Using molecular techniques to combine taxonomic and ecological data for fungi

Reviewing the Data Deficient fungi list, 2009

SCIENCE FOR CONSERVATION 306

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A B S T R A C T

A real problem with understanding the distribution of fungal species is a lack of specimens and associated collecting data. Few people can accurately identify fungi, and the effort required to collect and store a specimen is high, meaning even the most common species are poorly represented in New Zealand's fungal collections. This, in turn, creates a problem for biodiversity management, including the assessment of rarity, which requires species distribution data. The work reported here uses molecular techniques to supplement specimen-based distribution data with research data collected by fungal ecologists. We authenticate the ecological data through a molecular comparison with type specimens and other putatively authentic specimens. To test the practical value of this approach we focussed on Data Deficient fungi from the New Zealand Threat Classification System lists 2002, targeting ectomycorrhizal mushrooms, the subject of relatively intensive and ongoing ecological sampling. Data collected in this report indicate that 62 species of ectomycorrhizal mushrooms should be shifted from the Data Deficient category to a non-threatened category. At least one species should be considered for shifting to a higher threat category. However, balancing this is a recommendation to add 32 species to the Data Deficient category. Most of the additions represent newly described species, which were thus not considered during the 2002 assessment. The DNA sequence data accumulated during this project provide a first step in developing an authentic molecular data layer for New Zealand's fungi and, as such, have a high value beyond this project. They provide the means for non-experts to identify fungal species using a simple DNA sequence comparison. The data will also allow ecologists using t-RFLP sampling methods to match their samples to a species, and so continue to provide species distribution data through ecological projects. Previously, only three of the species treated had DNA sequence data available.

Keywords: fungi, ectomycorrhiza, Inocybe, Cortinarius, DNA, t-RFLP, molecular identification tools, New Zealand, threat classification

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1. Introduction

Effective biodiversity and biosecurity management requires accurate data on the distribution of species. A real problem with understanding the distribution of New Zealand's fungal species is a lack of specimens and associated collecting data. There are a small number of people who can accurately identify fungi, and the effort required to collect and store a specimen is high, meaning that even the most common species are poorly represented in New Zealand's fungal collections.

The fungal Threat Classification System lists (Hitchmough 2002), based on criteria from Molloy et al. (2002), were first developed following a workshop held during the New Zealand Fungal Foray at Haast in 2002, attended by a group of experienced mycologists from Landcare Research, Scion, and Otago University. The lack of distribution data for fungi is a major impediment to assessing the threat status of New Zealand's fungi and this is reflected in having more than 1400 species listed in the Data Deficient category.

Distribution records of fungi have traditionally relied solely on direct observation of the fruiting body. Fruiting bodies are short-lived, and their development depends on climatic and other conditions, which can lead to under-estimation of the actual distribution of a species. Practical and inexpensive DNA-based methods are now available to detect fungal species directly from hyphae, overcoming the need to observe fruiting bodies.

In this proof-of-concept project we use molecular methods to combine ecological and taxonomic datasets in an attempt to maximise the distribution data available for a group of Data Deficient fungal species. Ecological projects led by Ian Dickie (Landcare Research, sampling in beech and tea-tree in the South Island, e.g. Dickie et al. 2009) and Katrin Walbert (Scion, sampling in tea-tree in the North and South Islands) are accumulating t-RFLP-based genetic data on ectomycorrhizal fungal diversity directly from living roots of the host plants. This method is reviewed in Dickie & Fitzjohn (2007) and implemented in Fitzjohn & Dickie (2007), Dickie et al. (2009), and Dickie et al. (2010). The t-RFLP data measures changes in species diversity across sampled sites, but does not provide identification of those species. To identify the species represented in the ecological datasets requires a complementary set of molecular data from authentically identified herbarium specimens. This was provided through specimens in the New Zealand Fungal Herbarium (PDD), which represent 164 species of ectomycorrhizal mushrooms in the Data Deficient list.

We review the Data Deficient lists of ectomycorrhizal fungi on the basis of several other data sources, in addition to the ecological datasets. The publication of the fungal Threat lists provided a catalyst to encourage additions to the distribution records for the listed species. Many additional collections of Data Deficient species have been added to the New Zealand Fungal Herbarium (PDD) since 2002, through the collecting effort fostered by the annual New Zealand Fungal Forays (see Foray reports, www.funnz.org.nz). For example, the Fungal Forays in 2005, 2006, and 2007 reported 44, 57, and 32 Data Deficient species, respectively. Other projects, funded by the Terrestrial and Freshwater

Biodiversity Information System (TFBIS, see www.biodiversity.govt.nz/land/nzbs/tfbis/tfbis/), incorporated major historical collections of macrofungi into the New Zealand Fungal Herbarium and the NZFungi database[1]. These projects were based on specimens in the private collections of Marie Taylor, Barbara Segedin, and Greta Stephenson, and included 75 species on the Data Deficient lists. In addition, the exotic/indigenous status of several species on the original list has been reviewed (unpubl. data).

2. Objectives

The objective of this study was to review the ectomycorrhizal species on the Data Deficient fungi list (Hitchmough 2002) by:

- Utilising DNA data to integrate taxonomic and ecological datasets to maximise the distribution data available for these fungi.
- Reviewing all ectomycorrhizal species on the Data Deficient list on the basis of herbarium specimens deposited since 2002.

3. Methods

We used molecular methods to combine ecological and taxonomic datasets for a group of ectomycorrhizal fungal species listed as Data Deficient in Hitchmough (2002), so maximising distribution records for these species. Sequences and t-RFLP patterns from the ITS region are generated from reliably identified herbarium specimens. The t-RFLP patterns are used to recognise when these species appear in samples collected in ecological studies, allowing very high throughput assessment of species composition. Identifications can then be confirmed by direct sequencing or clone libraries in selected samples (e.g. Dickie et al. 2010). The sequences are used to provide identifications from sequences generated directly from ectomycorrhizal roots, as well as for collections of ectomycorrhizal fungi not identified to species level.

3.1 DATA SOURCES

3.1.1 Ecological datasets

Sampling of ectomycorrhizal diversity across ecological gradients using molecular techniques is being carried out in several independently funded projects (e.g. Dickie et al. 2009). The method used in these projects has been t-RFLP, which allows species diversity to be estimated from environmental samples comprising mycorrhizal tree roots and containing mixed DNA extracts of many

[1] 'Historically important mushroom collections added to the New Zealand Fungal Herbarium': www.landcareresearch.co.nz/research/research_details.asp?Research_Content_ID=265

species of fungi. Each species is characterised by a t-RFLP profile comprising a set of four fluorescently labelled DNA fragments of standard size. By matching t-RFLP profiles between samples, the presence or absence of individual species can be tracked. Detailed technical methods are provided in Dickie et al. (2009). To identify the species themselves requires a library of standard profiles to be generated from authentically identified specimens (see sections 3.1.2 and 3.2).

For the Data Deficient project we had access to 1,341 t-RFLP profiles generated from ecological samples gathered from three *Nothofagus* sites, two *Pinus* sites, and one *Kunzea* site in the Craigieburn, Granville, and Station Creek (Rakaia) areas of Canterbury, South Island. In addition, we were provided with 17 ITS sequences generated directly from *Leptospermum scoparium* root tips from four Canterbury high-country sites.

3.1.2 Dried herbarium specimens

To obtain DNA extracts to generate standard t-RFLP profiles for known species, putatively authentically identified dried herbarium specimens were selected to represent as many of the *Cortinarius* and *Inocybe* species on the Data Deficient list as possible. Whenever possible, DNA was extracted from type specimens. A few species from other genera were included, but *Cortinarius* and *Inocybe* were targeted because there are a large number of species of each on the Data Deficient list and because they are currently being actively studied taxonomically in complementary projects run by our international collaborators (K. Soop and E. Horak for *Cortinarius*; B. Matheny, University of Tennessee, and E. Horak for *Inocybe*). Because of the novelty of the DNA analysis approach we had no precedent from which to estimate the likely number of matches with the t-RFLP profiles, and thus included a few commonly collected species as a kind of control (see section 4.1). The specimens treated are listed in Appendix 1.

3.1.3 Tissue samples stored in CTAB buffer

At the same time as the Data Deficient project was running, we were given access to a set of about 230 tissue samples from ectomycorrhizal and saprobic mushrooms collected in 2001 and stored in CTAB buffer at the time of collecting. This storage method maximises the likelihood of successful extraction of high-quality DNA. Dried specimens of each of these specimens were recently deposited in the PDD herbarium. Many of these specimens were identified only to genus level. Based on these generic identifications, 104 of the tissue samples were from ectomycorrhizal species, based on the list of ectomycorrhizal genera provided by Orlovich & Cairney (2004). Of the specimens that had been identified to species level, eight represented Data Deficient ectomycorrhizal species. ITS and LSU sequences generated from the remaining unidentified specimens of ectomycorrhizal fungi showed that a further nine matched Data Deficient species. Molecular and distribution data from these specimens were incorporated into the project.

3.1.4 Updated collection data from PDD herbarium

The numbers of collections and the biostatus of all putatively ectomycorrhizal species on the 2002 Data Deficient list were updated from the current PDD collection data in the NZFungi database (http:\\nzfungi.landcareresearch.co.nz).

3.2 MOLECULAR METHODS

3.2.1 DNA extraction and amplification

DNA was extracted from herbarium specimens using REDExtract-N-Amp Plant PCR Kits (Sigma, USA) using the Corbett X-tractor Gene System (Corbett Robotics, Australia). Tiny pieces of mushroom tissue were ground in extraction buffer with a plastic pestle in the Eppendorf tube. Following this, DNA extraction and PCR were carried out following manufacturer's instructions. ITS and LSU sequences were obtained from each extract following the methods of Johnston & Park (2005). Amplification primers for ITS were ITS1F and ITS4 (White et al. 1990; Gardes & Bruns 1993) and for an approximately 950 bp fragment of the LSU were LROR and LR5 (Vilgalys & Hester 1990; Bunyard et al. 1994). Sequences have been deposited in Genbank, and accession numbers are provided in Appendix 1.

Standard t-RFLP fragment profiles were generated from the same DNA extracts using the methods of Dickie et al. (2009), to facilitate identification of the putative taxa in the ecological samples (see section 3.1.1).

3.2.2 DNA analysis

The ITS and LSU sequences were compared against data in Genbank using a BLAST search to check that they represented the fungus in the herbarium packet, rather than a chance contaminating mould. All reliable DNA sequences were aligned using Clustal X (Thompson et al. 1997), and phylogenetic trees generated from both the ITS and LSU datasets using a simple Neighbour Joining algorithm using PAUP* (Swofford 2002). The simple nature of these analyses means that the relative positions of the taxa in the trees has little phylogenetic significance, but they show clearly where there are multiple samples of the same species.

The t-RFLP patterns generated from the herbarium specimens were compared with those generated from the earlier ecological surveys, using the R based program TRAMPR (Dickie & FitzJohn 2007; FitzJohn & Dickie 2007).

4. Results

4.1 MOLECULAR DATA FROM HERBARIUM SPECIMENS

DNA was isolated from 102 dried herbarium specimens. A comparison of ITS and LSU sequences showed that 78 of these DNA samples represented the fungi expected, the remainder being various species of contaminating ascomycete fungi. The success rate for extracting good quality, reliable DNA lowered as the herbarium specimens got older, especially in those species with small, thin-fleshed fruiting bodies (Figs 1 & 2; Appendix 1). Most of the pre-1990 specimens from which good DNA was isolated were of species with large, robust fruiting bodies. Good quality DNA was successfully isolated from all of the tissue samples stored in CTAB. The specimens sequenced represented 74 different ectomycorrhizal species (Fig. 3). High-quality t-RFLP patterns were generated for all of these species. Several specimens represent *Inocybe* spp. in the process of being formally published and are listed as *Inocybe* sp. 1–12 in Appendix 1 and Fig. 3. DNA sequences for these species are not yet publicly available.

These data allowed us to:

- Recognise misidentified herbarium specimens (Table 1) through ITS sequence comparisons. In each case, the accuracy of the redetermination was confirmed by morphological examination.
- Generate additional distribution records for 13 Data Deficient species through matches to ecological samples using t-RFLP profiles.
- Recognise nine previously unidentified herbarium specimens as representing Data Deficient species, through matches to ITS sequences.
- Recognise that two species likely to be described in future taxonomic studies were common in the ecological samples. In most cases, recently described species of mushrooms in New Zealand taxonomic studies are based on one or a few herbarium specimens, the minimal data provided on their distribution and frequency meaning that they need to be added to the Data Deficient lists following publication.
- Recognise that the Data Deficient *Cortinarius cinnabarinus* was incorrectly recorded as present in New Zealand. The specimen on which this record was based has been redetermined as *Cortinarius veronicae* var. *dilutus* on the basis of an ITS sequence comparison.
- Question whether one of the common species included as a control, *Astrosporina asterospora*, in fact occurs in New Zealand. This fungus was originally described from north temperate regions, but Horak (1978) noted that although the Northern Hemisphere–New Zealand distribution is unusual, this species is found also in New Guinea, the Solomon Islands and Malaysia. However, the New Zealand specimen we sequenced is genetically distinct from Genbank records deposited as *A. asterospora* from Sweden and Denmark (Genbank accession numbers AJ889950, AJ889951, AM882897).

Figure 1. The proportion of herbarium specimens collected in each decade from 1960s to present, from which high-quality DNA was successfully isolated.

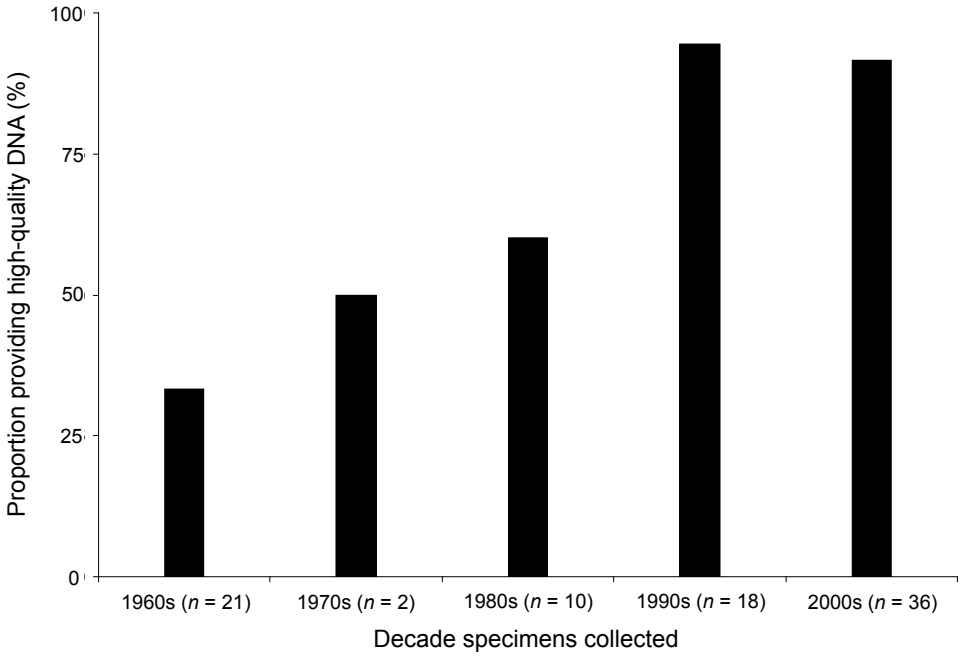
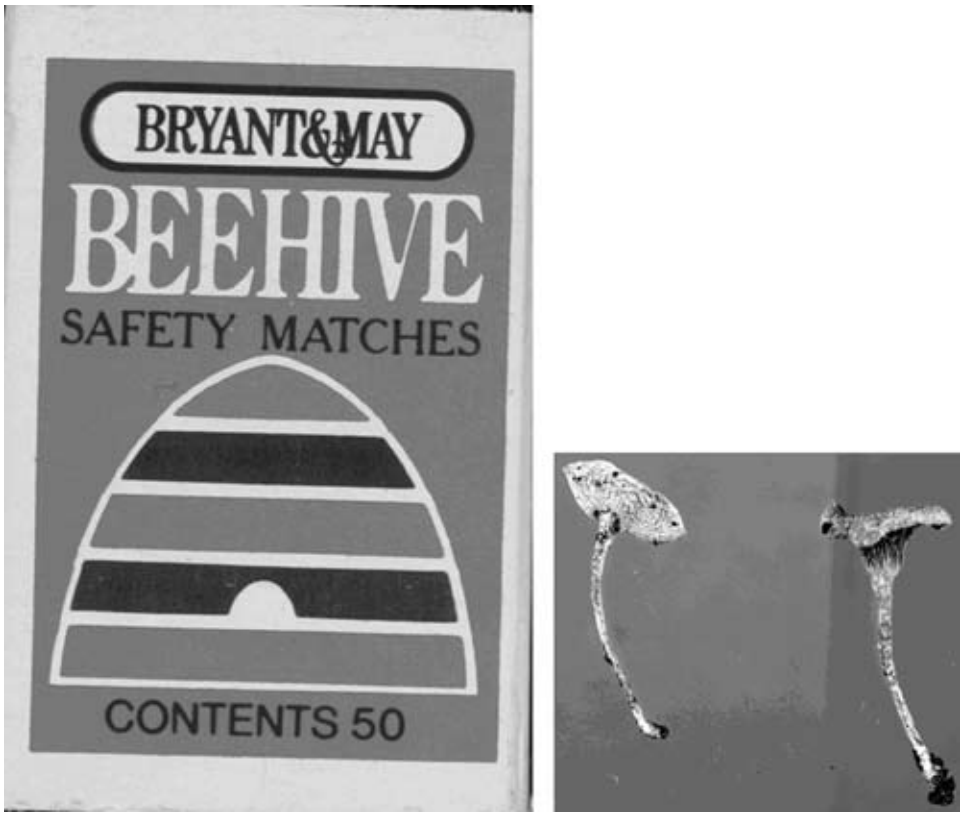


Figure 2. PDD 27072, the type specimen for *Camarophyllus patinicolor*, collected in 1968. This is one of the Data Deficient species with small, thin-fleshed fruiting bodies from which DNA was not successfully isolated.



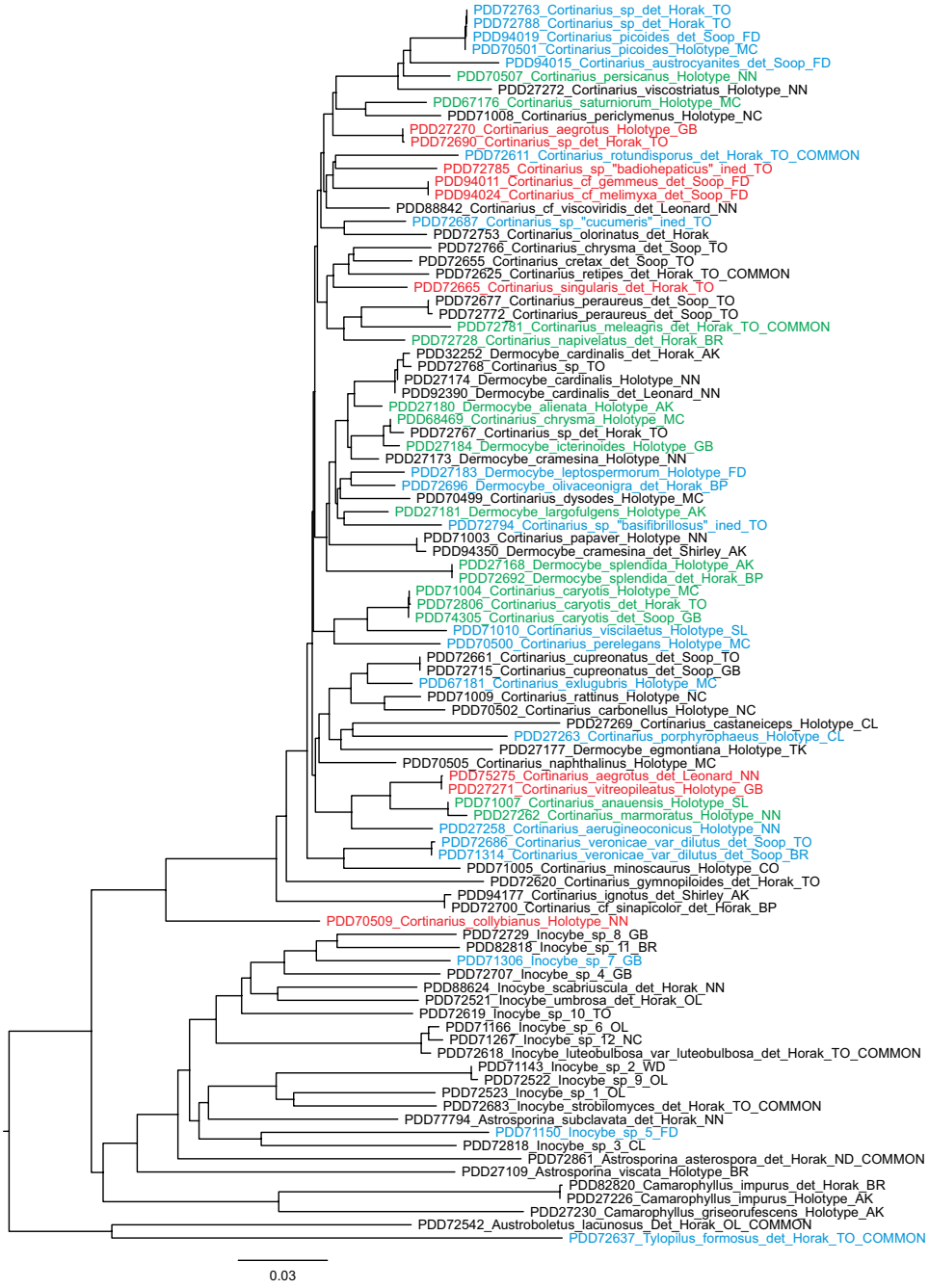


Figure 3. Neighbour joining tree based on ITS sequences. Species represented by their type specimens are indicated, as are the commonly collected species. Names in red are of species with clear matches to t-RFLP environmental samples; names blue have poorly defined matches with some parts of the profile low and difficult to distinguish from background signal; names in green match some t-RFLP profiles from environmental samples but are not unique for a single species. Geographic distribution of the specimens sampled is indicated by Crosby District codes (Crosby et al. 1998).

TABLE 1. EXAMPLES OF MISIDENTIFICATIONS OF SPECIES ON THE DATA DEFICIENT LIST REVEALED THROUGH DNA ANALYSIS.

PUTATIVE SPECIES SEQUENCED	IDENTITY BASED ON DNA SEQUENCE DATA
Dermocybe leptospermorum	PDD94202—another *Dermocybe* sp. with no DNA match
Cortinarius aegrotus	PDD75275—DNA match to *Cortinarius vitreopileatus*
Dermocybe cramesina	PDD94350—DNA match to *Cortinarius papaver*
Cortinarius chrysma	PDD94351—another *Cortinarius* sp. with no DNA match
Cortinarius ignotus	PDD94177—DNA match to *Cortinarius sinapicolor*
Cortinarius viscostriatus	PDD75709—another *Cortinarius* sp. with no DNA match
Camarophyllus impurus	PDD 81871—doubtfully *Camarophyllus*
Camarophyllus griseorufescens	PDD 87889—doubtfully *Camarophyllus*
Camarphyllus canus	PDD 88851—doubtfully *Camarophyllus*

4.2 MATCHING ECOLOGICAL DATA TO HERBARIUM SPECIMENS

Several of the environmental samples collected through the ecological projects had t-RFLP profiles matching those from herbarium specimens. However, many of the matches were tentative, being based on poorly defined profiles where one or more of the bands was doubtfully present, or where the profiles were not unique to a single species (Fig. 3). The putatively common species were no more often matched to the environmental samples than the supposedly rarer Data Deficient species.

Two of the species with multiple matches to environmental samples have yet to be formally described. These species are *Cortinarius* "*badiohepaticus*", and the species represented by the two collections labelled *Cortinarius* cf. *gemmeus* and *Cortinarius* cf. *melimyxa*. Even though they have not been formally described, the frequent matches to environmental samples suggest that both species are reasonably common.

None of the DNA sequences generated directly from mycorrhizal roots of *Leptospermum scoparium* matched any of those from herbarium specimens. Based on results from Genbank BLAST searches, these root samples comprised six *Cortinarius* spp., four *Tomentella* spp., two *Russula* spp., one *Clavulina* sp., and one *Tomentellopsis* sp. None closely matched any sequence deposited in Genbank.

4.3 REASSESSMENT OF SPECIES ON THE 'DATA DEFICIENT' LIST

Appendix 2 lists the 164 ectomycorrhizal species in the 2002 Data Deficient list for which specimens are available in the PDD herbarium. The appendix includes notes on those species to be removed from the Data Deficient category and placed in a higher threat category, those to be removed because they are now considered unthreatened, and those to be retained as Data Deficient.

Each name is assessed in relation to additional distribution records based on dried specimens gathered since 2002, as well as the additional distribution records provided by molecular matches to ecological samples. Several species are removed from the category for technical reasons—either they are now considered to be exotic, they are species now considered doubtfully present in New Zealand, or they are undescribed species deposited in the herbarium with informal, unpublished herbarium names and mistakenly categorised as Data Deficient in 2002.

In summary, we recommend that 62 of the originally listed species should be moved from the Data Deficient category to a non-threatened category. *Cortinarius pholiotellus* should be considered for a higher threat category—it has been collected only twice, each time from the same locality, beside a river and in a highly disturbed site with potential to be destroyed in the longer term. *Russula tapawera* could be recommended for adding to the Data Deficient category, but with all four collections from the same locality, should perhaps be considered for a higher threat status.

Counter-balancing the removal of species from the Data Deficient category is a recommendation to add 32 species (Appendix 3). Most of these species were described after the original 2002 assessment and are represented by less than four herbarium specimens.

5. Discussion and Conclusions

In addition to reviewing the status of 164 species of ectomycorrhizal fungi categorised as Data Deficient, this project provides a proof-of-concept test of the value that a basic, authentic molecular data layer can provide for understanding the distribution and diversity of New Zealand's fungi. This project combines data from ecological and taxonomic datasets, matching t-RFLP profiles from mycorrhizal root tips to those from authentic herbarium specimens of fruiting bodies of the Data Deficient species. On the basis of the DNA data generated we were able to provide more accurate identifications of herbarium specimens putatively representing Data Deficient species through ITS sequence comparisons, and provide additional distribution records for Data Deficient species through t-RFLP profile matches.

In a broader context, the project has provided a start to the accumulation of a barcode-like molecular data layer for the ectomycorrhizal fungi of New Zealand. The information gathered from authentic herbarium specimens will have value beyond the project. The power of molecular methods for understanding fungal distributions are three-fold—they allow non-experts to accurately identify specimens, they allow fungal species to be identified without the need to see fruiting bodies, and they provide the opportunity to combine data from diverse taxonomic and ecological projects.

Identification of mushrooms from fruiting body morphology relies also on the skills and accuracy of the observers. This project showed that even with skilled and experienced observers, many identifications are likely to be incorrect and that several past records of Data Deficient species have been based on incorrectly identified specimens (see Table 1). The DNA sequences now publicly available through Genbank (see Appendix 1) provide a greater resource for allowing molecular confirmation of an identification.

To date, the threat lists of fungi have been based only on data from herbarium specimens. It is well known that the diversity of fungi measured from the occurrence of fruiting bodies often does not reflect the diversity of fungal hyphae within that ecosystem (e.g. Gardes & Bruns 1996). To observe a fruiting body in an ecosystem requires a condition beyond the fungus being present—it also requires the environmental conditions to be suitable for that fungus to fruit. This reflects the pattern seen in this study, that species putatively common on the basis of fruit body observation are no more likely to be matched in datasets based on diversity of hyphae than are the much less commonly observed Data Deficient species. Therefore, as well as having the potential to provide huge numbers of records (the more than 1000 records from t-RFLP patterns available to us in this project for no cost would have taken a huge effort to generate as traditional

dried specimens), ecological datasets may more accurately reflect actual species diversity across the landscape. The molecular data gathered during this project will allow an ongoing accumulation of distribution data from ecological projects using both t-RFLP and direct sequencing methods.

The t-RFLP method used in this project is time and cost effective for generating large amounts of distribution data. A disadvantage is that even with a complete library of t-RFLP fragment sizes, it is unable to distinguish all species. Some putative matches are known to represent more than one species (e.g. the *Laccaria* spp. discussed in Dickie et al. 2009), and the quality of the signal from environmental samples may preclude a reliable identification (see Fig. 3 and Appendix 2). Using different or additional restriction enzymes may reduce this problem, but such a change would require whole new libraries of standard fragment sizes to be accumulated. Another disadvantage is that researchers from different labs must use exactly equivalent protocols and restriction enzymes for results to be combined.

One alternative is to sequence directly from individual mycorrhizal root fragments, but this method is not well suited to *Nothofagus* due to a high level (> 50%) of single root fragments being colonised by multiple fungal species (Dickie et al. 2010). While cloning can be used to separate DNA of multiple species, for cultural reasons, permission to clone DNA extracted from native environments in New Zealand is difficult to obtain. Tedersoo et al. (2008) attempted to avoid the problem of mixed DNA extracts by designing sets of taxon-specific primers targeting the kinds of fungi they expected to be present as mycorrhizas. However, this is a time-intensive approach that potentially biases results towards pre-selected groups of taxa.

Technological advances in pyrosequencing are starting to provide practical, time-efficient alternatives to t-RFLP and cloning. For example, the Life Sciences GS Junior 454 sequencing machines are suitable for small labs and for a few thousand dollars allow large numbers of sequences up to about 450 bp in length to be generated from mixed environmental DNA. Buée et al. (2009) used a similar system to investigate total fungal diversity in forest soils.

To realise the potential of any of these methods requires a much more complete library of DNA data matched to authentic specimens, irrespective of whether those data comprise t-RFLP band sizes, or DNA sequences. For example, in the 454-based study, Buée et al. (2009) were able to identify only 26 of the more than 1000 putative species detected from oak forest soils. O'Brien et al. (2005) used cloning to recognise 412 putative fungal taxa from soil on the basis of ITS sequences. Of the sequences representing Basidiomycota, 36% could not be identified even to the taxonomic level of Order.

Perhaps the most powerful advantage of a molecular approach is the ability to take data from a wide range of projects and reanalyse it for alternative purposes. Projects using DNA sequences rather than t-RFLP have greater potential for matching data between unrelated projects because the data generated in method-independent. Most ecological projects using DNA sequences to identify taxa target the ITS region. The ITS is currently regarded as having the best potential as a barcoding region for fungi (Seifert 2008) and is routinely collected as part of many ecological and taxonomic projects. Although not reliable for all groups, ITS sequences usually distinguish taxa at about the level of species, are easy to

generate from samples with even poor quality DNA, and can be searched against a rapidly expanding dataset. In this project, each case where a redetermination was based on an ITS sequence comparison was confirmed by a subsequent morphological examination.

This project provides a direction for the future. Modern fungal taxonomy demands a molecular approach, even for the most basic alpha-taxonomic study. The same will soon be true for any study asking questions about fungal diversity and distribution, rarity, threat, impact and recovery. Useful extensions of this project could include: generating a complete DNA data layer for New Zealand's rare and endangered fungi; developing more reliable protocols for extraction of DNA from old herbarium specimens; using DNA sequence data to develop probes to specifically target hyphae of fungal species in high threat categories to better understand their local, as well as their wider, distributions within ecosystems; expanding the dataset from ectomycorrhizal fungi to include wood-rotting fungi, which are also being targeted in ecological studies (D. Peltzer, Landcare Research, pers. comm.); and developing a web-based tool to allow broad user access to the authentic, New Zealand-focussed DNA layer being accumulated, so facilitating more accurate, DNA-based identification of specimens and avoiding data quality issues with Genbank (e.g. Nilsson et al. 2006; Pennisi 2008).

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Appendix 1

COLLECTIONS WITH MOLECULAR DATA (ITS AND LSU SEQUENCES, AND t-RFLP PATTERNS) GENERATED

SPECIES (BIOSTATUS)	PDD	HOLOTYPE	YEAR COLLECTED	t-RFLP, ITS, LSU DATA GENERATED (WITH GENBANK ACCESSION NUMBERS)	NOTES
Astrosporina aequalis (indigenous endemic)	32335	T	1974	Contaminating DNA	
Astrosporina amygdalina (indigenous endemic)	27114	T	1969	Contaminating DNA	
Astrosporina asterospora	72861*		2001	HM060326, HM060325	Common control species (see Methods)
Astrosporina manukanea (indigenous endemic)	27110	T	1968	Contaminating DNA	
Astrosporina paracerasphora (indigenous non-endemic)	27108	T	1967	Contaminating DNA	
Astrosporina straminea (indigenous endemic)	27112	T	1969	No DNA isolated	
Astrosporina subclavata (indigenous endemic)	27111	T	1969	Contaminating DNA	
Astrosporina subclavata (indigenous endemic)	77794		2003	GU233360	
Astrosporina viscata (indigenous endemic)	27109	T	1968	Contaminating DNA	
Austroboletus lacunosus (indigenous non-endemic)	72542*		2001	HM060327	Common control species (see Methods)
Boletus novae-zelandiae (indigenous endemic)	25896	T	1966	Contaminating DNA	
Camarophyllus canus (indigenous endemic)	27073	T	1969	Contaminating DNA	
Camarophyllus delicatus (indigenous endemic)	27087	T	1967	Contaminating DNA	
Camarophyllus griseorufescens (indigenous endemic)	27230	T	1981	GU233328, GU233423	
Camarophyllus impurus (indigenous endemic)	27226	T	1981	GU233327, GU233383	
Camarophyllus impurus (indigenous endemic)	75515		2002	GU233358, GU233410	
Camarophyllus impurus (indigenous endemic)	82820		2005	GU233363, GU233406	
Camarophyllus patinicolor (indigenous endemic)	27072	T	1968	Contaminating DNA	
Camarophyllus sp.?	81871		2004	Doubtful ID, ITS and LSU sequences but no tRFLP. GU233386, GU233362	Originally identified as *Camarophyllus impurus*
Camarophyllus sp.?	87889		2004	Doubtful ID, ITS and LSU sequences but no tRFLP. GU233364, GU233412	Originally identified as *Camarophyllus griseorufescens*
Camarophyllus sp.?	88851		2006	Doubtful ID, ITS and LSU sequences but no tRFLP. GU233366, GU233382	Originally identified as *Camarphyllus canus*
Cortinarius aegrotus (indigenous endemic)	27270	T	1981	GU233333, GU233389	
Cortinarius aerugineoconicus (indigenous endemic)	27258	T	1969	GU233329, GU233408	
Cortinarius anauensis (indigenous endemic)	71007	T	1999	GU233350, GU233381	
Cortinarius austrocyanites (indigenous non-endemic)	94015		2008	GU233370, GU233385	
Cortinarius carbonellus (indigenous endemic)	70502	T	1999	GU233343, GU233391	
Cortinarius caryotis (indigenous endemic)	71004	T	1999	GU233348, GU233407	
Cortinarius caryotis (indigenous endemic)	72806*		2001	Sequences not publicly available	Originally identified as *Cortinarius* cf. *retipes*
Cortinarius caryotis (indigenous endemic)	74305		2001	GU233356, GU233421	
Cortinarius castaneiceps (indigenous endemic)	27269	T	1981	GU233332	
Cortinarius chrysma (indigenous endemic)	68469	T	1997	GU233339, GU233393	
Cortinarius collybianus (indigenous endemic)	70509	T	1999	GU233346, GU233417	
Cortinarius dysodes (indigenous endemic)	70499	T	1999	GU233340, GU233394	

* DNA from tissue sample stored in CTAB buffer.

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Appendix 1 continued

SPECIES (BIOSTATUS)	PDD*	HOLOTYPE	YEAR COLLECTED	t-RFLP, ITS, LSU DATA GENERATED (WITH GENBANK ACCESSION NUMBERS)	NOTES
Cortinarius exlugubris (indigenous endemic)	67181	T	1997	GU233338, GU233409	
Cortinarius gemmeus (indigenous endemic)	27268	T	1981	No DNA isolated	
Cortinarius ? "*gymnopiloides*" (indigenous)	72620*		2001	Sequences not publicly available	Do not match sequences deposited in Genbank as *C. gymnopiloides*, an apparently unpublished name
Cortinarius ignotus (indigenous endemic)	27264	T	1981	No DNA isolated	
Cortinarius lubricanescens (indigenous endemic)	71006	T	1997	No DNA isolated	
Cortinarius marmoratus (indigenous endemic)	27262	T	1968	GU233330	
Cortinarius meleagris (indigenous)	72781*		2001	HM060324, HM060323	Common control species (see Methods)
Cortinarius melimyxa (indigenous endemic)	27267	T	1969	Contaminating DNA	
Cortinarius minoscaurus (indigenous endemic)	71005	T	1999	GU233349, GU233377	
Cortinarius naphthalinus (indigenous endemic)	70505	T	1999	GU233344, GU233401	
Cortinarius olorinatus (indigenous endemic)	27255	T	1981	No DNA isolated	
Cortinarius olorinatus (indigenous endemic)	72753*		2001	HM060330, HM060331	
Cortinarius papaver (indigenous non-endemic)	71003	T	1999	GU233347, GU233399	
Cortinarius papaver (indigenous non-endemic)	94350		2008	GU233375, GU233426	Originally identified as *Cortinarius cramesina*
Cortinarius perelegans (indigenous endemic)	70500	T	1999	GU233341, GU233398	
Cortinarius periclymenus (indigenous endemic)	71008	T	1999	GU233351, GU233379	
Cortinarius persicanus (indigenous non-endemic)	70507	T	1999	GU233345, GU233392	
Cortinarius phaeochlorus (indigenous endemic)	27265	T	1981	No DNA isolated	
Cortinarius pholiotellus (indigenous endemic)	68470	T	2008	Contaminating DNA	
Cortinarius picoides (indigenous endemic)	70501	T	1999	GU233342	
Cortinarius picoides (indigenous endemic)	94019		2008	GU233371, GU233424	
Cortinarius porphyrophaeus (indigenous endemic)	27263	T	1981	GU233331, GU233416	
Cortinarius rattinus (indigenous endemic)	71009	T	1999	GU233352, GU233419	
Cortinarius rotundisporus (indigenous non-endemic)	72611*		2001	HM060317, HM060316	Common control species (see Methods)
Cortinarius saturniorum (indigenous endemic)	67176	T	1997	GU233337, GU233388	
Cortinarius sinapicolor (indigenous non-endemic)	72700*		2001	HM060328, HM060329	
Cortinarius sinapicolor (indigenous non-endemic)	94177		2008	GU233373, GU233396	Originally identified as *Cortinarius ignotus*
Cortinarius sp. "*badiohepaticus*" ined. (indigenous)	72785*		2001	Sequences not publicly available	Unpublished herbarium name for an undescribed species
Cortinarius sp. "*basifibrillosus*" ined. (indigenous endemic)	72794*		2001	Sequences not publicly available	Unpublished herbarium name for an undescribed species

* DNA from tissue sample stored in CTAB buffer.

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Appendix 1 continued

SPECIES (BIOSTATUS)	PDD	HOLOTYPE	YEAR COLLECTED	t-RFLP, ITS, LSU DATA GENERATED (WITH GENBANK ACCESSION NUMBERS)	NOTES
Cortinarius sp. "*cucumeris*" ined. (indigenous)	72687*		2001	Sequences not publicly available	Unpublished herbarium name for an undescribed species; sometimes referred to using another unpublished name, *Myxacium cucumeris*
Cortinarius sp.	75709		2002	GU233359, GU233402	Originally identified as *Cortinarius viscostriatus*
Cortinarius sp.	94202		2008	GU233374, GU233411	Originally identified as *Cortinarius leptospermorum*
Cortinarius sp.	94351		2008	GU233376, GU233422	Originally identified as *Cortinarius chrysma*
Cortinarius sp. nov. (fide Soop, pers. comm.)	94011		2008	GU233369, GU233404	Originally identified as *Cortinarius* cf. *gemmeus*
Cortinarius sp. nov. (fide Soop, pers. comm.)	94024		2008	GU233372, GU233405	Originally identified as *Cortinarius* cf. *melimyxa*
Cortinarius veronicae var. *dilutus* (indigenous endemic)	71314		2000	GU233354, GU233400	Originally identified as *C. cinnabarinus*, a species doubtfully present in NZ
Cortinarius viscilaetus (indigenous endemic)	71010	T	1997	GU233353, GU233378	
Cortinarius viscostriatus (indigenous endemic)	27272	T	1968	GU233335	
Cortinarius viscovitridis (indigenous endemic)	27257	T	1969	Contaminating DNA	
Cortinarius viscovitridis (indigenous endemic)?	88842		2006	GU233390	Doubtfully authentic for this name
Cortinarius vitreopileatus (indigenous endemic)	27271	T	1981	GU233334, GU233397	
Cortinarius vitreopileatus (indigenous endemic)	75275		2004	GU233357, GU233403	Originally identified as *Cortinarius aegrotus*
Dermocybe alienata	27180	T	1969	GU233323, GU233384	
Dermocybe aurantiella (indigenous endemic)	27176	T	1968	Contaminating DNA	
Dermocybe cardinalis (indigenous endemic)	27174	T	1968	GU233321, GU233415	
Dermocybe cardinalis (indigenous endemic)	84245		1970	No DNA isolated	
Dermocybe cardinalis (indigenous endemic)	32252		1974	GU233336, GU233414	
Dermocybe cardinalis (indigenous endemic)	92390		2007	GU233368, GU233418	
Dermocybe cramesina (indigenous non-endemic)	27173	T	1968	GU233320, GU233420	
Dermocybe egmontiana (indigenous endemic)	27177	T	1968	GU233322	
Dermocybe icterinoides (indigenous endemic)	27184	T	1968	GU233326	
Dermocybe largofulgens (indigenous endemic)	27181	T	1969	GU233324	
Dermocybe leptospermorum (indigenous endemic)	27183	T	1969	GU233325, GU233395	
Dermocybe purpurata (indigenous endemic)	27171	T	1968	Contaminating DNA	
Dermocybe splendida (indigenous non-endemic)	27168	T	1969	GU233319, GU233387	
Inocybe cerea (indigenous endemic)	27122	T	1969	Contaminating DNA	

* DNA from tissue sample stored in CTAB buffer.

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Appendix 1 continued

SPECIES (BIOSTATUS)	PDD	HOLOTYPE	YEAR COLLECTED	t-RFLP, ITS, LSU DATA GENERATED (WITH GENBANK ACCESSION NUMBERS)	NOTES
Inocybe destruens (indigenous endemic)	27124	T	1968	Contaminating DNA	
Inocybe destruens (indigenous endemic)?	80480		2003	GU233361, GU233380	Doubtfully authentic for this name
Inocybe latericia (indigenous non-endemic)	27120	T	1969	Contaminating DNA	
Inocybe latericia (indigenous non-endemic)?	92382		2007	GU233367, GU233413	Doubtfully authentic for this name
Inocybe luteobulbosa var. *luteobulbosa* (Indigenous Endemic)	72618*		2001	HM060318	Common control species (see Methods)
Inocybe luteobulbosa var. *volvata* (indigenous endemic)	27130	T	1968	No DNA isolated	
Inocybe mendica (indigenous endemic)	27125	T	1968	Contaminating DNA	
Inocybe phaeosquarrosa (indigenous endemic)	27126	T	1969	Contaminating DNA	
Inocybe renispora (indigenous endemic)	27119	T	1968	Contaminating DNA	
Inocybe scabriuscula (indigenous endemic)	27127	T	1968	Contaminating DNA	
Inocybe scabriuscula (indigenous endemic)	88624		2006	GU233365 , GU233425	
Inocybe sp. 1 (indigenous endemic)	72523		2001	Sequences not publicly available	
Inocybe sp. 2 (indigenous endemic)	71143		2000	Sequences not publicly available	
Inocybe sp. 3 (indigenous endemic)	72818		2001	Sequences not publicly available	
Inocybe sp. 4 (indigenous endemic)	72707		2001	Sequences not publicly available	
Inocybe sp. 5 (indigenous endemic)	71150		2000	Sequences not publicly available	
Inocybe sp. 6 (indigenous endemic)	71166		2000	Sequences not publicly available	
Inocybe sp. 7 (indigenous endemic)	71306		2000	Sequences not publicly available	
Inocybe sp. 8 (indigenous endemic)	72729		2001	Sequences not publicly available	
Inocybe sp. 9 (indigenous endemic)	72522		2001	Sequences not publicly available	
Inocybe sp. 10 (indigenous non-endemic)	72619*		2001	Sequences not publicly available	
Inocybe sp. 11 (indigenous endemic)	82818		2005	Sequences not publicly available	
Inocybe sp. 12 (indigenous endemic)	71267		2000	Sequences not publicly available	
Inocybe strobilomyces (indigenous endemic)	72683*		2001	HM060322, HM060321	Common control species (see Methods)
Inocybe umbrosa	27131	T	1968	Contaminating DNA	
Inocybe umbrosa (indigenous endemic)	72521		2001	GU233355	
Phaeocollybia longipes (indigenous endemic)	27100	T	1968	No DNA isolated	
Phaeocollybia minuta (indigenous endemic)	27099	T	1969	Contaminating DNA	
Russula pudorina (indigenous endemic)	26574	T	1967	Contaminating DNA	
Tylopilus formosus (indigenous non-endemic)	72637*		2001	HM060320, HM060319	Common control species (see Methods)

* DNA from tissue sample stored in CTAB buffer.

Appendix 2

ALL SPECIES REVIEWED IN THIS PROJECT WITH NOTES ON RECOMMENDED CHANGES TO STATUS (IF ANY)

SPECIES	DNA DATA GENERATED?	NOTES
Astrosporina aequalis (indigenous endemic)		DNA not successfully amplified; retain
Astrosporina amygdalina (indigenous endemic)		DNA not successfully amplified
Astrosporina avellana (indigenous non-endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Astrosporina graveolens (indigenous endemic)		DNA not successfully amplified; several recent collections, but must be some doubt over their identity; retain on list
Astrosporina leptospermi (indigenous endemic)		Several collections referred to this species, but doubt about some of the identifications, retain on list
Astrosporina manukanea (indigenous endemic)		DNA not successfully amplified
Astrosporina paracerasphora (indigenous non-endemic)		Retain
Astrosporina straminea (indigenous endemic)		Retain
Astrosporina subclavata (indigenous endemic)	Y	No tRFLP matches; few collections, all collections in Nelson District; retain
Astrosporina viscata (indigenous endemic)		Retain
Boletellus ananas (exotic)		Exotic; **remove from Data Deficient category**
Boletus novae-zelandiae (indigenous endemic)		DNA not successfully amplified
Calocybe onychina (indigenous non-endemic)		Unpublished informal herbarium name; **remove from Data Deficient category**
Calocybe readiae (indigenous endemic)		Unpublished informal herbarium name; **remove from Data Deficient category**
Camarophyllus canus (indigenous endemic)		DNA not successfully amplified from type; some from a Pat Leonard collection identified as this species, but in strange place on ITS tree
Camarophyllus delicatus (indigenous endemic)		DNA not successfully amplified
Camarophyllus griseorufescens (indigenous endemic)	Y	No tRFLP matches; both collections from AK
Camarophyllus impurus (indigenous endemic)	Y	No tRFLP matches; four widespread collections in N and S Islands; **remove from Data Deficient category**
Camarophyllus patinicolor (indigenous endemic)		Another recent AK collection, now five in PDD; **remove from Data Deficient category**
Clavaria musculospinosa (indigenous endemic)		Perhaps only three authentically identified collections; retain
Clavaria phoenicea var. *persicina* (indigenous non-endemic)		Four collections, but perhaps doubt over accuracy of some identifications; retain
Clavulina alutaceosiccescens (indigenous endemic)		Retain
Clavulina brunneocinerea (Indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Clavulina copiosocystidiata (indigenous endemic)		Retain
Clavulina cristata var. *zealandica* (indigenous endemic)		Now three possible collections; retain
Clavulina geoglossoides (indigenous non-endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Clavulina humilis (indigenous non-endemic)		Retain
Clavulina leveillei var. *atricha* (indigenous non-endemic)		Retain
Clavulina purpurea (indigenous endemic)		Retain
Clavulina samuelsii (indigenous endemic)		Several recent collections, but doubt over identity of some; retain
Clavulina septocystidiata (indigenous endemic)		Retain
Clavulina urnigerobasidiata (indigenous endemic)		Retain
Cortinarius aegrotus (indigenous endemic)	Y	Numerous t-RFLP matches; also another DNA-based match; **remove from Data Deficient category**
Cortinarius aerugineoconicus (indigenous endemic)	Y	Some uncertain t-RFLP matches
Cortinarius anauensis (indigenous endemic)	Y	Numerous t-RFLP matches, doubtfully distinct from *C. marmoratus*; retain until taxonomy resolved

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Appendix 2 continued

SPECIES	DNA DATA GENERATED?	NOTES
Cortinarius aurantioferreus (indigenous endemic)		Now five widespread collections from both N and S Islands; **remove from Data Deficient category**
Cortinarius austrocyanites (indigenous non-endemic)	Y	One tentative t-RFLP match; now four collections widespread in S Island; **remove from Data Deficient category**
Cortinarius bellus (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Cortinarius carbonellus (indigenous endemic)	Y	Some doubtful t-RFLP matches; retain
Cortinarius caryotis (indigenous endemic)	Y	Tentative tRFLP matches, but perhaps not unique; recent collections from Taupo (previously misidentified, redetermined on the basis of DNA) and Gisborne; **remove from Data Deficient category**, more than four collections, from several Crosby districts
Cortinarius castaneiceps (indigenous endemic)	Y	No tRFLP matches; known only from near the summit of Hauturu/Little Barrier Island
Cortinarius chrysma (indigenous endemic)	Y	*Dermocybe alienata* and *C. chrysma* with same tRFLP, many matches to this; *C. chrysma* also matches PDD 72767 (TO) on basis of DNA comparison, with at least three other collections in PDD, and Soop (2006) noted as common; **remove from Data Deficient category**
Cortinarius collybianus (indigenous endemic)	Y	One tRFLP match, several recent collections; Soop (2006) considered it common; **remove from Data Deficient category**; PDD 72718 identified by Horak as *C. collybianus* is something else; PDD 89033, identified (incorrectly?) as *C. veronicae* matches by t-RFLP
Cortinarius cremeolinus (indigenous endemic)		Numerous recent collections; **remove from Data Deficient category**
Cortinarius cucumeris (indigenous endemic)	Y	Unpublished informal herbarium name (sometimes referred to as *Myxacium cucmeris*, another informal, unpublished name); several doubtful t-RFLP matches; several recent collections from many regions have been tagged with this name; **remove from Data Deficient category**
Cortinarius cupreonatus (indigenous endemic)	Y	No authentic DNA, no tRFLP matches, but Soop (pers. comm.) identified two additional Horak specimens (ex TO, GB); another nine collections in PDD; **remove from Data Deficient category**
Cortinarius cycneus (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Cortinarius dysodes (indigenous endemic)	Y	No t-RFLP matches; only two authentic collections; retain
Cortinarius exlugubris (indigenous endemic)	Y	Some doubtful tRFLP matches; only one authentic collection in PDD
Cortinarius gemmeus (indigenous endemic)		No authentic DNA; a *Cortinarius* cf. *gemmeus* collection represents a distinct, undescribed species
Cortinarius ignotus (indigenous endemic)		DNA doubtfully authentic (got none from the type); retain
Cortinarius indolicus (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Cortinarius lubricanescens (indigenous endemic)		DNA not successfully amplified; but now five collections in PDD widespread through S Island; **remove from Data Deficient category**
Cortinarius marmoratus (indigenous endemic)	Y	Numerous t-RFLP matches, but doubtfully distinct from *C. anauensis*; retain until taxonomy resolved
Cortinarius melimyxa (indigenous endemic)		No authentic DNA (a *Cortinarius* cf. *melimyxa* collection represents an undescribed species)
Cortinarius minoscaurus (indigenous endemic)	Y	No t-RFLP matches; **perhaps move to higher threat category**
Cortinarius naphthalinus (indigenous endemic)	Y	No t-RFLP matches; single collection; retain
Cortinarius olorinatus (indigenous endemic)	Y	DNA not successfully amplified from Type, but some from a specimen identified as this by Horak (not yet in PDD); no tRFLP matches; several collections in PDD but some doubtful identifications; retain
Cortinarius papaver (indigenous non-endemic)	Y	No t-RFLP matches; one additional collection from AK (redetermination based on DNA comparison); retain
Cortinarius peraureus (indigenous endemic)		No authentic DNA; many recent collections (including on ex Horak identified by Soop, pers. comm.); **remove from Data Deficient category**
Cortinarius perelegans (indigenous endemic)	Y	One tentative tRFLP match; Soop (2006) noted as 'fairly common', but few authentically identified collections in PDD; retain on list

Continued next page

SPECIES	DNA DATA GENERATED?	NOTES
Cortinarius periclymenus (indigenous endemic)	Y	No t-RFLP matches; Soop (2006) noted as 'fairly common' but only two collections in PDD; retain
Cortinarius persicanus (indigenous non-endemic)	Y	Several t-RFLP matches, but could be one of several species; only two collections PDD; retain
Cortinarius phaeochlorus (indigenous endemic)		Single AK collection
Cortinarius pholiotellus (indigenous endemic)		Two collections from a single locality; **perhaps move to higher threat status**
Cortinarius picoides (indigenous endemic)	Y	Two possible t-RFLP matches, another DNA match to two Taupo specimens, together with four other South Island collections; **remove from Data Deficient category**
Cortinarius porphyrophaeus (indigenous endemic)	Y	One doubtful t-RFLP match; known from two far-apart Crosby Districts; few authentically identified collections; retain for now
Cortinarius rattinus (Indigenous endemic)	Y	No t-RFLP matches; a single authentic collection; retain
Cortinarius rotundisporus subsp. *nothofagi* (indigenous endemic)		Now called *C. tessiae*; seven collections in PDD; Soop (2006) regarded as common; **remove from Data Deficient category**
Cortinarius saturniorum (indigenous endemic)	Y	Several t-RFLP matches, but cannot distinguish *Dermocybe largofulgens* and *C. saturniorum*; now five collections in PDD; **remove *C. saturniorum* from Data Deficient category** and leave *D. largofulgens* (the Type, from AK, is the only known specimen)
Cortinarius sinapicolor (indigenous non-endemic)	Y	One collection identified by Horak, DNA matches another collection misidentified as *C. ignotus*; no t-RFLP matches; retain
Cortinarius taylorianus (indigenous endemic)		Six collections from N and S Islands, Soop (2006) taged as 'fairly common'; **remove from Data Deficient category**
Cortinarius ursus (indigenous endemic)		Several recent collections, but few identified by Soop; Soop (2006) regarded as rare; retain for meantime
Cortinarius veronicae (indigenous non-endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Cortinarius viscilaetus (indigenous endemic)	Y	One doubtful tFRLP match; retain
Cortinarius viscostriatus (indigenous endemic)	Y	No t-RFLP match; now two collections; retain
Cortinarius viscovirdis (indigenous endemic)		Doubtful that DNA is authentic; no tRFLP matches; few reliably identified collections; retain
Cortinarius vitreopileatus (indigenous endemic)	Y	One t-RFLP match; several recent collections, another misidentification revealed by DNA sequences; **remove from Data Deficient category**
Dermocybe alienata (indigenous endemic)	Y	*Dermocybe alienata* and *Cortinarius chrysma* with same t-RFLP; many matches to this; *D. alienata* from six collections from five Crosby Districts; **remove from Data Deficient category**
Dermocybe aurantiella (indigenous endemic)		Two collections, authenticity of one is doubtful; retain
Dermocybe aurata (indigenous)		Unpublished informal herbarium name; **remove from Data Deficient category**
Dermocybe cardinalis (indigenous endemic)	Y	No t-RFLP matches, but other DNA comparisons reveal several recent collections, in several Crosby districts; **remove from Data Deficient category**
Dermocybe cinnabarina (indigenous non-endemic)	Y	Recent collections referred to this (European) name identified by Soop (pers. comm.) as *Cortinarius veronicae* var. *dilutus*; **remove *D. cinnabarina* from Data Deficient category**; occurrence in New Zealand uncertain.
Dermocybe cramesina (indigenous non-endemic)	Y	No t-RFLP matches; retain
Dermocybe egmontiana (indigenous endemic)	Y	No t-RFLP matches; retain
Dermocybe icterinoides (indigenous endemic)	Y	Several doubtful t-RFLP matches; single collection in PDD; retain
Dermocybe indotata (indigenous endemic)		Several recent collections; **remove from Data Deficient category**
Dermocybe largofulgens (indigenous endemic)	Y	t-RFLP cannot distinguish *D. largofulgens* and *Cortinarius saturniorum*; one additional *D. largofulgens* collection ex TO (ID based on DNA comparison of unidentified *Cortinarius*) to add to the type ex AK
Dermocybe leptospermorum (indigenous endemic)	Y	t-RFLP not distinct; no extra data; retain

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Appendix 2 continued

SPECIES	DNA DATA GENERATED?	NOTES
Dermocybe olivaceonigra (indigenous endemic)	Y	Several tentative t-RFLP matches; several recent collections from North and South Islands; **remove from Data Deficient category**
Dermocybe purpurata (indigenous endemic)		DNA not successfully amplified but now four collections widespread around New Zealand; **remove from Data Deficient category**
Dermocybe splendida (indigenous non-endemic)	Y	Several t-RFLP matches but not unique (*D. largofulgens* the same); four collections, all in North Island, perhaps North Island restricted, so further collecting effort needed; retain
Dermocybe vinicolor (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Dermocybe viscida (indigenous)		Unpublished informal herbarium name; **remove from Data Deficient category**
Dermoloma hemisphaericum (indigenous endemic)		Retain
Dermoloma murinum (indigenous endemic)		Now three collections, doubt about identity of some; retain
Gigasperma cryptica (indigenous endemic)		Retain
Gliophorus fumosogriseus (indigenous endemic)		Retain
Gliophorus graminicolor (indigenous non-endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Gliophorus lilacipes (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Gliophorus luteoglutinosus (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Gliophorus ostrinus (indigenous endemic)		Only two authentically identified collections; retain
Gliophorus subheteromorphus (indigenous non-endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Gliophorus viscaurantius (indigenous endemic)		Now five collections in PDD; **remove from Data Deficient category**
Hebeloma mediorufum (indigenous endemic)		Many recent collections; **remove from Data Deficient category**
Hygrocybe blanda (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Hygrocybe cerinolutea (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Hygrocybe elegans (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Hygrocybe fuliginata (indigenous endemic)		Now five collections in PDD; geographically widespread; **remove from Data Deficient category**
Hygrocybe fuscoaurantiaca (indigenous endemic)		Now five collections in PDD; **remove from Data Deficient category**
Hygrocybe julietae (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Hygrocybe miniata (indigenous non-endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Hygrocybe miniceps (indigenous endemic)		At least six collections in PDD; **remove from Data Deficient category**
Hygrocybe striatolutea (indigenous endemic)		Retain
Hygrophorus carcharias (indigenous endemic)		DNA not successfully amplified; retain
Hygrophorus gloriae (indigenous endemic)		Retain
Hygrophorus involutus (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Hygrophorus segregatus (indigenous endemic)		Retain
Hygrophorus waikanaensis (indigenous endemic)		Retain
Hysterangium neglectum (indigenous non-endemic)		Redetermination of the single NZ collection, now no evidence that this species occurs in NZ; **remove from Data Deficient category?**
Hysterangium rupticutis (exotic)		Exotic; **remove from Data Deficient category**
Inocybe cerea (indigenous endemic)		DNA not successfully amplified; several collections but doubt about several of the identifications

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SPECIES	DNA DATA GENERATED?	NOTES
Inocybe destruens (indigenous endemic)		No authentic DNA; one collection ex Clive Shirley
Inocybe latericia (indigenous non-endemic)		DNA from Leonard collection only, doubtfully authentic; no tRFLP cutting sites; three collections but two doubtfully identified
Inocybe luteobulbosa var. *luteobulbosa* (indigenous endemic)	Y	No t-RFLP matches; many recent collections; **remove from Data Deficient category**
Inocybe luteobulbosa var. *volvata* (indigenous endemic)		DNA not successfully amplified; retain
Inocybe mendica (indigenous endemic)		Known from type only; retain
Inocybe phaeosquarrosa (indigenous endemic)		Retain
Inocybe renispora (indigenous endemic)		Retain
Inocybe scabriuscula (indigenous endemic)	Y	No t-RFLP matches; retain
Inocybe umbrosa (indigenous endemic)	Y	DNA not successfully amplified from Type, but good from another Horak specimen; no tRFLP matches; some doubt over identification of some collections; retain
Neohygrocybe innata (indigenous endemic)		Retain
Neohygrocybe squarrosa (indigenous endemic)		Retain
Phaeocollybia longipes (indigenous endemic)		Several recent, widespread collections, retain for now, will probably be removed later
Phaeocollybia minuta (indigenous endemic)		Several recent collections but doubts over identifications; retain
Pleurocollybia cremea (indigenous endemic)		Several recent collections; **remove from Data Deficient category**
Porpoloma amyloideum (indigenous endemic)		DNA not successfully amplified
Ramaria ambigua (indigenous endemic)		Several recent collections; perhaps doubt about identification; retain
Ramaria anziana (indigenous endemic)		Retain
Ramaria gigantea f. *tenuispora* (indigenous endemic)		Several recent collections from North and South Islands; **remove from Data Deficient category**
Ramaria pancaribbea (indigenous non-endemic)		Three collections, geographically widespread; retain for meantime
Ramaria perfluopunicea (indigenous endemic)		Retain
Ramaria purpureopallida (indigenous endemic)		Six PDD collections; **remove from Data Deficient category**
Ramaria rotundispora (indigenous endemic)		Retain
Ramaria samuelsii (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Ramaria zippelii f. *aeruginosa* (indigenous non-endemic)		Retain
Ramaria zippelii f. *zippelii* (indigenous non-endemic)		Several recent PDD collections; **remove from Data Deficient category**
Ramariopsis agglutinata (indigenous endemic)		Retain
Ramariopsis alutacea (indigenous endemic)		Retain
Ramariopsis cremicolor (indigenous endemic)		Retain
Ramariopsis crocea (indigenous non-endemic)		Now three collections; retain
Ramariopsis depokensis f. *persicina* (indigenous endemic)		Retain
Ramariopsis junquillea (indigenous endemic)		Retain
Ramariopsis longipes (indigenous endemic)		Retain
Ramariopsis ovispora (indigenous endemic)		Retain
Ramariopsis ramarioides (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Rhodocybe antipoda (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**

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Appendix 2 continued

SPECIES	DNA DATA GENERATED?	NOTES
Rhodocybe conchata (indigenous endemic)		Retain
Rhodocybe dingleyae (indigenous endemic)		Perhaps now two collections; retain
Rhodocybe fulginea (indigenous non-endemic)		Retain
Rhodocybe piperita (exotic)		Exotic; **remove from Data Deficient category**
Russula multicystidiata (indigenous endemic)		DNA not successfully amplified; many recent collections; **remove from Data Deficient category**
Thaxterogaster viola (indigenous endemic)		Retain
Timgrovea reticulata (indigenous non-endemic)		Now two collections; retain

Appendix 3

ADDITIONS TO THE DATA DEFICIENT LIST

Most of these species were described after the original 2002 assessment. In most cases, recently described species of mushrooms in New Zealand taxonomic studies are based on one or a few herbarium specimens.

SPECIES	DNA DATA?	NOTES
Cortinarius anisodorus (indigenous endemic)		Three collections; **add to Data Deficient category**
Cortinarius atrolazulinus (indigenous endemic)		Three collections, North and South Islands; Soop (2006) considered it rare; **add to Data Deficient category**
Cortinarius atroviolaceus (indigenous non-endemic)		One or two collections; **add to Data Deficient category**
Cortinarius coneae (indigenous endemic)		One or two collections; **add to Data Deficient category**
Cortinarius dulciolens (indigenous endemic)		Only two collections; **add to Data Deficient category**
Cortinarius dulciorum (indigenous endemic)		Single collection; **add to Data Deficient category**
Cortinarius flavidulus (indigenous endemic)		Single collection; **add to Data Deficient category**
Cortinarius gymnocephalus (indigenous endemic)		Two collections; **add to Data Deficient category**
Cortinarius incensus (indigenous endemic)		Only two collections; **add to Data Deficient category**
Cortinarius lamproxanthus (indigenous endemic)		Two collections, one area; **add to Data Deficient category**
Cortinarius leucocephalus (indigenous non-endemic)		Few collections, **add to Data Deficient category**
Cortinarius luteobrunneus (indigenous endemic)		Few collections; **add to Data Deficient category**
Cortinarius melleomitis (indigenous non-endemic)		Three collections; **add to Data Deficient category**
Cortinarius myxenosma (indigenous endemic)		Two collections; **add to Data Deficient category**
Cortinarius nivalis (indigenous endemic)		Few collections; **add to Data Deficient category**
Cortinarius ohauensis (indigenous endemic)		Few collections; **add to Data Deficient category**
Cortinarius orixanthus (indigenous)		Few certain IDs; **add to Data Deficient category**
Cortinarius paraonui (indigenous endemic)		Three collections; **add to Data Deficient category**
Cortinarius pectochelis (indigenous endemic)		Three collections; **add to Data Deficient category**
Cortinarius pisciodorus (indigenous endemic)		Two collections; **add to Data Deficient category**
Cortinarius sarchinochrous (indigenous endemic)		Now four collections; because these truffle-like species are intensively sought but rarely found; **add to Data Deficient category**
Cortinarius suecicolor (indigenous endemic)		Four collections, some uncertainty over identity of some; **add to Data Deficient category**
Cortinarius thaumastus (indigenous endemic)		Few collections; **add to Data Deficient category**
Cortinarius tigrellus (indigenous endemic)		Few collections; **add to Data Deficient category**
Cortinarius violaceovolvatus (indigenous endemic)		Three collections; **add to Data Deficient category**
Inocybe strigiceps (indigenous non-endemic)	Y	No t-RFLP matches; two, perhaps three, collections in PDD; **add to Data Deficient category**
Labyrinthomyces varius (indigenous non-endemic)		Three collections; **add to Data Deficient category**
Laccaria amethystina (indigenous non-endemic)		Several collections PDD, but uncertain identifications; **add to Data Deficient category**
Ramaria sclerocarnosa (indigenous endemic)		Few collections; **add to Data Deficient category**
Ramariopsis luteotenerrima (indigenous non-endemic)		Several collections, but doubt over identifications; **add to Data Deficient category**
Ramariopsis novohibernica (indigenous non-endemic)		Few collections; **add to Data Deficient category**
Russula pudorina (indigenous endemic)		Five collections, all in AK; **add to Data Deficient category**
Russula rubrolutea (indigenous endemic)		Three collections; **add to Data Deficient category**
Russula tapawera (indigenous endemic)		Four collections but all from same locality; **either add to Data Deficient category or consider for higher threat status;** macroscopically distinctive species of intensively targeted group

Novel taxonomic methods enable better understanding of fungal distributions for threat classification system lists

Molecular methods were used to combine ecological and taxonomic data sets for a group of ectomycorrhizal fungal species. Sequences and t-RFLP patterns from the ITS region were generated from reliably identified herbarium specimens and used to recognise when these species appeared in samples collected in ecological studies, so providing large numbers of additional distribution records for these species. A new listing of Data Deficient ectomycorrhizal fungal species was produced.

Johnston, P.; Park, D.; Dickie. I.; Walbert, K. 2010: Using molecular techniques to combine taxonomic and ecological data for fungi: reviewing the Data Deficient fungi list, 2009. *Science for Conservation 306*. Department of Conservation, Wellington. 31 p.