A new species of *Cryptohymenium* (*Leotiomycetes, Helotiales*) from New Caledonia

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Abstract: The newly described *Cryptohymenium meruu* from New Caledonia is the second species in a genus previously thought to be endemic to New Zealand. *Cryptohymenium* is a soil-inhabiting, morphologically bizarre inoperculate discomycete (*Leotiomycetes*). It is a member of the order *Helotiales*, with a sister relationship to *Sclerotiniaceae* and *Cenangiaceae*.

Keywords: *Ascomycota*, New Zealand, taxonomy, Zealandia, 1 new taxon.

Résumé : *Cryptohymenium meruu*, espèce nouvelle décrite de Nouvelle-Calédonie, est la deuxième espèce d'un genre que l'on croyait endémique de Nouvelle-Zélande. Les *Cryptohymenium* sont des discomycètes inoperculés (*Leotiomycetes*) poussant sur le sol et morphologiquement déroutants. Ils sont membres de l'ordre des *Helotiales* et sont apparentés aux *Sclerotiniaceae* et aux *Cenangiaceae*.

Mots-clés : *Ascomycota*, Nouvelle-Zélande, taxinomie, Zélandia, 1 nouveau taxon.

Introduction

New Zealand and New Caledonia are the largest above-sea parts of the largely submerged continent Zealandia (Mortimer *et al.*, 2017). These two land masses share many animal and plant lineages, a relationship sometimes explained as reflecting a shared geological history. Long thought to have been driven by ancient links from the Gondwana continent, more recent geological studies have shown that entire New Caledonian landmass was submerged during the Palaeocene and Eocene (Grandcolas *et al.*, 2008), the present-day biota a result of long-distance dispersal events. The historical occurrence of chains of islands along what are now undersea ridges between New Zealand and New Caledonia may have helped facilitate such dispersal (see discussion and references in Gibbs, 2016).

Much of the unique diversity of New Caledonian biota is due to recently evolved endemic species (Grandcolas *et al.*, 2008). Although the diversity of the fungi of New Caledonia has barely been investigated, Jourand *et al.* (2014) and Carriconde *et al.* (2019) suggest a similar mechanism for the evolution in ultramafric soils of local populations of *Pisolithus albus*-like fungi (*Boletales*, *Sclerodermataceae*) and of the mushroom genus *Cortinarius* (*Agaricales*, *Cortinariaceae*). Recent environmental DNA (eDNA)-based surveys of soil fungi evoke plant community diversity as a major driver of fungal diversity in New Caledonia (Bordez *et al.*, 2016; Gourmelon *et al.*, 2016).

Prior to this publication, *Cryptohymenium* was considered endemic to New Zealand. The only known New Zealand species is widespread through the country. Here we describe a second species in the genus, from New Caledonia. These morphologically distinctive fungi are phylogenetically isolated within *Helotiales*, with a sister relationship to *Sclerotiniaceae* and *Cenangiaceae* (Johnston *et al.*, 2019; Haelewaters *et al.*, 2021).

Methods

Fungarium specimens of the New Caledonian and New Zealand species of *Cryptohymenium* were rehydrated in 3% KOH, gently crushed, and hymenial elements mounted in Melzer's reagent, or rehydrated in water and mounted in Lugol's iodine. Entire apothecia of *C. pycnidiophorum* Samuels & L.M. Kohn were sectioned at about 10 µm using a freeze microtome, the sections mounted in lactic acid. Specimens are deposited in the New Zealand Fungarium (PDD) and Herbarium of the Muséum national d'Histoire Naturelle, Paris (PC).

For sequencing, DNA was extracted from ground, dried apothecia using the QIAamp DNA Mini kit (QIAGEN, US) on the QIAcube nucleic acid extraction robot (QIAGEN, US). Amplification primers used for each of the genes were: for ITS, ITS1F, ITS3, and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993); for LSU, LR0R and LR5 (Hopple, 1994; Bunyard *et al.*, 1994; Vilgalys & Hester, 1990). Sequences have been deposited in GenBank.

The *Cryptohymenium* ITS and LSU sequences were placed in alignments with selected *Sclerotiniaceae*, *Rutstroemiaceae*, and *Cenangiaceae*, with *Chlorociboriaceae* and *Polydesmia* as outgroups. Taxon selection was based on the multilocus analysis from Johnston *et al.* (2019). Sequences (Table 1) were aligned with MAFFT and then concatenated using Geneious 10. Relationships were inferred by maximum likelihood analysis, using IQ-TREE (Nguyen *et al.*, 2015; Chernomor *et al.*, 2016) with models selected by ModelFinder (Kalyaanamoorthy *et al.*, 2017) for each partitioned gene (TIM2+F+I+G4 for ITS; TN+F+R3 for LSU), and branch support estimated using ultrafast bootstrapping with 1000 replicates (Hoang *et al.*, 2018).

Taxonomy

Cryptohymenium meruu P.R. Johnst. & Eyssart., *sp. nov*. Fig. 1
Mycobank: MB 844779

Diagnosis: Differs from *Cryptohymenium pycnidiophorum* in having a green rather than black hymenium when fresh, and shorter ascospores, average less than 20 µm long versus more than 20 µm long.

Holotype: New Caledonia, commune du Mont Dore, Bois du Sud, 22.1653° S, 166.7398° E, amongst litter and soil beneath *Arillastrum gummiferum*, coll. C. Lauderau, 10 June 2018 (holotype PDD 116651, isotype PC); GenBank accession numbers ON951723 (ITS), ON951722 (LSU).

Etymology: From méruu, the word for green in the Kanak Numèè language of the region in which the type specimen was collected, reflecting the colour of the hymenium when fresh.

Classification: *Leotiomycetes*, *Helotiales*, *incertae sedis.*

Ascomata comprising multiple, stalked apothecia arising from a common stromatic base buried in litter and soil. **Apothecia** up to 4.5–8.5 mm tall, with a long, scaly, dark grey-brown stipe and an apical flaring cap 2–3 mm diam. Cap of mature apothecium comprising flattened, greenish hymenium (black when dry) wrapped around the stalk, with a grooved join down one side of hymenium, and with a smooth, globose, black, multiloculate pycnidium at the apex. Immature apothecia not seen, but the remains of what was probably a sterile, pale grey-brown layer that covered the developing hymenium remain visible along both edges of the hymenium. **Excipulum** comprises an outer layer of short-cylindric to subglobose cells with dark, thick walls, an internal layer comprising cells long-cylindric with walls slightly thickened, more or less hyaline, and a subhymenium of *textura intricata*, cells with thin walls partially encrusted with dark brown material. **Paraphyses** 1.5–2.5 µm diam., swelling to 4–6 µm at the clavate apex, sometimes branched near apex, about

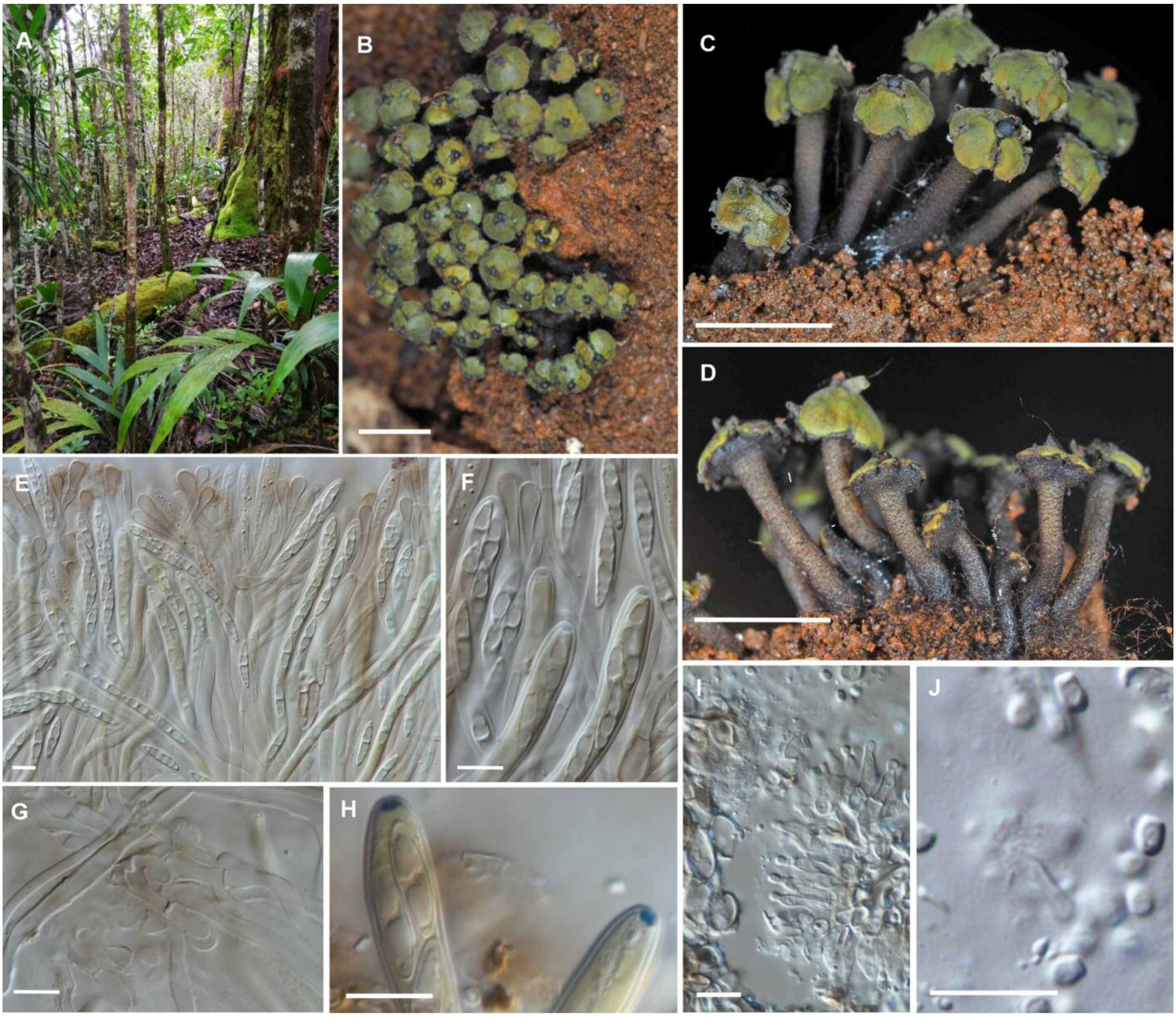

Fig. 1 – *Cryptohymenium meruu* (Holotype, PDD 116651). A: Type locality. B–D: Fresh apothecia. E: Asci, ascospores, paraphysis apex. F: Ascus apex and ascospores. G: Crozier. H: Ascus apex (water + Lugol's iodine). I: Conidiogenous cells. J: Conidia. E–G, I–J mounted in 3% KOH + Melzer's reagent; H mounted in water + Lugol's iodine. Scale bars: B–D = 5 mm; E–J = 10 µm. Photographs A–D: C. Laudereau.

same length as asci, apex surrounded by brownish gel. **Asci** 150–220 × 7.5–10 µm, cylindric, with croziers at base, tapering gradually towards rounded apex, wall thickened at apex with diffuse amyloid reaction across apical pore, pore broader towards inside of wall, 8-spored, spores in upper 60–80 µm of ascus. **Ascospores** 15.5–23.5 × 3.5–5 µm (average 18.6 × 4.2 µm), fusoid, tapering to both narrow-rounded ends, slightly flattened on one side, not curved, 0-septate, hyaline, smooth-walled. **Pycnidia** with multiple locules lined with conidiogenous cells (5.5–) 8–11 × 2.5–3 (–3.5) µm, solitary, cylindric to flask-shaped, walls thickened at single apical conidiogenous locus, wall sometimes flaring at apex, sometimes proliferating percurrently with 1–2 annellidic thickenings. **Conidia** 2.5–3 × 2–2.5 µm (average 2.7 × 2.3 µm), subglobose to short and broad cylindric, apex rounded, base truncate, 0–septate, hyaline.

Other species examined: *Cryptohymenium pycnidiophorum*, New Zealand: Westland, Haast Pass, Blue Pools Track, 44.1985° S 169.2520° E, amongst moss on soil under *Nothofagus* spp., coll. P.R. Johnston (D2514) & M. Padamsee, 16 May 2018 (PDD 111534); GenBank accession numbers MH578491 (ITS), MH985296 (LSU), MH986710 (MCM7), MH986707 (RPB1), MH986703 (RPB2). Taupo, Tongariro National Park, Lake Rotopounamu, 39.0276° S 175.7304° E, coll. P.R. Johnston, 7 May 1987 (PDD 54580); GenBank accession number ON951724 (ITS). Gisborne, Urewera National Park, near forest headquarters, 38.4698° S 176.7103° E, on soil under *Nothofagus* spp., coll. G.J. Samuels (GJS 83-107), P.R. Johnston, T. Matsushima & A.Y. Rossman, 29 May 1983 (PDD 46298).

Discussion

The genus *Cryptohymenium* was previously regarded as endemic to New Zealand (Samuels & Kohn, 1987), known from a single species *C. pycnidiophorum* (Fig. 2). *Cryptohymenium meruu* extends the generic range to New Caledonia, geologically part of the ancient continent Zealandia (Mortimer *et al.*, 2017) and perhaps reflecting an ancient vicariant distribution of the ancestor of the New Zealand and New Caledonian species. The genus has an isolated position within *Helotiales*, with a sister relationship to *Sclerotiniaceae* and *Cenangiaceae* (Johnston *et al.*, 2019; Haelewaters *et al.*, 2021). The two species of *Cryptohymenium* form a well-supported clade in a molecular phylogenetic analysis using concatenated ITS and LSU sequences (Fig. 3). The structure of the ascoma and the individual apothecia of *C. meruu* matches that of *C. pycnidiophorum* (Samuels

Fig. 2 – *Cryptohymenium pycnidiophorum* (A–B: PDD 46298; C–G: PDD 111534). A: Dry apothecium, sterile layer still covering hymenium. B: Dry apothecium, sterile covering layer mostly lost, with hymenium exposed. C: Ascoma in vertical section, rounded pycnidium to right, hymenial layer extending down stipe, tissue within stipe broken apart. D: Detail of top of ascoma. E: Detail of upper margin of hymenial layer. F: Detail of lower margin of hymenial later. G: Detail of pycnidial locule. H: Asci and paraphyses. I: Ascospores. J: Ascus apex (water + Lugol's iodine). C–G mounted in lactic acid; H–I mounted in 3% KOH + Melzer's reagent; J mounted in water + Lugol's iodine . Scale bars: A–B = 1 mm; C = 500 µm; D–F = 100 µm; G–I = 10 µm.

& Kohn, 1987), but the position of the hymenial layer differs. In *C. pycnidiophorum* the hymenium extends as a sleeve-like layer down the stipe, whereas in *C. meruu* it flares outwards and extends only a short distance down the stipe. The ascospores of *C. pycnidiophorum* are larger, (21–) 23–28 (–40) × (3–) 4–5.4 (–6) µm (Samuels & Kohn, 1987). Additional illustrations of *C. meruu* are in the AscoFrance website, see http://www.ascofrance.com/forum/54148/cf-cryptohymenium#.

Known from a single specimen, it is not known whether *C. meruu* is widespread in New Caledonia. It was not detected in the soil eDNA data of Gourmelon *et al.* (2016) or Bordez *et al.* (2016), available from NCBI through the SRA accession numbers SRP052988 and SRR4289165. Although morphologically very distinctive, this small fungus is not easily seen and there may even be additional species awaiting discovery in New Caledonia. Based on knowledge of other fungi of the South Pacific regions (Johnston, 2010) the genus may be expected to occur also in eastern Australia or southern Asia.

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Authors' contribution

Peter Johnston prepared the initial draft of the manuscript, prepared the phylogenies and plates, accessioned the specimens into PDD and the sequences into GenBank. The initial manuscript was reviewed and modified by Guillaume Eyssartier, who arranged for accession of isotype in PC. Duckchul Park carried out the DNA sequencing of *C. pycnidiophorum*. Philippe Bourdeau facilitated access to specimens of *C. meruu*, on behalf of the Société Mycologique de Nouvelle-Calédonie.

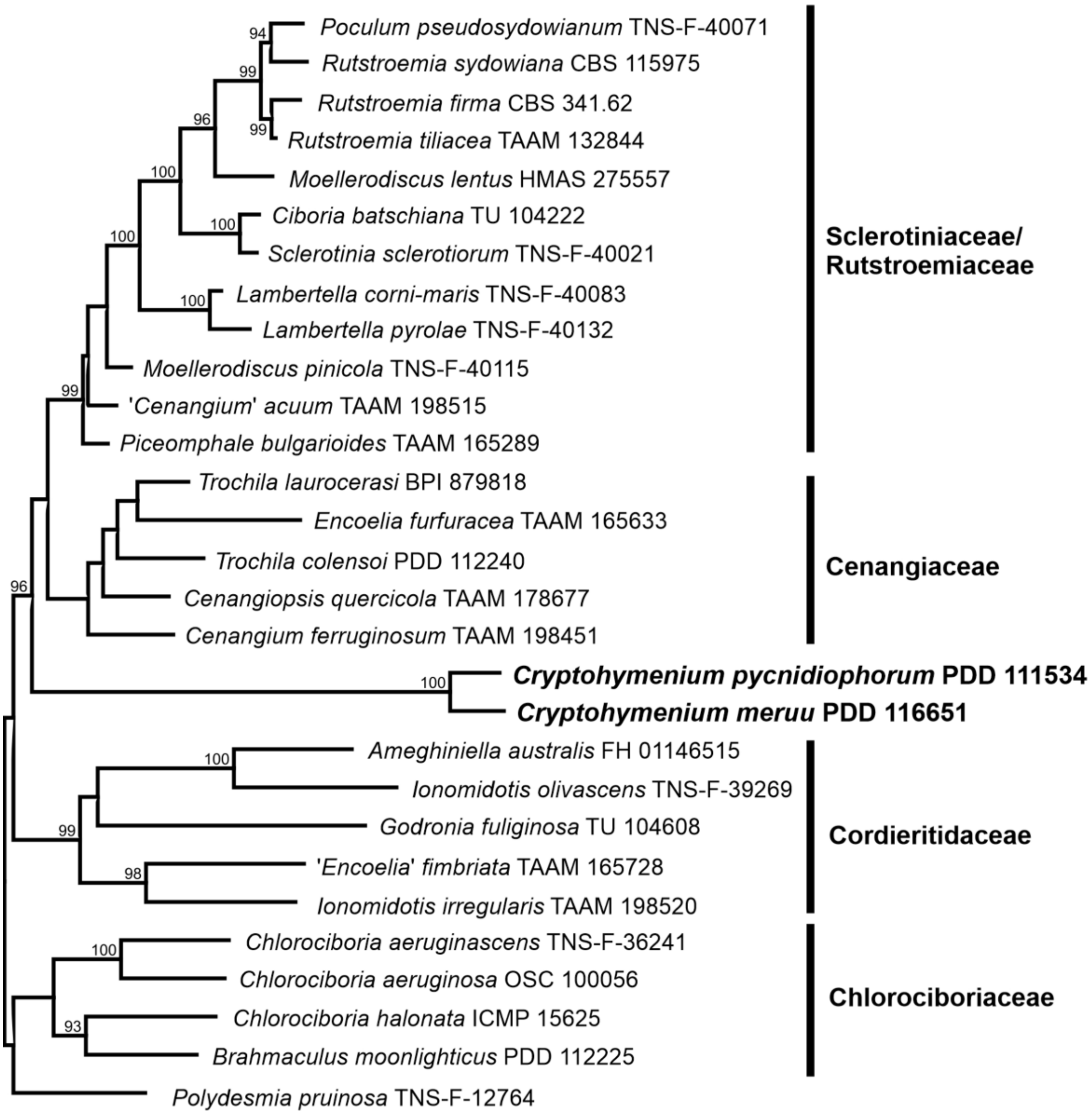

Fig. 3 – Maximum likelihood phylogeny based on concatenated ITS and LSU sequences. Bootstrap values indicated when greater than 90%. The relationships in this tree match those of the multigene analysis of Johnston *et al.* (2019).

Table 1 – Details of specimens used for the phylogeny in Fig. 3.

Species	Voucher	Collector	ITS	LSU
Ameghiniella australis	FH 01146515	D.H. Pfister	MH752070	KX090841
Brahmaculus moonlighticus	PDD 112225	A. Chinn	MK248036	MK248011
Cenangiopsis quercicola	TAAM 178677	–	LT158425	KX090811
'Cenangium' acuum	TAAM 198515	–	LT158439	KX090822
Cenangium ferruginosum	TAAM 198451	–	LT158471	KX090840
Chlorociboria aeruginascens	TNS F-36241	T. Hosoya	LC425045	LC429376
Chlorociboria aeruginosa	OSC 100056	–	DQ491501	AY544669
Chlorociboria halonata	ICMP 15625	P.R. Johnston	AY755355	JN939933
Ciboria batschiana	TU 104222	–	LT158466	MH748088
Cryptohymenium meruu	PDD 116651	C. Lauderau	ON951723	ON951722
Cryptohymenium pycnidiophorum	PDD 111534	P.R. Johnston	MH578491	MH985296
'Encoelia' fimbriata	TAAM 165728	G. Marson	MH752063	KX090800
Encoelia furfuracea	TAAM 165633	–	LT158416	KX090798
Godronia fuliginosa	TU 104608	L.G. Krieglsteiner	MH752064	MH748090
Ionomidotis irregularis	TAAM 198520	U. Roffler	MH752065	KX090804
Ionomidotis olivascens	TNS F-39269	T. Hosoya	MH752068	KX090833
Lambertella corni-maris	TNS F-40083	T. Hosoya	AB926069	AB926139
Lambertella pyrolae	TNS F-40132	Yan-jie Zhao	AB926081	AB926164
Moellerodiscus lentus	HMAS 275557	–	KU668566	MH729337
Moellerodiscus pinicola	TNS F-40115	Yan-jie Zhao	AB926078	AB926162
Piceomphale bulgarioides	TAAM 165289	–	LT158483	KX090797
Poculum pseudosydowianum	TNS F-40071	Yan-jie Zhao	AB904505	AB926136
Polydesmia pruinosa	TNS F-12764	T. Hosoya	JN033453	JN086753
Rutstroemia firma	CBS 341.62	P. Berthet	KF545334	DQ470963
Rutstroemia tiliacea	TAAM 132844	H.-O. Baral	LT158423	KX090808
Rutstroemia sydowiana	CBS 115975	G. Verkley	KF545331	from genome SAMN02903521
Sclerotinia sclerotiorum	TNS F-40021	Yan-jie Zhao	AB926054	LC429380
Trochila colensoi	PDD 112240	P.R. Johnston	MH921874	MH985297
Trochila laurocerasi	BPI 879818	–	LT158460	KX090835

References

Bordez L., Jourand P., Ducousso M., Carriconde F., Cavaloc Y., Santini S., Claverie J.M., Wantiez L., Leveau A. & Amir H. 2016. — Distribution patterns of microbial communities in ultramafic landscape: a metagenetic approach highlights the strong relationships between diversity and environmental traits. *Molecular Ecology*, 25: 2258–2272. doi: 10.1111/mec.13621

Bunyard B.A., Nicholson M.S. & Royse D.J. 1994. — A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. *Mycologia*, 86: 762–772. doi: 10.1080/00275514.1994.12026481

Carriconde F., Gardes M., Bellanger J.M., Letellier K., Gigante S., Gourmelon V., Ibanez T., McCoy S., Goxe J., Read J. & Maggia L. 2019. — Host effects in high ectomycorrhizal diversity tropical rainforests on ultramafic soils in New Caledonia. *Fungal Ecology*, 39: 201–212. doi: 10.1016/j.funeco.2019.02.006

Chernomor O., von Haeseler A. & Minh B.Q. 2016. — Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, 65: 997–1008. doi: 10.1093/sysbio/syw037

Gardes M. & Bruns T.D. 1993. — ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2: 113–118. doi: 10.1111/j.1365-294X.1993.tb00005.x

Gibbs G. 2016. — *Ghosts of Gondwana*. Nelson, Potton & Burton, 367 p.

Gourmelon V., Maggia L., Powell J.R., Gigante S., Hortal S., Gueunier C., Letellier K. & Carriconde F. 2016. — Environmental and geographical factors structure soil microbial diversity in New Caledonian ultramafic substrates: A metagenomic approach. *PLoS ONE*, 11(12): e0167405. doi: 10.1371/journal.pone.0167405

Grandcolas P., Murienne J., Robillard T., Desutter-Grandcolas L., Jourdan H., Guilbert E. & Deharveng L. 2008. — New Caledonia: a very old Darwinian island? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363: 3309–3317. doi: 10.1098/rstb.2008.0122

Haelewaters D., Park D. & Johnston P.R. 2021. — Multilocus phylogenetic analysis reveals that *Cyttariales* is a synonym of *Helotiales*. *Mycological Progress*, 20: 1323–1330. doi: 10.1007/s11557-021-01736-2

Hoang D.T., Chernomor O., Haeseler A. (von), Minh B.Q. & Vinh L.S. 2018. — UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35: 518–522. doi: 10.1093/molbev/msx281

Hopple J.S. 1994. — *Phylogenetic investigations in the genus Coprinus based on morphological and molecular characters*. Ph.D. Dissertation, Duke University, Durham, NC, USA.

Johnston P.R. 2010. — Causes and consequences of changes to New Zealand's fungal biota. *New Zealand Journal of Ecology*, 341: 175–184.

Johnston PR, Quijada L, Smith CA, Baral H.-O., Hosoya T., Baschien C., Pärtel K., Zhuang W.-Y., Haelewaters D., Park D., Carl S., López-Giráldez F., Wang Z. & Townsend J.P. 2019. — A multigene phylogeny toward a new phylogenetic classification for the *Leotiomycetes*. *IMA Fungus*, 10 (1): 1–22. doi: 10.1186/s43008-019-0002-x

Jourand P., Carriconde F., Ducousso M., Majorel C., Hannibal L., Prin Y. & Lebrun M. 2014. — Abundance, distribution, and dunction of Pisolithus albus and other ectomycorrhizal fungi of ultramafic soils in New Caledonia. *In*: Ba A.M., McGuire K.L. & Diedhiou A.G. (eds.) *Ectomycorrhizal Symbioses in Tropical and Neotropical Forests*. Boca Raton, CRC Press: 100–125.

Kalyaanamoorthy S., Minh B.Q., Wong T.F.K., von Haeseler A. & Jermiin L.S. 2017. — ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14: 587–589. doi: 10.1038/nmeth.4285

Mortimer N., Campbell H.J., Tulloch A.J., King P.R., Stagpoole, V.M., Wood R.A., Rattenbury M.S., Sutherland R., Adams C.J., Collot J & Seton M. 2017. — Zealandia: Earth's Hidden Continent. *GSA Today*, 27: 27–35. doi: 10.1130/GSATG321A.1

Nguyen L.-T., Schmidt H.A., von Haeseler A. & Minh B.Q. 2015. — IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution*, 32:268–274. doi: 10.1093/molbev/msu300

Samuels G.J. & Kohn L.M. 1987. — Ascomycetes of New Zealand. 7. Some bizarre, inoperculate discomycetes. *Sydowia*, 39: 202–216.

Vilgalys R. & Hester M. 1990. — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172: 4238–4246. doi: 10.1128/JB.172.8.4238-4246.1990

White T.J., Bruns T., Lee S. & Taylor J.W. 1990. — Amplification of direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis M.A., Gelfand D.H., Sninsky J.J. & White T.J. (eds). *PCR Protocols: A Guide to Methods and Applications*. San Diego, Academic Press: 315–322. doi: 10.1016/B978-0-12-372180-8.50042-1

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