Comparing morphological species as used in ecological surveys against molecular barcoding with the ITS region: a case study of *Cortinarius* in the south-west of Western Australia

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Introduction

Studies of fungal communities usually use only one method of identification: either morphological or molecular. For macrofungi, there are potential problems with both methods — use of morphology requires presence of fruit-bodies and considerable experience and molecular identification relies on existence of a comprehensive barcode database. In addition, both methods rely on meaningful delimitation of species prior to identification of field samples. *Cortinarius* is the most diverse mushroom genus worldwide, and in Australia. A large series of collections of this genus is available from biodiversity surveys in Western Australia. These collections allow testing of morphological species as used in field surveys against molecular species as distinguished by use of the ITS region, which has recently been confirmed as the barcode region for fungi.

Methods

One hundred and seventy eight collections of *Cortinarius* (inclusive of *Dermocybe*) from regular surveys of permanent plots in jarrah (*Eucalyptus marginata*) forests in south-west Western Australia were assigned to 118 morpho-species groups (MG), recognisable on field characters. Genomic DNA was isolated for all collections and the ITS region of the rDNA was amplified and sequenced. The number of molecular species was determined by assembling the ITS sequences at a threshold of 98% of similarity. The NCBI GenBank database was queried using BLASTn to match each molecular species identified as described above with the closest ITS sequences available in the database. A Bayesian phylogenetic tree was also built based on the ITS sequences to graphically display the clades containing groups of sequences with no more than 2% of sequence dissimilarities.

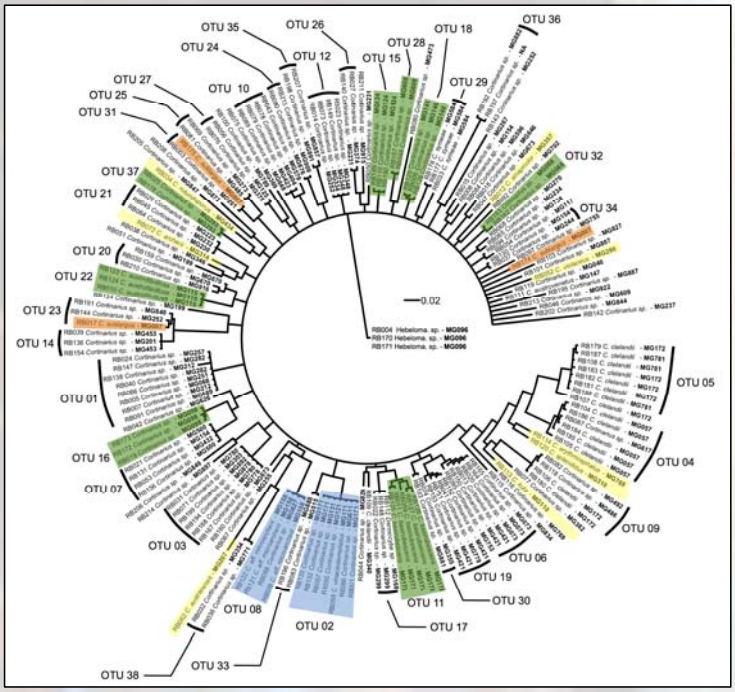

Figure 1: Tree based on phylogenetic analysis of ITS sequences of 178 collections of *Cortinarius* (including outgroup, *Hebeloma* sp. MG96). Each collection has collection (RB) number followed by morpho-species group (MG) designation. Eight of the multi-collection morpho-species formed an exclusive clade, such as *C. austrofibrillosus*, *C. basirubescens* and *C.* spp. including MG98, MG124 and MG293 [green highlight]; 10 multi-collection morpho-species were mixed with other species or singletons, as in clade 2 (which consisted of the four collections of *C. vinaceolamellatus*, along with the single collections of each of *C.* spp. MG121, MG131 and MG611) and clade 8 (which consisted of the three collections of *C. microarcheri* along with the single collection of C. sp. MG627) [blue highlight]; and the remaining 13 multi-collection morpho-species were split across more than one clade (as in *C. sublargus* and *C.* sp. MG421) or formed several singletons (as in *C.* sp. MG199) [orange highlight]. About half of the original 86 singleton morpho-species remained as singletons in the molecular analysis, such as *C. archeri*, *C. australiensis*, *C. erythrocephalus*, *C. kula*, *C. splendidus*, *C. rotundisporus*, *C. sinapicolor*, *C. violaceus* [yellow highlight].

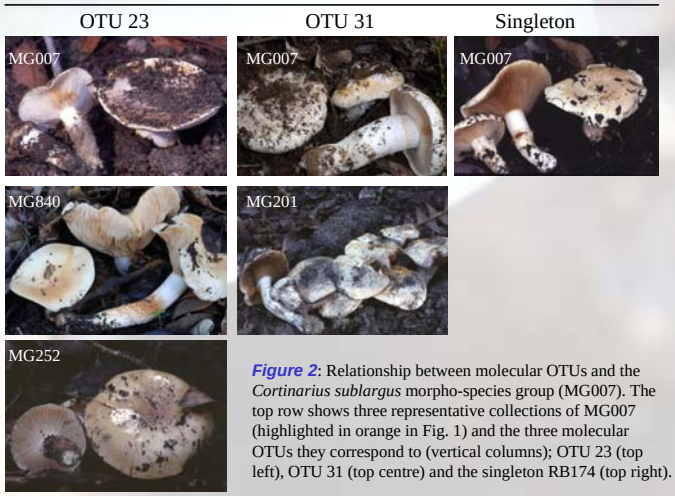

Figure 2: Relationship between molecular OTUs and the *Cortinarius sublargus* morpho-species group (MG007). The top row shows three representative collections of MG007 (highlighted in orange in Fig. 1) and the three molecular OTUs they correspond to (vertical columns); OTU 23 (top left), OTU 31 (top centre) and the singleton RB174 (top right).

Results

The 178 sequences yielded a phylogenetic tree with 37 clades (with two or more sequences) and 57 singleton collections (Fig. 1); making a total of 94 molecular species.

Of 32 morpho-species with more than one collection, eight were recovered exactly in the molecular analysis, 10 formed coherent clades and 13 were found in two or three places in the tree. When morpho-species were split, the segregate groups were often closely related. A few, however, were found in very different parts of the tree; in particular, each of the three collections of *Cortinarius sublargus* were unrelated (Fig. 2).

Overall, less than half of the morpho-species were recovered exactly in the molecular analysis. Some 45% were fused with other morpho-species and 11% were split into two or three molecular species.

We could only name 14% of morpho-species from the literature. Only 18% of molecular species matched named sequences in GenBank at a 98% similarity threshold (such as for *Cortinarius austrovenetus* and *C. erythrocephalus*). A further 11% of molecular species matched un-named environmental sequences from a study of ectomycorrhizal fungi in Tasmania.

Conclusions

This analysis:

- Confirms the high level of diversity in *Cortinarius* apparent from field observations of morphology.
- Allows calibration of morphological identification, suggesting that morpho-species as used in current surveys in south-west Western Australia are over-splitting observed variation in the majority of cases.
- Identifies a large set of species suitable for more detailed investigation of phylogeny (such as by other more phylogenetically informative loci such as *rpb*1) with specimens available for further sequencing.
- Establishes a databank for future molecular identification, both of fruit-bodies, but also of environmental sequences

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