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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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