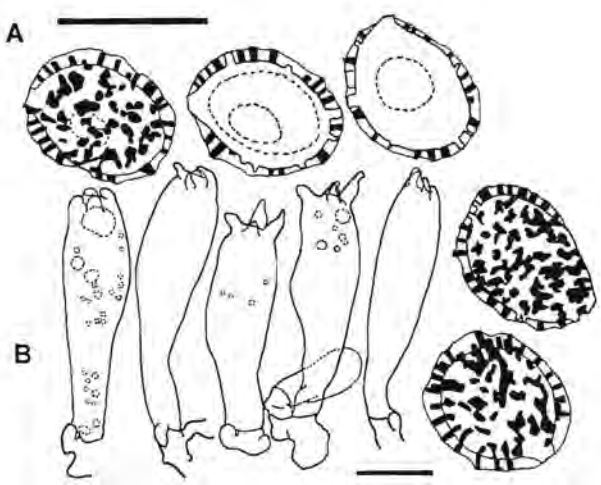


Figure 5: *Dermocybe globuliformis* **A** Spores. Note the asymmetric shape of the spores. H0359, scale bar = 10 µm. **B** Basidia. H0359, scale bar = 10 µm.

Spores pale yellowish (in KOH), asymmetric, heterotropic (and ballistosporic), elliptical to subglobose in profile, subglobose in face view, 9–12 × 6–9 µm, mean of 35 spores 10 ± 0.5 × 7 ± 0.5 µm, Q = (1.27) 1.45 ± 0.11 (1.71) in KOH, ornamented with rods and short ridges both to 1 µm tall in profile; perisporium pale yellow, inconspicuous, adhering closely to ornamentation; spores not aggregating; hilar appendix small, inconspicuous, tapering to truncate, entire; spores thick-walled, inamyloid, faintly dextrinoid (dark brown in Melzer's

solution); apex rounded and ornamented. *Basidia* hyaline, clavate, 4-spored, 20–30 × 6–10 µm. *Cystidia* absent. *Hymenium* palisade, of hyaline to pale red-brown (KOH), non-gelatinised, clavate to cylindrical, thin-walled, 17.5–21.5 × 6–8.0 µm elements. *Subhymenium* a narrow, hyaline to pale red-brown (KOH) layer of non-gelatinised, cylindrical, thin-walled, 2–3 µm broad, much branched hyphae. *Hymenophoral trama* parallel of pale pink (KOH), non-gelatinised, more or less cylindrical, up to 12 µm broad hyphae. *Peridiopellis* (in longitudinal section) of a single layer, relatively broad, a repent cutis of red-brown to pale red-brown (pigmentation concentrated in the outer region of the peridiopellis, in KOH), non-gelatinised, cylindrical, thin-walled, 2–5 µm broad hyphae. *Clamp connections* present in peridiopellis and hymenium.

Habitat and distribution: Abundant throughout southern Australia in a variety of soils and eucalypt-dominated vegetation communities. Often seated within bright yellow mycelium at or near the soil surface. Fruiting July and August.

Collections examined: **W.A.**: Amphion, Mycorrhizal Plots, Dwellingup, under *Eucalyptus marginata,* 5 July 1983, coll. *N. Bougher s.n.* isotype H0354. Shannon River, Pemberton side of Northcliffe, under *Corymbia* [*Eucalyptus*] *calophylla, E. marginata* and associated understorey ('teatree' Myrtaceae), 18 June 1981, coll. *N. Malajczuk s.n.* H0121. East side of Dwellingup Collie Road just south of Harvey turnoff, 13 Aug. 1981, coll. *N. Malajczuk s.n.* H0201. Cobiac, Jarrahdale, under *Eucalyptus marginata* and *Corymbia* [*E.*] *calophylla*, 20 July 1983, coll. *N. Malajczuk s.n.* H0358. 7 Day Road, Manjimup, under *Eucalyptus marginata*, *Corymbia* [*E.*] *calophylla*, 4 Aug. 1983, coll. *N. Bougher s.n.* H0359. Lockwood Road, Bickley, under *Eucalyptus marginata*, *Corymbia* [*E.*] *calophylla*, 29 June 1992, coll. *M. Pearce s.n.* H0654. Mundaring Shire Ashendon Rd, under *Eucalyptus marginata* and *Corymbia* [*E.*] *calophylla*, 18 July 1993, coll. *M. Castellano* Trappe 14637 (PERTH). Mundaring Ashendon Rd, under *Eucalyptus marginata,* 18 July 1993, coll. *J. Trappe* Trappe 14643 (PERTH). Jarrahdale Serpentine National Park, under *Eucalyptus marginata,* 19 July 1993, coll. *M. Castellano* Trappe 14660 (PERTH). Brockman Highway 40.3 km from Nannup Brook, under *Eucalyptus marginata*, 21 July 1993, coll. *N. Bougher* Trappe 14814 (PERTH). Off Narrogin Rd to Williams Rd on Contine Rd, under *Eucalyptus wandoo*, 23 July 1993, coll. *J. Trappe* Trappe 14943 (PERTH). Williams to York Rd 4.6 km from Wandering Rd junction Dryandra State Forest, under *Eucalyptus accedens*, 23 July 1993, coll. *W. Colgan* Trappe 14955 (PERTH). Amphion Block, Dwellingup, under *Eucalyptus marginata*, 12 Aug. 1993, coll. *N. Malajczuk, N. Bougher & J. Tommerup s.n.* H6371. Plavins Block, Murray Road, east of Dwellingup, under *Eucalyptus marginata*, 12 July 1994, coll. *s.n.* H6640. Cemetery Road, Walpole-Nornalup National Park, under *Eucalyptus jacksonii*, 13 July 1994, coll. *N Bougher s.n.* H6677. Hilltop Road Walpole-Nornalup National Park, under *Eucalyptus jacksonii, E. diversicolor*, 15 July 1994, coll. *N. Bougher M. Brundrett s.n.* H7002. Amphion Block, near Murray Road, about 15 km east of Dwellingup, under *Eucalyptus marginata*, 4 July 1995, coll. *W. Dunstan s.n.* H7260. Amphion Forest Block, off Murray River road, Dwellingup, under *Eucalyptus marginata*, 2 July 1996, coll. *J. Catchpole & S. Bolsenbroek s.n.* H7327.

Etymology: In reference to the short squat appearance of the basidiomes.

Discussion: *Dermocybe globuliformis* was transferred from *Cortinarius* into the genus *Dermocybe* by Bougher & Trappe (2002) on the basis of its bright pigmentation (a yellow pigment which reacts characteristically with KOH) and phylogenetic position (rDNA sequence data of Peintner *et al.* 2001 accession numbers AF388870, AF388794, AF388775 and AF325582). More collections of *Dermocybe globuliformis* have been lodged in herbaria in Australia than of any other cortinarioid sequestrate species with the possible exception of *Descomyces albus*, probably because of the former's conspicuous colouration and shallow position in the soil. Examination of the range of variation in the Western Australian collections of this species yielded a greater range in spore sizes and more predominantly ellipsoidal spores (Fig. 5A) than indicated previously (Bougher & Malajczuk 1986). The macroscopic red reaction of the peridium with 15% KOH is also seen as soon as sections are placed in 3% KOH and microscopically as a prominent colouration of the tissues of the hymenial elements, subhymenium, hymenophoral trama and peridiopellis.

3. *Descomyces*

Key to species of *Descomyces* currently recorded from Western Australia.

1 Inner peridiopellis a polycystoderm of inflated elements. **2. *Descomyces albus***

1: Inner peridiopellis not a polycystoderm, inflated end cells scattered and intermingled with predominantly cylindrical hyphae.

2 Thick-walled golden hyphae copious on peridiopellis; spores citriform, subfusoid or ellipsoidal, with a prominent, often extended rostrum not obscured by the perisporium. **1. *D. albellus***

2: Thick-walled golden hyphae sparse on peridiopellis; spores consistently ellipso-fusoid to subfusoid with a small rostrum frequently obscured by the perisporium. **3. *D. angustisporus***

1. *Descomyces albellus* (Massee & Rodway) Bougher & Castellano, *Mycologia* 85: 282 (1993) (Figure 6, Plate 1D)

= *Hymenogaster albellus* Massee & Rodway *in* Massee, *Kew Bull. Misc. Inform.* 1898: 126 (1898).
Hymenogaster zeylanicus Petch, *Ann. Roy. Bot. Gard. (Peradeniya)* 6: 207 (1971).
Hymenogaster maideni Rodway, *Pap. & Proc. Roy. Soc. Tasmania* 1920: 157 (1921).

Basidiomes hypogeous, growing singly or in groups amongst litter, gastroid, 5–30 mm diam., globose, subglobose or irregular. *Peridium* white or cream with yellow tufts or fibrils initially quite dense becoming scattered or disappearing, dry, fibrillose, not bruising, usually without much adhering debris, thin (< 0.5 mm), of two layers, outer layer yellow patchy and more or less fibrillose to tufted, inner layer continuous and white. *Gleba* loculate, initially pale cream or grey becoming dark brown, dry, not rapidly disintegrating after maturity, locules to 1 mm long, empty, no obvious radial arrangement. *Columella/sterile tissue* usually absent, if present a sterile basal pad or dendroid or truncate columella, small, more or less central, variable shape in cross-section, white to cream, dry, of a single layer, solid, base tapering; basal mycelium usually inconspicuous, white. *Macrochemical tests* not recorded. *Odour* and *taste* mildly fungoid but not distinctive.

Spores bright yellow-brown (KOH), symmetrical, orthotropic, citriform, subfusoid or ellipsoidal, 15–21 × 6.5–10.5 µm, mean of 79 spores 18.0 ± 1.5 × 8.5 ± 0.86 µm, Q = 1.78–2.57, 2.11 ± 0.19 (KOH), irregularly, ornamented with rods and short ridges both to 1 µm tall in profile, appearing somewhat irregular and crowded in face view; perisporium yellow-brown (in KOH), conspicuous, may be loose and flaring; spores not usually clumping; hilar appendix large though sometimes obscured by the ornamentation, truncate tapering, entire; spores thick-walled, inamyloid, inconsistently dextrinoid; apex rostrate and unadorned. *Basidia* hyaline but encrusted with a yellow pigment when necrotic, clavate but collapsing soon after maturity, one- and two-spored, 23–40 × 6–10 µm. *Cystidia* absent. *Hymenium* a palisade, of hyaline, non-gelatinised, clavate, thin-walled, to 23 × 13 µm broad basidia and basidioles. *Subhymenium* narrow, poorly differentiated, of hyaline, non-gelatinised, more or less cylindrical though often branching, thin-walled, to approximately 12 µm broad hyphae. *Hymenophoral trama* narrow, parallel to slightly interwoven, of hyaline or pale yellow (in KOH), non-gelatinised, cylindrical, thin-walled, 3 or 4 µm broad hyphae. *Peridiopellis* (in longitudinal section) of two layers, outer layer a relatively thin cutis of golden, non-gelatinised, cylindrical, much-branched, thin- to thick-walled (to 1 µm thick), 4–15 µm broad hyphae; inner layer broader, of interwoven, hyaline, non-gelatinised, thin-walled, cylindrical hyphae (3–7 µm broad) with cylindrical or less commonly vesiculose or pyriform end-cells (up to 35 × 15 µm), the end cells forming a trichoderm as they gradually merge with the outer layer. *Clamp connections* present and conspicuous in all tissues.

Habitat and distribution: Common among *Eucalyptus* dominated forests and woodlands of south Western Australia and in Western Australian plantings under a variety of local and eastern Australian eucalypts. In Australia, fruiting from June to October.

Collections examined: **W.A.**: Inglehope (near Dwellingup), under *Pinus* sp., 25 June 1981, coll. *N. Malajczuk s.n.* H0145. Cobiac, Jarrahdale, under *Eucalyptus marginata*, *Corymbia* [*Eucalyptus*] *calophylla*, 8 Sept. 1981, coll. *N. Malajczuk s.n.* H0213. Dieback replant area, Inglehope, near Dwellingup, under *Eucalyptus microcorys*, *Eucalyptus obliqua*, 7 July 1982, coll. *N. Malajczuk s.n.* H0278. Inglehope Arboretum, under *Eucalyptus obliqua*, *E. saligna*, 7 July 1982, coll. *N. Malajczuk s.n.* H0280. Gleneagle Forest, 11 Sept. 1982, coll.

N. Malajczuk s.n. H4904. CSIRO Glasshouse, Floreat Park, under *Eucalyptus camaldulensis,* 4 Oct. 1991, coll. *N. Bougher s.n.* H0608. Collie, Nanga Rd, under *Eucalyptus marginata*, 19 July 1993, coll. *J. Trappe* Trappe 14679 (PERTH). Junction of Murray Valley Rd and Nanga Rd, under *Eucalyptus marginata*, 20 July 1993, coll. *J. Trappe* Trappe 14742 (PERTH). Porongurup National Park junction of Woodlands Rd and Scenic Drv, under *Corymbia* [*Eucalyptus*] *calophylla* and *E. redacta*, 22 July 1993, coll. *M. Castellano, T. Lebel & W. Colgan III* Trappe 14905 (PERTH). The Knoll Walpole Nornalup National Park, 14 July 1994, coll. *T. Lebel s.n.* H7021. **Other**: Tas.: McRobies Gully, July 1920, coll. *Rodway s.n.* designated as isotype of *Hymenogaster maidenii* HO 100573. Near Hobart, McRobies Gully, date?, coll. *L. Rodway* Rodway 117 designated as isotype of *Hymenogaster albellus* HO 100580 and K. Georges Plain, 3 May 1990, coll. *J. Trappe s.n.* H1425. Sri Lanka, Hakgala, Mar. 1922, coll. *T. Petch s.n.* designated as isotype of *Hymenogaster zeylanicus* PDD 8277.

Etymology: In reference to the off-white peridium.

Discussion: As in *Descomyces albus* the mean spore dimensions of the examined Western Australian collections of *D. albellus* fall within the range of values (13–20 × 7–13 µm) initially given in Bougher & Castellano (1993). Some spores, however, are considerably both longer and wider (15–21 × 6.5–10.5 µm) than the largest spores referred to by Bougher & Castellano (1993). Again, as in *Descomyces albus*, we believe that this species accommodates a broader range of variation in spore size than previously reported. Because of such variation the presence of a polycystoderm is of primary importance in distinguishing *Descomyces albus* and *albellus*. This can be difficult if the peridium is degraded or the section is suboptimal. *Descomyces albellus* and *D. albus* can co-

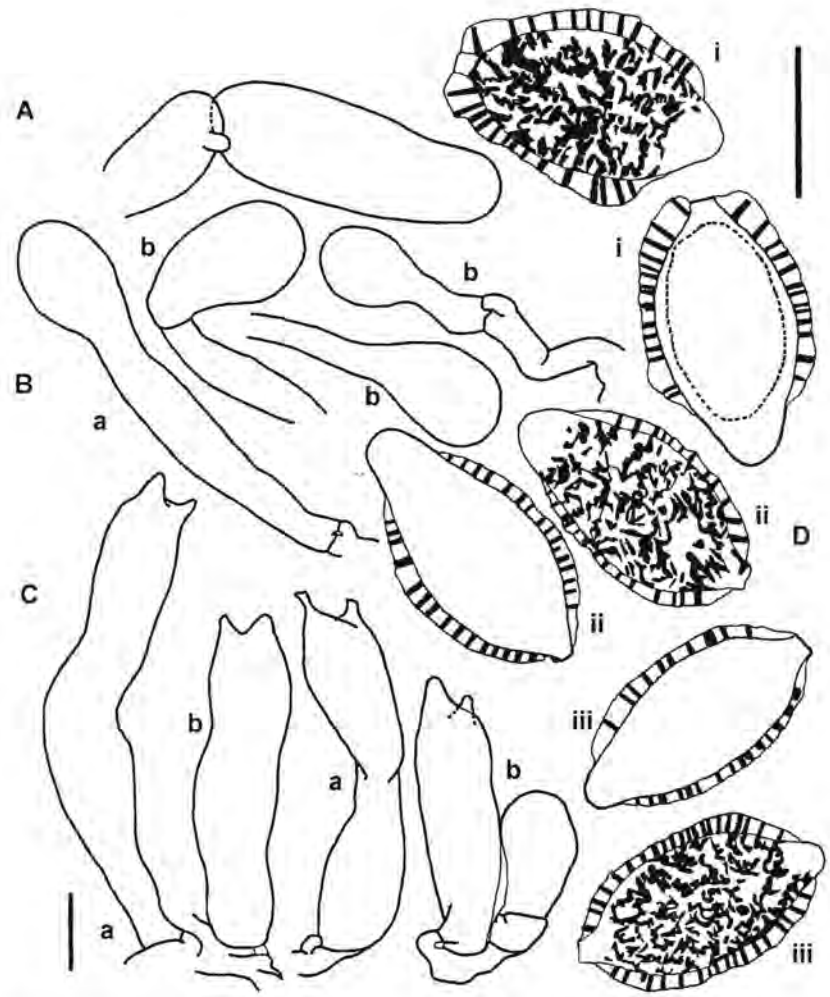


Figure 6: *Descomyces albellus* **A** Thin-walled golden hyphae of the outer peridium. **B** Inflated elements of the inner peridium **a** H7021 **b** H1425. **C** Basidia **a** H1425 **b** H0721, scale bar for A, B and C = 10 µm. **D** Spores **i** H0145 **ii** H7021 **iii** H0213, scale bar = 10 µm.

occur in eucalypt plantations and in natural ecosystems, providing ample opportunities for populations to interbreed. The extent of genetic isolation is unknown for these undoubtedly closely related species.

2. *Descomyces albus* (Klotzsch) Bougher & Castellano, *Mycologia* 85: 280 (1993)
(Figure 7, Plate 1C)

= *Hymenangium album* Klotzsch *apud* A. Dietr., *Fl. Regn. Boruss.* 7: 466 (1839).
= *Hymenogaster albus* (Klotzsch) Berk. & Broome, *Ann. Mag. Nat. Hist. ser. 1* 13: 349 (1844).
= *Hymenogaster klotzschii* Tul., *Fung. Hypogeal.*: 64 (1851).
= *Splanchnomyces albus* Corda *emend* Zobel *apud* Corda, *Icones Fungorum* 6: 40 (1854).
Hymenogaster maurus Maire, *Bull. Soc. Hist. Nat. Afrique. N.* 22: 18 (1931).
Hymenogaster weiblianus Maire, *Bull. Soc. Hist. Nat. Afrique N.* 22: 20 (1931).

Basidiomes hypogeous, growing singly or in groups under litter, gastroid, 5–20 mm diam., globose, subglobose or irregular. *Peridium* white or cream with yellow stains or fibrils initially quite dense becoming scattered or disappearing, dry, fibrillose, not bruising, not much adhering debris, thin, of two layers, outer layer yellow patchy and more or less fibrillose the inner continuous and white. *Gleba* loculate, initially white becoming pale cream or grey finally cinnamon brown, dry, not rapidly disintegrating after maturity, locules to 1 mm long, empty, no obvious radial arrangement. *Columella/sterile tissue* usually absent, if present a sterile basal pad or dendroid or small truncate columella, if dendroid then narrow (strands ± 1 mm broad), more or less central, variable shape in cross-section, white to cream, dry, of a single layer, solid, base tapering; basal mycelium usually inconspicuous, white. *Macrochemical tests* not recorded. *Odour* and *taste* not recorded.

Spores yellow-brown (in KOH), symmetrical, orthotropic, citriform, 14–23.0 × 5.5–16 µm, mean of 40 spores 17.5 ± 1.5 × 9.0 ± 1.1, Q = 1–2.67, 2.01 ± 0.27 (KOH), irregularly though quite closely ornamented with rods and short ridges, both to 3 µm tall in profile, appearing irregular and in some specimens crowded; perisporium yellow-brown (KOH), conspicuous, usually adhering relatively closely; spores generally not aggregating; hilar appendix reasonably large, conspicuous though may be obscured by the ornamentation, tapering and truncate, entire; spores thick-walled, inamyloid, inconsistently dextrinoid; apex rostrate and unadorned. *Basidia* hyaline though encrusted with a yellow pigment when necrotic, clavate though collapsing soon after maturity, one- and two-spored, 25–31 × 7–10 µm. *Cystidia* absent. *Hymenium* a palisade of hyaline, non-gelatinised, clavate to ellipsoidal, thin-walled, to 17 µm broad elements. *Subhymenium* variable, from narrow and undifferentiated to a broad hyaline pseudoparenchymatous layer, more or less hyaline, non-gelatinised, cylindrical to inflated cells, thin-walled, to 12 µm broad. *Hymenophoral trama* parallel to subparallel, hyaline (KOH), non-gelatinised, cylindrical, to 10 µm broad hyphae. *Peridiopellis* (in longitudinal section) of two layers, outer layer a relatively thin cutis of golden, non-gelatinised, cylindrical, much-branched, thick-walled (1 µm), 5–15 µm broad hyphae, inner layer broader, interwoven and forming a polycystoderm, of hyaline, non-gelatinised, cylindrical to inflated hyphae with end cells commonly clavate, vesiculose or pyriform, thin-walled, to 20–35 × 14–17 µm. *Clamp connections* present and conspicuous in all tissues.

Habitat and distribution: Common among *Eucalyptus* dominated natural forests and woodlands of southern Western Australia and also found growing in Western Australia under a variety of eucalypts introduced from eastern Australia. Fruiting in Australia from May to November.

Collections examined: **W.A.**: CSIRO glasshouse, Floreat Park '*Descomyces albus* from *Eucalyptus globulus* pot cultures 5 months old', (in glasshouse for 4 months) inoculated with isolate E1160, 14 Nov. 1990, coll. *I. Tommerup s.n.* H0574. Channybearup Plantation Manjimup, *Eucalyptus viminalis,* 20 June 1991, coll. *N. Malajczuk & G. Hardy s.n.* H0584. CSIRO near Workshop next to Cooling Tower, Floreat, *Eucalyptus marginata,* 14 July 1991, coll. *N. Bougher, I. Tommerup & S. Snelling s.n.* H0596. Lot 406 Denmark, *Eucalyptus patens*, *Corymbia* [*E.*] *calophylla*, *Allocasuarina decussata*, 27 June 1992, coll. *K. Syme s.n.* H0653. CSIRO grounds, near the old workshop, Floreat, *Eucalyptus marginata*, 13 June 2001, coll. *S Bolsenbroek s.n.* H0716. Channybearup W.A. MH159014, *Eucalyptus* spp., 9 Sept. 1986, collector unknown *s.n.* H1056. Quininup, 9 Sept. 1982, coll. *J. & M. Trappe s.n.* H4903. Walpole North, Dawson Plantation, Hull Road, 30-year-old mixed Eastern States *Eucalyptus* sp., 9 June 1993, coll. *N. Bougher s.n.* H6355. Walpole-Nornalup National Park, Tingle East Drive, *Eucalyptus jacksonii*, *E. diversicolor*, 11 June 1993, coll. *N. Bougher s.n.* H6363. **Other**: Tas.: Ritters Tree Farm, 5 May 1990, coll. *M. Castellano s.n.* H1490. Sheffield Rd, 4 May 1990, coll.

J. Trappe & M. Castellano s.n. H1447. Great Britain, Glasgow, 1830, coll. *J.D. Hooker* (designated as isotype of *Hymenogaster albus* K).

Etymology: In reference to the white peridium.

Discussion: The spores of the Western Australian representatives of *Descomyces albus* show considerable variation in spore size, shape and ornamentation and in the degree of inflation of the hyphae of the polycystoderm. The mean dimensions of the spores of the Western Australia collections examined fell within the range of sizes detailed in Bougher & Castellano (1993); however, both the length and the width of the largest spores

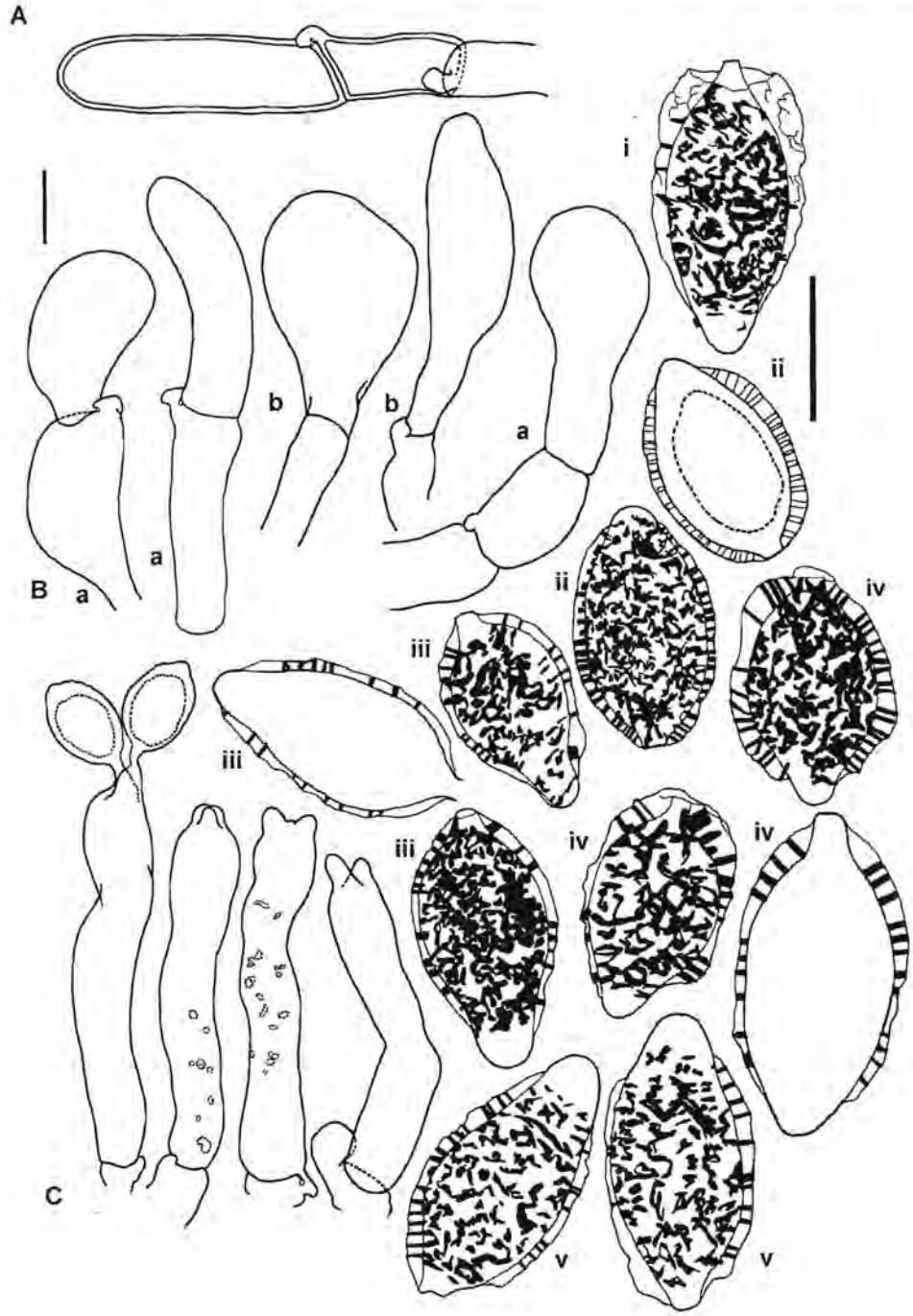


Figure 7: *Descomyces albus* **A** Thick-walled golden hypha of the outer peridium, H0584. **B** Inflated elements of the polycystoderm. **a** H1490 **b** H4903. **C** Basidia. H1490, scale bar for A, B and C = 10 µm. **D** Spores. Note the large variation in spore size encapsulated within the species concept of *Descomyces albus*. **i** H0584 **ii** H1490 **iii** H0574 **iv** H0635 **v**. H4903, scale bar = 10 µm.

measured (spores 14–23.0 × 5.5–16 µm) were considerably greater than the extremes given in the 1993 description (13–19 × 7–11 µm). Despite this variation, the combined presence of a polycystoderm and overlying golden hyphae leads us to suggest a greater range of variation in spore size in *Descomyces albus* than has been previously recognised.

3. ***Descomyces angustisporus*** A.A. Francis & N.L. Bougher *sp. nov.*
(Figure 8, Plate 1B)

Descomyces albellus primo adspectu maxime simile, sed extra-peridiopellis hyphis aureis crassiparietis sparsioribus, et sporis sat uniformiter ellipso-fusiformibus earum rostris relative obtusis et ab perisporiis fere obtectis.
Typus hic designatus PERTH 06234615.

Basidiomes hypogeous, fruiting in small groups within the first 15 cm of soil, gastroid, to 15 × 8 mm, ellipsoidal, almost globose to flattened. *Peridium* white (only sparse yellow fibrils), dry, slightly fibrillose (glistening under lens), not bruising, without much adhering debris, thin, fragile, macroscopically of a single layer. *Gleba* loculate, pale fawn when young maturing to bright brown, dry, not rapidly disintegrating after maturity, locules to 1 mm diam., empty, arranged irregularly. *Columella/sterile tissue* usually absent, if present a small sterile base; basal mycelium inconspicuous. *Macrochemical tests* 15% KOH, no reaction on gleba or peridium. *Odour* and *taste* not distinctive.

Spores yellow-brown (KOH), symmetrical to slightly asymmetrical, orthotropic, ellipso-fusoid to subfusoid in both face view and profile though asymmetrical spores slightly adaxially applanate in profile, 15.5–22.5 × 7.0–10.5 µm, mean of 40 spores 18.5 ± 2.0 × 8.5 ± 0.2, Q = 1.84–2.54, 2.14 ± 0.2 (KOH), finely ornamented with rods to 1.5 µm tall in profile, in face view markings appear crowded and irregular; perisporium brown-yellow (KOH), conspicuous, adhering quite closely to the ornamentation; spores not aggregating; hilar appendix to 1 µm, prominent though may be obscured by perisporium and ornamentation, tapering and truncate, entire; spores thick-walled, inamyloid, faintly dextrinoid (not deep red); apex more or less rostrate and unadorned, characteristically obscured by the perisporium. *Basidia* hyaline, initially long, cylindrical and droplet-filled becoming shorter, more clavate and loosing droplets with maturity, 4-spored, 35–69 × 9–13.5 µm. *Cystidia* absent. *Hymenium* palisade, of hyaline, non-gelatinised, clavate, ellipsoidal or pyriform, thin-walled, 28 × 9 µm end-cells. *Subhymenium* reasonably broad relative to the hymenophoral trama, pseudoparenchymatous, of hyaline, non-gelatinised, inflated, isodiametric, thin-walled, to 12.5 µm broad hyphae. *Hymenophoral trama* fairly narrow, subparallel, of hyaline, non-gelatinised, cylindrical, thin-walled, 4–9 µm broad hyphae. *Peridiopellis* (in longitudinal section) of two layers, outer layer: very thin and scattered layer of repent to semi-

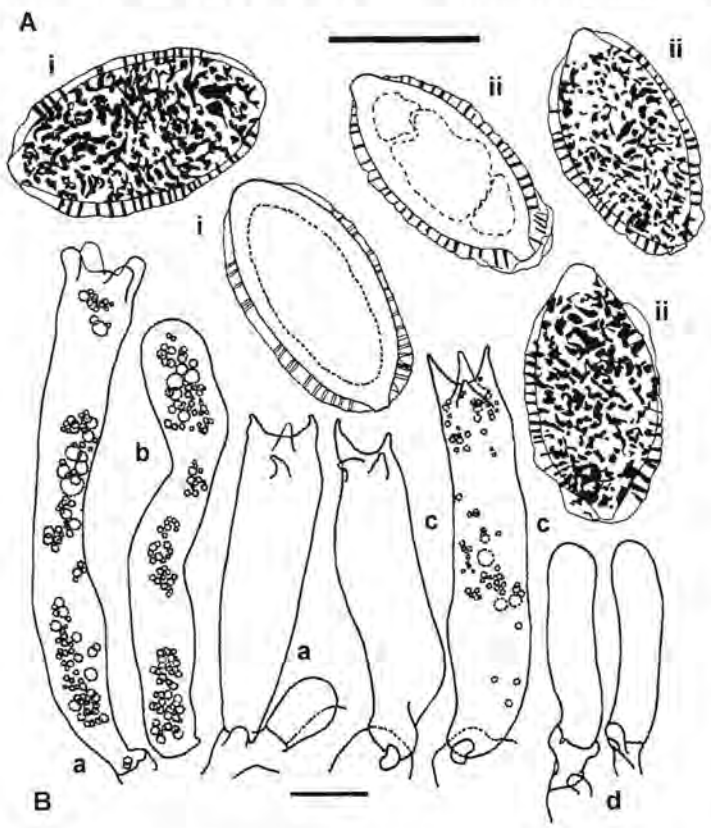


Figure 8: *Descomyces angustisporus* sp. nov. **A** Spores. Note the perisporium usually almost covers the rostrate apex of the spores that in some cases can cause the spores to appear almost oblong in shape. **i** H7282, **ii** H7216, scale bar = 10 µm. **B** Basidia, basidiole and hymenial elements. **a** basidia H7282, **b** basidiole H7282, **c** basidia PERTH 06234615, **d** hymenial elements PERTH 06234615, scale bar = 10 µm.

erect, hyaline to golden brown, non-gelatinised, cylindrical, thin to thick-walled, 1.5–3 µm broad hyphae; inner layer: much broader than outer layer, of interwoven, hyaline to slightly yellow-brown, non-gelatinised, cylindrical, thin-walled, 2–5 µm broad hyphae. *Clamp connections* present in both layers of the peridiopellis though more conspicuous in the outer layer, also present in the hymenium.

Habitat and distribution: *Descomyces angustisporus* is fairly widely distributed throughout the south-west of Western Australia, particularly in coastal vegetation and in woodlands of the wheatbelt region, fruiting among *Eucalyptus astringens*, *E. gomphocephala*, *E. wandoo* and *E. accedens* in association with *Casuarina*, *Gastrolobium*, *Acacia* and *Melaleuca* species. Fruiting June to September.

Collections examined: **W.A.**: Kawana Rd, Dryandra State Forest, off Wandering Narrogin Road, 21 June 1995, coll. *N. Bougher s.n.* (holotype here designated as PERTH 06234615, isotype H7216). Dryandra State Forest, *Eucalyptus astringens*, 19 Aug. 1986, coll. *D. Murray s.n.* H2027. Rottnest Island, 25 Sept. 1982, coll. *N. Malajczuk* Trappe 6985 (OSC #82231). Ludlow National Park, Laymans picnic area, under *Melaleuca* sp. and *Eucalyptus gomphocephala*, 20 July 1993, coll. *J. Trappe* Trappe 14763 (PERTH). Off Narrogin to Williams Rd on Contine Rd, under mixed *Eucalyptus wandoo* and *E. accedens*, 29 July 1993, coll. *T. Lebel, W. Colgan, M. Castellano & J. Trappe* Trappe 14948 (PERTH). Off Narrogin to Williams Rd on Cowecker Rd , under *Eucalyptus* sp. and *Casuarina fraseriana*, 23 July 1993, coll. *M. Castellano* Trappe 14951 (PERTH). Site 18, grazed (tree 3), 31 km along Bencubbin-Kellerberrin Rd, north of Kellerberrin, *Eucalyptus wandoo*, 6 July 1995, coll. *W. Dunstan & S. Bolsenbroek s.n.* H7272. Site 17B, Higginson Road, 16 km along Bencubbin-Kellerberrin Rd, North of Kellerberrin, *Eucalyptus wandoo* and *Gastrolobium*, 19 July 1995, coll. *I. Tommerup s.n.* H7282. Site 17B, Higginson Road, 16 km along Bencubbin-Kellerberrin Rd, North of Kellerberrin, *Eucalyptus wandoo*, *Gastrolobium* and *Acacia* sp., 19 July 1995, coll. *N. Bougher s.n.* H7283. Site 290, (Tree 8, ungrazed), near Pullen Rd, of Bencubbin-Kellerberrin Rd, 35 km north of Kellerberrin, *Eucalyptus wandoo*, 7 Sept. 1995, coll. *W. Dunstan s.n.* H7322. Site 290, T 10, ungrazed), near Pullen Rd, off Bencubbin-Kellerberrin Rd, 35 km north of Kellerberrin, *Eucalyptus wandoo*, 7 Sept. 1995, coll. *I. Tommerup, W. Dunstan & S. Bolsenbroek s.n.* H7325. Site 17, ungrazed, off Higginson Rd, off Bencubbin-Kellerberrin Rd, Kellerberrin, *Eucalyptus wandoo*, 1 Aug. 1996, coll. *I. Tommerup s.n.* H7350.

Etymology: In reference to the narrow spores of this species.

Discussion: The macroscopic appearance and spore morphology of *Descomyces angustisporus* is typical of the genus *Descomyces*. When fresh, the gleba is bright chestnut brown and the peridium is a 'cleaner' white than those of *Descomyces albus* and *D. albellus* (presumably because of the relative sparseness of the golden, veil-remnant hyphae that are prominent in the latter two species (Bougher & Castellano 1993)). *Descomyces angustisporus* is considered most closely related to *D. albellus* on the basis of its peridiopellis and spore structure. As in *Descomyces albellus*, the peridium of *D. angustisporus* contains only few and scattered (if any) swollen elements; however, in the latter species the spores are consistently ellipso-fusoid (with the rostrum much less prominent/elongated than in *D. albellus c.f.* Figures 8A and 6D) and the ornamentation and perisporium, frequently extend quite a long way towards the apex and surround the hilar appendix. Field observations suggest that *Descomyces angustisporus* may replace *D. albellus* and *D. albus* to some degree in the more arid regions of the Western Australian wheatbelt (though *D. angustisporus* is also known to occur much further south and in coastal regions). The testing of this hypothesis will require further sampling to accurately establish distributions. The genus *Descomyces* has been represented by *D. albellus* in the molecular studies of both Glen *et al.* (2001) and Peintner *et al.* (2001); however, as yet no published phylogeny has examined multiple collections of more than one species of the genus. Such research should provide more information on the reliability of characteristics such as spore and peridiopellis morphology in differentiating these fungi.

4. *Protoglossum*

Key to species of *Protoglossum* currently recorded from Western Australia.

1: Peridium yellow, orange to copper red; spores broadly ellipsoidal to subglobose, 9.5–14.5 × 7–13 µm. **1. *Protoglossum luteum***

1: Peridium violet fading to greyish violet or greyish brown; spores broadly ellipsoidal, 9.5–15.5 × 6.5–9 µm. **2. *P. violaceum***

1. *Protoglossum luteum* Massee, *Grevillea* 19: 94 (1891)
(Figure 9, Plate 2D, E)

= *Hymenogaster luteus* (Massee) G. Cunn., *Proc. Linn. Soc. New South Wales* 59: 169 (1934) *non* Vittad. 1931.
= *Cortinomyces luteus* (Massee) Bougher & Castellano, *Mycologia* 85: 277 (1993).
Hysterangium atratum Rodway, *Pap. & Proc. Roy. Soc. Tasmania* 1919: 112 (1920).
Hymenogaster atratus (Rodway) Zeller & C.W. Dodge *in* C.W. Dodge & Zeller, *Ann. Missouri Bot. Gard.* 21: 656 (1934).
Hymenogaster effodiendus G. Cunn., *Trans. Roy. Soc. South Australia* 75: 14 (1952).
Cortinomyces effodiendus (G. Cunn.) Bougher & Castellano, *Mycologia* 85: 279 (1993).

Basidiomes subhypogeous, often growing singly, protruding from the ground, gastroid, 10–50 × 10–20 mm, globose, subglobose, cylindrical or irregular, may be taller than broad in some specimens. *Peridium* colour is apparently dependent on exposure and method of preservation (May 1995), underground portions may be yellowish, while exposed portions tend to be orange to copper red, peridium viscid, smooth, not bruising, without much adhering debris, thin, of two layers, outer layer gelatinised, inner layer pale in longitudinal section. *Gleba* loculate, tan (near 5C6) then brown (near 6F6), dry, not rapidly disintegrating after maturity, locules approx. 1 mm diam., empty, not radially arranged. *Columella/sterile tissue* usually absent, if present a small sterile basal pad or narrowly tapering truncate columella, to 1.5 mm thick at the base, essentially central, terete (though very small), pale to cream; basal mycelium inconspicuous. *Macrochemical tests* not recorded. *Odour* and *taste* not recorded.

Spores dark rust brown (in KOH), symmetrical, orthotropic, broadly ellipsoidal to subglobose, 9.5–14.5 × 7–13 µm, mean of 63 spores 13.0 ± 1.0 × 9.5 ± 1.0 µm, Q = 0.93–1.61, 1.36 ± 0.26 (KOH), irregular rods and short ridges sometimes tapering towards their apices but not spinose, up to 1.5 µm tall; perisporium pale yellow-brown (KOH), conspicuous, closely adhering, covering apex; mature spores not aggregating; hilar appendix 1.5 × 1 µm, conspicuous, tapering truncate, entire; spores thick-walled, inamyloid, non-dextrinoid; apex rounded and ornamented. *Basidia* hyaline to yellow and necrotic, clavate, (2-) 4-spored, 32–56.5 × 6–10.5 µm. *Cystidia* absent. *Hymenium* palisade, of basidia and hyaline, non-gelatinised, clavate, thin-walled, 20–45 × 8–10.0 µm broad elements. *Subhymenium* narrow (only one or two isodiametric cells subtending each hymenial element)

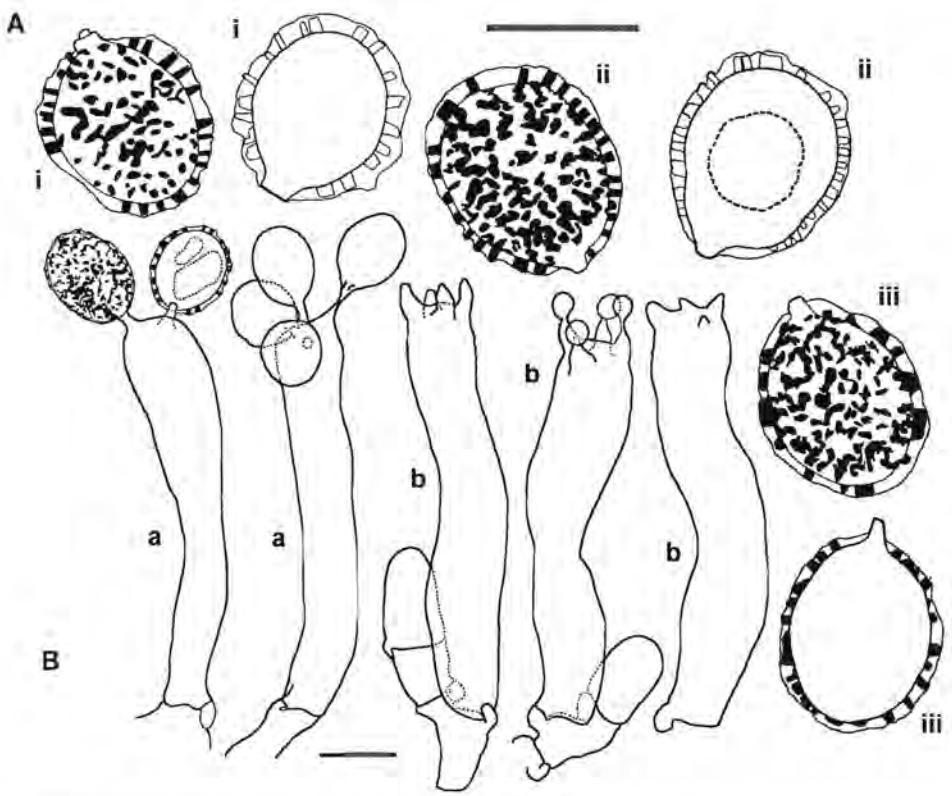
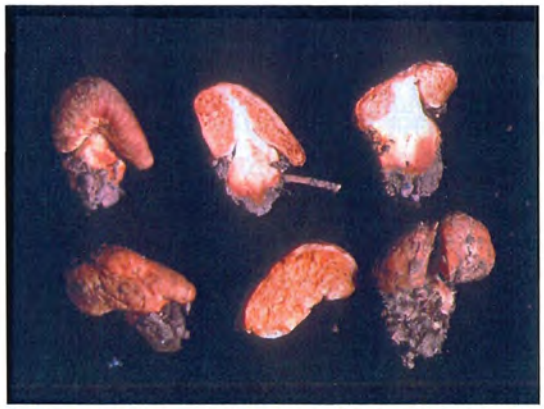


Figure 9: *Protoglossum luteum* **A.** Spores **i** H0175. **ii** H7660. **iii** H7259, scale bar = 10 µm. **B** Basidia. **a** H7259. **b** H7660, scale bar = 10 µm.


A

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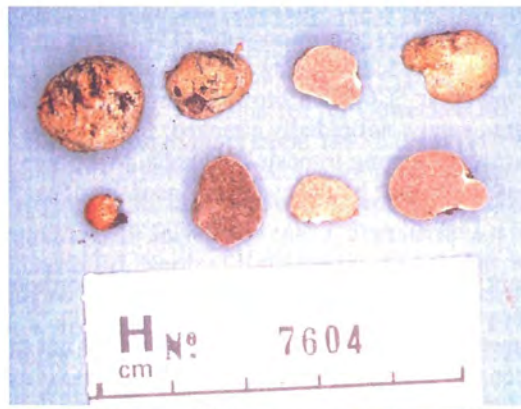

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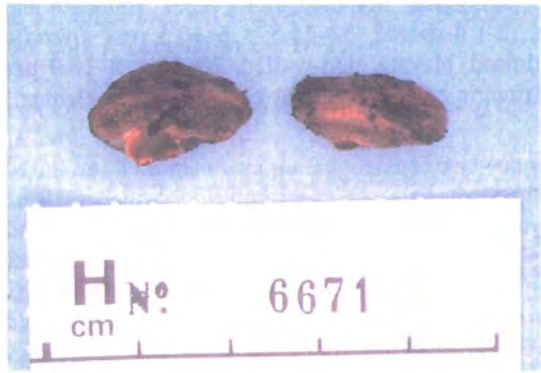
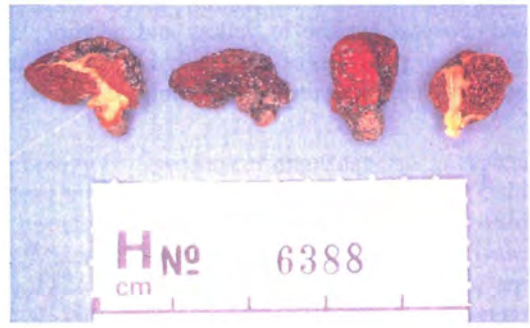


E



F

G

Plate 1. Basidiomes of: **A** *Setchelliogaster australiensis* H1023, scale bar = 2 cm. **B** *Descomyces angustisporus*. **C** *Descomyces albus*. **D** *Descomyces albellus*. **E** *Quadrispora oblongispora*. **F** *Quadrispora tubercularis sp. nov.* **G** *Cortinarius walpolensis*.

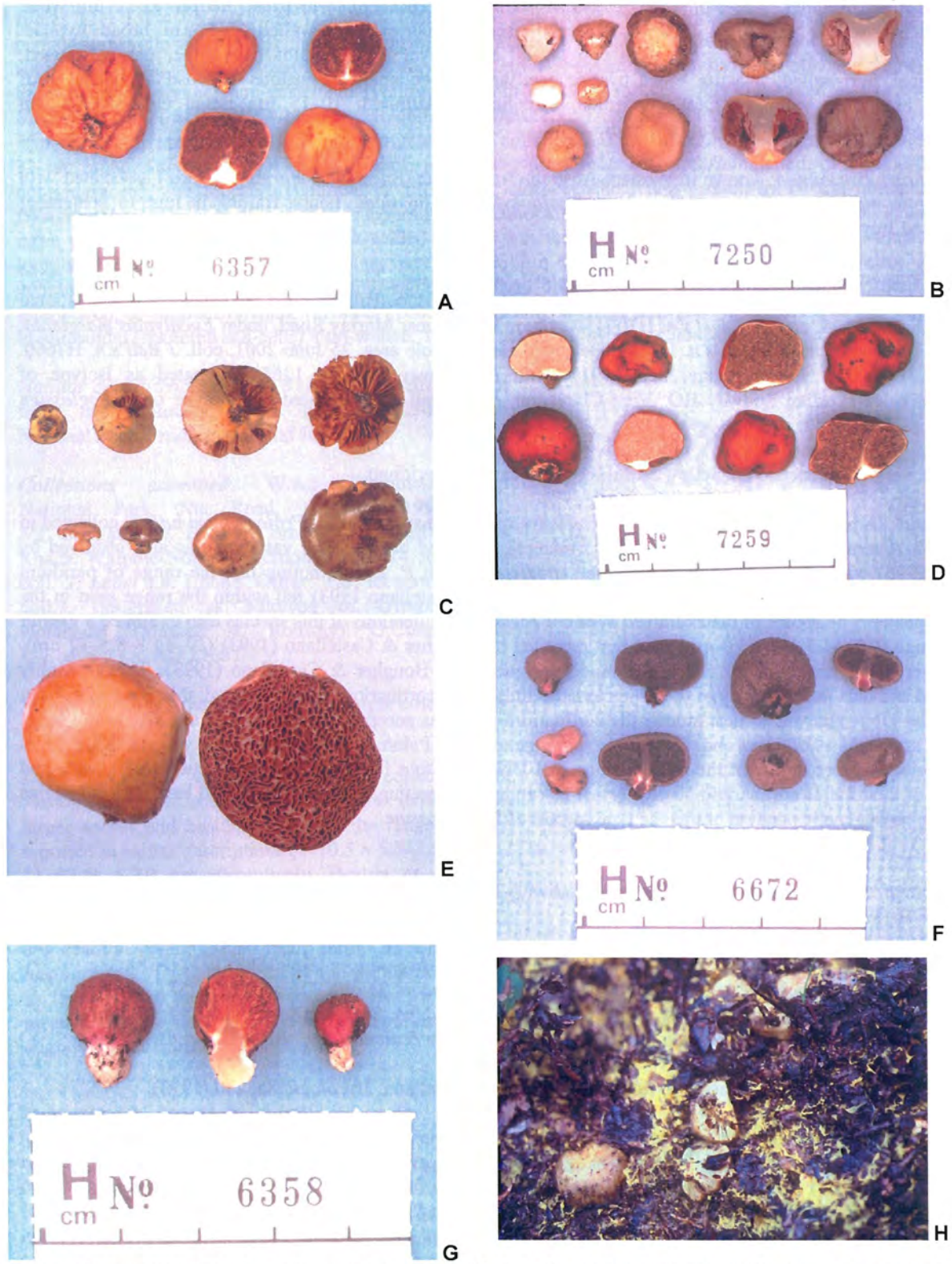


Plate 2. Basidiomes of: **A** *Cortinarius luteirufescens* H6357. **B** *Cortinarius sebosus*. **C** *Dermocybe globuliformis*, H0359, scale bar = 2 cm. **D & E** *Protoglossum luteum* **D** H7259, **E** H0175, scale bar = 2 cm. **F** *Cortinarius basipurpureus* compare **G** *Protoglossum violaceum,* Note the truncate stipe-columella in these specimens. **H** The prominent (yellow) mycelium of *Dermocybe globuliformis* in the field.

pseudoparenchymatous to hyphal in appearance, of more or less hyaline, non-gelatinised, more or less short cylindrical to irregular, thin-walled, to 22 µm broad hyphae. *Hymenophoral trama* narrow to 20 µm wide, parallel, of yellow or brown (KOH), non-gelatinised, cylindrical, thin-walled, 2–8 µm broad hyphae. *Peridiopellis* (in longitudinal section) of two layers, outer layer a broad cutis of hyaline, non-gelatinised, cylindrical, thin-walled, 2–5 µm broad hyphae overlying a pseudoparenchymatous or broad hyphal layer of golden brown or yellow (KOH) pigmented, sometimes gelatinised, 10–15 µm broad elements. *Clamp connections* present though rare in the peridium.

Habitat and distribution: Known in Western Australia from jarrah (*Eucalyptus marginata*) forest associated with *Eucalyptus marginata*, *Corymbia* [*Eucalyptus*] *calophylla* and *Bossiaea*. Found fruiting in June to September (June and July in Western Australia).

Collections examined: **W.A.**: Pine Creek Road near Scatter Road, Manjimup, under *Corymbia* [*Eucalyptus*] *calophylla*, *Bossiaea*, 27 July 1981, coll. *N. Malajczuk s.n.* H0175. Hilltop Road Walpole-Nornalup National Park, 15 July 1994, coll. *T. Lebel s.n.* H7004. Amphion Block, near Murray Road, under *Eucalyptus marginata*, 4 July 1995, coll. *W. Dunstan s.n.* H7259. Delany Walk, Walpole area, 25 June 2001, coll. *J Bell s.n.* H7660. **Other**: Tas.: Mt Nelson Range, Sept. 1919, coll. *L. Rodway* Rodway 1265 designated as isotype of *Hysterangium atratum* Rodway HO. Vic.: Clarendon, date?, coll. ?, designated as holotype of *Protoglossum luteum* Massee K859.

Etymology: In reference to the yellowish colour of the exposed peridium.

Discussion: A broad range of peridium colours occurs among the specimens of *Protoglossum luteum* collected in Western Australia, ranging from yellowish to orange-brown and brown. Such variation was also observed by May (1995) who synonymised *Hymenogaster effodiendus* with *P. luteum* noting that the range of peridium colours used to distinguish *H. effodiendus* (*c.f.* Bougher & Castellano 1993) fell within the range seen in the latter species. The spores of the examined Western Australian collections of this species also exhibited a greater size range (9.5–14.5 × 7–13.0 µm) than that recorded by Bougher & Castellano (1993) (10–14 × 8.5–12 µm). However, the mean sizes lie within the range designated by Bougher & Castellano (1993). Though highly reduced and thus lacking many of the characteristics linking the cortinarioid agaricoid fungi, this fungus has been found by DNA analysis to nest among the cortinarioid fungi, as accepted by Peintner *et al.* (2001), in a clade with *Cuphocybe melliolens* and *Cortinarius corrugatus*. The Peintner *et al.* (2001) analysis of the Internal Transcribed Spacer region of the nuclear ribosomal DNA lead to a four-way polytomy between this clade and others of their major cortinarioid clade. Further research incorporating other regions should lead to new insights into the phylogenetic position of this the type species of *Protoglossum*.

2. ***Protoglossum violaceum*** (Massee & Rodway) T.W. May, *Muelleria* 8: 288 (1995)
(Figure 10, Plate 2G)

= *Hymenogaster violaceus* Massee & Rodway *in* Massee, *Bull. Misc. Inform. Kew* 1898: 127 (1898).
= *Arcangeliella violacea* (Massee & Rodway) C.W. Dodge, *Compar. Morph. Fungi* 487 (1928).
= *Dendrogaster violaceus* (Massee & Rodway) G. Cunn., *Proc. Linn. Soc. New South Wales* 59: 172 (1934).
= *Gymnoglossum violaceum* (Massee & Rodway) G. Cunn., *New Zealand J. Sci. Technol.*, ser. B, 22: 300 (1941).
= *Cortinomyces violaceus* (Massee & Rodway) Bougher & Castellano, *Mycologia* 85: 280 (1993).

Basidiomes hypogeous, found growing singly or in small groups under litter, gastroid, 13–30 × 10–20 mm, globose, broadly to irregularly ellipsoidal, with a stipe that is generally not taller than the peridium. *Peridium* violet (16E7–17B6) fading to greyish violet (17B3) or greyish brown (near 6E4), glutinous becoming less-so with age, smooth to slightly lumpy, not bruising, without much adhering debris, approximately 1 mm thick, two-layered, outer layer gelatinised, inner layer not gelatinised and pale in longitudinal section. *Gleba* loculate, initially cream (near 8B3) becoming brown (near 7E8), dry, not rapidly disintegrating after maturity, locules to 1 mm long, empty, irregular to radially arranged. *Columella/sterile tissue* a truncate stipe-columella, 3.5 × 2 mm, essentially central, terete, columella truncate, stipe portion may be concolourous with the peridium or white, dry, smooth, not forming distinct layers, solid, flesh cream to white, sometimes violet in immature specimens, base rounded protruding up to 4.5 mm below peridium; basal mycelium inconspicuous. *Macrochemical tests* 15% KOH darkens peridium, $FeSO_4$ gleba darkening slightly. *Odour* yeasty, *taste* not distinctive.

Spores dull brown (KOH), symmetrical, orthotropic, broadly ellipsoidal, 9.5–15.5 × 6.5–9 µm, mean of 61 spores 12 ± 1.0 × 7.5 ± 0.5 µm, Q = 1.52–1.82, 1.66 ± 0.08 (KOH), ornamented with small warts and ridges; perisporium yellowish (KOH) though inconspicuous, adhering closely; mature spores not aggregating; hilar appendix 0.5 µm, generally conspicuous but may be obscured by ornamentation, subequal truncate, entire; spores thin-walled, inamyloid, non-dextrinoid (yellow-brown in Melzer's solution); apex rounded and ornamented. *Basidia* pale yellowish, yellow-brown when collapsed, clavate, (2-) 4-spored, 31–53 × 9–13 µm. *Cystidia* absent. *Hymenium* palisade, of hyaline (KOH), non-gelatinised, cylindrical to clavate, thin-walled, broad elements 15.5–33 µm broad. *Subhymenium* 11–30 µm thick, subcellular to more or less undifferentiated, of hyaline (KOH), non-gelatinised, subglobose, cylindrical or irregularly short-branched, thin-walled, to 19 µm broad hyphae. *Hymenophoral trama* 25–40 µm thick, subparallel to pseudoparenchymatous, of hyaline (KOH), non-gelatinised, more or less cylindrical, thin- to thick-walled (to 1.5 µm thick), to 45 µm broad hyphae. *Peridiopellis* (in longitudinal section) of two layers, outer layer to 208 µm, a loosely interwoven cutis, of hyaline (KOH), gelatinised, more or less cylindrical, thin-walled, 2–4 µm broad hyphae, inner layer to 42 µm, more tightly interwoven, of yellowish hyphae (in water) becoming hyaline in KOH, non-gelatinised, more or less cylindrical to ellipsoidal (scattered end-cells), thin-walled, 10–17.5 µm broad hyphae. *Clamp connections* none seen.

Habitat and distribution: In Western Australia, known from two collections from the Walpole-Nornalup National Park. Fruiting June and July.

Collections examined: **W.A.**: Walpole-Nornalup National Park, Nut Road, 10 June 1992, coll. *N.L. Bougher s.n.* H6358. Corner of Nut and Ficifolia Roads, Walpole-Nornalup National Park, 14 July 1994, coll. *T. Lebel s.n.* H6688. **Other**: Tas. location?, date?, coll.?, designated as holotype of *Gymnoglossum violaceum* (Massee & Rodway) G.Cunn., coll. *L. Rodway s.n.* AD 22607.

Etymology: In reference to the violet/purple colour of the peridium.

Discussion: The Western Australian collections of *Protoglossum violaceum* reported here exhibit both larger spores and basidia sizes than the range of sizes reported in earlier descriptions (7–10.5 × 5.5–7.5 µm and 11–42 × 4–10 µm respectively, Beaton *et al.* 1985, Dodge & Zeller 1936, Massee 1898, Rodway 1924 and Singer & Smith 1960). Mature basidiomes of this species are readily distinguished from those of *Cortinarius basipurpureus* by the absence of the purple colouration in the flesh of the base of the pseudostipe, the less prominent spore ornamentation and perisporium and the slightly longer spores.

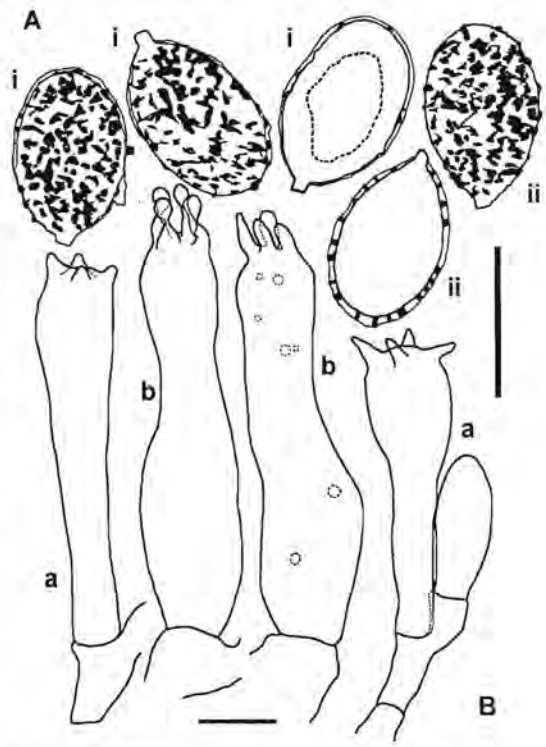


Figure 10: *Protoglossum violaceum* **A.** Spores **i.** H6688. **ii.** H6358, scale bar = 10 µm. **B.** Basidia. **a** H6688. **b** H6358, scale bar = 10 µm.

5. *Quadrispora*

Key to species of *Quadrispora* currently recorded from Western Australia.

1 Spores ellipsoidal to oblong, 13–18 × 7–10 µm, ornamented with crowded, irregular tubercles (to approximately 1.5 µm in diam.) and ridges (to approximately 3 µm long), ornaments to 1 µm tall in profile. **1. *Quadrispora oblongispora***

1: Spores subobovoid, 12.5–17 × 7–10 µm, coarsely ornamented with irregular tubercles (to approximately 3 µm in diam.) and ridges (to approximately 6 µm long), ornaments to 2 µm tall in profile. **2. *Q. tubercularis***

1. *Quadrispora oblongispora* (G.W. Beaton, Pegler & T.W.K. Young) Bougher & Castellano, *Mycologia* 85: 286 (1993)
(Figure 11, Plate 1E)

= *Hymenogaster oblongisporus* G.W. Beaton, Pegler & T.W.K. Young, *Kew Bulletin* 40: 188 (1985).

Basidiomes hypogeous found fruiting in small groups underneath the litter layer, gastroid, 5–20 mm diam., subglobose or ellipsoidal, slightly tapering at the base. *Peridium* warm brown (near 7E8) to apricot yellow, drying to greyish yellow, initially viscid and smooth, drying with a silky sheen, not bruising, without much adhering debris, thick, of one gelatinised layer. *Gleba* loculate, grey to dark brown (near 7E7), dry, not rapidly disintegrating after maturity, locules to 0.5–1.5 mm diam., empty, no radial pattern evident. *Columella/sterile tissue* absent or a protruding basal pad/pseudostipe[1], 5 × 5 mm, cream or brown in longitudinal section, central, more or less terete, surface obscured by the thick gelatinous layer that appears to be continuous with that covering the peridium, not forming distinct layers, solid or with a narrow hollow centre, base emergent, narrow to bulbous; basal mycelium inconspicuous. *Macrochemical tests* not recorded. *Odour* not distinctive and *taste* not recorded.

Spores dark yellow-brown (KOH), asymmetrical, heterotropic, adaxially applanate, ellipsoidal or oblong, 14.5–17.5 × 5–7.5 µm, mean of 20 spores 16.0 ± 1.0 × 6.5 ± 0.5 µm, Q = 2.21–3, 2.54 ± 0.27 (KOH), ornamented with warts or small tubercles (to approximately 1.5 µm in diam.) and ridges (to approximately 3 µm long), ornaments to 1 µm tall in profile, crowded and irregular in face view; perisporium pale yellow (KOH), conspicuous, more or less flaring, continuous between spores; spores for the most part remaining aggregated in tetrads after release from the basidium; hilar appendix up to 1.5 µm long, conspicuous though may be obscured by ornamentation and perisporium, tapering and truncate, entire; spores thin-walled, non-dextrinoid, inamyloid, non-dextrinoid (orange-brown in Melzer's solution); apex rounded and ornamented. *Basidia* hyaline, broadly clavate, 4-spored, 25–35 × 8.5–11 µm. *Cystidia* absent. *Hymenium* palisade, hyaline, non-gelatinised, elements clavate to pyriform, thin-walled. *Subhymenium* a broad, pseudoparenchymatous, hyaline, non-gelatinised, layer of inflated, thin-walled, to 12 µm broad cells. *Hymenophoral trama* parallel or slightly interwoven, hyphae hyaline, non-gelatinised, 3–13 µm broad. *Peridiopellis* (in longitudinal section) of one layer, to 600 µm broad, of interwoven, hyaline, gelatinised or intact, narrow, cylindrical, sometimes dissolved and irregular, thin-walled, 1–4 µm broad hyphae. *Clamp connections* present throughout the peridium and hymenium but small and inconspicuous.

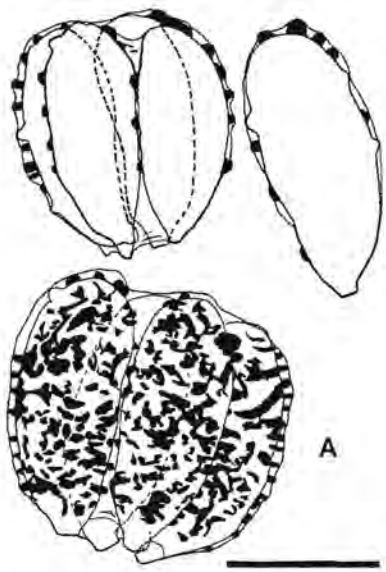


Figure 11: *Quadrispora oblongispora* H6671. **A** Spores. The spores of this species are often released in tetrads held together by the gelatinous perispore, scale bar = 10 µm.

Habitat and distribution: Known in Western Australia from a single record from the Walpole region, fruiting in woodlands close to *Eucalyptus jacksonii*. Fruiting June–August.

Collections examined: **W.A.**: Cemetery Road, Walpole-Nornalup National Park, under *Eucalyptus jacksonii*, 13 July 1994, coll. *T. Lebel s.n.* H6671. **Other**: Vic.: Rubicon, 30 June 1974, coll. *G. Crichton & G. Beaton Beaton 64* designated as holotype of *Hymenogaster oblongisporus* K. Macedon Regional Park, 31 Aug. 1982, coll. *A.C. Beauglehole Beaton 76* designated as iso-paratype of *Hymenogaster oblongisporus* K.

Etymology: In reference to the ellipsoidal to oblong shape of the spores.

Discussion: *Quadrispora oblongispora* is distinguished from *Q. musispora* Bougher & Castellano (not known in Western Australia) by the colour of the peridium (apricot yellow as compared with violet in *Q. musispora*) and host plants (*Eucalyptus* species versus *Nothofagus* species). *Quadrispora oblongispora* is differentiated from *Quadrispora tubercularis* by having longer, less prominently ornamented spores, and a single layered peridium (see discussion for *Q. tubercularis*).

[1] The term pseudostipe as used by Beaton *et al.* (1985) refers to the external stipe-like protrusion of a sterile base rather than the extension of a columella (either percurrent or truncate).

2. *Quadrispora tuberculariis* N.L. Bougher & A.A. Francis *sp. nov.*
(Figure 12, Plate 1F)

Species sporarum ornamentis tuberculariibus grandioribus aquilioribus (in KOH) et peridiopelli bistrata a congeneribus diversa.
Typus hic designatus PERTH 0090403.

Basidiomes subhypogeous, a small collection found protruding from the sand, gastroid, to 12 mm diam., subglobose to pyriform. *Peridium* brown (when dried), viscid or gelatinous when fresh, smooth, not bruising, without much adhering debris, thin, of two layers, outer layer broader and gelatinised, inner layer cream in section and not gelatinised. *Gleba* loculate, bright orange-brown, dry, not disintegrating rapidly after maturity, locules to 1 mm broad, empty, not noticeably radially arranged. *Columella/sterile tissue* absent to present as a percurrent columella, to 6.5 × 2 mm within the gleba, cream to yellowish in section, central, more or less finely terete, of one layer, solid, with an emergent angular-bulbous base protruding up to 5 mm below the peridium; basal mycelium not seen. *Macrochemical tests* not tested. *Odour* and *taste* not tested.

Spores dark orange-brown (KOH), symmetrical to very slightly adaxially flattened, heterotropic, subobovoid, 12.5–17 × 7–10 µm, mean of 20 spores 14.0 ± 1.0 × 8.0 ± 0.5 µm, Q = 1.65–2, 1.81 ± 0.11 (KOH), ornamented with quite large tubercles (to approximately 3 µm in diam.) and ridges (to approximately 6 µm long), ornaments to 2 µm tall in profile, the ornaments are irregular and less crowded than those of *Quadrispora oblongispora*; perisporium yellowish in KOH, conspicuous, generally adhering closely to ornamentation but sometimes fragmenting or flaring; spores aggregating in tetrads; hilar appendix short, inconspicuous (short, broad and commonly obscured by ornamentation), tapering and truncate, entire; thin-walled, inamyloid, non-dextrinoid (light yellow brown in Melzer's solution); apex rounded and ornamented. *Basidia* hyaline, yellow when collapsed, clavate (collapsing soon after maturity), 4-spored, quite variable in size (actual range undetermined due to almost all basidia having collapsed). *Cystidia* absent. *Hymenium* palisade, hyaline but for necrotic basidia, non-gelatinised though badly degraded, vesiculose broadly obpyriform to cylindrical hymenial elements, thin-walled, 18–29 µm broad. *Subhymenium* collapsed. *Hymenophoral trama* interwoven to subparallel, hyaline to bright yellow-brown in KOH, non-gelatinised, 5–12 µm broad. *Peridiopellis* (in longitudinal section) of two layers, the outer broader layer of interwoven, hyaline, partly gelatinised, cylindrical, thin-walled, 1.5–5 µm broad clamped hyphae embedded in a hyaline matrix, abruptly meeting the inner layer of tightly interwoven, yellow-encrusted, non-gelatinised, inflated, thin-walled hyphae (appearing as interlocking polygonal cells), 10 to 60 µm broad. *Clamp connections* present in the outer peridiopellis and hymenophoral trama.

Figure 12: *Quadrispora pyriformis sp. nov.* PERTH 00960403. **A** Spores. Note the broader, more coarsely ornamented spores of this species compared with those of *Quadrispora oblongispora*, scale bar = 10 µm. **B** Basidia, scale bar = 10 µm.

Habitat and distribution: Known from a single collection from among south western coastal heath/open woodland of Two Peoples Bay. Found fruiting in July.

Collection examined: **W.A.**: Two Peoples Bay Nature Reserve, West Gully number 7 off Mt Gairdner, vegetation type not recorded, *G. Smith s.n.* (holotype here designated as PERTH 0090403, isotype H6388).

Etymology: Latin, in reference to the coarse tuberculate spore ornamentation that distinguishes this species from others in the genus.

Discussion: The much more prominent spore ornamentation, the much darker spores (in KOH) and the presence of a two-layered peridiopellis in *Quadrispora tubercularis* distinguishes this species from others in the genus described by Bougher & Castellano (1993). The spores of *Q. tubercularis* are also wider than those of *Q. musispora*. A current project by the authors aims to use molecular attributes to assess the phylogenetics of the known species of *Quadrispora* including *Q. tubercularis*. Further collections of *Q. tubercularis* are needed to characterise the extent of morphological variability in the features of this species as the specimens studied did not revive well from the air-dried state.

6. *Setchelliogaster*

Setchelliogaster australiensis G.W. Beaton, Pegler & T.W.K. Young, *Kew Bull.* 40: 169 (1985)
(Figure 13, Plate 1A)

Basidiomes hypogeous to subhypogeous, fruiting in small groups within the first 10 cm of soil, secotioid, 10–13 mm diam., conical, subglobose to flattened-ellipsoidal but with a thick, short stipe from which the peridium pulls away exposing the gleba. *Peridium* light orange (5A5) with darker brown discolourations (to 8F8), becoming greyish orange when air-dried, dry, glabrous, not bruising, without much adhering debris, to 1 mm thick, of a single layer which is pale in longitudinal section. *Gleba* labyrinthoid to sublamellate, light yellow to reddish golden (2A2 to 6C7), dry, not rapidly disintegrating after maturity, locules 0.5–1 mm diam., sometimes showing strong radial arrangement towards the base and with the gleba adnate to the stipe. *Columella/sterile tissue* a percurrent stipe-columella, 4–6 × 1–2 mm within the gleba, central, cylindrical terete, light yellow (M.3Y/8.7/4.4), dry, smooth, not forming distinct layers, solid, base bulbous, protruding up to 1 cm below the peridium; basal mycelium inconspicuous. *Macrochemical tests* not recorded. *Odour* and *taste* not recorded.

Spores yellow-brown (KOH), asymmetric, heterotropic, amygdaliform to subfusoid to narrowly citriform (spore shape variable both within and between collections (see Fig. 13A), 8.5–19 × 7–9.5 µm, mean of 59 spores 15.5 ± 2.0 × 8.0 ± 0.5 Q = 1–2.27, 1.92 ± 0.2 (KOH), ornamentated with small warts, rods or ridges, to 1.5 µm tall, less prominent towards base and apex; perisporium hyaline (KOH), reasonably conspicuous, though usually closely adhering may become fragmented and wrinkled; mature spores not aggregating; hilar appendix to 1 µm, conspicuous, tapering and truncate, entire; spores thick-walled (to 1.5 µm), inamyloid, non- to faintly dextrinoid (spores orange-brown to pinkish brown or reddish brown in Melzer's solution); apex rostrate and unadorned. *Basidia* hyaline, though becoming slightly yellow-brown when collapsed, cylindro-clavate, 4-spored, 35–50 × 8–11 µm. *Cystidia* absent. *Hymenium* palisade, of hyaline, non-gelatinised, clavate to inflated, thin-walled, to 19 µm broad elements. *Subhymenium* 18–25 µm broad, pseudoparenchymatous, of hyaline, non-gelatinised, isodiametric, thin-walled, to 19 µm broad elements. *Hymenophoral trama* 200–275 µm broad, parallel, of pale brown, non-gelatinised, cylindrical, thin-walled, 3–10 µm broad hyphae. *Peridiopellis* (in longitudinal section) of a single layer, up to 180 µm broad, forming a stratified epithelium of brown, gelatinised, inflated, thin-walled, 20–55 × 15–37 µm elements. *Clamp connections* present in hymenial tissues.

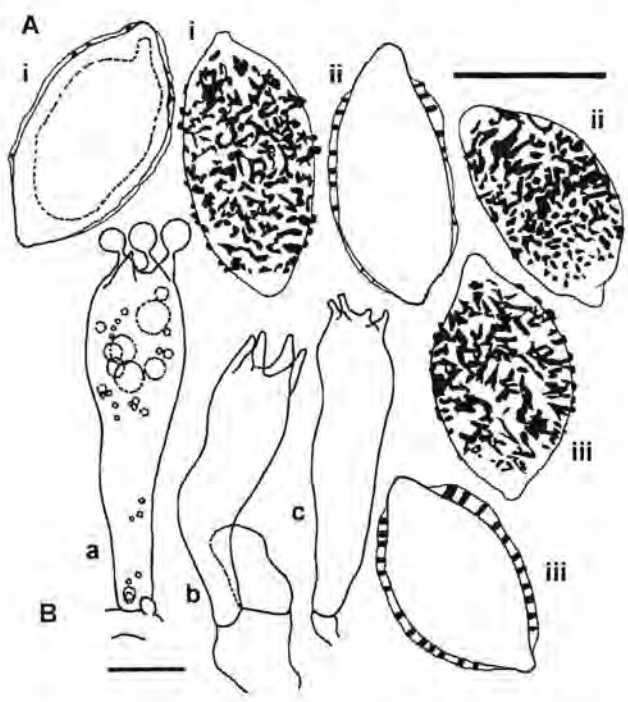


Figure 13: *Setchelliogaster australiensis* **A** Spores. Note the strongly amygdaliform to citriform shape. **i** H7317, **ii** *G. Beaton 39*, **iii** H1023, scale bar = 10 µm. **B** Basidia **a** H1023 **b** *G. Beaton 39* **c** H7317. Scale bar = 10 µm.

Habitat and distribution: This species is not currently considered a Western Australian native. It is only known in Western Australia from collections amongst nursery stock and inoculated plantations of *Eucalyptus globulus*, and has not

been recorded in forests or woodlands. Found fruiting July to September.

Collections examined: **W.A.**: CSIRO Perth W.A., in glasshouse pots with *Eucalyptus globulus* seedlings inoculated with H1023 from Tasmania (see below), 20 Aug. 1987, coll. *N. Bougher NB182* (H1023). Five Acre Nursery W.A., MH143062, in pots with *Eucalyptus globulus* seedlings inoculated with H1023 from Tasmania (see below), 20 July 1990, coll. *B. Thomson s.n.* (H1573). Northcliffe (Bebes site), in *Eucalyptus globulus* plantation inoculated with H1023 from Tasmania (see below), 8 Sept. 1990, coll. *N. Malajczuk s.n.* (H4317). **Other**: Vic.: Apollo Bay end of Turtons track, partially exposed under *Eucalyptus* debris, 19 July 1982, coll. *K. & G. Beaton Beaton 39* (isotype MELU). Tas.: Lone Star Provenance Trial TAS.EQ244375, in *Eucalyptus globulus* plantation, 10 July 1986, coll. *N. Malajczuk & T. Burgess s.n.* (H1023).

Etymology: Beaton *et al.* (1985) allocated the epithet '*australiensis*', noting that this was the first *Setchelliogaster* species recorded from Australia.

Discussion: Lago *et al.* (2001) in examining this species found veil remnants resembling those of *Descomyces* on the stipe of young *Setchelliogaster australiensis* specimens. The current authors did not see such hyphae but given the relatedness of the two genera (*e.g.* Peintner *et al.* 2001) we do not find it surprising that such hyphae should occur in *Setchelliogaster*. All Western Australian collections of *Setchelliogaster australiensis* available for study can be traced back to a fungus isolated from Tasmania (H1023). The apparent absence of naturally occurring representatives of this species in Western Australia is somewhat surprising in view of the abundance of this species in other parts of Australia (Lago *et al.* 2001). Also the apparent absence of *Setchelliogaster tenuipes* in Western Australia is also quite unexpected as it is widely distributed in eucalypt forests in other parts of Australia and in eucalypt plantations throughout many parts of the world (Lago *et al.* 2001).

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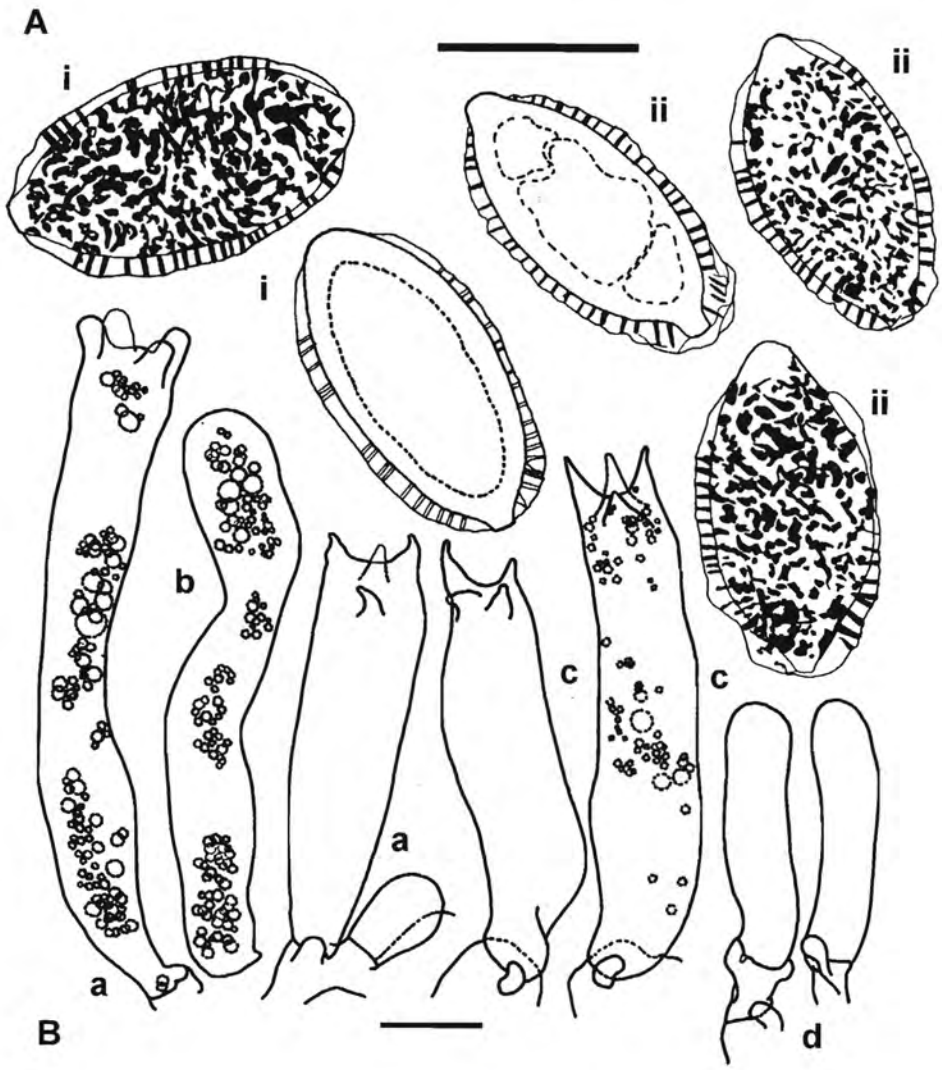

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