Phylogenetic relationships of eight new Dacrymycetes collected from New Zealand

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Abstract Dacrymycetes, sister to Agaricomycetes, is a noteworthy lineage for studying the evolution of wood-decaying basidiomycetes; however, its species diversity and phylogeny are largely unknown. Species of Dacrymycetes previously used in molecular phylogenetic analyses are mainly derived from the Northern Hemisphere, thus insufficient knowledge exists concerning the Southern Hemisphere lineages. In this study, we investigated the species diversity of Dacrymycetes in New Zealand. We found 11 previously described species, and eight new species which were described here: Calocera pedicellata, Dacrymyces longistipitatus, D. pachysporus, D. stenosporus, D. parastenosporus, D. cylindricus, D. citrinus, and D. cyrtosporus. These eight newly described species and seven of the known ones, namely, Calocera fusca, C. cf. gu.epinoioides, C. lutea, Dacrymyces flabelliformis, D. intermedius, D. subantarcticensis, and Heterotextus miltinus, have rarely or never been recorded from the Northern Hemisphere. In a molecular-based phylogeny, these New Zealand strains were scattered throughout the Dacrymycetaceae clade. Sequences obtained from specimens morphologically matching C. gu.epinoioides were separated into three distant clades. Because no obvious morphological differences could be discerned between the specimens in each clade and no sequence exists from the type specimen, a C. gu.epinoioides s.str. clade could not be determined. This survey of dacrymycetous species in the Southern Hemisphere has increased taxon sampling for phylogenetic analyses that can serve as a basis for the construction of a stable classification of Dacrymycetes.

INTRODUCTION

Dacrymycetes, one of the early-diverging wood decomposers in Basidiomycota, is sister to Agaricomycetes. Although consequently a noteworthy lineage for studying the evolution of wood-decaying basidiomycetes, its species diversity and phylogeny remain poorly understood. Morphology-based classifications of dacrymycetous species from the 1960s and 1970s (McNabb 1964, 1965a–e, 1966, 1973, Lowy 1971, Reid 1974) are only recently beginning to be reassessed using DNA-based phylogenies. To date, the species used for molecular phylogenetic analyses have been mainly collected from the Northern Hemisphere (Weiß & Oberwinkler 2001, Shirouzu et al. 2007, 2009, 2013a); consequently, insufficient knowledge exists about the phylogenetic relationships of the Southern Hemisphere Dacrymycetes. The major host trees of dacrymycetous species in the Northern Hemisphere belong to Pinaceae and Fagaceae, whereas forests in the Southern Hemisphere are characterised by families such as Nothofagaceae, Myrtaceae, Podocarpaceae, and Araucariaceae. Confers in the Southern Hemisphere have different evolutionary histories than those in the Northern Hemisphere (Leslie et al. 2012). In some Agaricomycetes mushrooms, distributed species or lineages are different between the hemispheres (e.g. Coetzee et al. 2001, Hosaka et al. 2008). Because of the dissimilarities of host trees and geographical background, Dacrymycetes distributed in the Southern Hemisphere are predicted to include phylogenetically different lineages from those in the Northern Hemisphere. The species diversity of Dacrymycetes from the Southern Hemisphere has been described in taxonomic studies by McNabb (McNabb 1964, 1965a–e, 1966, 1973) and Lowy (1971). Nevertheless, many dacrymycetous species from the Southern Hemisphere have not been included in any molecular phylogenetic analysis and samples have not been preserved for DNA extraction. Because it tends to degrade with time (e.g. Erkens et al. 2008, Hosaka & Uno 2013), DNA is difficult to obtain from specimens collected more than 50 years ago, therefore field collection of fresh material is needed. The acquisition of newly collected specimens from the Southern Hemisphere will help remove the current geographic bias in taxon sampling and will likely improve our understanding of phylogenetic relationships within Dacrymycetes.

In this study, field expeditions were conducted in New Zealand to collect dacrymycetous fruiting bodies as an initial step in the investigation of Dacrymycetes species in the Southern Hemisphere. We then conducted a molecular phylogenetic analysis and taxonomic classification of New Zealand Dacrymycetes and compared species compositions between Southern and Northern Hemispheres.

MATERIALS AND METHODS

Fruiting body collection and identification

From 2011 to 2015, fruiting bodies of Dacrymycetes were collected at 74 sites in the North and South Islands of New Zealand. For species identification, collected specimens were morphologically examined with a stereomicroscope and a light microscope (Shirouzu et al. 2009). Genus- and species-level identifications were conducted according to a classification system based on morphological characteristics (Olive 1958, McNabb 1965a, d, 1973, Lowy 1971, McNabb & Talbot 1973, Reid 1974, Oberwinkler 1993, 2014, Shirouzu et al. 2009). Although some genera based on these criteria are not mono-
phyletic (Shirouzu et al. 2013a), we retained those generic concepts because no phylogenetic-based classification system has yet been established for Dacrymycetes. In similar situations, new species have been described according to the traditional system based on morphological criteria (Shirouzu et al. 2009, 2013b, Wu et al. 2011, Delivorias et al. 2012).

Fruiting bodies were dried with a food dehydrator (58 °C for 12 h) and deposited in the Fungal and Plant Disease Collection (PDD) in New Zealand and the National Museum of Nature and Science (TNS) in Japan. Pure cultures were isolated from fresh fruiting bodies by multi-basidiospore isolation on 2.5 % malt agar (MA; Nissui, Tokyo, Japan) plates and preserved in sealed vials containing commenal agar (0.2 % CMA, Nissui) + MA medium (0.2 % CMA, 8.5 g, 2.5 % MA (22.5 g), 1 g yeast extract, and 1 L distilled water). The isolated cultures were deposited in the International Collection of Micro-organisms from Plants (ICMP) in New Zealand (Table 1).

DNA sequencing and phylogenetic analysis

Fresh tissues of fruiting bodies were soaked at 4 °C in DMSO buffer (Seutin et al. 1991) containing 100 mM Tris-HCl (pH 8.0) and 0.1 M sodium sulphate (Na2SO4) until extraction. Soaked tissue samples were then ground in liquid nitrogen using a mortar and pestle. After grinding, samples were immediately transferred to 1.5 mL tubes along with 1 000 µL of 2× CTAB buffer (Doyle & Doyle 1987) followed by the addition of 0.1 M NaCl. Samples were incubated at 65 °C for 1 h and then centrifuged at 13 500 × g for 5 min. The aqueous phase was transferred to a new tube and the precipitated tissue debris was discarded. After the addition of an equal volume of chloroform : isoamyl alcohol (24 : 1) and vigorous mixing for 2 min, the mixture was centrifuged at 13 500 × g for 15 min. Using a pipette, the aqueous phase was transferred to a new tube. To c. 300 µL of the aqueous phase, 1 000 µL of 6 M sodium iodine buffer (6 M NaI, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, and 0.1 M Na2SO4) was added and mixed gently for 1 min. Twenty-five microlitres of a silica mixture prepared following the protocol of Rogstad (2003) was added to the samples. Samples were incubated at 55 °C for 1 h and then centrifuged at 13 500 × g for c. 10 s. The supernatant was discarded and 750 µL of wash buffer (10 µL Tris-HCl (pH 7.4), 1 mM EDTA, 100 mM NaCl, and 50 % ETOH) was added and mixed briefly, followed by centrifugation at full speed for c. 5 s. This washing step was repeated twice. After washing, the samples were centrifuged at 13 500 × g for 10 s; the remaining wash buffer was removed by pipetting, and the precipitated silica was dried at room temperature for 30 min to 1 h. Final elution was performed by adding 100 µL of ultrapure water with brief mixing, followed by incubation at 65 °C for 15 min. Samples were centrifuged at 13 500 × g for 1 min. The supernatant layer was then transferred to a new tube and stored at -20 °C until PCR was performed.

DNA sequences from the holotype — LC131375 (LSU), and ITS1 and ITS4 (White et al. 1990). The sequences determined in this study were deposited in the DNA Data Bank of Japan (DDBJ; Table 1).

Multiple sequence alignment of a combined dataset comprising the sequences obtained in this study and available sequences of Dacrymycetes and Agaricomycetes species downloaded from DDBJ was carried out with MAFFT v. 7 (mafft.cbrc.jp/alignment/software; Katoh & Standley 2013). Poorly aligned sequence regions were removed prior to subsequent analysis. Molecular phylogenetic analysis of LSU and ITS sequences was performed in RAXML v. 8.1.15 (Stamatakis 2014) under a GTR+Γ model. The dataset was partitioned to allow different parameters for each gene region (LSU, ITS1, 5.8S, and ITS2). Maximum likelihood bootstrap percentages and the tree were obtained by simultaneously running rapid bootstrap analyses of 1 000 pseudoreplicates followed by a search for the most likely tree. The aligned dataset was uploaded to TreeBASE under ID S19007 (http://purl.org/phylo/treebase/phylows/study/TB2:S19007).

RESULTS

As a result of field collections, 441 specimens of fruiting bodies were obtained and 281 cultures were isolated. Immature or overmature fruiting bodies were omitted from subsequent observations and the molecular analysis. Using the sequences obtained from collected samples and downloaded from DDBJ (Table 1), a phylogenetic tree was estimated in RAxML (Fig. 1). A total of 524 (LSU) and 824 (ITS) characters (including gaps) were used for the phylogenetic analysis.

Sequences of the New Zealand samples obtained in this study were widely distributed within the Dacrymycetaceae clade, but were not found in Cerinomycetaceae and Unilacrymales clades (Fig. 1).

As described below, eight new and 11 known species were identified on the basis of morphological observations and the molecular phylogenetic analysis.

TAXONOMY

New species

**Calocera pedicellata** Shirouzu, sp. nov. — MycoBank MB817692; Fig. 2a, 3

Differs from *Calocera cornea* by the basidiocarps consistently having stipes and by the presence of irregularly shaped terminal cells on the sterile surfaces.

Etymology. From the Latin ‘pedicellatus’ = pedicellate, referring to the stipitate basidiocarps.

Type. NEW ZEALAND, South Island, Denniston, Coalbrookdale Walk, on dead branches of a woody plant, 27 May 2015, T. Shirouzu (holotype PDD 107926, isotype TNS-F-65489, culture ex-type ICMP 21230).

DNA sequences from the holotype — LC131375 (LSU), LC131416 (ITS).

**Basidiocarps** scattered, cylindrical, subulate, sometimes pal- mate, simple or branched, stipitate-pileate, bearing cylindrical or subulate, sometimes rugose pilei, pale yellow to orange, soft-cartilaginous, 1–6 mm high, 0.5–1 mm diam, in transverse section through the pileus showing an organization into three zones, i.e. a central core of compact parallel hyphae surrounded by a zone of loosely interwoven hyphae enclosed by a hymenium. **Internal hyphae** branched, sepalate, thin- or thick-walled, hyaline, 2–5 µm diam, without clamp connections. **Margin**
Table 1  Specimen, culture, and sequence accession numbers and localities of samples used in molecular phylogenetic analyses.

| Name                   | Locality         | Specimen no. | Culture no. | DDBJ accession no. |
|------------------------|------------------|--------------|-------------|--------------------|
|                        |                  | 1            | 2           | LSU                | ITS               |
| Calocera arborea       | Brazil           | INPA 241458  |             | AB723514           |  –                |
|                        | Brazil           | INPA 241457  |             | AB723513           |  –                |
| Calocera bambusicola   | Taiwan           | Wu 9910-12   |             | –                  | FJ195751          |
| Calocera cornea        | New Zealand      | PDD 104991   | ICMP 20465  | LC131362           | LC131403          |
|                        | New Zealand      | PDD 107847   | ICMP 21223  | LC131363           | LC131404          |
|                        | Japan            | TNS-F-21061  | MAFF 241196 | AB472722           | –                 |
|                        | Japan            | TNS-F-21065  | MAFF 241188 | AB472725           | –                 |
|                        | USA              | –            | CBS 125.84  | AB472739           | –                 |
|                        | Canada           | –            | CBS 124.84  | AB472738           | AB712437          |
| Calocera fusca         | New Zealand      | PDD 107930   |             | LC131364           | LC131405          |
|                        | New Zealand      | PDD 107972   | ICMP 21238  | LC131365           | LC131406          |
| Calocera glossoides (= Dacryomitra pusilla) | New Zealand | PDD 107932 | – | – | |
|                        | New Zealand      | PDD 107972   | ICMP 21238  | LC131365           | LC131406          |
| Calocera lutea         | New Zealand      | PDD 107842   | ICMP 21222  | LC131373           | LC131414          |
|                        | Australia        | –            | CBS 291.82  | AB712379           | AB712438          |
| Calocera pedicellata   | New Zealand      | PDD 107830   |             | LC131374           | LC131415          |
|                        | New Zealand      | PDD 107925   | ICMP 21230  | LC131375           | LC131416          |
| Calocera sinensis      | Taiwan           | Wu 0703-6    |             | –                  | FJ195754          |
|                        | Taiwan           | JCH 07026    |             | –                  | FJ195755          |
| Calocera viscosa       | Japan            | TNS-F-15704  | MAFF 240119 | AB299048           | AB712439          |
|                        | Canada           | –            | CBS 292.82  | AB472740           | –                 |
| Cerinomyces albosporus | Japan            | TNS-F-15706  | MAFF 240121 | AB299050           | AB712440          |
| Cerinomyces canadensis | Japan            | TNS-F-21034  | MAFF 241162 | AB472696           | AB712441          |
| Cerinomyces ceraceus   | USA              | –            | HBB-8969    | AB712422           | AB712442          |
| Cerinomyces crustulinus| Canada           | –            | TUF7 30545  | AB712423           | AB712443          |
| Cerinomyces grandinoides| USA              | –            | HBB-9608    | AB712424           | AB712444          |
| Cerinomyces lagerheimii| USA              | –            | RLG-13487   | AB712425           | AB712445          |
| Cerinomyces pallidus   | Japan            | TNS-F-21064  |             | AB472724           | –                 |
|                        | Belize           | –            | F150848     | AB712426           | AB712446          |
| Dacryomyces adpressus  | Japan            | TNS-F-21045  | MAFF 241172 | AB472707           | AB712447          |
|                        | Japan            | TNS-F-21069  | MAFF 241191 | AB472729           | –                 |
| Dacryomyces ancyclus   | Japan            | TNS-F-21051  | MAFF 241177 | AB472713           | AB712448          |
| Dacryomyces aureosporus| Japan            | TNS-F-15711  | MAFF 240126 | AB299057           | AB712449          |
|                        | Japan            | TNS-F-21074  | MAFF 241195 | AB472734           | –                 |
| Dacryomyces capitatus  | Japan            | TNS-F-15709  | MAFF 240124 | AB299055           | –                 |
|                        | Japan            | TNS-F-21062  | MAFF 241187 | AB472723           | –                 |
|                        | Canada           | –            | CBS 293.82  | AB472741           | AB712450          |
| Dacryomyces chrysocomus| UK               | –            | CBS 280.84  | AB712427           | AB712451          |
| Dacryomyces chrysospermus | Japan        | TNS-F-15712  | MAFF 240127 | AB299073           | AB712452          |
|                        | Japan            | TNS-F-21060  | MAFF 241185 | AB472721           | –                 |
| Dacryomyces citrinus   | New Zealand      | PDD 107915   | ICMP 21227  | LC131376           | LC131417          |
|                        | New Zealand      | PDD 107979   | ICMP 21239  | LC131377           | LC131418          |
| Dacryomyces cylindricus| New Zealand      | PDD 105052   | ICMP 20517  | LC131378           | LC131419          |
|                        | New Zealand      | PDD 107989   | –            | LC131379           | LC131420          |
| Dacryomyces cyrtosporus| New Zealand      | PDD 107952   | –            | LC131380           | LC131421          |
|                        | New Zealand      | PDD 107980   | –            | LC131381           | LC131422          |
| Dacryomyces dendostralami | Japan          | TNS-F-38903  | TUF7 13914  | AB712428           | AB712453          |
| Dacryomyces dictyosporus | USA             | –            | HBB-8618    | AB712429           | AB712454          |
| Dacryomyces flabelliformis | New Zealand   | PDD 107863   | ICMP 21225  | LC131382           | LC131423          |
|                        | New Zealand      | PDD 107944   | ICMP 21233  | LC131383           | LC131424          |
|                        | New Zealand      | PDD 76696    | HHB-18308   | AB712430           | AB712455          |
| Dacryomyces intermedius| New Zealand      | PDD 107851   | ICMP 21224  | LC131384           | –                 |
|                        | New Zealand      | PDD 107939   | ICMP 21232  | LC131385           | –                 |
| Dacryomyces lacrymalis | Japan            | TNS-F-15719  | MAFF 240134 | AB299069           | AB712456          |
|                        | Japan            | TNS-F-21040  | MAFF 241167 | AB472702           | –                 |
|                        | Japan            | TNS-F-21042  | MAFF 241169 | AB472704           | –                 |
Table 1 (cont.)

| Name                             | Locality      | Specimen no. 1 | Culture no. 2 | DDBJ accession no. |
|----------------------------------|---------------|----------------|---------------|-------------------|
| Dacrymyces longistipitatus       | New Zealand   | PDD 107996     | ICMP 21241    | LC131386 LC131425 |
|                                  | New Zealand   | PDD 107997     | ICMP 21242    | LC131387 LC131426 |
| Dacrymyces cf. microsporus       | New Zealand   | PDD 104992     | ICMP 20466    | LC131388 –        |
|                                  | New Zealand   | PDD 104993     | ICMP 20467    | LC131389 –        |
| Dacrymyces microsporus           | Japan         | TNS-F-21049    | MAFF 241175   | AB472711 –        |
|                                  | Japan         | TNS-F-21050    | MAFF 241176   | AB472712 AB712457 |
| Dacrymyces minor                 | Japan         | TNS-F-15720    | MAFF 240135   | AB299059 –        |
|                                  | Japan         | TNS-F-15721    | MAFF 240136   | AB299063 AB712458 |
| Dacrymyces minutus               | Japan         | TNS-F-15722    | MAFF 240137   | AB299070 –        |
|                                  | Japan         | TNS-F-21073    | –             | AB472733 AB712459 |
| Dacrymyces novae-zelandiae       | New Zealand   | PDD 107892     | –             | LC131390 LC131427 |
|                                  | New Zealand   | PDD 107953     | ICMP 21235    | LC131391 LC131428 |
| Dacrymyces pachysporus           | New Zealand   | PDD 105064     | ICMP 20479    | LC131392 LC131429 |
|                                  | New Zealand   | PDD 107916     | ICMP 21228    | LC131393 LC131430 |
| Dacrymyces parastenosporus       | New Zealand   | PDD 104960     | ICMP 20433    | LC131394 LC131431 |
|                                  | New Zealand   | PDD 104963     | ICMP 20436    | LC131395 LC131432 |
| Dacrymyces pinacearum            | Japan         | TNS-F-21056    | MAFF 241182   | AB472718 AB712461 |
| Dacrymyces punctiformis          | Japan         | TNS-F-15723    | MAFF 240138   | AB299052 AB712462 |
|                                  | Japan         | TNS-F-15725    | MAFF 240140   | AB299071 –        |
| Dacrymyces san-augustini          | Japan         | TNS-F-15726    | MAFF 240141   | AB299081 AB712463 |
|                                  | Japan         | TNS-F-21075    | MAFF 241196   | AB472735 –        |
| Dacrymyces stenosporus           | New Zealand   | PDD 105018     | ICMP 20488    | LC131396 LC131433 |
|                                  | New Zealand   | PDD 107970     | ICMP 21237    | LC131397 LC131434 |
| Dacrymyces cf. stillatus         | New Zealand   | PDD 105038     | ICMP 20505    | LC131398 –        |
| Dacrymyces stillatus             | Japan         | TNS-F-15727    | MAFF 240142   | AB299061 AB712464 |
|                                  | Japan         | TNS-F-21052    | MAFF 241178   | AB472714 –        |
|                                  | Germany       | FO28136        | –             | AF291309 –        |
| Dacrymyces subalpinus            | Japan         | TNS-F-15730    | MAFF 240145   | AB299060 AB712465 |
|                                  | Japan         | TNS-F-21071    | MAFF 241193   | AB472731 –        |
| Dacrymyces subantarcticensis     | New Zealand   | PDD 107948     | ICMP 21234    | LC131399 LC131435 |
|                                  | New Zealand   | PDD 107988     | –             | LC131400 LC131436 |
| Dacrymyces subantarcticus        | New Zealand   | PDD 76679      | HHB-18220     | AB712431 AB712466 |
| Dacrymyces varisporus            | Japan         | TNS-F-21067    | –             | AB472727 AB712467 |
|                                  | Japan         | TNS-F-21076    | –             | AB472736 –        |
| Dacrypinax elegans               | USA           | –              | HHB-18731     | AB712433 AB712471 |
| Dacrypinax indacocheae           | Venezuela     | –              | CRM-72        | AB712434 AB712472 |
| Dacrypinax spathularia           | Japan         | TNS-F-15736    | MAFF 240151   | AB299079 –        |
|                                  | Japan         | TNS-F-21048    | MAFF 241174   | AB472710 AB712473 |
| Dacrypinax sphenocarpa           | Japan         | TNS-F-21046    | MAFF 241173   | AB472708 AB712474 |
|                                  | Japan         | TNS-F-21066    | MAFF 241189   | AB472726 –        |
| Dacryoscyphus chrysochilus       | China         | KUN F45014     | –             | AY604567 –        |
| Dililacryma haasi                | Germany       | RoKi100        | –             | AF291314 –        |
| Femajonia peziziformis           | Japan         | TNS-F-15737    | MAFF 240152   | AB299080 AB712476 |
|                                  | Germany       | FO25100        | –             | AF291330 –        |
| Guepiniopsis buccina             | Japan         | TNS-F-15738    | MAFF 240153   | AB299085 AB712477 |
|                                  | USA           | AFTOL-ID 888   | –             | AY745711 DQ206986 |
| Heterotextus mitinus             | New Zealand   | PDD 104962     | ICMP 20435    | LC131401 LC131437 |
|                                  | New Zealand   | PDD 107924     | ICMP 21229    | LC131402 LC131438 |
| Unilacryma unispora              | Japan         | TNS-F-15731    | MAFF 240146   | AB299074 AB712468 |
|                                  | Japan         | TNS-F-38904    | –             | AB712432 AB712469 |
| Coprinus comatus                 | USA           | –              | AY635772      | AY854066 –        |
| Suillus pictus                   | USA           | AFTOL-ID 717   | –             | AY684154 AY854069 |

Newly described species as well as specimens, cultures, and sequences obtained in this study are shown in **bold**.

1. PDD, Fungal and Plant Disease Collection (New Zealand).
2. ICMP, International Collection of Micro-organisms from Plants (New Zealand).
Fig. 1 Phylogenetic tree of Dacrymyces estimated in RAxML using concatenated LSU and ITS sequences. Maximum likelihood bootstrap percentages ≥ 50% are shown above or below branches, with bolded branches indicating ≥ 80% support. Newly described species and collected samples in this study are shown in bold. Southern Hemisphere strains are highlighted in grey. Asterisks denote clades comprising only New Zealand species. TreeBASE ID: S19007.
Fig. 2 Basidiocarps. a. Calocera pedicellata PDD 107925; b. Dacrymyces longistipitatus PDD 107997; c. Dacrymyces pachysporus PDD 107916; d. Dacrymyces stenosporus PDD 107970; e. Dacrymyces paraestenosporus PDD 104963; f. Dacrymyces cylindricus PDD 105052; g. Dacrymyces citrinus PDD 107915; h. Dacrymyces cyrtosporus PDD 107980. — Scale bars = 5 mm.
Calocera pedicellata is characterised by cylindrical stipitate-pileate basidiocarps, irregularly shaped terminal cells, and small 1-septate basidiospores. This species is similar to Calocera cornea. Calocera pedicellata is distinguished from C. cornea on the basis of the presence of cylindrical basidiocarps, hyphae without clamp connections, and small 1–septate basidiospores (McNabb 1965a). Calocera pedicellata is distinguished from C. cornea on the basis of the characteristics of the basidiocarps consistently having stipes and by irregularly shaped terminal cells on the sterile surfaces. Calocera pedicellata is phylogenetically distant from the samples accepted here as C. cornea (Fig. 1).

Dacrymyces longistipitatus Shirouzu, sp. nov. — MycoBank MB817693; Fig. 2b, 4

Differ from Dacrymyces capitatus by the basidiocarps having longer stipes and by its thicker-walled basidiospores.

Etymology. From the Latin 'longus' = long and 'stipitatus' = stipitate, referring to the basidiocarps with long stipes.

Type. New Zealand, North Island, Korototo Forest Park, Kiriwhakapapa Road, on dead branches of a woody plant, 6 June 2015, T. Shirouzu, PDD 107995, culture ICMP 21246; South Island, Catlins Forest Park, Catlins River Track, on dead branches of Pinus radiata, 3 May 2015, T. Shirouzu, PDD 107830 (TNS-F-65488); Lake Brunner, AorO Te Kinga, on dead branches of a woody plant, 18 May 2015, T. Shirouzu, PDD 107990, culture ICMP 21243.

Notes — Dacrymyces longistipitatus is characterised by cylindrical to turbinate stipitate-pileate basidiocarps, irregularly shaped terminal cells, and thick-walled 3-septate basidiospores. This species is similar to D. capitatus and D. dacryomitriformis in having stipitate-pileate basidiocarps, hyphae lacking clamp connections, and 3-septate basidiospores. Compared with D. longistipitatus, D. capitatus has shorter-stipitae basidiocarps and thinner-walled basidiospores (McNabb 1973). Dacrymyces longistipitatus is phylogenetically distant from specimens accepted here as D. capitatus (Fig. 1). In contrast to D. longistipitatus, D. dacryomitriformis has simple or sparingly branched dikaryophyses, relatively long probasidia (35–60 × 3.5–5 µm), and thin-walled basidiospores with thick septa (McNabb 1973).
Dacrymyces pachysporus Shirouzu, sp. nov. — MycoBank MB817694; Fig. 2c, 5

Diffsers from Dacrymyces sichuanensis by the presence of longer basidiospores and the absence of branched dikaryophyses.

_Etymology._ From the Greek ‘_pachy_’ = thick and ‘_sporus_’ = spore, referring to the thick-walled basidiospores.

**Type.** _New Zealand_, South Island, Nelson Lakes National Park, Lake Rotoroa, on dead branches of _Coprosma robusta_, 9 May 2014, T. Shirouzu (holotype PDD 105018; isotype TNS-F-65510, culture ex-type ICMP 20488).

DNA sequences from the holotype — LC131392 (LSU), LC131429 (ITS).

**Basidiocarps** scattered or gregarious, sometimes coalesced, pustulate to pulvinate, sessile, orange, firm-gelatinous, 0.5–1 mm high, 0.5–2 mm diam. _Internal hyphae_ branched, septate, thick-walled, hyaline, 2–3 µm diam, with clamp connections. _Marginal hyphae_ on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with irregularly shaped thin-walled terminal cells of 30–45 × 3–5 µm. _Hymenium_ limited to the ventral surface of the basidiocarp, amphiogenous, composed of basidia and simple cylindrical dikaryophyses. _Probasidia_ cylindrical to clavate, pale yellow, 35–55 × 5 µm, with basal clamp connections, becoming bifurcate. _Basidiospores_ cylindrical to reniform, straight or curved, with an apiculum at the base, thick-walled, hyaline to pale yellow, 16–19 × 6–7 µm (17 × 6 µm on average, n = 10), l/w 2.3–3.2 (2.8 on average), 0–3-septate.

**Specimens examined.** _New Zealand_, South Island, Victoria Forest Park, Mt Haast Route, on dead branches of a woody plant, 22 May 2015, T. Shirouzu, PDD 107916 (TNS-F-65507), culture ICMP 21228.

Notes — _Dacrymyces pachysporus_ is characterised by its small pustulate to pulvinate basidiocarps, hyphae with clamp connections, and long thick-walled 0–3-septate basidiospores. This species is similar to _D. sichuanensis_ and _D. stiltatus_ in having small pustulate to pulvinate sessile basidiocarps and 0–3-septate thick-walled basidiospores. _Dacrymyces sichuanensis_ has shorter basidiospores (12.5–15.6 × 4.5–6.5 µm, Liu & Fan 1990) and branched dikaryophyses, the latter discerned based on a line drawing in Liu & Fan (1990). _Dacrymyces stiltatus_ has no clamp connections on hyphae (McNabb 1973, Shirouzu et al. 2009). _Dacrymyces pachysporus_ is also similar to _D. punctiformis_ in having small pustulate to pulvinate sessile basidiocarps and clamp connections on hyphae, but _D. punctiformis_ has thin-walled smaller basidiospores (10–15 × 3.5–5 µm, as _Dacrymyces tortus_, McNabb 1973; 7–13 × 4–6 µm, Shirouzu et al. 2009). Samples accepted here as _D. punctiformis_ and _D. stiltatus_ are phylogenetically distant from _D. pachysporus_ (Fig. 1).
samples accepted here as *D. lacrymalis* and *D. minor* are phylogenetically distant from *D. stenosporus*. *Dacrymyces neoalbidus* has white fruiting bodies and larger basidiospores (21–22 × 5–6 μm, as *Dacrymyces albidos*, Kobayasi 1954, 1955).

**Dacrymyces parastenosporus** Shirouzu, sp. nov. — MycoBank MB817696; Fig. 2e, 7

Diffs from *D. stenosporus* by having longer probasidia.

*Etymology.* From the Greek 'paras' = near and the epithet 'stenosporus', referring to its similarity to *D. stenosporus*.

**Type.** New Zealand, South Island, Arthur’s Pass National Park, Waimekariri River, on dead branches of a woody plant, 4 May 2014, T. Shirouzu (holotype PDD 104963; isotype TNS-F-65509, culture ex-type ICMP 20436).

DNA sequences from the holotype — LC131395 (LSU), LC131432 (ITS).

**Basidiocarps** scattered or gregarious, coalesced, postulate to pulvinate, gyrose, sessile, orange yellow, firm-gelatinous to soft-cartilaginous, 2–4 mm high, 1–4 mm diam. *Internal hyphae* branched, septate, thin-walled, hyaline, 2–5 μm diam, without clamp connections. *Marginal hyphae* on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with cylindrical thin-walled terminal cells of 20–30 × 2–3 μm. *Hymenium* limited to the ventral surface of the basidiocarp, amphigenous, composed of basidia and simple cylindrical dikaryophyses. *Probasidia* cylindrical to clavate, pale yellow, 40–50 × 5 μm, with basal clamp connections, becoming bifurcate. *Basidiospores* cylindrical, straight or slightly curved, with an apiculum at the base, thick-walled, hyaline, 8–10 × 4–5 μm (9 × 4 μm on average, n = 10), l/w 2.8–4.3 (3.4 on average), 0–3-septate.

**Specimens examined.** New Zealand, North Island, Tararua Forest Park, Kirihakapapa Road, on dead branches of a broad-leaved tree, 6 June 2015, T. Shirouzu, PDD 107962; South Island, Craigieburn Forest Park, Dracophyl- ium Flat Track, on dead branches of Pinus radiata, 4 May 2014, T. Shirouzu, PDD 104960 (TNS-F-65508), culture ICMP 20433; Fiordland National Park, Lake Hauroko, on dead branches of a broad-leaved tree, 8 May 2015, T. Shirouzu, PDD 107843; Victoria Forest Park, Waimakariri Valley, on dead branches of a broad-leaved tree, 19 May 2015, T. Shirouzu, PDD 107895.

Notes — *Dacrymyces parastenosporus* is characterised by its postulate to pulvinate basidiocarps and slender 0–3-septate basidiospores. This species is similar to *Dacrymyces stenosporus*, but the latter species has shorter probasidia (30–40 × 4 μm). These two species are phylogenetically distant from one another (Fig. 1).

**Dacrymyces cylindricus** Shirouzu, sp. nov. — MycoBank MB817697; Fig. 2f, 8

Diffs from *Dacrymyces ancyileus* by the presence of smaller thick-walled basidiospores.

*Etymology.* From the Latin 'cylindricus' = cylindrical, referring to the shape of the basidiocarps.

**Type.** New Zealand, South Island, Kahurangi National Park, Kaituna Track, on dead branches of a broad-leaved tree, 15 May 2014, T. Shirouzu (holotype PDD 105052; isotype TNS-F-65492, culture ex-type ICMP 20517).

DNA sequences from the holotype — LC131378 (LSU), LC131419 (ITS).

**Basidiocarps** scattered, cylindrical to subulate, simple, stipitate-pilose, bearing a cylindrical to subglobose, sometimes subulate pileus, white to pale yellow, firm-gelatinous to soft-cartilaginous, 2–4 mm high, 2–3 mm diam. *Internal hyphae* branched, septate, thin-walled, hyaline, 2–4 μm diam, with clamp connections. *Marginal hyphae* on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with irregularly shaped thin-walled terminal cells of 25–35 × 3 μm. *Hymenium* limited to the surface of the pileus, amphigenous, composed of basidia. *Probasidia* cylindrical to clavate, pale yellow, 40–50 × 4 μm, with basal clamp connections, becoming bifurcate. *Basidiospores* cylindrical to reniform, straight or curved, with an apiculum at the base, thick-walled, hyaline, 8–10 × 4–5 μm (9 × 4 μm on average, n = 10), l/w 2–2.5 (2.3 on average), 0–1-septate.

**Specimens examined.** New Zealand, North Island, Tararua Forest Park, Kirihakapapa Road, 6 June 2015, T. Shirouzu, PDD 107960, culture ICMP 21247; Tongariro National Park, Rotopounamu Walk, on dead branches of a woody plant, 18 June 2015, T. Shirouzu, PDD 107989 (TNS-F-65493); South Island, Mt Richmond Forest Park, Pelorus Bridge, on dead branches of a woody plant, 30 May 2015, T. Shirouzu, PDD 107933; Nelson Lakes National Park, Lake Rotolli, on dead branches of a broad-leaved tree, 1 June 2015, T. Shirouzu, PDD 107945.

Notes — *Dacrymyces cylindricus* is characterised by its cylindrical to subulate basidiocarps, hyphae with clamp connections, and small thick-walled 1-septate basidiospores. The irregularly shaped terminal cells are also diagnostic characters of this species. *Dacrymyces cylindricus* has cylindrical to subglobose and the hymenium is amphigenous. Consequently, this fungus should be assigned to the genus *Dacrymyces*. *Dacrymyces cylindricus* is similar to *D. ancyileus*.
and *D. flabelliformis* in having stipitate-pileate basidiocarps and clamp connections on hyphae. *Dacrymyces ancyloides* has larger thin-walled basidiospores (10.5–19.5 × 4–9 μm, Shirouzu et al. 2009). *Dacrymyces flabelliformis* has spathulate to flabelliform basidiocarps and larger thin-walled 0–3-septate basidiospores (12.5–14 × 5–6 μm, Burdsall & Laursen 2004). These two species are phylogenetically distant from *D. cylindricus* (Fig. 1).

*Dacrymyces citrinus* Shirouzu, *sp. nov.* — MycoBank MB817698; Fig. 2g, 9

Differs from *Dacrymyces enatus var. macrosporus* by its wider basidiospores and the absence of branched dikaryophyses.

**Etymology.** From the Latin ‘citrinus’ = pale yellow, referring to the colour of the basidiocarps.

**Type.** New Zealand, South Island, Victoria Forest Park, Mt Haast Route, on dead branches of a woody plant, 22 May 2015, T. Shirouzu (holotype PDD 107915, isotype TNS-F-65490, culture ex-type ICMP 21227).

DNA sequences from the holotype — LC131376 (LSU), LC131417 (ITS).

Basidiocarps scattered or gregarious, sometimes coalesced, pustulate to pulvinate, sessile, pale yellow to yellow, firm-gelatinous, 0.5–1 mm high, 1–5 mm diam. Internal hyphae branched, septate, thin-walled, hyaline, 2–5 μm diam, with clamp connections. Marginal hyphae on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with cylindrical thin-walled terminal cells of 20–40 × 2–5 μm. Hymenium limited to the ventral surface of the basidiocarp, amphigenous, composed of basidia and simple cylindrical dikaryophyses. Probasidia cylindrical to clavate, pale yellow, 35–45 × 5–6 μm, with basal clamp connections, becoming bifurcate. Basidiospores cylindrical to reniform, straight, with an apiculum at the base, thick-walled, hyaline to pale yellow, 11–14 × 7–9 μm (13 ± 8 μm on average, n = 10), l/w 1.5–2 (1.7 on average), 0–3-septate.

Specimens examined. New Zealand, North Island, Waiohine Gorge, on dead branches of a broad-leaved tree, 5 June 2015, T. Shirouzu, PDD 107934; South Island, Fiordland National Park, Lake Hauroko, on dead branches of a broad-leaved tree, 7 May 2015, T. Shirouzu, PDD 107837; Kahurangi National Park, Wanganeka Track, on dead branches of Leptospermum scoparium, 31 May 2015, T. Shirouzu, PDD 107934.

Notes — *Dacrymyces citrinus* is characterised by the presence of pulvinate yellow basidiocarps, hyphae with clamp connections, and wide, thick-walled, 3-septate basidiospores. This species is similar to *D. enatus var. macrosporus*, *D. paraphysatus*, *D. sichuanensis*, and *D. pachysporus* in having pulvinate basidiocarps, hyphae with clamp connections, and 3-septate thick-walled basidiospores. *Dacrymyces enatus var. macrosporus* has thinner basidiospores (11–15.5 × 4.5–6.5 μm), branched dikaryophyses, and dark basidiocarps (McNabb 1973). *Dacrymyces paraphysatus* has longer basidiospores (13.5–21 × 5–7 μm) and branched dikaryophyses (McNabb 1973). *Dacrymyces sichuanensis* has smaller basidiocarps (1–2 mm diam), narrower basidiospores (12.5–15.6 × 4.5–6.5 μm), and branched dikaryophyses as discerned from a line drawing in Liu & Fan (1990). *Dacrymyces pachysporus* has smaller basidiocarps (0.5–2 mm diam), longer basidiospores (16–19 × 6–7 μm), and irregularly shaped terminal cells (Fig. 4). *Dacrymyces citrinus* is phylogenetically distant from *D. pachysporus* (Fig. 1). Some specimens of *D. citrinus* have slightly slender basidiospores (e.g. 13–14 × 6–7 μm, l/w 1.9–2.3, PDD 107979) but are phylogenetically indistinguishable from those with wider spores (Fig. 1).

*Dacrymyces cyrtosporus* Shirouzu, *sp. nov.* — MycoBank MB817699; Fig. 2h, 10

Differs from *D. sichuanensis* by the absence of branched dikaryophyses.

**Etymology.** From the Greek ‘cyrtos’ = bent or curved and ‘sporus’ = spore, referring to the curved basidiospores.

**Type.** New Zealand, North Island, Whanganui National Park, Atene Viewpoint Walk, on dead branches of a woody plant, 13 June 2015, T. Shirouzu (holotype PDD 107980; isotype TNS-F-65495).

DNA sequences from the holotype — LC131381 (LSU), LC131422 (ITS).

Basidiocarps scattered or gregarious, sometimes coalesced, pustulate to pulvinate, sessile, pale yellow to olive, firm-gelatinous, 0.5 mm high, 0.5–2 mm diam. Internal hyphae branched, septate, thin-walled, hyaline, 2–3 μm diam, with clamp connections. Marginal hyphae on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with cylindrical thin-walled terminal cells of 40–80 × 2–3 μm. Hymenium limited to the ventral surface of the basidiocarp, amphigenous, composed of basidia and simple cylindrical dikaryophyses. Probasidia cylindrical to clavate, hyaline, 30–50 × 5–6 μm, with clamp connections.
Calocera cornea

Known species

Calocera cornea (Batsch) Fr., Stirp. Agri Fems. 5: 67. 1827
Type locality. Germany.

Specimens examined. New Zealand, South Island, Fiordland National Park, Lake Hauroko, on dead branches of a woody plant, 8 May 2015, T. Shirouzu, PDD 107847, culture ICMP 21223; Granville Road, on dead branches of a woody plant, 6 May 2014, T. Shirouzu, PDD 104991, culture ICMP 20465.

Notes — Calocera cornea was morphologically identified with reference to McNabb (1965a), Reid (1974), and Shirouzu et al. (2009). The sequences obtained in this study formed a clade with Japanese (TNS-F-21061, 21065) and North American (CBS 124.84, 125.84) strains identified as Calocera cornea. The geographical and phylogenetic distributions of Calocera cornea seem wide and diverse, suggesting that it could be a species complex.

Calocera fusca Lloyd, Mycol. Writings 7 (75): 1357. 1925
Type locality. Canterbury, New Zealand.

Specimens examined. New Zealand, North Island, Whanganui National Park, Pipiriki, on dead branches of a broad-leaved tree, 11 June 2015, T. Shirouzu, PDD 107972, culture ICMP 21238; South Island, Mt Richmond Forest Park, Pelorus Bridge, on dead branches of a broad-leaved tree, 30 May 2015, T. Shirouzu, PDD 107930.

Notes — Calocera fusca was morphologically identified with reference to McNabb (1965a). This species has also been recorded from the Juan Fernández Islands (McNabb 1965a). The sequence obtained in this study is the first DNA sequence data provided for C. fusca.

Calocera cf. guepiniioides Berk., London J. Bot. 4: 61. 1845
Type locality. Swan River, West Australia.

Specimens examined. New Zealand, North Island, Kaimanawa Forest Park, Clements Mill Road, on dead branches of a woody plant, 16 June 2015, T. Shirouzu, PDD 107981, culture ICMP 21240; Tararua Forest Park, Waio-tau Track, on dead branches of a woody plant, 8 June 2015, T. Shirouzu, PDD 107969, culture ICMP 21236; South Island, Kahurangi National Park, Heaphy Track, on dead branches of a woody plant, 25 May 2015, T. Shirouzu, PDD 107929, culture ICMP 21231; Mt Aspiring National Park, Haast Pass Lookout, on dead branches of a woody plant, 15 May 2015, T. Shirouzu, PDD 107874, culture ICMP 21226; Nelson, Fringed Hill, on dead branches of a woody plant, 12 May 2014, T. Shirouzu, PDD 105033, culture ICMP 20502; Nelson Lakes National Park, Lake Rotorito, on dead branches of a woody plant, 6 May 2014, T. Shirouzu, PDD 105005, culture ICMP 20480.

Notes — These specimens were morphologically identified with reference to McNabb (1965a). Phylogenetic analysis separated the sequences obtained from the samples into three clades (Calocera cf. guepiniioides 1, 2, and 3; Fig. 1). The specimens constituting each clade could not be morphologically distinguished, and the true clade of Calocera cf. guepiniioides could not be confirmed because DNA from the type strain was not included in this study. Calocera cf. guepiniioides has already been recorded from New Zealand (McNabb 1965a). This species has originally been described from Western Australia; the inclusion of samples from such areas is critically needed in phylogenetic and taxonomic studies.

Calocera lutea (Massee) McNabb, New Zealand J. Bot. 3: 46. 1965
Type locality. Tasmania, Australia.

Specimens examined. New Zealand, South Island, Fiordland National Park, Lake Hauroko, on dead branches of a woody plant, 8 May 2015, T. Shirouzu, PDD 107841, culture ICMP 21221; PDD 107842, culture ICMP 21222.

Notes — Calocera lutea, originally described from Tasmania, was morphologically identified with reference to McNabb (1965a). This species has already been recorded from New Zealand (McNabb 1965a). The sequences obtained in this study formed a clade with an Australian strain (CBS 291.82; Fig. 1). Seifert (1983) has reported that a decomposition test using the Australian strain of Calocera lutea revealed features of white rot, but our specimens collected in New Zealand showed characteristics of brown rot, such as brown discoloration and cracking into roughly cubical pieces of wood.

Dacrymyces flabelliformis Burds. & Laursen, Mem. New York Bot. Gard. 89: 109. 2004
Type locality. Auckland Islands, New Zealand.

Specimens examined. New Zealand, South Island, Fiordland National Park, Lake Hauroko, on dead branches of a broad-leaved tree, 12 May 2015, T. Shirouzu, PDD 107863, culture ICMP 21225; Nelson Lakes National Park, Lake Rotorito, on dead branches of a broad-leaved tree, 1 June 2015, T. Shirouzu, PDD 107944, culture ICMP 21233.

Notes — Dacrymyces flabelliformis was morphologically identified with reference to the original description (Burdsall & Laursen 2004). The sequences obtained in this study were closely related to the ex-type strain collected from New Zealand (HHB-18308; Fig. 1). This species is presumably endemic to New Zealand.

Dacrymyces cyrtosporus (Batsch) Fr., Stirp. Agri Fems. 5: 67. 1827

Known species

Dacrymyces cyrtosporus var. macrosporus Liu & Fan (1990). Dacrymyces enatus var. macrosporus has branched dikaryophyses as discerned from a line drawing in Liu & Fan (1990). Dacrymyces enatus var. macrosporus has larger, dark basidiocarps (3–4 mm diam) and branched dikaryophyses (McNabb 1973). Dacrymyces parahysatus has branched dikaryophyses and yellowish brown, larger basidiocarps (13.5–21 × 5–7 µm, McNabb 1973). Dacrymyces pachysporus has irregularly shaped terminal cells (Fig. 5) and longer basidiocarps (16–19 × 6–7 µm). Dacrymyces cinereus has larger basidiocarps (1–5 mm diam) and wider, straight basidiospores (11–14 × 7–9 µm). Dacrymyces cyrtosporus is phylogenetically distant from D. pachysporus and D. cinereus (Fig. 1).
**Dacrymyces intermedium** L.S. Olive, Bull. Torrey Bot. Club 85: 108. 1958

*Type locality.* Tahiti.

*Specimens examined.* **New Zealand,** South Island, Fiordland National Park, Kepler Track, on dead branches of a woody plant, 10 May 2015, T. Shirouzu, PDD 107851, culture ICMP 21224; Kahurangi National Park, Wangapakea Track, on dead branches of a broad-leaved tree, 31 May 2015, T. Shirouzu, PDD 107939, culture ICMP 21232.

*Notes* — **Dacrymyces intermedium,** originally described from Tahiti, was morphologically identified with reference to the original description (Olive 1958) and that in McNabb (1973). The species has not been reported from any other regions of the world, and no other DNA sequence data are available.

**Dacrymyces** cf. *microsorus* P. Karst., Bidrag Kannedom Finlands Natur Folk 48: 459. 1889

*Type locality.* Mustiala, Finland.

*Specimens examined.* **New Zealand,** South Island, Granville Ecological Area, Granville Road, on dead branches of a woody plant, 6 May 2014, T. Shirouzu, PDD 104992, culture ICMP 20466; PDD 104993, culture ICMP 20467.

*Notes* — These specimens were morphologically identified with reference to McNabb (1973) and Shirouzu et al. (2009). The sequences obtained from the New Zealand specimens were related to that of a Japanese strain (TNS-F-21049); however, a second Japanese strain (TNS-F-20150), although morphologically similar, was genetically distinct (Fig. 1). Sequences from the type specimen or authentically identified specimens from the type locality are needed to clarify the taxonomy of this species.

**Dacrymyces novae-zelandiae** McNabb, New Zealand J. Bot. 11: 493. 1973

*Type locality.* Auckland, New Zealand.

*Specimens examined.* **New Zealand,** North Island, Tararua Forest Park, Kiriwhakapapa Road, on dead branches of a broad-leaved tree, 6 June 2015, T. Shirouzu, PDD 107953, culture ICMP 21235; South Island, Greymouth, Point Elizabeth, on dead branches of a broad-leaved tree, 18 May 2015, T. Shirouzu, PDD 107892.

*Notes* — **Dacrymyces novae-zelandiae,** described on the basis of a New Zealand type, was morphologically identified with reference to the original description (McNabb 1973). The sequences obtained in this study were closely related to a New Zealand strain (CBS 295.82) collected near the type locality, but a morphologically similar Japanese strain was genetically distinct (TNS-F-21038; Fig. 1). This species is presumably endemic to New Zealand.

**Dacrymyces** cf. *stillatus* Nees, Syst. Mycol. 2: 250. 1822

*Type locality.* Europe.

*Specimens examined.* **New Zealand,** South Island, Farewell Spit, on dead branches of a woody plant, 13 May 2014, T. Shirouzu, PDD 105038, culture ICMP 20669.

*Notes* — This specimen was morphologically identified with reference to McNabb (1973), Reid (1974), and Shirouzu et al. (2009). The sequence obtained in this study was very close to that from a German strain (AF291309; Weiß & Oberwinkler 2001) and close to but distinct from Japanese strains identified as *D. stillatus* (TNS-F-15727) and *D. minor* (TNS-F-15720, 15721; Fig. 1). According to McNabb (1973), *D. stillatus* can be distinguished from *D. minor* by its larger basidio-caps and thicker-walled basidiospores. **Dacrymyces stillatus** is a common species of *Dacrymycetes* and has been recorded worldwide (Lowy 1971, McNabb 1973, Reid 1974, Shirouzu et al. 2009).

**Dacrymyces subantarcticensis** Burds. & Laursen, Mem. New York Bot. Gard. 89: 107. 2004

*Type locality.* Campbell Island, New Zealand.

*Specimens examined.* **New Zealand,** North Island, Tongariro National Park, Rotopounamu Walk, on dead branches of a woody plant, 18 June 2015, T. Shirouzu, PDD 107988; South Island, Nelson Lakes National Park, Lake Rototoi, on dead branches of a woody plant, 1 June 2015, T. Shirouzu, PDD 107948, culture ICMP 21234.

*Notes* — **Dacrymyces subantarcticensis** was morphologically identified with reference to the original description (Burdsall & Laursen 2004). The sequences obtained in this study were closely related to the type strain collected from Campbell Island (HHB-18220; Fig. 1). This species is presumably endemic to New Zealand.

**Heterotextus miltinus** (Berk.) McNabb, New Zealand J. Bot. 3: 220. 1966

*Type locality.* Tasmania, Australia.

*Specimens examined.* **New Zealand,** South Island, Arthur’s Pass National Park, Wainamakari River, on dead branches of Nothofagus solandri, 4 May 2014, T. Shirouzu, PDD 104962, ICMP 20435; Denniston, Coaltbrookdale Walk, on dead branches of a broad-leaved tree, 27 May 2015, T. Shirouzu, PDD 107924, culture ICMP 21229.

*Notes* — **Heterotextus miltinus,** originally described from Tasmania, was morphologically identified with reference to McNabb (1965d). This species has already been recorded from New Zealand (McNabb 1965d). The sequences referred to *H. miltinus* in this study were genetically somewhat divergent but in a close sister relationship (Fig. 1). One of the isolates exactly matched a New Zealand strain (ICMP 16702, isolated from PDD 89156) from the North Island (Fig. 1).

**DISCUSSION**

**Dacrymycetes species in the Southern Hemisphere**

The phylogenetic hypothesis of *Dacrymycetes* was updated by the addition of eight new taxa as well as specimens referable to previously described species with no available DNA sequence data, namely, *C. fusca,* *C. cf. guepinioides,* and *D. intermedius.* Two monophyletic groups, one comprising *D. longistipitatus* and *D. pachysporus* and the other consisting of *D. cylindricus,* *D. citrinus,* and *D. cyrtosporus,* were each composed only of New Zealand species (Fig. 1). These clades might be unique lineages useful for characterisation of the dacrymycetous mycoflora of New Zealand.

Although specimens identified as *Dacrymyces* cf. *stillatus,* *Dacrymyces* cf. *microsorus,* and *C. cornea* were morphologically and phylogenetically related to strains from the Northern Hemisphere, unique species characterising New Zealand or the Southern Hemisphere *Dacrymycetes* were also collected in this study. The eight newly described taxa as well as seven known species, i.e., *C. fusca,* *C. cf. guepinioides,* *C. lutea,* *D. flabelliformis,* *D. intermedius,* *D. subantarcticensis,* and *H. miltinus* — which have been collected from New Zealand, Australia, Tahiti, and the Juan Fernández Islands (McNabb 1965a, d, 1973, Burdsall & Laursen 2004), have rarely or never been reported from the Northern Hemisphere. These known species were identified on the basis of morphology with the exception of *D. flabelliformis* and *D. subantarcticensis,* for which sequences from type specimens or authentically identified specimens from type localities are lacking. We believe that these eight new and
seven known species reflect the unique Dacrymycetes mycoflora in the Southern Hemisphere and complement existing knowledge of the species diversity of this class. Two new species, <i>C. pedicellata</i> and <i>D. parastenosporus</i>, were collected from dead branches of <i>Pinus radiata</i>, a conifer introduced from the west coast of the United States. We believe, however, that these dacrymycetous species are native to New Zealand, as they have never been reported from the original habitats of <i>P. radiata</i> and were additionally found on dead branches of unidentified local trees in the collection sites.

**Morphologically indistinguishable species**

Among the newly described taxa, six species – <i>D. citrinus</i>, <i>D. cylindricus</i>, <i>D. cyrtosporus</i>, <i>D. longistipitatus</i>, <i>D. pachysporus</i>, and <i>D. pedicellata</i> – were morphologically similar to each other, but were described as two different species because they were phylogenetically distant from one another (Fig. 1). <i>Dacrymyces parastenosporus</i> can be distinguished from <i>D. stenosporus</i> in having longer probasidia. Although the size of probasidia has not been considered to be a significant criterion compared with characteristics such as shape and size of basidiocarps, basidiospores, and marginal hyphae, it might be a useful feature to distinguish some dacrymycetous species.

The molecular phylogenetic analysis separated the sequences obtained from <i>C. cf. guarpinioides</i> specimens into three clades (<i>C. cf. guarpinioides</i> 1, 2, and 3; Fig. 1). These specimens share the morphological features of small and typically spathulate basidiocarps, 1–3-septate spores, and clamp connections on hyphae that characterize <i>C. guarpinioides</i> (McNabb 1965a). The clade corresponding to <i>C. guarpinioides</i> s.str. could not be identified because no morphological differences were found among the three clades and no sequence exists from the type specimen. This species displays wide variation in the shape of basidiocarps (McNabb 1965a) and therefore might be separated into two or more species.

**Higher classification in Dacrymycetes**

Familial and generic classifications in <i>Dacrymycetes</i> are based on morphological criteria such as the shape and internal hyphal structure of basidiocarps, the position of the hymenium, and presence or absence of developed marginal hyphae (McNabb 1964, 1965a–e, 1966, 1973, McNabb & Talbot 1973, Reid 1974, Jülich 1981). However, this morphology-based classification has often conflicted with the results of molecular phylogenetic analyses, and Calocera, Cerinomycetes, Dacrymyces, and Dacryopinax have been shown to be non-monophyletic genera (Fig. 1; Shirouzu et al. 2009, 2013a). As a result, <i>Dacrymeceteae</i> and <i>Cerinomycetaceae</i>, the two families in <i>Dacrymecetes</i>, are also revealed to be non-monophyletic in various phylogenetic trees. No useful phenotypic features have been found for classification of families and genera that reflect their phylogenetic relationships.

The phylogenetic heterogeneity of the studied genera and families became even more obvious upon the addition of the sequences of New Zealand specimens. The polyphyletic nature of <i>Dacrymyces</i> and <i>Calocera</i> was particularly evident (Fig. 1). The genus <i>Dacrymyces</i> is mainly characterised by sessile pulvinate, turbinate, or sometimes stipitate basidiocarps, a homogeneous intra-structure of fruiting bodies, and an amphigenous hymenium (McNabb 1973), but its delineation has often been obscure (e.g. Reid 1974). The results of molecular phylogenetic analyses have supported this ambiguity (Shirouzu et al. 2007, 2009, 2013a), and <i>Dacrymyces</i> appears to be the most phylogenetically scattered genus in the <i>Dacrymeceteae</i> clade (Fig. 1). The genus <i>Calocera</i> is characterised by cylindrical basidiocarps, a three-zoned intra-structure of fruiting bodies, and an amphigenous hymenium (McNabb 1965a). Because previous studies have demonstrated the sister relationship of <i>C. cornea</i> and <i>C. viciosa</i> (Weiß & Oberwinkler 2001, Shirouzu et al. 2007, 2009), the genus <i>Calocera</i> has been considered to be a monophyletic taxon. In the present phylogenetic tree, however, many of the <i>Calocera</i> species used in this study, such as <i>C. arborea</i>, <i>C. bambusicola</i>, <i>C. fusca</i>, <i>C. cf. guarpinioides</i>, <i>C. glossoides</i>, <i>C. lutea</i>, <i>C. pedicellata</i>, and <i>C. sinensis</i>, were found to be dispersed throughout the <i>Dacrymeceteae</i> clade (Fig. 1), suggesting the convergent evolution of calocera-like cylindrical basidiocarps in this family.

Our field investigations in New Zealand have improved the current knowledge of the diversity and phylogeny of Southern Hemisphere <i>Dacrymecetes</i>. In this class, however, taxon sampling is still insufficient to estimate a reliable phylogeny and establish a higher classification system (Shirouzu et al. 2013a). In addition, a recent study has suggested the existence of hidden dacrymycetous lineages that rarely or perhaps never produce visible fruiting bodies – the structures providing almost all morphological criteria used for classification purposes (Shirouzu et al. 2016). To unveil the whole range of phylogenetic diversity of <i>Dacrymecetes</i>, mycelium strains not associated with basidiocarps as well as lineages with visible fruiting bodies must be incorporated. Our survey of the diversity of <i>Dacrymecetes</i> in the Southern Hemisphere has increased taxon sampling and thus improves the reliability of phylogenetic analyses that can serve as a basis for establishing a stable classification of <i>Dacrymecetes</i>.

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