



Annabella australiensis gen. & sp. nov. (Helotiales, Cordieritidaceae) from South Australian mangroves

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Abstract

A new genus of helotialean fungi, *Annabella* gen. nov. (Cordieritidaceae), is described to accommodate *Annabella australiensis* sp. nov. This species was collected on attached decaying wood of *Avicennia marina*, a common mangrove species found in protected waters of southern Australia. *Annabella* is distinctive among Cordieritidaceae in having relatively small perithecioid hyaline to yellowish apothecia and by the absence of an ionomidotic reaction. The apothecial shape and size of *Annabella* is most similar to *Skyttea*. The molecular phylogenetic analysis of a concatenated dataset of three ribosomal nuclear loci confirms the placement of *Annabella* within Cordieritidaceae, as a sister clade to *Skyttea*.

Keywords Ascomycota · Australia · Marine fungi · New taxa · Taxonomy

Introduction

Mangroves are salt-tolerant evergreen forests distributed throughout tropical, subtropical, and warm temperate latitudes. They grow in intertidal zones of sheltered shores, estuaries, tidal creeks, backwaters, lagoons, marshes, and mudflats. A total of 73 species of mangrove trees and shrubs are known, covering 15.2 million hectares—0.4% of all forests worldwide (Sandilyan and Kathiresan 2012). Mangrove forests in South Australia are comprised of a single tree species, *Avicennia marina*, which can grow between 2 and 5 m in height and is confined to sheltered shores in the Gulf of Saint Vincent and Spencer Gulf as well as protected bays of the Eyre Peninsula (Harbison 2008).

Although fungi growing on mangroves have been reported since the 1920s (Stevens 1920), it was not until the work of

Cribb and Cribb (1955, 1956) in Queensland, Australia, that research on mangrove-inhabiting fungi gained momentum. In their worldwide checklist of fungi associated with mangroves, Schmit and Shearer (2003) reported a total of 600 fungi, including 279 ascomycetes, 277 mitosporic (asexual) fungi, 3 chytrids, 12 zygomycetes, and 29 basidiomycetes. Despite a worldwide interest in mangrove fungi and marine fungi more generally, there has been surprisingly little research on the marine and mangrove fungi in southern Australia. There are currently no records for fungi on mangroves in South Australia.

During ongoing surveys of marine and freshwater fungi in Australia, an undescribed helotialean ascomycete was collected from attached, decaying mangrove wood. Based on morphological and molecular phylogenetic characters, a new genus is described within the family Cordieritidaceae Sacc. and compared with related taxa.

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Materials and methods

Collection details and examination

Twenty decayed, attached, partially decorticated branches were collected from the upper intertidal region of mangrove plants (*Avicennia marina*) on 15 April 2012. Samples were placed into individual plastic zip-lock bags and taken to the laboratory where they were incubated at room temperature in

individual, sterile plastic containers. The samples were examined regularly over 6 months using a Leica MZ75 dissecting microscope. Any fungi observed under the dissecting microscope were further examined using a compound microscope (Leica DMLS with phase contrast and Nikon Eclipse Ni with differential interference contrast). Images were captured using either a Sony RX100 or a Lumenera Infinity 3 camera. Line drawings were made with the assistance of a drawing tube (Nikon Y-IDT) attached to a Nikon Eclipse Ni. Apothecia of *Annabella australiensis* were discovered on 9 June 2012 and 18 June 2012. Initial morphological analysis was done immediately, but more detailed analysis was done after the specimens were dried. Mounting media used for microscopic work were H₂O, 2% KOH, 5% KOH, 10% KOH, Congo Red, Lugol's solution, and Melzer's reagent. All measurements were taken when specimens were mounted in water. Numerous attempts at culturing this species involved squashed apothecia in sterile water including expelled ascospores as outlined by Devadatha et al. (2017).

PCR amplification and sequencing

Numerous attempts to culture this species were unsuccessful. As a result, DNA was amplified directly from apothecia, without DNA extraction. Primers ITS1 (White et al. 1990) and LR5 (Vilgalys and Hester 1990) were used to amplify sequences of the internal transcriber spacer (ITS) and large subunit (LSU) of the nuclear ribosomal RNA genes. PCR amplification was performed in an Applied Biosystems 2720 Thermo Cycler. Three apothecia were placed into a PCR tube with PCR reagents. Reaction mixtures contained 10 µl 5X HF Phusion buffer, 1 µl 10 mM DNTP, 12.5 pmol of each primer, 0.5 µl Phusion High-Fidelity DNA polymerase (New England Biolabs), and the total volume was adjusted to 50 µL with sterile deionized H₂O. Cycling conditions for PCR were initial denaturation at 98 °C for 5 min; followed by 40 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 15 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 5 min. PCR products were purified using a PCK-1 kit (AdBiotec) and sequenced in both directions by the Australian Genome Research Facility using the primers ITS1 and ITS4 for the ITS locus (White et al. 1990), and LR0R and LR5 for the LSU locus (Rehner and Samuels 1994; Vilgalys and Hester 1990). Raw sequence reads were assembled, examined, and edited using Sequencher v.4.10.1 (Gene Codes Corporation). Newly generated sequences were submitted to NCBI GenBank under accession numbers MK328475 (ITS) and MK328476 (LSU).

Sequence alignment and phylogenetic analysis

To find a placement for the fungus among Helotiales, a concatenated ITS+LSU dataset was constructed (Haelewaters

2019). Taxonomic sampling covered 25 families in the order Helotiales (Arachnopezizaceae, Calloriaceae, Cenangiaceae, Chaetomellaceae, Chlorociboriaceae, Cordieritidaceae, Dermateaceae, Drepanopezizaceae, Gelatinodiscaceae, Godroniaceae, Helotiaceae, Heterosphaeriaceae, Hyaloscyphaceae, Lachnaceae, Leptodontidiaceae, Loramycetaceae, Mitrulaceae, Mollisiaceae, Pezizellaceae, Ploettnerulaceae, Roesleriaceae, Rutstroemiaceae, Sclerotiniaceae, Vibrisseaceae) and a number of taxa without clear affinities. *Sarea difformis* and *S. resiniae* (Lecanoromycetes, Baeomycetales) served as outgroup taxa. Sequences were aligned using Muscle v3.7 (Edgar 2004) implemented on the Cipres Science Gateway (Miller et al. 2010). Ambiguously aligned regions and uninformative positions were removed using the command line version of trimAl v1.2 (Capella-Gutiérrez et al. 2009) with gap threshold (-gt)=0.6 and minimal coverage (-cons)=0.5. The data for both loci were concatenated in MEGA7 (Kumar et al. 2016). Nucleotide substitution models were selected with the help of jModelTest2 (Darriba et al. 2012) by considering the Akaike Information Criterion (AIC). Maximum likelihood (ML) inference of the two-locus dataset was carried out using IQ-tree (Nguyen et al. 2015) on the Command Prompt of Windows under partitioned models (Chernomor et al. 2016): GTR+I+G for ITS (-lnL = 23,662.1757) and TIM3+I+G for LSU (-lnL = 14,446.7323). Ultrafast bootstrap (BS) analysis was implemented with 1000 replicates (Hoang et al. 2017).

Next, a concatenated SSU+ITS+LSU dataset was constructed covering 11 genera in Cordieritidaceae (*Ameghiniella*, *Cordierites*, *Diplocarpa*, *Diplolaeviopsis*, *Ionomidotis*, *Llimoniella*, *Macroskyttea*, *Rhymbocarpus*, *Skyttea*, *Thamnogalla*, *Unguiculariopsis*), an unnamed member of Cordieritidaceae (isolate G.M. 2015-05-16-1), “*Encoelia*” *fimbriata* and “*E.*” *heteromera* (sensu Pärtel et al. 2017). Alignments were constructed and trimmed using the same methods as those described above. The ML analysis of the three-locus dataset was run using IQ-tree (Chernomor et al. 2016; Nguyen et al. 2015) from the command line. Nucleotide substitution models were selected under the Akaike Information Criterion corrected for small sample size (AICc) with the help of jModelTest2 in Cipres. For the SSU dataset, the TIM1+I+G model was selected (-lnL = 4819.7076); for ITS, the TIM1+I+G model (-lnL = 5292.1487), and for LSU, the TIM2+I+G model (-lnL = 6249.4892). ML was inferred under partitioned models. Ultrafast bootstrap analysis was implemented with 1000 replicates (Hoang et al. 2017).

Bayesian analyses were also performed on the three-locus data. These were run using a Markov chain Monte Carlo (MCMC) coalescent approach implemented in BEAST (Drummond et al. 2012) with a strict molecular clock assuming a constant rate of evolution across the tree. The Birth-Death Incomplete Sampling speciation model (Stadler 2009) was selected as tree prior and the appropriate substitution models

(TIM1+I+G for SSU and ITS, TIM2+I+G for LSU) were selected by jModelTest 2 (under AICc). Four independent runs were performed from a random starting tree for 40 million generations with a sampling frequency of 4000. The setting of priors was entered in BEAUti (Drummond et al. 2012) to generate an XML file, which was run using BEAST on XSEDE in Cipres (3 runs) and locally from the command line (1 run). The resulting log files were entered in Tracer (Rambaut et al. 2014) to check trace plots for convergence and to adjust burn-in. Burn-in values were changed for each log file to achieve effective sample sizes of ≥ 200 for the majority of sampled parameters. Whilst removing a portion of each run as burn-in, log files and trees files were combined in LogCombiner v.1.8.4. TreeAnnotator v.1.8.4 was used to generate consensus trees (0% burn-in) and to infer the Maximum Clade Credibility tree, with the highest product of individual clade posterior probabilities. Final ML phylogenetic reconstructions with bootstrap values (BS) and Maximum Clade Credibility trees with posterior probabilities (pp) were visualized in FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

Nucleotide alignment datasets and phylogenetic inferences

The concatenated ITS+LSU dataset of Helotiales included 1396 characters and 155 isolates representing 25 families. Of all characters, 612 were constant whereas 627 were parsimony-informative. The concatenated SSU+ITS+LSU dataset of Cordieritidaceae included 40 isolates and 2467 characters. Of these, 1532 were constant and 678 were parsimony-informative. *Annabella australiensis* was placed within the Cordieritidaceae family with maximum support in both the ITS+LSU dataset of Helotiales (Haelewaters 2019) and the three-locus dataset of Cordieritidaceae (Fig. 1). For the Cordieritidaceae phylogenetic reconstruction, all isolates with rDNA sequences available in GenBank were included (Table 1). Maximum support was found at the family level. The relationships between genera are not well resolved. However, the new taxon *A. australiensis* was retrieved as sister to the *Skyttea* clade with high support (BS = 89, pp = 1.00) (Fig. 1).

Taxonomy

Annabella Fryar, Haelew., & D.E.A. Catches., gen. nov.

Mycobank number: MB 829170.

Etymology: Honoring Annabelle Daniel, who works tirelessly with local communities to establish Women's Community Shelters across New South Wales, Australia.

Type species: *Annabella australiensis* Fryar, Haelew. & D.E.A. Catches.

Sexual morph: Ascomata minute, perithecioid, solitary or gregarious. Ectal excipulum thin, composed of cells of textura angularis-epidermoidea. Paraphyses septate, sparsely branched, protruding further than the asci, hyaline, thin ($\sim 2 \mu\text{m}$ wide), attached at base, ends free. Asci 8-spored, unitunicate, arising from croziers, ascospores uniseriate, no apical apparatus, rounded apices, inamyloid, cylindrical. Ascospores highly variable in shape, ellipsoid to fusoid to oblong, hyaline, aseptate, no appendages or sheath. Asexual morph: undetermined.

Notes: This new genus is proposed to accommodate *Annabella australiensis* from mangrove wood. *Annabella* shares morphological features with other members of the family Cordieritidaceae, including inamyloid asci with rounded apices. Currently there are 12 described genera within the Cordieritidaceae: *Ameghiniella* Speg., *Austrocenangium* Gamundí, *Cordierites* Mont., *Diplocarpa* Masee, *Diplolaeviopsis* Giralt & D. Hawksw., *Ionomidotis* E.J. Durand ex Thaxt., *Llimoniella* Hafellner & Nav.-Ros., *Macroskyttea* Etayo et al., *Rhymbocarpus* Zopf, *Skyttea* Sherwood, D. Hawksw. & Coppins, *Thamnogalla* D. Hawksw., and *Unguiculariopsis* Rehm (Baral in Jaklitsch et al. 2016). The newly described genus, *Annabella*, is significantly different in morphology from all other members of this family by having perithecioid hyaline to yellowish apothecia and the absence of ionomidotic reaction.

Annabella australiensis Fryar, Haelew., & D.E.A. Catches., sp. nov., Figs. 2 and 3.

Mycobank number: MB 829171.

Etymology: Named after the country where the holotype was collected.

Material examined: Australia, Port River, 34.755°S, 138.509°E, on intertidal decaying attached decorticated branch of *Avicennia marina* incubated for 2 months, 15 Apr 2012, S.C. Fryar, (AD283531, holotype; AD283532, isotype).

Saprobic on attached, decorticated, dead branch of *Avicennia marina* (Forssk.) Vierh. (Lamiales, Acanthaceae) in the upper intertidal region. Sexual morph: Ascomata minute, apothecial, hyaline to pale yellowish, superficial, perithecioid, with a broad ostiolar opening when mature, solitary or gregarious, (175)188–225(250) μm high, (88)100–123(188) μm diam. (\bar{x} = 210 \times 123 μm , n = 8), initially closed, then opening to a maximum of 100 μm wide, tissues not ionomidotic. Ectal excipulum thin, composed of cells of textura angularis-epidermoidea ((6)9–20(30) \times 4–13 μm , \bar{x} = 15 \times 7 μm , n = 20). Paraphyses septate, hyaline, 1.5–2.5 μm wide (\bar{x} = 2 μm , n = 9), sparsely branched (mostly unbranched, occasionally forked towards the tip), protruding beyond the asci, growing from subhymenium, upper ends free. Asci 8-spored, cylindrical, unitunicate, arising from croziers, ascospores uniseriate, no apical wall thickening or ring structure, rounded apices, inamyloid, ascospores expelled through a split at or near the apex, (72.5)80–130(150) \times (5)6–7.5(9) μm (\bar{x} = 99 \times 7.5 μm , n =

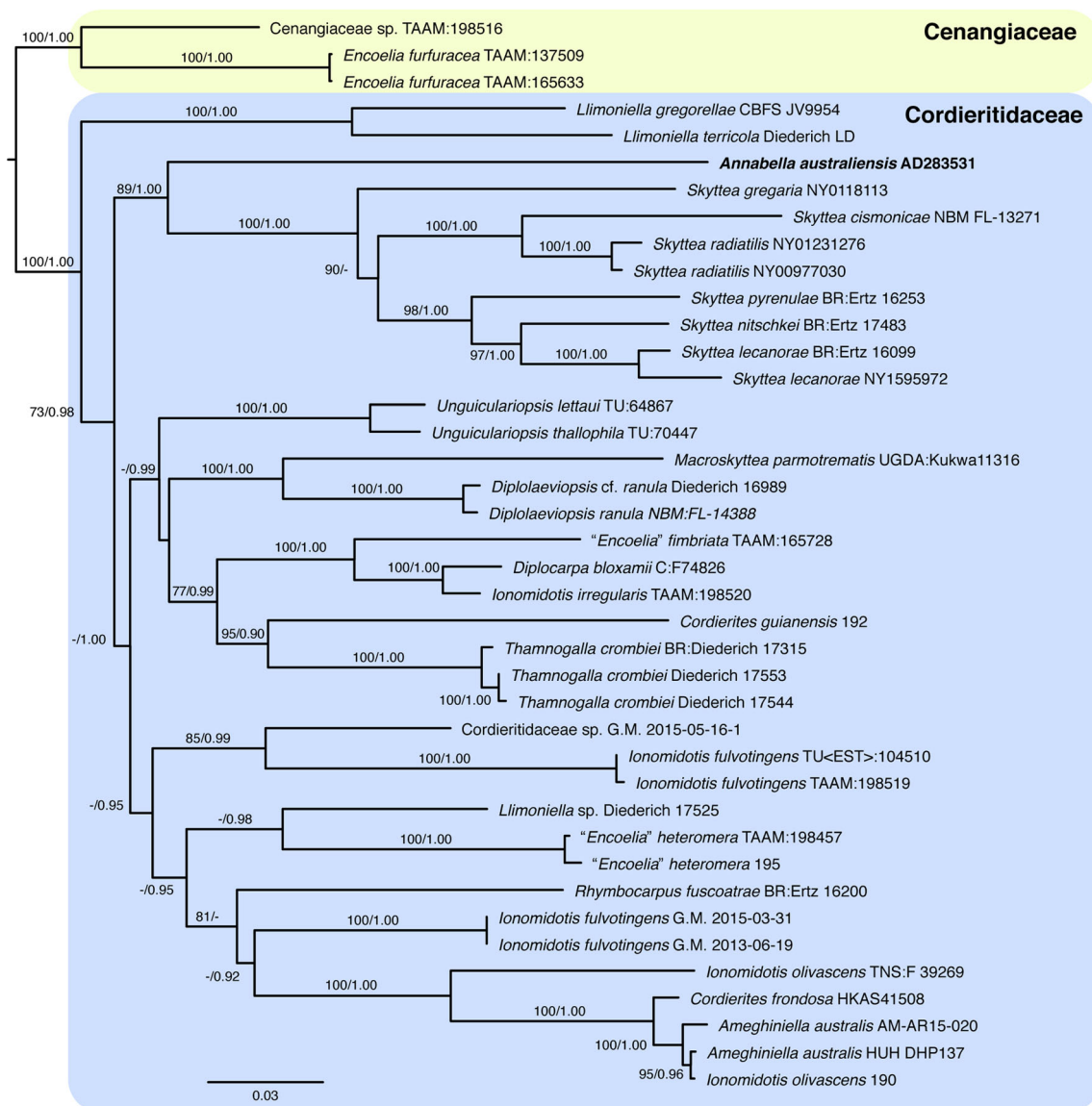


Fig. 1 Phylogeny of Cordieritidaceae reconstructed from the concatenated SSU+ITS+LSU dataset. The topology is the result of maximum likelihood inference performed with IQ-TREE. Only ML bootstrap support values $\geq 70\%$ and Bayesian posterior probabilities ≥ 0.90 are presented for each node

22), contents turning rusty-red in Melzer's reagent and faintly yellow/brown in Melzer's reagent when pretreated with 2–5% KOH, ascus wall inamyloid. Ascospores not overlapping in the asci and straight, highly variable in shape, ellipsoid to fusoid to oblong, hyaline, two, occasionally one large oil drop per ascospore, aseptate, $(7.5)9\text{--}14(17.5) \times (4)5\text{--}6(7.5) \mu\text{m}$ ($\bar{x} = 11 \times 5.5 \mu\text{m}$, $n = 30$), no appendages or sheath, sometimes budding outside the asci. Asexual morph: undetermined.

Notes: *Annabella australiensis* is unique in the family Cordieritidaceae in having a marine habitat. Similarities with other taxa within this family are the inamyloid asci with rounded apices and an association with wood. The apothecial shape of *A. australiensis* is most similar to that of *Skyttea* and *Thamnogalla* (Diederich and Etayo 2000; Hawksworth 1980).

Annabella australiensis differs from other taxa within Cordieritidaceae in having hyaline to yellowish perithecioid apothecia, contrasting with the usually dark-colored apothecia of other members of the family. Due to this absence of pigment, *Annabella australiensis* also differs from other taxa within the Cordieritidaceae by the absence of reaction to 5% or 10% KOH solution (non-ionomidotic). Although the ionomidotic reaction is common among Cordieritidaceae, *A. australiensis* is not alone in lacking this reaction. It shares this feature with e.g., *Cordierites guianensis* Mont., and *C. boedijnii* W.Y. Zhuang (Zhuang 1988). As with some other members of the Helotiales (Spooner 1987; Pärtel et al. 2017), the ascospores of *A. australiensis* are occasionally observed budding, particularly in over-mature specimens. Material was

Table 1 Taxa used in the phylogenetic analysis and their GenBank accession numbers. Newly generated sequences are indicated in bold

Species	Strain/voucher	GenBank accession number			Reference
		SSU	ITS	LSU	
<i>Ameghiniella australis</i>	AM-AR15-020		KY462281		Truong et al. 2017
<i>Ameghiniella australis</i>	KL391/HUH DHP137	KX090893		KX090841	Pärtel et al. 2017
<i>Annabella australiensis</i>	AD283531		MK328475	MK328476	This paper
Cenangiaceae sp.	KL244/TAAM:198516 /H.B. 9008a	KX090874	LT158440	KX090823	Pärtel et al. 2017
<i>Cordierites frondosa</i>	HKAS41508	AY789353	AY789355	AY789354	Wang et al. 2005
<i>Cordierites guianensis</i>	192	EU107262		EU107270	Peterson and Pfister 2010
Cordieritidaceae sp.	G.M. 2015-05-16-1		KY462806	KY462806	Baral and Marson unpubl.
<i>Diplocarpa bloxamii</i>	KL317/C:F74826	KX090885		KX090834	Pärtel et al. 2017
<i>Diplolaeviopsis</i> cf. <i>ranula</i>	Diederich 16989	KX090896	KJ559532	KJ559554	Suija et al. 2015
<i>Diplolaeviopsis ranula</i>	NBM:FL-14388		KP984782	KP984785	Etayo et al. 2015
“ <i>Encoelia</i> ” <i>fimbriata</i>	KL111/TAAM:165728	KX090852		KX090800	Pärtel et al. 2017
<i>Encoelia furfuracea</i>	KL92/TAAM:137509	KX090847	LT158482	KX090796	Pärtel et al. 2017
<i>Encoelia furfuracea</i>	KL107/TAAM:165633	KX090850	LT158416	KX090798	Pärtel et al. 2017
“ <i>Encoelia</i> ” <i>heteromera</i>	KL164/TAAM:198457	KX090861		KX090809	Pärtel et al. 2017
“ <i>Encoelia</i> ” <i>heteromera</i>	195	EU107204		EU107233	Peterson and Pfister 2010
<i>Ionomidotis fulvotagens</i>	G.M. 2015-03-31		KY462808	KY462808	Baral and Marson unpubl.
<i>Ionomidotis fulvotagens</i>	G.M. 2013-06-19		KY462807	KY462807	Baral and Marson unpubl.
<i>Ionomidotis fulvotagens</i>	KL231/TU<EST>:104510	KX090870		KX090819	Pärtel et al. 2017
<i>Ionomidotis fulvotagens</i>	KL239/TAAM:198519 /H.B. 9632	KX138403		KX138407	Pärtel et al. 2017
<i>Ionomidotis irregularis</i>	KL154/TAAM:198520 /H.B. 8233	KX090856		KX090804	Pärtel et al. 2017
<i>Ionomidotis olivascens</i>	190	EU107263		EU107271	Peterson and Pfister 2010
<i>Ionomidotis olivascens</i>	KL301/TNS:F 39269	KX090883		KX090833	Pärtel et al. 2017
<i>Llimoniella gregorellae</i>	CBFS JV9954	KJ559581	KJ559531	KJ559553	Suija et al. 2015
<i>Llimoniella terricola</i>	LL95/Diederich LD	KX090895		KX090842	Pärtel et al. 2017
<i>Macroskyttea parmotrematis</i>	UGDA:Kukwa 11316	KP984790	KP984784	KP984788	Etayo et al. 2015
<i>Rhymbocarpus fuscoatrae</i>	BR:Ertz 16200	KJ559593	KJ559549	KJ559571	Suija et al. 2015
<i>Skyttea cisonicae</i>	NBM FL-13271		KP984783	KP984786	Etayo et al. 2015
<i>Skyttea gregaria</i>	NY0118113		KJ559537	KJ559559	Suija et al. 2015
<i>Skyttea lecanorae</i>	NY1595972		KJ559539	KJ559561	Suija et al. 2015
<i>Skyttea lecanorae</i>	BR:Ertz 16099	KJ559597		KJ559574	Suija et al. 2015
<i>Skyttea nitschkei</i>	BR:Ertz 17483	KJ559595		KJ559577	Suija et al. 2015
<i>Skyttea pyrenulae</i>	BR:Ertz 16253	KJ559596		KJ559575	Suija et al. 2015
<i>Skyttea radiatilis</i>	NY01231276		KJ559538	KJ559560	Suija et al. 2015
<i>Skyttea radiatilis</i>	NY00977030		KJ559536	KJ559558	Suija et al. 2015
<i>Thamnogalla crombiei</i>	BR:Diederich 17315	KJ559594	KJ559550	KJ559578	Suija et al. 2015
<i>Thamnogalla crombiei</i>	Diederich 17553	KJ559583	KJ559535	KJ559557	Suija et al. 2015
<i>Thamnogalla crombiei</i>	Diederich 17544	KJ559582	KJ559534	KJ559556	Suija et al. 2015
<i>Unguiculariopsis lettaui</i>	TU:70447	KP984789		KP984787	Etayo et al. 2015

dried before thorough examination, and, as a result, we have been unable to verify the absence of refractive vacuoles in the paraphyses. *Annabella australiensis* appears to be uncommon, growing on only 2 of the 20 samples collected.

Discussion

The new genus *Annabella* falls with high support within the Cordieritidaceae family. Pärtel et al. (2017) resurrected and

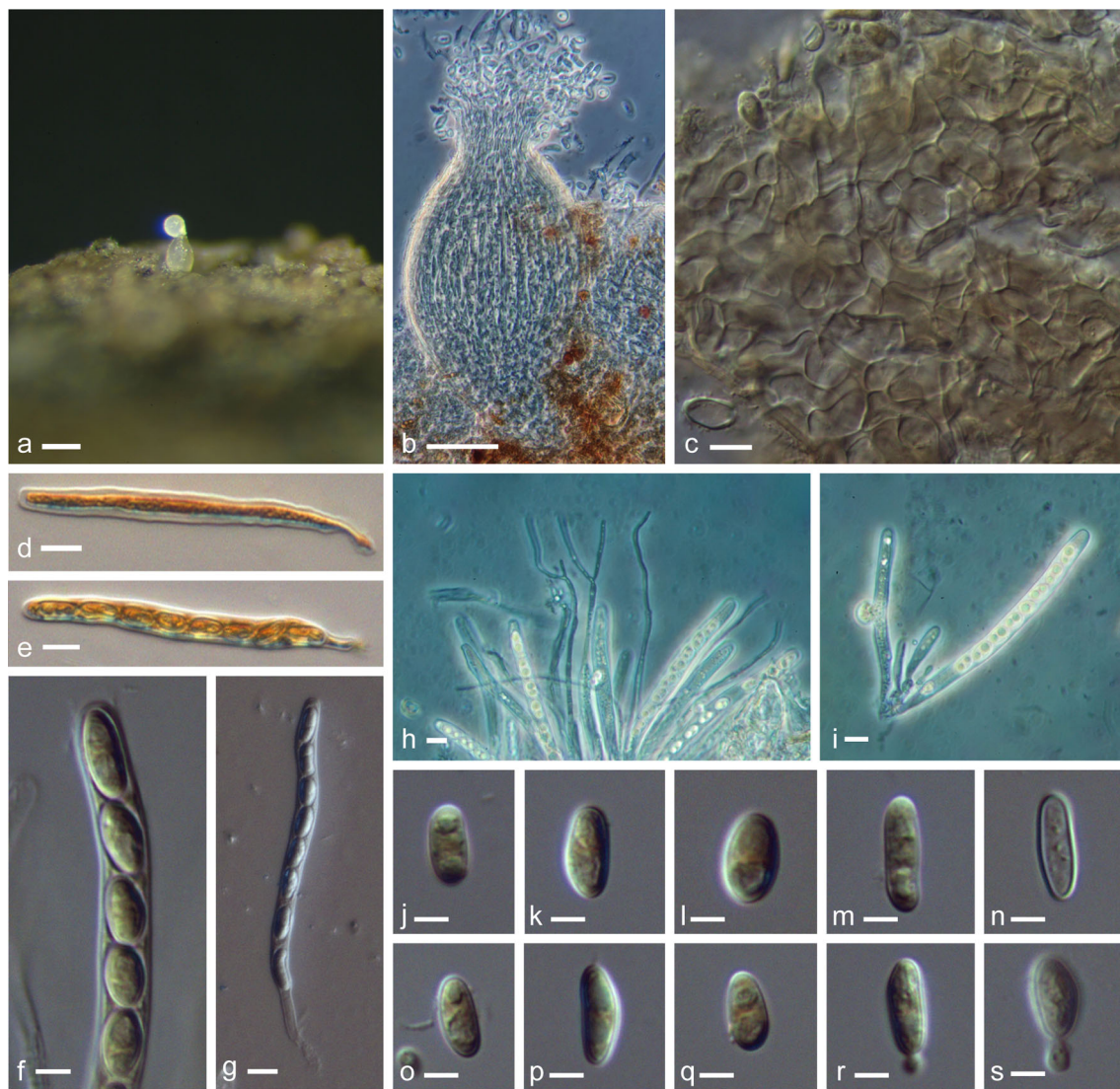


Fig. 2 *Annabella australiensis* (AD283531, holotype). **a** Appearance of ascomata on the host surface, showing a mass of expelled ascospores at the top of the apothecium. **b** Apothecium in optical median section. **c** Ectal excipulum in squash mount pretreated with 5% KOH followed by Lugol's solution. **d** and **e** asci stained in Melzer's reagent without

pretreatment. **f** Upper part of ascus. **g** Ascus. **h, i** Mature and immature asci, paraphyses. **j–s** Ascospores (germinating in **r** and **s**). Tissues mounted in water unless otherwise stated. Scale bars: **a** 200 μm , **b** 50 μm , **c–e, g–i** 10 μm , **f, j–s** 5 μm

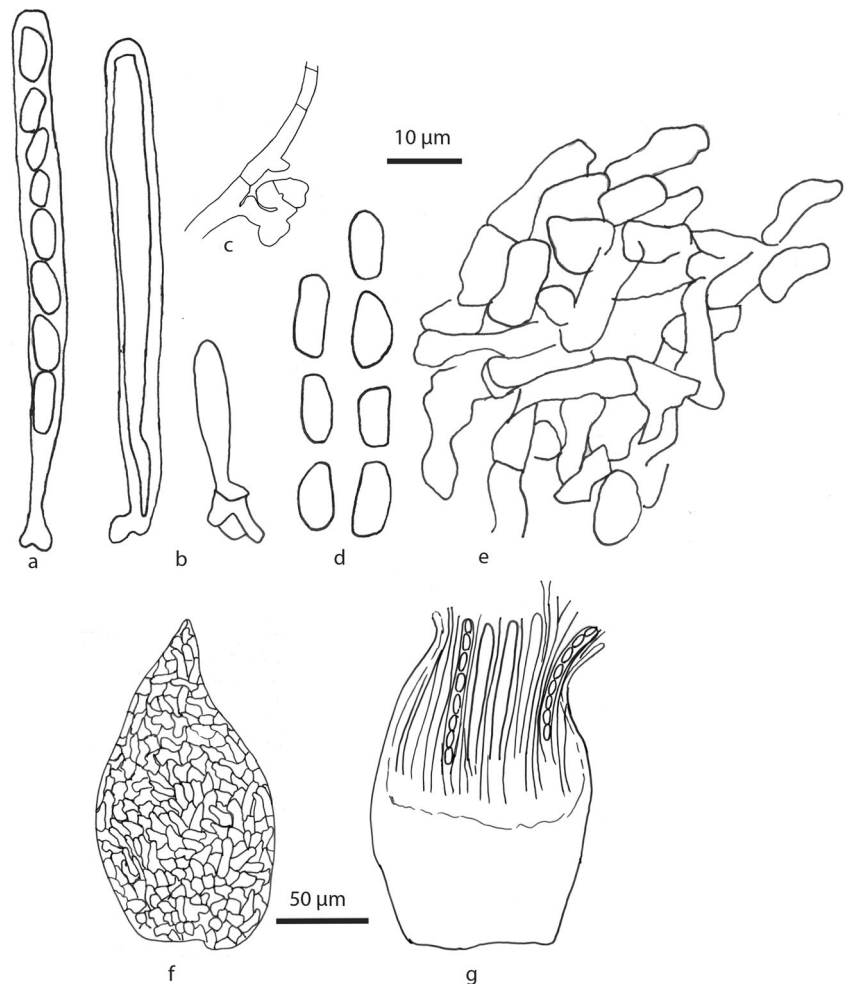
expanded the concept of this family. Members of Cordieritidaceae are characterized by apothecial ascomata and inamyloid asci with a rounded apex. They often have a roughened or pustulate, pigmented ectal excipulum, sometimes with distinct hairs, and are either lichenicolous or corticolous (Pärtel et al. 2017). The family Cordieritidaceae includes genera in which apothecia vary in size from minuscule to 10 cm in diameter (Pärtel 2016).

Annabella is retrieved as a sister taxon to *Skyttea*. *Annabella* and *Skyttea* share some morphological characters including relatively small perithecioid apothecia. However, *Annabella* is distinctly different to other members of the Cordieritidaceae, which typically have larger and dark(er) pigmented, apothecial ascomata with either a positive

ionomidotic reaction or excipular pigments changing color in aqueous KOH (Pärtel et al. 2017). *Annabella* concurs with all other members of the Cordieritidaceae in having inamyloid asci with a typically rounded apex, but it lacks the rough or pustulate ectal excipulum or distinct hairs that some other Cordieritidaceae possess.

The family Cordieritidaceae was circumscribed by Pärtel et al. (2017) as monophyletic and our analyses confirm this, showing that the genera *Ameghiniella*, *Annabella*, *Cordierites*, *Diplocarpa*, *Diplolaeviopsis*, *Ionomidotis*, *Llimoniella*, *Macroskyttea*, *Thamnogalla*, and *Ungiuculariopsis* form a well-supported monophyletic clade. The same authors also recognized that the genus *Encoelia* (Fr.) P. Karst. is highly polyphyletic and found that the type species, *Encoelia furfuracea*,

Fig. 3 *Annabella australiensis* (AD283531, holotype). **a** Mature ascus. **b** Immature asci. **c** Crozier. **d** Ascospores. **e** Ectal excipulum external view. **f** Immature apothecium showing external surface. **g** Mature apothecium showing internal structures



was placed in the Cenangiaceae, whereas *Encoelia heteromera* and *E. fimbriata* were revealed to be in different clades within the Cordieritidaceae. These findings were replicated in this study and indicate the need for taxonomic revision of *Encoelia*.

Annabella australiensis is unique among the Cordieritidaceae in having a marine habitat. Indeed, it is unusual to find a member of the Leotiomyces on a mangrove or in a marine habitat, where Dothideomycetes and Sordariomycetes are more common (Jones et al. 2015). Of the 805 species of marine Ascomycota, just 17 of these are in the Leotiomyces (Jones et al. 2015). Marine fungi have significant ecological roles as decomposers (Sridhar 2012), endophytes (Osorio et al. 2017), mycorrhizae (Sengupta and Chaudhuri 2002), and pathogens (Liu et al. 2016; Osorio et al. 2016). They show enormous potential for new natural products (Cicatiello et al. 2016; Oliveira et al. 2012), including cancer-inhibiting (Liu et al. 2017), antibacterial, and antifungal drugs (Xu et al. 2015). Despite these important ecosystem functions and potential applications, relatively little research has been done into the ecology of these organisms (Raghukumar 2017).

During the late 1980s and 1990s, several mycologists were active in Australia describing marine fungi (e.g., Hyde 1992;

Kohlmeyer and Volkmann-Kohlmeyer 1990). The majority of these studies were focused on Queensland with limited research in other regions of Australia. Since that time, there has been very little research on the marine fungi of Australia. *Annabella australiensis* represents the first record of a fungus growing on mangroves from South Australia. Only ten marine species of fungi have been previously recorded from South Australia, mostly from seaweeds (Bebout et al. 1987; Boyd and Kohlmeyer 1982; Cribb and Cribb 1960; Inderbitzin et al. 2004; Kohlmeyer 1971, 1972, 1983, 1984; Kohlmeyer and Kohlmeyer 1975, 1979).

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References

- Bebout B, Schatz S, Kohlmeyer J, Haibach M (1987) Temperature-dependent growth in isolates of *Corollospora maritima* Werdern. (Ascomycetes) from different geographical regions. *J Exp Mar Biol Ecol* 106:203–210. [https://doi.org/10.1016/0022-0981\(87\)90093-1](https://doi.org/10.1016/0022-0981(87)90093-1)

- Boyd P, Kohlmeyer J (1982) The influence of temperature on the seasonal and geographic distribution of three marine fungi. *Mycologia* 74: 894–902. <https://doi.org/10.2307/3792718>
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Chernomor O, Von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Syst Biol* 65:997–1008. <https://doi.org/10.1093/sysbio/syw037>
- Cicatiello P, Gravagnuolo AM, Gnavi G, Varese GC, Giardina P (2016) Marine fungi as source of new hydrophobins. *Int J Biol Macromol* 92:1229–1233. <https://doi.org/10.1016/j.ijbiomac.2016.08.037>
- Cribb AB, Cribb JW (1955) Marine fungi from Queensland 1. *Pap Dept Bot Univ Queensl* 3:77–81
- Cribb AB, Cribb JW (1956) Marine fungi from Queensland. 2. *Pap Dept Bot Univ Queensl* 3:97–105
- Cribb AB, Cribb JW (1960) Some marine fungi on algae in European herbaria. *Pap Dept Bot Univ Queensl* 4:45–48
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772. <https://doi.org/10.1038/nmeth.2109>
- Devadatha B, Sarma VV, Wanasinghe DN, Hyde KD, Jones EBG (2017) Introducing the new Indian mangrove species, *Vaginatisspora microarmatisspora* (Lophiostomataceae) based on morphology and multigene phylogenetic analysis. *Phytotaxa* 329:139–149. <https://doi.org/10.11646/phytotaxa.329.2.4>
- Diederich P, Etayo J (2000) A synopsis of the genera *Skyttea*, *Llimoniella* and *Rhymbocarpus* (Lichenicolous Ascomycota, Leotiales). *Lichenologist* 32:423–485. <https://doi.org/10.1006/lich.2000.0290>
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29:1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Etayo J, Flakus A, Suija A, Kukwa M (2015) *Macroskyttea parmotrematis* gen. et sp. nov. (Helotiales, Leotiomyces, Ascomycota), a new lichenicolous fungus from Bolivia. *Phytotaxa* 224:247–257. <https://doi.org/10.11646/phytotaxa.224.3.3>
- Haelewaters D (2019) Data from "*Annabella australiensis* gen. & sp. nov. (Helotiales, Cordieritidaceae) from South Australian mangroves". <https://doi.org/10.6084/m9.figshare.c.4414700.v3> Accessed 25 February 2019
- Harbison P (2008) Mangroves. In: Shepherd SA, Bryars S, Kirkegaard I, Harbison P, Jennings JT (eds) *Natural history of Gulf St. Vincent*. Royal Society of South Australia, Inc, Adelaide, pp 95–105
- Hawksworth DL (1980) Notes on British lichenicolous fungi: III Notes from the Royal Botanical Garden Edinburgh 38: 165–183
- Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol* 35:518–522. <https://doi.org/10.1093/molbev/msx281>
- Hyde KD (1992) *Julella avicenniae* (Borse) comb. nov. (Thelennellaceae) from intertidal mangrove wood and miscellaneous fungi from the North East Coast of Queensland. *Mycol Res* 96:939–942
- Inderbitzin P, Lim S, Volkmann-Kohlmeyer B, Kohlmeyer J, Berbee M (2004) The phylogenetic position of *Spathulospora* based on DNA sequences from dried herbarium material. *Mycol Res* 108:737–748
- Jaklitsch W, Baral HO, Lücking R, Lumbsch HT (2016) Ascomycota. In: Frey W (ed) *Engler's syllabus of plant families*, 13th edn. Borntraeger Science Publisher, Stuttgart, Germany, pp 1–322
- Jones EBG, Suetrong S, Sakayaroj J, Bahkali AH, Abdel-Wahab MA, Boekhout T, Pang K-L (2015) Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. *Fungal Divers* 73:1–72. <https://doi.org/10.1007/s13225-015-0339-4>
- Kohlmeyer J (1971) Fungi from the Sargasso Sea. *Mar Biol* 8:344–350. <https://doi.org/10.1007/BF00348012>
- Kohlmeyer J (1972) Parasitic *Haloguignardia oceanica* (Ascomycetes) and hyperparasitic *Sphaceloma cecidii* sp. nov. (Deuteromycetes) in drift Sargassum in North Carolina. *J Elisha Mitch Sci S* 88:255–258
- Kohlmeyer J (1983) Geography of marine fungi. *Aust J Bot, Suppl. Ser.* 10:67–76
- Kohlmeyer J (1984) Tropical marine fungi. *PSZNI Mar Ecol* 5:329–378. <https://doi.org/10.1111/j.1439-0485.1984.tb00130.x>
- Kohlmeyer J, Kohlmeyer E (1975) Biology and geographical distribution of *Spathulospora* species. *Mycologia* 67:629–637. <https://doi.org/10.2307/3758398>
- Kohlmeyer J, Kohlmeyer E (1979) *Marine mycology: the higher fungi*. Academic press. In: New York. San Fransisco, London
- Kohlmeyer J, Volkmann-Kohlmeyer B (1990) New species of *Koralionastes* (Ascomycotina) from the Caribbean and Australia. *Can J of Bot* 68:1554–1559. <https://doi.org/10.1139/b90-199>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Liu Y, Li Y, Lin Q, Zhang Y (2016) Assessment of the pathogenicity of marine *Cladosporium* spp. towards mangroves. *Forest Pathol* 47:12322. <https://doi.org/10.1111/efp.12322>
- Liu X, Wu X, Ma YF, Zhang WZ, Hu L, Feng XW, Li XY, Tang XD (2017) Endophytic fungi from mangrove inhibit lung cancer cell growth and angiogenesis in vitro. *Oncol Rep* 37:1793–1803. <https://doi.org/10.3892/or.2017.5366>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)* 14 Nov. 2010: 1–8. New Orleans, Louisiana
- Nguyen L-T, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol Biol Evol* 32:268–274. <https://doi.org/10.1093/molbev/msu300>
- Oliveira ALL d, de FR, Debonsi HM (2012) Marine natural products: chemical and biological potential of seaweeds and their endophytic fungi. *Rev Bras Farmacogn* 22:906–920. <https://doi.org/10.1590/S0102-695X2012005000083>
- Osorio JA, Wingfield MJ, Roux J (2016) A review of factors associated with decline and death of mangroves, with particular reference to fungal pathogens. *S Afr J Bot* 103:295–301. <https://doi.org/10.1016/j.sajb.2014.08.010>
- Osorio JA, Crous CJ, de Beer ZW, Wingfield MJ, Roux J (2017) Endophytic Botryosphaeriaceae, including five new species, associated with mangrove trees in South Africa. *Fungal Biol* 121:361–393. <https://doi.org/10.1016/j.funbio.2016.09.004>
- Pärtel K (2016) Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. *Dissertation*, University of Tartu
- Pärtel K, Baral H-O, Tamm H, Pöldmaa K (2017) Evidence for the polyphyly of *Encoelia* and *Encoelioidae* with reconsideration of respective families in Leotiomyces. *Fungal Divers* 82:183–219. <https://doi.org/10.1007/s13225-016-0370-0>
- Peterson KR, Pfister DH (2010) Phylogeny of *Cyttaria* inferred from nuclear and mitochondrial sequence and morphological data. *Mycologia* 102:1398–1416. <https://doi.org/10.3852/10-046>
- Raghukumar S (2017) *Fungi in coastal and oceanic marine ecosystems*. Springer, Cham, Switzerland. <https://doi.org/10.1007/978-3-319-54304-8>
- Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) *Tracer v1.6*. <http://tree.bio.ed.ac.uk/software/tracer/> Accessed 22 December 2018
- Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol Res* 98:625–634. [https://doi.org/10.1016/S0953-7562\(09\)80409-7](https://doi.org/10.1016/S0953-7562(09)80409-7)

- Sandilyan S, Kathiresan K (2012) Mangrove conservation: a global perspective. *Biodivers Conserv* 21:3523–3542. <https://doi.org/10.1007/s10531-012-0388-x>
- Schmit J, Shearer CA (2003) A checklist of mangrove-associated fungi, their geographical distribution and known host plants. *Mycotaxon* 85:423–477
- Sengupta A, Chaudhuri S (2002) Arbuscular mycorrhizal relations of mangrove plant community at the Ganges river estuary in India. *Mycorrhiza* 12:169–174. <https://doi.org/10.1007/s00572-002-0164-y>
- Spooner BM (1987) Helotiales of Australasia: Geoglossaceae, Orbiliaceae, Sclerotiniaceae, Hyaloscyphaceae. *Bibliotheca Mycologica*. 116, J. Cramer, Berlin, Stuttgart
- Sridhar KR (2012) Decomposition of materials in the sea. In: Jones EBG, Pang K-L (eds) *Marine fungi: and fungal-like organisms*. De Gruyter, Berlin, Boston, pp 475–500 <https://www.degruyter.com/view/product/177990>
- Stadler T (2009) On incomplete sampling under birth-death models and connections to the sampling-based coalescent. *J Theor Biol* 261:58–66. <https://doi.org/10.1016/j.jtbi.2009.07.018>
- Stevens FL (1920) New or noteworthy Puerto Rican fungi. *Bot Gaz* 70: 399–402
- Suija A, Ertz D, Lawrey JD, Diederich P (2015) Multiple origin of the lichenicolous life habit in Helotiales, based on nuclear ribosomal sequences. *Fungal Divers* 70:55–72. <https://doi.org/10.1007/s13225-014-0287-4>
- Truong C, Mujic AB, Healy R, Kuhar F, Furci G, Torres D, Niskanen T, Sandoval-Leiva PA, Fernández N, Escobar JM, Moretto A, Palfner G, Pfister D, Nouhra E, Swenie R, Sánchez-García M, Matheny PB, Smith ME (2017) How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. *New Phytol* 214:913–919. <https://doi.org/10.1093/mmy/myv083>
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172(8):4238–4246
- Wang Z, Binder M, Hibbett DS (2005) Life history and systematics of the aquatic discomycete *Mitrula* (Helotiales, Ascomycota) based on cultural, morphological, and molecular studies. *Am J Bot* 92: 1565–1574. <https://doi.org/10.3732/ajb.92.9.1565>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322
- Xu L, Meng W, Cao C, Wang J, Shan W, Wang Q (2015) Antibacterial and antifungal compounds from marine fungi. *Mar Drugs* 13:3479–3513. <https://doi.org/10.3390/md13063479>
- Zhuang W-Y (1988) Studies on some discomycete genera with an ionomidotic reaction: *Ionomidotis*, *Poloniodiscus*, *Cordierites*, *Phyllomyces*, and *Ameghiniella*. *Mycotaxon* 31:261–298

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