



# Phylogenetic relationships of eight new *Dacrymycetes* collected from New Zealand

T. Shirouzu<sup>1</sup>, K. Hosaka<sup>1</sup>, K.-O. Nam<sup>1</sup>, B.S. Weir<sup>2</sup>, P.R. Johnston<sup>2</sup>, T. Hosoya<sup>1</sup>

## Key words

*Dacrymycetes*  
New Zealand  
phylogeny  
Southern Hemisphere  
taxonomy

**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. paratenosporus*, *D. cylindricus*, *D. citrinus*, and *D. cyrtosporus*. These eight newly described species and seven of the known ones, namely, *Calocera fusca*, *C. cf. guepinoides*, *C. lutea*, *Dacrymyces flabelliformis*, *D. intermedius*, *D. subantarcticensis*, and *Heterotextus miltnus*, 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. guepinoides* 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. guepinoides* 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*.

**Article info** Received: 20 July 2016; Accepted: 11 January 2017; Published: 8 March 2017.

## 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*. Conifers 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-

<sup>1</sup> Department of Botany, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan;  
corresponding author e-mail: shirouzy@gmail.com.

<sup>2</sup> Landcare Research, Private Bag 92170, Auckland, New Zealand.

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 cornmeal 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 ( $\text{Na}_2\text{SO}_3$ ) 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  $\mu\text{L}$  of 2 $\times$  CTAB buffer (Doyle & Doyle 1987) followed by the addition of 0.1 M  $\text{Na}_2\text{SO}_3$ . Samples were incubated at 65 °C for 1 h and then centrifuged at 13 500  $\times g$  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  $\times g$  for 15 min. Using a pipette, the aqueous phase was transferred to a new tube. To c. 300  $\mu\text{L}$  of the aqueous phase, 1 000  $\mu\text{L}$  of 6 M sodium iodine buffer (6 M NaI, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, and 0.1 M  $\text{Na}_2\text{SO}_3$ ) 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  $\times g$  for c. 10 s. The supernatant was discarded and 750  $\mu\text{L}$  of wash buffer (10  $\mu\text{L}$  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  $\times g$  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  $\mu\text{L}$  of ultrapure water with brief mixing, followed by incubation at 65 °C for 15 min. Samples were centrifuged at 13 500  $\times g$  for 1 min. The supernatant layer was then transferred to a new tube and stored at -20 °C until PCR was performed.

DNA sequence data were obtained from large subunit (LSU) and internal transcribed spacer (ITS1-5.8S-ITS2, ITS) regions of nuclear rRNA. The primer combinations LR0R/LR5 (Vilgalys & Hester 1990) and ITS5/ITS4 (White et al. 1990) were used. PCR amplifications were carried out in 20  $\mu\text{L}$  reaction volumes containing 1  $\mu\text{L}$  genomic DNA, 1  $\mu\text{L}$  dNTPs (4 mM), 1  $\mu\text{L}$  of each primer (8 mM), 0.5 units of *Taq* polymerase (Takara, Kusatsu, Japan), 2  $\mu\text{L}$   $\text{MgCl}_2$  (25 mM), and 2  $\mu\text{L}$  bovine serum albumin (10 mg/mL). Cycling parameters were 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 51 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 15 min. PCR products were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and directly sequenced using a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Norwalk, CT, USA) following the manufacturer's instructions. The primers used for cycle sequencing were LR0R and LR5 (Vilgalys & Hester

1990) 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+ $\Gamma$  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/phyloids/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 107925; isotype TNS-F-65489, culture ex-type ICMP 21230).

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

*Basidiocarps* scattered, cylindrical, subulate, sometimes palmate, 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, septate, thin- or thick-walled, hyaline, 2–5  $\mu\text{m}$  diam, without clamp connections. *Marginal*

**Table 1** Specimen, culture, and sequence accession numbers and localities of samples used in molecular phylogenetic analyses.

Name	Locality	Specimen no. <sup>1</sup>	Culture no. <sup>2</sup>	DDBJ accession no.	
				LSU	ITS
<i>Calocera arborea</i>	Brazil	INPA 241458 (holotype)	–	AB723514	–
	Brazil	INPA 241457	–	AB723513	–
<i>Calocera bambusicola</i>	Taiwan	Wu 9910-12	–	–	FJ195751
<i>Calocera cornea</i>	New Zealand	<b>PDD 104991</b>	<b>ICMP 20465</b>	<b>LC131362</b>	<b>LC131403</b>
	New Zealand	<b>PDD 107847</b>	<b>ICMP 21223</b>	<b>LC131363</b>	<b>LC131404</b>
	Japan	TNS-F-21061	MAFF 241186	AB472722	–
	Japan	TNS-F-21065	MAFF 241188	AB472725	–
	USA	–	CBS 125.84	AB472739	–
	Canada	–	CBS 124.84	AB472738	AB712437
<i>Calocera fusca</i>	New Zealand	<b>PDD 107930</b>	–	<b>LC131364</b>	<b>LC131405</b>
	New Zealand	<b>PDD 107972</b>	<b>ICMP 21238</b>	<b>LC131365</b>	<b>LC131406</b>
<i>Calocera glossoides</i> (= <i>Dacryomitra pusilla</i> )	Germany	FO38346	–	AJ406406	–
<i>Calocera</i> cf. <i>guelpinoides</i>	New Zealand	<b>PDD 105005</b>	<b>ICMP 20480</b>	<b>LC131366</b>	<b>LC131407</b>
	New Zealand	<b>PDD 105033</b>	<b>ICMP 20502</b>	<b>LC131367</b>	<b>LC131408</b>
	New Zealand	<b>PDD 107874</b>	<b>ICMP 21226</b>	<b>LC131368</b>	<b>LC131409</b>
	New Zealand	<b>PDD 107929</b>	<b>ICMP 21231</b>	<b>LC131369</b>	<b>LC131410</b>
	New Zealand	<b>PDD 107969</b>	<b>ICMP 21236</b>	<b>LC131370</b>	<b>LC131411</b>
	New Zealand	<b>PDD 107981</b>	<b>ICMP 21240</b>	<b>LC131371</b>	<b>LC131412</b>
<i>Calocera lutea</i>	New Zealand	<b>PDD 107841</b>	<b>ICMP 21221</b>	<b>LC131372</b>	<b>LC131413</b>
	New Zealand	<b>PDD 107842</b>	<b>ICMP 21222</b>	<b>LC131373</b>	<b>LC131414</b>
	Australia	–	CBS 291.82	AB712379	AB712438
<i>Calocera pedicellata</i>	New Zealand	<b>PDD 107830</b>	–	<b>LC131374</b>	<b>LC131415</b>
	New Zealand	<b>PDD 107925 (holotype)</b>	<b>ICMP 21230</b>	<b>LC131375</b>	<b>LC131416</b>
<i>Calocera sinensis</i>	Taiwan	Wu 0703-6	–	–	FJ195754
	Taiwan	JCH 070726	–	–	FJ195755
<i>Calocera viscosa</i>	Japan	TNS-F-15704	MAFF 240119	AB299048	AB712439
	Canada	–	CBS 292.82	AB472740	–
<i>Cerinomyces albosporus</i>	Japan	TNS-F-15706	MAFF 240121	AB299050	AB712440
<i>Cerinomyces canadensis</i>	Japan	TNS-F-21034	MAFF 241162	AB472696	AB712441
	Japan	TNS-F-21035	MAFF 241163	AB472697	–
<i>Cerinomyces ceraceus</i>	USA	–	HHB-8969	AB712422	AB712442
<i>Cerinomyces crustulinus</i>	Canada	–	TUFC 30545	AB712423	AB712443
	Taiwan	–	–	AY600248	–
<i>Cerinomyces grandinioides</i>	USA	–	HHB-6908	AB712424	AB712444
<i>Cerinomyces lagerheimii</i>	USA	–	RLG-13487	AB712425	AB712445
<i>Cerinomyces pallidus</i>	Japan	TNS-F-21064	–	AB472724	–
	Belize	–	FP150848	AB712426	AB712446
<i>Dacrymyces adpressus</i>	Japan	TNS-F-21045	MAFF 241172	AB472707	AB712447
	Japan	TNS-F-21069	MAFF 241191	AB472729	–
<i>Dacrymyces ancyleus</i>	Japan	TNS-F-21051 (holotype)	MAFF 241177	AB472713	AB712448
<i>Dacrymyces aureosporus</i>	Japan	TNS-F-15711	MAFF 240126	AB299057	AB712449
	Japan	TNS-F-21074	MAFF 241195	AB472734	–
<i>Dacrymyces capitatus</i>	Japan	TNS-F-15709	MAFF 240124	AB299055	–
	Japan	TNS-F-21062	MAFF 241187	AB472723	–
	Canada	–	CBS 293.82	AB472741	AB712450
<i>Dacrymyces chrysocomus</i>	UK	–	CBS 280.84	AB712427	AB712451
<i>Dacrymyces chrysospermus</i>	Japan	TNS-F-15712	MAFF 240127	AB299073	AB712452
	Japan	TNS-F-21060	MAFF 241185	AB472721	–
<i>Dacrymyces citrinus</i>	New Zealand	<b>PDD 107915 (holotype)</b>	<b>ICMP 21227</b>	<b>LC131376</b>	<b>LC131417</b>
	New Zealand	<b>PDD 107979</b>	<b>ICMP 21239</b>	<b>LC131377</b>	<b>LC131418</b>
<i>Dacrymyces cylindricus</i>	New Zealand	<b>PDD 105052 (holotype)</b>	<b>ICMP 20517</b>	<b>LC131378</b>	<b>LC131419</b>
	New Zealand	<b>PDD 107989</b>	–	<b>LC131379</b>	<b>LC131420</b>
<i>Dacrymyces cyrtosporus</i>	New Zealand	<b>PDD 107952</b>	–	<b>LC131380</b>	<b>LC131421</b>
	New Zealand	<b>PDD 107980 (holotype)</b>	–	<b>LC131381</b>	<b>LC131422</b>
<i>Dacrymyces dendrocalami</i>	Japan	TNS-F-38903	TUFC 13914	AB712428	AB712453
<i>Dacrymyces dictyosporus</i>	USA	–	HHB-8618	AB712429	AB712454
<i>Dacrymyces flabelliformis</i>	New Zealand	<b>PDD 107863</b>	<b>ICMP 21225</b>	<b>LC131382</b>	<b>LC131423</b>
	New Zealand	<b>PDD 107944</b>	<b>ICMP 21233</b>	<b>LC131383</b>	<b>LC131424</b>
	New Zealand	PDD 76696 (holotype)	HHB-18308	AB712430	AB712455
<i>Dacrymyces intermedius</i>	New Zealand	<b>PDD 107851</b>	<b>ICMP 21224</b>	<b>LC131384</b>	–
	New Zealand	<b>PDD 107939</b>	<b>ICMP 21232</b>	<b>LC131385</b>	–
<i>Dacrymyces lacrymalis</i>	Japan	TNS-F-15719	MAFF 240134	AB299069	AB712456
	Japan	TNS-F-21040	MAFF 241167	AB472702	–
	Japan	TNS-F-21042	MAFF 241169	AB472704	–

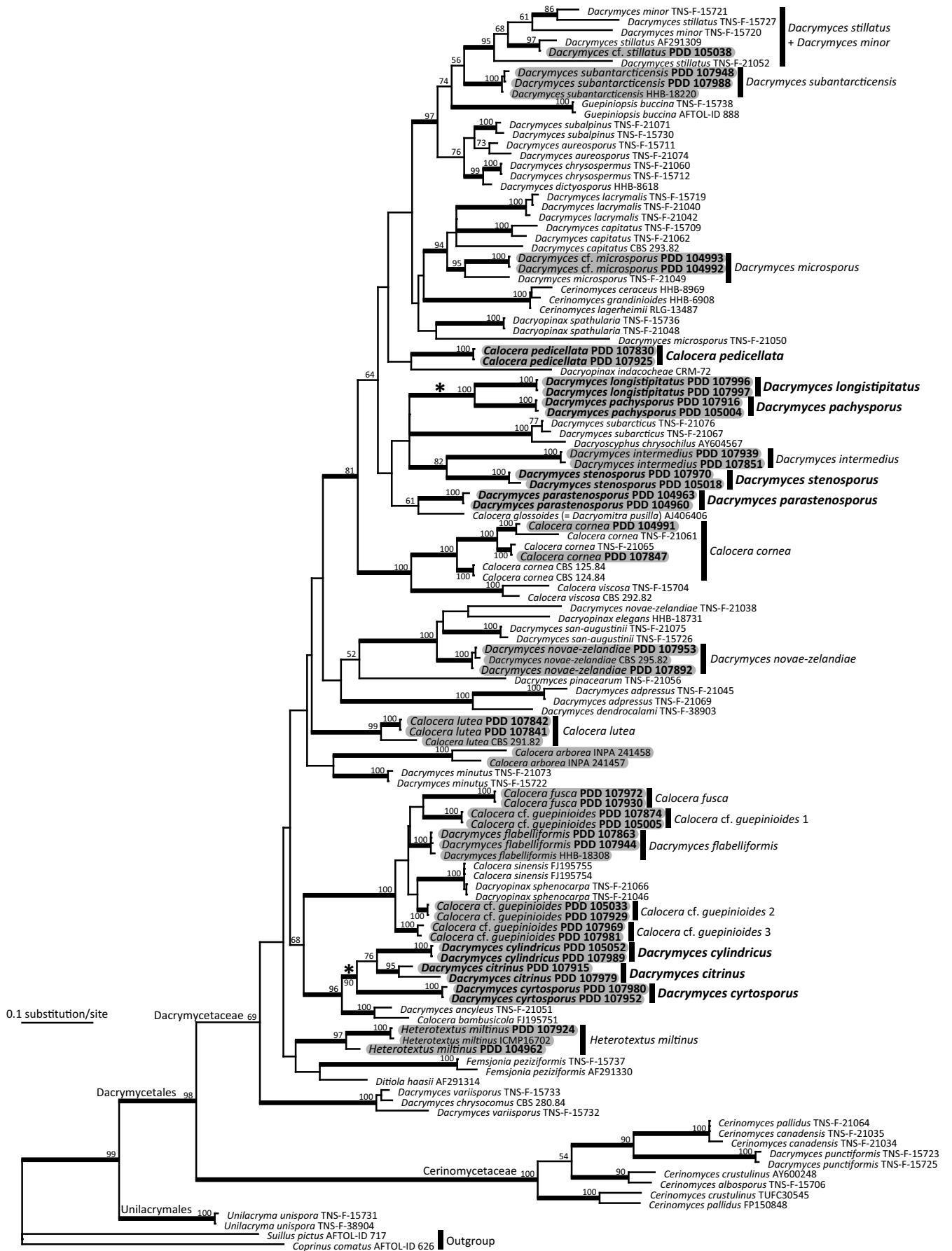
**Table 1** (cont.)

Name	Locality	Specimen no. <sup>1</sup>	Culture no. <sup>2</sup>	DBJ accession no.	
				LSU	ITS
<i>Dacrymyces longistipitatus</i>	New Zealand	<b>PDD 107996</b>	<b>ICMP 21241</b>	<b>LC131386</b>	<b>LC131425</b>
	New Zealand	<b>PDD 107997 (holotype)</b>	<b>ICMP 21242</b>	<b>LC131387</b>	<b>LC131426</b>
<i>Dacrymyces</i> cf. <i>microsporus</i>	New Zealand	<b>PDD 104992</b>	<b>ICMP 20466</b>	<b>LC131388</b>	–
	New Zealand	<b>PDD 104993</b>	<b>ICMP 20467</b>	<b>LC131389</b>	–
<i>Dacrymyces microsporus</i>	Japan	TNS-F-21049	MAFF 241175	AB472711	–
	Japan	TNS-F-21050	MAFF 241176	AB472712	AB712457
<i>Dacrymyces minor</i>	Japan	TNS-F-15720	MAFF 240135	AB299059	–
	Japan	TNS-F-15721	MAFF 240136	AB299063	AB712458
<i>Dacrymyces minutus</i>	Japan	TNS-F-15722	MAFF 240137	AB299070	–
	Japan	TNS-F-21073	–	AB472733	AB712459
<i>Dacrymyces novae-zelandiae</i>	New Zealand	<b>PDD 107892</b>	–	<b>LC131390</b>	<b>LC131427</b>
	New Zealand	<b>PDD 107953</b>	<b>ICMP 21235</b>	<b>LC131391</b>	<b>LC131428</b>
	Japan	TNS-F-21038	MAFF 241165	AB472700	AB712460
	New Zealand	–	CBS 295.82	AB472742	–
<i>Dacrymyces pachysporus</i>	New Zealand	<b>PDD 105004 (holotype)</b>	<b>ICMP 20479</b>	<b>LC131392</b>	<b>LC131429</b>
	New Zealand	<b>PDD 107916</b>	<b>ICMP 21228</b>	<b>LC131393</b>	<b>LC131430</b>
<i>Dacrymyces parastenosporus</i>	New Zealand	<b>PDD 104960</b>	<b>ICMP 20433</b>	<b>LC131394</b>	<b>LC131431</b>
	New Zealand	<b>PDD 104963 (holotype)</b>	<b>ICMP 20436</b>	<b>LC131395</b>	<b>LC131432</b>
<i>Dacrymyces pinacearum</i>	Japan	TNS-F-21056 (holotype)	MAFF 241182	AB472718	AB712461
<i>Dacrymyces punctiformis</i>	Japan	TNS-F-15723	MAFF 240138	AB299052	AB712462
	Japan	TNS-F-15725	MAFF 240140	AB299071	–
<i>Dacrymyces san-augustinii</i>	Japan	TNS-F-15726	MAFF 240141	AB299081	AB712463
	Japan	TNS-F-21075	MAFF 241196	AB472735	–
<i>Dacrymyces stenosporus</i>	New Zealand	<b>PDD 105018 (holotype)</b>	<b>ICMP 20488</b>	<b>LC131396</b>	<b>LC131433</b>
	New Zealand	<b>PDD 107970</b>	<b>ICMP 21237</b>	<b>LC131397</b>	<b>LC131434</b>
<i>Dacrymyces</i> cf. <i>stillatus</i>	New Zealand	<b>PDD 105038</b>	<b>ICMP 20505</b>	<b>LC131398</b>	–
<i>Dacrymyces stillatus</i>	Japan	TNS-F-15727	MAFF 240142	AB299061	AB712464
	Japan	TNS-F-21052	MAFF 241178	AB472714	–
	Germany	FO28136	–	AF291309	–
<i>Dacrymyces subalpinus</i>	Japan	TNS-F-15730	MAFF 240145	AB299060	AB712465
	Japan	TNS-F-21071	MAFF 241193	AB472731	–
<i>Dacrymyces subantarcticensis</i>	New Zealand	<b>PDD 107948</b>	<b>ICMP 21234</b>	<b>LC131399</b>	<b>LC131435</b>
	New Zealand	<b>PDD 107988</b>	–	<b>LC131400</b>	<b>LC131436</b>
	New Zealand	PDD 76679 (holotype)	HHB-18220	AB712431	AB712466
<i>Dacrymyces subarcticus</i>	Japan	TNS-F-21067 (holotype)	–	AB472727	AB712467
	Japan	TNS-F-21076	–	AB472736	–
<i>Dacrymyces variisporus</i>	Japan	TNS-F-15732	MAFF 240147	AB299067	AB712470
	Japan	TNS-F-15733	MAFF 240148	AB299072	–
<i>Dacryopinax elegans</i>	USA	–	HHB-18731	AB712433	AB712471
<i>Dacryopinax indacocheae</i>	Venezuela	–	CRM-72	AB712434	AB712472
<i>Dacryopinax spathularia</i>	Japan	TNS-F-15736	MAFF 240151	AB299079	–
	Japan	TNS-F-21048	MAFF 241174	AB472710	AB712473
<i>Dacryopinax sphenocarpa</i>	Japan	TNS-F-21046 (holotype)	MAFF 241173	AB472708	AB712474
	Japan	TNS-F-21066	MAFF 241189	AB472726	–
<i>Dacryoscyphus chrysochilus</i>	China	KUN F45014 (holotype)	–	AY604567	–
<i>Ditiola haasii</i>	Germany	RoKi100	–	AF291314	–
<i>Femsjonia peziziformis</i>	Japan	TNS-F-15737	MAFF 240152	AB299080	AB712476
	Germany	FO25100	–	AF291330	–
<i>Guepiniopsis buccina</i>	Japan	TNS-F-15738	MAFF 240153	AB299085	AB712477
	USA	AFTOL-ID 888	–	AY745711	DQ206986
<i>Heterotextus miltinus</i>	New Zealand	<b>PDD 104962</b>	<b>ICMP 20435</b>	<b>LC131401</b>	<b>LC131437</b>
	New Zealand	<b>PDD 107924</b>	<b>ICMP 21229</b>	<b>LC131402</b>	<b>LC131438</b>
	New Zealand	–	ICMP 16702	AB712436	AB712478
<i>Unilacryma unisporea</i>	Japan	TNS-F-15731	MAFF 240146	AB299074	AB712468
	Japan	TNS-F-38904	–	AB712432	AB712469
<i>Coprinus comatus</i>	USA	AFTOL-ID 626	–	AY635772	AY854066
<i>Suillus pictus</i>	USA	AFTOL-ID 717	–	AY684154	AY854069

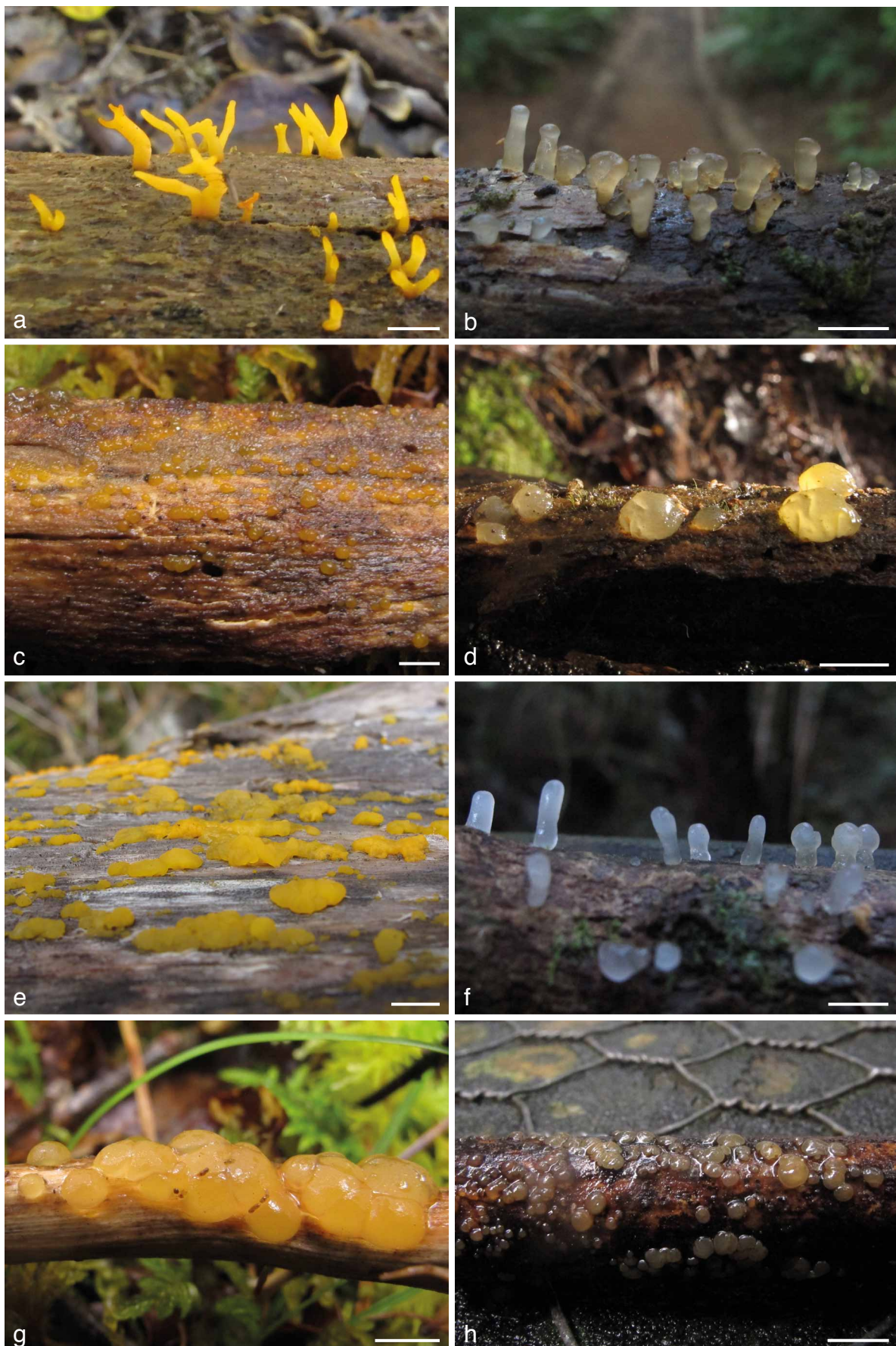
Newly described species as well as specimens, cultures, and sequences obtained in this study are shown in **bold**.

<sup>1</sup> PDD, Fungal and Plant Disease Collection (New Zealand).

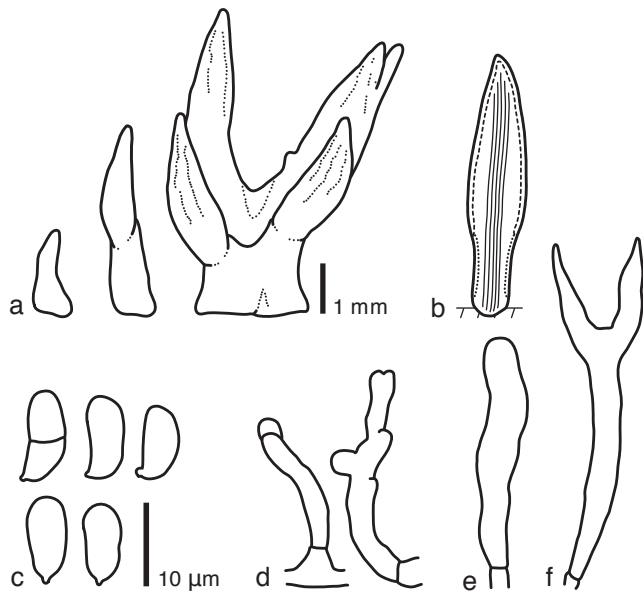
<sup>2</sup> ICMP, International Collection of Micro-organisms from Plants (New Zealand).



**Fig. 1** Phylogenetic tree of *Dacrymycetaceae* estimated in RAxML using concatenated LSU and ITS sequences. Maximum likelihood bootstrap percentages  $\geq 50\%$  are shown above or below branches, with **bolded** branches indicating  $\geq 80\%$  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 parastenosporus* PDD 104963; f. *Dacrymyces cylindricus* PDD 105052; g. *Dacrymyces citrinus* PDD 107915; h. *Dacrymyces cyrtosporus* PDD 107980. — Scale bars = 5 mm.



**Fig. 3** *Calocera pedicellata* PDD 107925. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (solid lines: central core of compact parallel hyphae; dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium; f. basidium.

*hyphae* on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with irregularly shaped thin-walled terminal cells of  $10\text{--}30 \times 2\text{--}3 \mu\text{m}$ . *Hymenium* limited to the surface of the pileus, amphigenous, composed of basidia and simple cylindrical dikaryophyses. *Probasidia* cylindrical to clavate, pale yellow,  $25\text{--}40 \times 4\text{--}6 \mu\text{m}$ , without basal clamp connections, becoming bifurcate. *Basidiospores* cylindrical to reniform, straight or curved, with an apiculum at the base, thin-walled, hyaline,  $9\text{--}12 \times 4\text{--}6 \mu\text{m}$  ( $10.5 \times 5 \mu\text{m}$  on average,  $n = 10$ ),  $l/w$   $1.8\text{--}2.5$  ( $2.1$  on average),  $0\text{--}1$ -septate.

*Specimens examined.* NEW ZEALAND, North Island, Tararua Forest Park, Kirihakapapa Road, on dead branches of a woody plant, 6 June 2015, *T. Shirouzu*, PDD 107959, 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, Ara O Te Kinga, on dead branches of a woody plant, 18 May 2015, *T. Shirouzu*, PDD 107890, culture ICMP 21243.

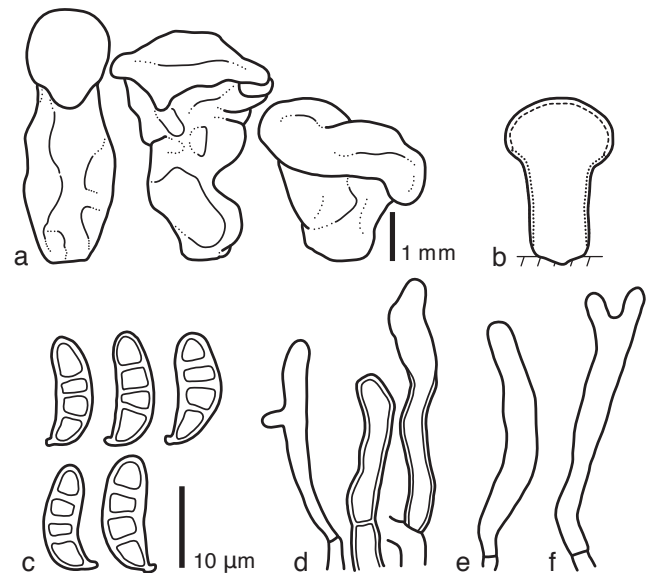
**Notes** — *Calocera pedicellata* is characterised by cylindrical stipitate-pileate basidiocarps, irregularly shaped terminal cells, and small 1-septate basidiospores. This species is assigned to the genus *Calocera* on the basis of the presence of cylindrical basidiocarps, three-zoned internal structures, and amphigenous hymenia. The most similar species to *C. pedicellata* is *C. cornea*. These two species share the characteristics of small cylindrical basidiocarps, hyphae without clamp connections, and small  $0\text{--}1$ -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

Differs 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, Coromandel Forest Park, Rangihau Track, on dead branches of a broad-leaved tree, 22 June 2015, *T. Shirouzu* (holotype PDD 107997; isotype TNS-F-65501, culture ex-type ICMP 21242).



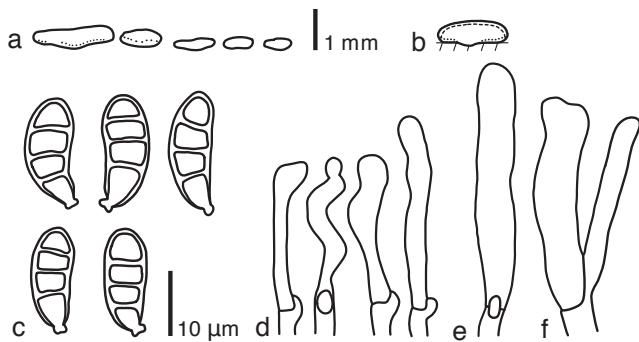
**Fig. 4** *Dacrymyces longistipitatus* PDD 107997. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium; f. developing basidium.

DNA sequences from the holotype — LC131387 (LSU), LC131426 (ITS).

*Basidiocarps* scattered, cylindrical to turbinate, simple, stipitate-pileate, bearing a cylindrical to subglobose, sometimes discoid pileus, pale yellow to pale olive, firm-gelatinous to soft-cartilaginous,  $2\text{--}6 \text{ mm}$  high,  $1\text{--}3 \text{ mm}$  diam. *Internal hyphae* branched, septate, thin-walled, hyaline,  $2\text{--}4 \mu\text{m}$  diam, without clamp connections. *Marginal hyphae* on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with irregularly shaped thin- or slightly thick-walled terminal cells of  $20\text{--}30 \times 3\text{--}4 \mu\text{m}$ . *Hymenium* limited to the surface of the pileus, amphigenous, composed of basidia. *Probasidia* cylindrical to clavate, pale yellow,  $30\text{--}40 \times 3\text{--}4 \mu\text{m}$ , without basal clamp connections, becoming bifurcate. *Basidiospores* cylindrical to reniform, curved, with an apiculum at the base, thick-walled, hyaline to pale yellow,  $12\text{--}15 \times 4\text{--}5 \mu\text{m}$  ( $14 \times 4.5 \mu\text{m}$  on average,  $n = 10$ ),  $l/w$   $2.4\text{--}3.8$  ( $3$  on average),  $0\text{--}3$ -septate.

*Specimens examined.* NEW ZEALAND, North Island, Coromandel Forest Park, Rangihau Track, on dead branches of a woody plant, 22 June 2015, *T. Shirouzu*, PDD 107996 (TNS-F-65500), culture ICMP 21241; Whenuakite Block, on dead branches of a conifer, 22 June 2015, *T. Shirouzu*, PDD 107995; South Island, Westland Tai Poutini National Park, Fox Glacier, on dead branches of a woody plant, 16 May 2015, *T. Shirouzu*, PDD 107885; Lake Brunner, Ara O Te Kinga, on dead branches of a woody plant, 18 May 2015, *T. Shirouzu*, PDD 107889.

**Notes** — *Dacrymyces longistipitatus* is characterised by cylindrical to turbinate stipitate-pileate basidiocarps, irregularly shaped slightly thick-walled terminal cells, and thick-walled 3-septate basidiospores. This species is similar to *D. capitatus* and *D. dacryomitriiformis* in having stipitate-pileate basidiocarps, hyphae lacking clamp connections, and 3-septate basidiospores. Compared with *D. longistipitatus*, *D. capitatus* has shorter-stiped 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. dacryomitriiformis* has simple or sparingly branched dikaryophyses, relatively long probasidia ( $35\text{--}60 \times 3.5\text{--}5 \mu\text{m}$ ), and thin-walled basidiospores with thick septa (McNabb 1973).



**Fig. 5** *Dacrymyces pachysporus* PDD 105004. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium; f. developing basidium and dikaryophysis.

***Dacrymyces pachysporus* Shirouzu, sp. nov.** — MycoBank MB817694; Fig. 2c, 5

Differs 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.

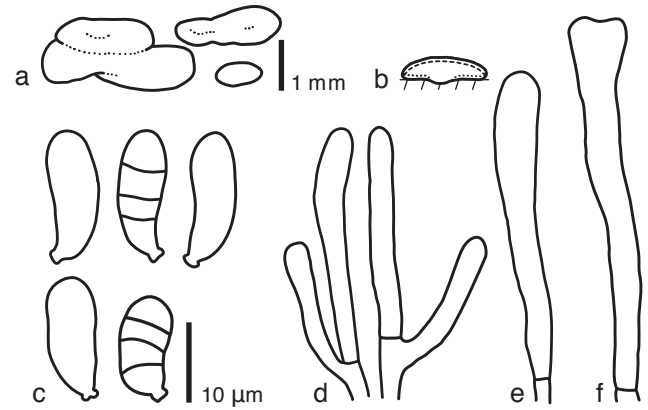
**Type.** NEW ZEALAND, South Island, Nelson Lakes National Park, Lake Lotoiti, on dead branches of *Leptospermum* sp. or *Kunzea* sp., 8 May 2014, T. Shirouzu (holotype PDD 105004; isotype TNS-F-65506, culture ex-type ICMP 20479).

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, thin-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, amphigenous, 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. stillatus* 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 stillatus* 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. stillatus* are phylogenetically distant from *D. pachysporus* (Fig. 1).



**Fig. 6** *Dacrymyces stenosporus* PDD 105018. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium; f. developing basidium.

***Dacrymyces stenosporus* Shirouzu, sp. nov.** — MycoBank MB817695; Fig. 2d, 6

Differs from *Dacrymyces lacrymalis* by its longer basidiospores.

**Etymology.** From the Greek 'stenos' = narrow and 'sporus' = spore, referring to the slender 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 — LC131396 (LSU), LC131433 (ITS).

**Basidiocarps** scattered or gregarious, sometimes coalesced, pulvinate to irregularly discoid, sometimes gyrose, sessile, pale to orange yellow, firm-gelatinous, 1–2 mm high, 1–5 mm diam. **Internal hyphae** branched, septate, thin-walled, hyaline, 2–3 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, 30–40 × 4 µm, without basal clamp connections, becoming bifurcate. **Basidiospores** cylindrical to reniform, curved, with an apiculum at the base, thin-walled, hyaline to pale yellow, 13–17 × 5–6 µm (15 × 5.5 µm on average,  $n = 10$ ), l/w 2.5–3.2 (2.8 on average), 0–3-septate.

**Specimens examined.** NEW ZEALAND, North Island, Tongariro National Park, Rotopounamu Walk, on dead branches of a broad-leaved tree, 18 June 2015, T. Shirouzu, PDD 107990; Whanganui National Park, Pipiriki, on dead branches of a broad-leaved tree, 11 June 2015, T. Shirouzu, PDD 107970 (TNS-F-65511), culture ICMP 21237; South Island, Fiordland National Park, Kepler Track, on dead branches of a woody plant, 10 May 2015, T. Shirouzu, PDD 107852; Kahurangi National Park, Wangapeka Track, on dead branches of a woody plant, 10 May 2014, T. Shirouzu, PDD 105022, culture ICMP 20491.

**Notes** — *Dacrymyces stenosporus* is characterised by its pulvinate to irregularly discoid basidiocarps and slender 0–3-septate basidiospores. This species is similar to *D. lacrymalis*, *D. minor*, *D. neoalbidus*, and *D. subantarcticensis* in having pulvinate sessile basidiocarps, hyphae without clamp connections, and 0–3-septate thin-walled basidiospores. *Dacrymyces lacrymalis* (10–15.5 × 4.5–6 µm, McNabb 1973; 9.5–15 × 3.5–6 µm, Shirouzu et al. 2009) and *D. subantarcticensis* (10–13 × 4.5–6 µm, Burdsall & Laursen 2004) have shorter basidiospores, and *D. minor* is characterized by having smaller basidiocarps (0.5–2 mm diam, McNabb 1973; 1–2 mm diam, Shirouzu et al. 2009). *Dacrymyces subantarcticensis* and



samples accepted here as *D. lacrymalis* and *D. minor* are phylogenetically distant from *D. stenosporus* (Fig. 1). *Dacrymyces neoalbidus* has white fruiting bodies and larger basidiospores ( $21\text{--}22 \times 5\text{--}6 \mu\text{m}$ , as *Dacrymyces albidus*, Kobayasi 1954, 1955).

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

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

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

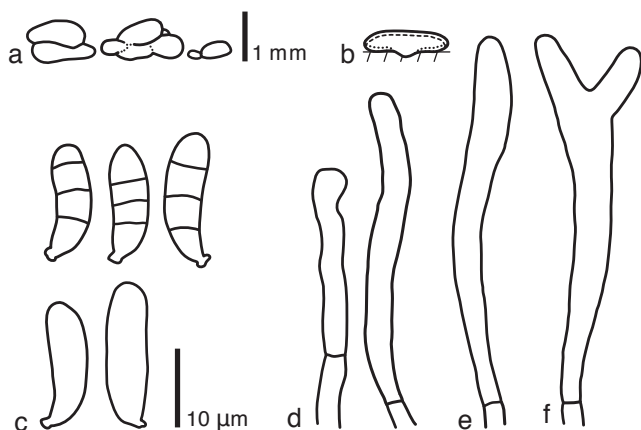
*Type.* NEW ZEALAND, South Island, Arthur's Pass National Park, Waimakariri River, on dead branches of a woody plant, 4 May 2014, *T. Shirouzu* (holotype PDD 104963; isotype TNS-F-65509, culture ex-type ICMP 20436).

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

*Basidiocarps* scattered or gregarious, coalesced, pustulate to pulvinate, gyrose, sessile, orange yellow, firm-gelatinous, 0.5–1 mm high, 1–4 mm diam. *Internal hyphae* branched, septate, thin-walled, hyaline, 2–5  $\mu\text{m}$  diam, without clamp connections. *Marginal hyphae* on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with cylindrical thin-walled terminal cells of  $20\text{--}30 \times 2\text{--}3 \mu\text{m}$ . *Hymenium* limited to the ventral surface of the basidiocarp, amphigenous, composed of basidia and simple cylindrical dikaryophyses. *Probasidia* cylindrical to clavate, pale yellow,  $40\text{--}50 \times 5 \mu\text{m}$ , without basal clamp connections, becoming bifurcate. *Basidiospores* cylindrical, straight or slightly curved, with an apiculum at the base, thin-walled, hyaline to pale yellow,  $14\text{--}17 \times 4\text{--}6 \mu\text{m}$  ( $16 \times 5 \mu\text{m}$  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, Kiriwhakapapa Road, on dead branches of a broad-leaved tree, 6 June 2015, *T. Shirouzu*, PDD 107962; South Island, Craigieburn Forest Park, Dracophyllum 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 pustulate to pulvinate basidiocarps and slender 0–3-septate basidiospores. This species is similar to *Dacrymyces stenosporus*, but the latter species has shorter probasidia ( $30\text{--}40 \times 4 \mu\text{m}$ ). These two species are phylogenetically distant from one another (Fig. 1).



**Fig. 7** *Dacrymyces parastenosporus* PDD 104963. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium; f. developing basidium.

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

Differs from *Dacrymyces ancyleus* by the presence of smaller thick-walled basidiospores.

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

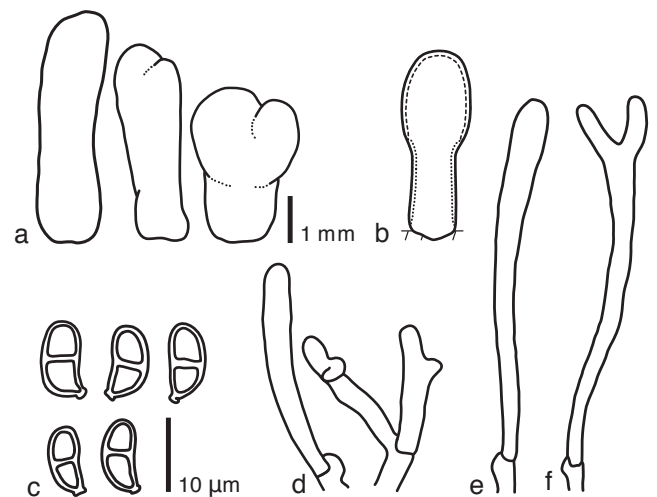
*Type.* NEW ZEALAND, South Island, Kahurangi National Park, Kaituna Track, on dead branches of a broad-leaved tree, 15 May 2014, *T. Shirouzu* (holotype PDD 105052; isotype TNS-F-65492, culture ex-type ICMP 20517).

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

*Basidiocarps* scattered, cylindrical to subulate, simple, stipitate-pileate, 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  $\mu\text{m}$  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  $25\text{--}35 \times 3 \mu\text{m}$ . *Hymenium* limited to the surface of the pileus, amphigenous, composed of basidia. *Probasidia* cylindrical to clavate, pale yellow,  $40\text{--}50 \times 4 \mu\text{m}$ , with basal clamp connections, becoming bifurcate. *Basidiospores* cylindrical to reniform, straight or curved, with an apiculum at the base, thick-walled, hyaline,  $8\text{--}10 \times 4\text{--}5 \mu\text{m}$  ( $9 \times 4 \mu\text{m}$  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, Kiriwhakapapa 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 Rotoiti, 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 subulate basidiocarps, but its fruiting bodies lack the three-zoned internal structure of species in the genus *Calocera*. Furthermore, this new species is not placed in *Dacryopinax* because the pileus is cylindrical to subglobose and the hymenium is amphigenous. Consequently, this fungus should be assigned to the genus *Dacrymyces*. *Dacrymyces cylindricus* is similar to *D. ancyleus*



**Fig. 8** *Dacrymyces cylindricus* PDD 105052. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium; f. developing basidium.

and *D. flabelliformis* in having stipitate-pileate basidiocarps and clamp connections on hyphae. *Dacrymyces ancyleus* has larger thin-walled basidiospores ( $10.5\text{--}19.5 \times 4\text{--}9 \mu\text{m}$ , Shirouzu et al. 2009). *Dacrymyces flabelliformis* has spatulate to flabelliform basidiocarps and larger thin-walled 0–3-septate basidiospores ( $12.5\text{--}14 \times 5\text{--}6 \mu\text{m}$ , 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.

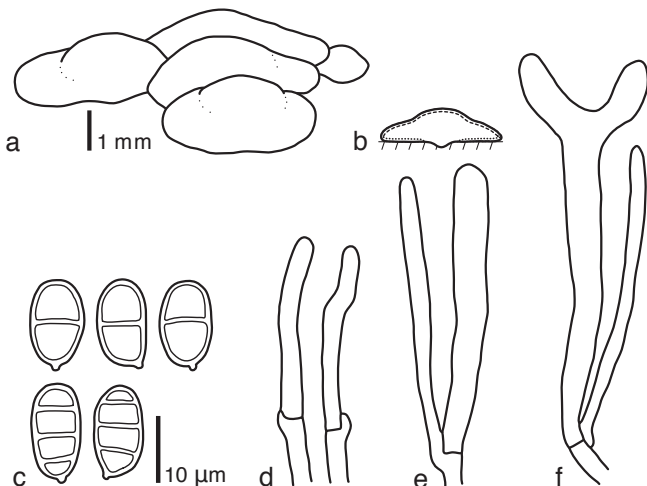
**Etymology.** From the Latin '*citrinus*' = pale yellow, referring to the colour of the basidiocarps.

**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  $\mu\text{m}$  diam, with clamp connections. **Marginal hyphae** on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with cylindrical thin-walled terminal cells of  $20\text{--}40 \times 2\text{--}5 \mu\text{m}$ . **Hymenium** limited to the ventral surface of the basidiocarp, amphigenous, composed of basidia and simple cylindrical dikaryophyses. **Probasidia** cylindrical to clavate, pale yellow,  $35\text{--}45 \times 5\text{--}6 \mu\text{m}$ , with basal clamp connections, becoming bifurcate. **Basidiospores** cylindrical to reniform, straight, with an apiculum at the base, thick-walled, hyaline to pale yellow,  $11\text{--}14 \times 7\text{--}9 \mu\text{m}$  ( $13 \times 8 \mu\text{m}$  on average,  $n = 10$ ), l/w 1.5–2 (1.7 on average), 0–3-septate.

**Specimens examined.** NEW ZEALAND, North Island, Tararua Forest Park, Waiohine Gorge, on dead branches of a broad-leaved tree, 5 June 2015, *T. Shirouzu*, PDD 107949; Whanganui National Park, Atene Viewpoint Walk, on dead branches of a woody plant, 12 June 2015, *T. Shirouzu*, PDD 107979 (TNS-F-65491), culture ICMP 21239; 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, Wangapeka Track, on dead branches of *Leptospermum scoparium*, 31 May 2015, *T. Shirouzu*, PDD 107934.



**Fig. 9** *Dacrymyces citrinus* PDD 107915. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium and dikaryophysis; f. developing basidium and dikaryophysis.

**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 ( $11\text{--}15.5 \times 4.5\text{--}6.5 \mu\text{m}$ ), branched dikaryophyses, and dark basidiocarps (McNabb 1973). *Dacrymyces paraphysatus* has longer basidiospores ( $13.5\text{--}21 \times 5\text{--}7 \mu\text{m}$ ) and branched dikaryophyses (McNabb 1973). *Dacrymyces sichuanensis* has smaller basidiocarps (1–2 mm diam), narrower basidiospores ( $12.5\text{--}15.6 \times 4.5\text{--}6.5 \mu\text{m}$ ), 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 ( $16\text{--}19 \times 6\text{--}7 \mu\text{m}$ ), 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.  $13\text{--}14 \times 6\text{--}7 \mu\text{m}$ , 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

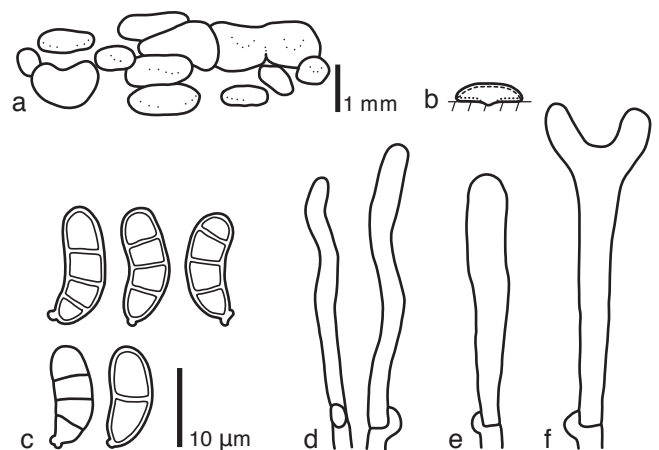
Differs from *D. sichuanensis* by the absence of branched dikaryophyses.

**Etymology.** From the Greek '*cyrto*' = 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  $\mu\text{m}$  diam, with clamp connections. **Marginal hyphae** on sterile surfaces of basidiocarps cylindrical, straight or flexuous, septate, hyaline, with cylindrical thin-walled terminal cells of  $15\text{--}45 \times 2\text{--}3 \mu\text{m}$ . **Hymenium** limited to the ventral surface of the basidiocarp, amphigenous, composed of basidia and simple cylindrical dikaryophyses. **Probasidia** cylindrical to clavate, hyaline,  $30\text{--}50 \times 5\text{--}6 \mu\text{m}$ , with



**Fig. 10** *Dacrymyces cyrtosporus* PDD 107980. a. Basidiocarps; b. diagram showing longitudinal section of basidiocarp (dashed line: hymenium; dotted line: sterile marginal part); c. basidiospores; d. marginal hyphae; e. probasidium; f. developing basidium.

basal clamp connections, becoming bifurcate. *Basidiospores* cylindrical, curved, with an apiculum at the base, thick-walled, hyaline, 13–15 × 5–6 µm (14 × 5.5 µm on average,  $n = 10$ ), l/w 2.2–3 (2.6 on average), 0–3-septate.

*Specimens examined.* NEW ZEALAND, North Island, Tararua Forest Park, Kiriwhakapapa Road, on dead branches of a woody plant, 6 June 2015, *T. Shirouzu*, PDD 107957, culture ICMP 21245; Tararua Forest Park, Waiohine Gorge, on dead branches of a broad-leaved tree, 5 June 2015, *T. Shirouzu*, PDD 107951, culture ICMP 21244; PDD 107952 (TNS-F-65494); South Island, Fiordland National Park, Borland Nature Walk, on dead branches of a woody plant, 12 May 2015, *T. Shirouzu*, PDD 107867.

**Notes** — *Dacrymyces cyrtosporus* is characterised by its pustulate to pulvinate basidiocarps, hyphae with clamp connections, and curved thick-walled 3-septate basidiospores. This species is similar to *D. enatus* var. *macrosporus*, *D. paraphysatus*, *D. sichuanensis*, *D. pachysporus*, and *D. citrinus* in having pulvinate basidiocarps, hyphae with clamp connections, and 3-septate thick-walled basidiospores. However, *D. sichuanensis* 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 paraphysatus* has branched dikaryophyses and yellowish brown, larger basidiospores (13.5–21 × 5–7 µm, McNabb 1973). *Dacrymyces pachysporus* has irregularly shaped terminal cells (Fig. 5) and longer basidiospores (16–19 × 6–7 µm). *Dacrymyces citrinus* 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. citrinus* (Fig. 1).

### 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 Ecological Area, 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 *C. cornea* (Fig. 1). *Calocera cornea* is a common species of *Dacrymycetetes* and has been recorded worldwide (McNabb 1965a, Lowy 1971, Reid 1974, Shirouzu et al. 2009). The geographical and phylogenetic distributions of *C. 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. *guepinioides*** 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-tauru 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, 28 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 Rotoiti, on dead branches of a woody plant, 8 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. *guepinioides* 1, 2, and 3; Fig. 1). The specimens constituting each clade could not be morphologically distinguished, and the true clade of *C. guepinioides* could be not confirmed because DNA from the type strain was not included in this study. *Calocera guepinioides* 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*** (Masse) 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 *C. 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, Borland Nature Walk, on dead branches of a broad-leaved tree, 12 May 2015, *T. Shirouzu*, PDD 107863, culture ICMP 21225; Nelson Lakes National Park, Lake Rotoiti, 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 intermedius*** 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, Wangapeka Track, on dead branches of a broad-leaved tree, 31 May 2015, *T. Shirouzu*, PDD 107939, culture ICMP 21232.

Notes — *Dacrymyces intermedius*, 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. *microsporus*** 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 20505.

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 basidiospores 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 Rotoiti, 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. 1965

*Type locality.* Tasmania, Australia.

*Specimens examined.* NEW ZEALAND, South Island, Arthur's Pass National Park, Waimakariri River, on dead branches of *Nothofagus solandri*, 4 May 2014, *T. Shirouzu*, PDD 104962, ICMP 20435; Denniston, Coalbrookdale 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. guepinoides*, 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. *microsporus*, 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. guepinoides*, *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, *C. pedicellata* and *D. parastenosporus*, were collected from dead branches of *Pinus radiata*, 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 *P. radiata* 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 – *D. citrinus*, *D. cylindricus*, *D. cyrtosporus*, *D. longistipitatus*, *D. pachysporus*, and *D. pedicellata* – were morphologically and phylogenetically distinct from other species. Two new species, *D. stenosporus* and *D. parastenosporus*, were morphologically similar to each other, but were described as two different species because they were phylogenetically distant from one another (Fig. 1). *Dacrymyces parastenosporus* can be distinguished from *D. stenosporus* 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 *C. cf. guepiniooides* specimens into three clades (*C. cf. guepiniooides* 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 *C. guepiniooides* (McNabb 1965a). The clade corresponding to *C. guepiniooides* 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 *Dacrymycetes* 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*, *Cerinomyces*, *Dacrymyces*, and *Dacryopinax* have been shown to be non-monophyletic genera (Fig. 1; Shirouzu et al. 2009, 2013a). As a result, *Dacrymycetaceae* and *Cerinomycetaceae*, the two families in *Dacrymycetales*, 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 *Dacrymyces* and *Calocera* was particularly evident (Fig. 1). The genus *Dacrymyces* 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 *Dacrymyces* appears to be the most phylogenetically scattered genus in the *Dacrymycetales* clade (Fig. 1).

The genus *Calocera* 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 *C. cornea* and *C. viscosa* (Weiß & Oberwinkler 2001, Shirouzu et al. 2007, 2009), the genus *Calocera* has been considered to be a monophyletic taxon. In the present phylogenetic tree, however, many of the *Calocera* species used in this study, such as *C. arborea*, *C. bambusicola*, *C. fusca*, *C. cf. guepiniooides*, *C. glossooides*, *C. lutea*, *C. pedicellata*, and *C. sinensis*, were found to be dispersed throughout the *Dacrymycetaceae* 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 *Dacrymycetes*. 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 *Dacrymycetes*, mycelium strains not associated with basidiocarps as well as lineages with visible fruiting bodies must be incorporated. Our survey of the diversity of *Dacrymycetes* 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 *Dacrymycetes*.

**Acknowledgements** We are grateful to Drs Renee Johansen, Peter Buchanan, Roy E. Halling, Teresa Lebel, and Gregory Bonito for their help during field trips. This work was supported by the Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for JSPS Fellows (25-9680), Young Scientists (A) (24680085), and Scientific Research (B) (24300314).

#### REFERENCES

- Burdsall HH, Laursen GA. 2004. Fungi of New Zealand's subarctic islands I: Two new species of *Dacrymyces* (Basidiomycota). In: Miller OK, Cripps CL (eds), *Fungi in forest ecosystems: systematics, diversity and ecology*: 107–111. New York Botanical Garden, USA.
- Coetzee MPA, Wingfield BD, Bloomer P, et al. 2001. Phylogenetic relationships of Australian and New Zealand Armillaria species. *Mycologia* 93: 887–896.
- Delivouras P, Gonou-Zagou Z, Kapsanaki-Gotsi E. 2012. A new species of *Guepinioopsis* (*Dacrymycetes*) from Greece. *Sydowia* 64: 19–27.
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.
- Erkens RHJ, Cross H, Maas JW, et al. 2008. Assessment of age and greenness herbarium specimens as predictors for successful extraction and amplification of DNA. *Blumea* 53: 407–428.
- Hosaka K, Castellano MA, Spatafora JW. 2008. Biogeography of Hysterangiales (Phallomycetidae, Basidiomycota). *Mycological Research* 112: 448–462.
- Hosaka K, Uno K. 2013. Assessment of the DNA quality in mushroom specimens: a recovery of the whole ITS sequence from fragmented DNA of the type specimen. *Bulletin of the National Museum of Nature and Science. Series B, Botany* 39: 53–60.
- Jülich W. 1981. Higher taxa of Basidiomycetes. *Bibliotheca Mycologica* 85: 1–845.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Kobayasi Y. 1954. Monographic studies of Japanese Tremellaceous fungi VI. *Nagaoa* 4: 36–47.
- Kobayasi Y. 1955. Correction of scientific name. *Nagaoa* 5: 60.
- Leslie AB, Beaulieu JM, Rai HS, et al. 2012. Hemisphere-scale differences in conifer evolutionary dynamics. *Proceedings of the National Academy of Sciences of the United States of America* 109: 16217–16221.

- Liu B, Fan L. 1990. New species and new variety of *Dacrymycetaceae* in China. *Acta Mycologica Sinica* 9: 12–19.
- Lowy B. 1971. Tremellales. *Flora Neotropica* 6: 1–153.
- McNabb RFR. 1964. Taxonomic studies in the *Dacrymycetaceae* I. *Ceriniomyces* Martin. *New Zealand Journal of Botany* 2: 415–424.
- McNabb RFR. 1965a. Taxonomic studies in the *Dacrymycetaceae* II. *Calocera* (Fries) Fries. *New Zealand Journal of Botany* 3: 31–58.
- McNabb RFR. 1965b. Taxonomic studies in the *Dacrymycetaceae* III. *Dacryopinax* Martin. *New Zealand Journal of Botany* 3: 59–72.
- McNabb RFR. 1965c. Taxonomic studies in the *Dacrymycetaceae* IV. *Guepiniopsis* Patouillard. *New Zealand Journal of Botany* 3: 159–169.
- McNabb RFR. 1965d. Taxonomic studies in the *Dacrymycetaceae* V. *Heterotextus* Lloyd. *New Zealand Journal of Botany* 3: 215–222.
- McNabb RFR. 1965e. Taxonomic studies in the *Dacrymycetaceae* VI. *Femsonia* Fries. *New Zealand Journal of Botany* 3: 223–228.
- McNabb RFR. 1966. Taxonomic studies in the *Dacrymycetaceae* VII. *Ditiola* Fries. *New Zealand Journal of Botany* 4: 546–558.
- McNabb RFR. 1973. Taxonomic studies in the *Dacrymycetaceae* VIII. *Dacrymyces* Nees ex Fries. *New Zealand Journal of Botany* 11: 461–524.
- McNabb RFR, Talbot PHB. 1973. *Holobasidiomycetidae: Exobasidiales, Brachybasidiales, Dacrymycetales*. In: Ainsworth GC, Sparrow FK, Sussman AS (eds), *The fungi*, Vol. IV B: 317–325. Academic Press, USA.
- Oberwinkler F. 1993. Genera in a monophyletic group: The *Dacrymycetales*. *Mycologia Helvetica* 6: 35–72.
- Oberwinkler F. 2014. *Dacrymycetes*. In: McLaughlin DJ, Spatafora JW (eds), *The Mycota, systematics and evolution*, vol. 7A: 357–372. Springer, Germany.
- Olive LS. 1958. The lower *Basidiomycetes* of Tahiti (continued). *Bulletin of the Torrey Botanical Club* 85: 89–110.
- Reid DA. 1974. A monograph of the British *Dacrymycetales*. *Transactions of the British Mycological Society* 62: 433–494.
- Rogstad SH. 2003. Plant DNA extraction using silica. *Plant Molecular Biology Reporter* 21: 463a–463g.
- Seifert KA. 1983. Decay of wood by the *Dacrymycetales*. *Mycologia* 75: 1011–1018.
- Seutin G, White BN, Boag PT. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69: 82–90.
- Shirouzu T, Hirose D, Oberwinkler F, et al. 2013a. Combined molecular and morphological data for improving phylogenetic hypothesis in *Dacrymycetes*. *Mycologia* 115: 1110–1125.
- Shirouzu T, Hirose D, Tokumasu S. 2007. Sequence analyses of the 28S rRNA gene D1/D2 region suggest *Dacrymyces* (*Heterobasidiomycetes, Dacrymycetales*) is polyphyletic. *Mycoscience* 48: 388–394.
- Shirouzu T, Hirose D, Tokumasu S. 2009. Taxonomic study of the Japanese *Dacrymycetes*. *Persoonia* 23: 16–34.
- Shirouzu T, Ishikawa NK, Hirose D, et al. 2013b. A new Amazonian species of *Calocera* with dendroid and multi-headed basidiocarp. *Mycoscience* 54: 252–256.
- Shirouzu T, Uno K, Hosaka K, et al. 2016. Early-diverging wood-decaying fungi detected using three complementary sampling methods. *Molecular Phylogenetics and Evolution* 98: 11–20.
- Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.
- Weiß M, Oberwinkler F. 2001. Phylogenetic relationships in *Auriculariales* and related groups – hypotheses derived from nuclear ribosomal DNA sequences. *Mycological Research* 105: 403–415.
- White TJ, Bruns T, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al. (eds), *PCR protocols*: 315–322. Academic Press, USA.
- Wu S-H, Shih K, Yu S-Y. 2011. *Calocera bambusicola* sp. nov. and *C. sinensis* newly recorded from Taiwan. *Mycotaxon* 115: 163–169.