

Dyfrlolomycetaceae, a new family in the Dothideomycetes, Ascomycota

Author(s): Ka-Lai Pang , Kevin D. Hyde Siti Aisyah Alias , Satinee Suetrong ,
Sheng-Yu Guo , Rizman Idid & E.B. Gareth Jones

Source: Cryptogamie, Mycologie, 34(1):223-232. 2013.

Published By: Association des Amis des Cryptogames

DOI: <http://dx.doi.org/10.7872/crym.v34.iss3.2013.223>

URL: <http://www.bioone.org/doi/full/10.7872/crym.v34.iss3.2013.223>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Dyfolomycetaceae, a new family in the Dothideomycetes, Ascomycota

Ka-Lai PANG^a, Kevin D. HYDE^{b,*}, Siti Aisyah ALIAS^c, Satinee SUETRONG^d,
Sheng-Yu GUO^c, Rizman IDID^c & E.B. Gareth JONES^e

^a Institute of Marine Biology and Center of Excellence for the Oceans,
National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224,
Taiwan (R.O.C.)

^b Institute of Excellence in Fungal Research, and School of Science,
Mae Fah Luang University, Chiang Rai, Thailand

* email: kdhyde3@gmail.com

^c Institute of Ocean and Earth Sciences, University Malaya,
50603 Kuala Lumpur, Malaysia

^d Mycology Laboratory (BMYC), Bioresources Technology Unit (BTU),
National Center for Genetic Engineering and Biotechnology (BIOTEC),
113 Thailand Science Park, Phahonyothin Rd., Khlong Nueng,
Khlong Luang, Pathum Thani 12120, Thailand

^e Department of Botany and Microbiology, College of Science,
King Saud University, P.O. Box 2455 Riyadh 11451, Kingdom of Saudi Arabia

Abstract – A new mangrove fungus collected in Tioman Island, Malaysia, is morphologically similar to marine species of *Saccardoella*. It also phylogenetically groups with *Saccardoella rhizophorae* in the *Dothideomycetes*, based on combined analysis of partial SSU, LSU rRNA and TEF1 gene sequences. The new fungus and *S. rhizophorae* form a well-supported clade with *Acrospermum* spp. in the *Acrospermaceae*. Both species therefore do not belong in *Saccardoella*, a genus with unitunicate asci. A new genus, *Dyfolomyces*, is established to accommodate the new fungus (*Dyfolomyces tiomanensis*) while the three marine *Saccardoella* species (*S. mangrovei*, *S. marinospora*, *S. rhizophorae*) are transferred to the new genus. *Dyfolomyces* is characterized by forming a clypeus on substrates, with immersed perithecial ascomata, bitunicate/fissitunicate asci and multi-septate ascospores with/without a sheath. Since *D. rhizophorae* and *D. tiomanensis* do not cluster with any known families in the *Dothideomycetes*, a new family, *Dyfolomycetaceae*, is introduced to accommodate the *Dyfolomyces* species.

Ascomycota / marine fungi / *Saccardoella* / *Sordariomycetes* / *Xylariales*

INTRODUCTION

Saccardoella Speg. was introduced by Spegazzini (1879) and was typified by *Saccardoella montellica* Speg., which was described from *Quercus* sp. in Italy. *Saccardoella montellica* is characterized by having large ascomata with erumpent papillae, long, cylindrical, unitunicate asci with an apical ring and uniseriate

ascospores having numerous septa and a sheath or polar appendage (Petraik 1962; Hyde 1992). There has been confusion over the nature of the asci as they are neither typically unitunicate nor bitunicate, thus the placement of the genus has been problematic (Mathiassen 1989; Hyde 1992). *Saccardoella* has been variously classified in the *Clypeosphaeriaceae*, *Xylariales* (Barr 1989), the *Pleurotrema-taceae*, a family with an unknown higher taxonomic position (Barr 1994) and 'Unitunicate *Ascomycota* genera *incertae sedis*' (Jones *et al.*, 2009).

Currently, there are 22 species epithets referred to *Saccardoella* (Index Fungorum 2013) with most described from aquatic environments. Hyde (1992) described the marine species including *S. mangrovei* K.D. Hyde, *S. marinospora* K.D. Hyde and *S. rhizophorae* K.D. Hyde from wood collected in mangroves of Australia and Thailand. Species from freshwater environments were subsequently described: *S. allequashensis* Fallah & Shearer, *S. aquatic* K.M. Tsui *et al.*, *S. horizontalis* Fallah & Shearer, *S. lacustris* Fallah & Shearer and *S. minuta* L. Cai & K.D. Hyde (Tsui *et al.*, 1998; Fallah and Shearer 2001; Cai *et al.*, 2002) and these have been recollected repeatedly (Cai *et al.*, 2003; Luo *et al.*, 2004). Suetrong *et al.* (2009) investigated the phylogeny of marine *Dothideomycetes* and included sequences of an isolate of *S. rhizophorae* from Oahu, Hawaii. Surprisingly, this fungus did not show any affinities to members of the *Sordariomycetes*, but grouped within the *Dothideomycetes* although it did not cluster with any known families and orders in the class.

Recently, a morphologically similar ascomycete to the three described marine *Saccardoella* species was collected in a mangrove area of Tioman Island, Malaysia. The LSU rRNA gene sequence of this fungus grouped with that of *S. rhizophorae* from GenBank and therefore confirmed the phylogeny of *S. rhizophorae* as a dothideomycetous fungus. As a result, a new genus, *Dyfratomyces*, is introduced to accommodate the three marine species of *Saccardoella* in the new family *Dyfratomycetaceae*, *Pleosporomycetidae*, *Dothideomycetes*. The fungus from Tioman Island is described here as a new species in *Dyfratomyces* as it is different in having spindle-shaped ascospores with 20-24 septa.

MATERIALS AND METHODS

Collection, identification and isolation: Driftwood/trapped wood was collected in a mangrove area of Tioman Island on 13 July 2010. Wood samples were placed in large Zip-lock plastic bags and incubated at room temperature in the laboratory. Ascospores of the new fungus (*D. tiomanensis*) on wood were cut open by a razor blade under an Olympus SZ61 stereomicroscope (Tokyo, Japan). Centrum material was transferred to a drop of sterile natural seawater on a glass slide. The morphology of asci and ascospores was observed under an Olympus BX51 microscope (Tokyo, Japan) and photographs taken with an Olympus DP20 Microscope Camera (Tokyo, Japan).

For isolation, a spore suspension of *D. tiomanensis* was made by transferring centrum material to a drop of sterile natural seawater on a sterilized glass slide. Spore mass was dispersed evenly in the drop of seawater with a sterilized forceps and its identification confirmed by observing under the compound microscope. More sterile natural seawater was added and dispensed onto the surface of a cornmeal seawater agar (CMAS) plate (Difco). The plate was incubated at 25°C for 1-3 days. Germinated single spores were picked up and

transferred to new CMAS plates. Cultures are deposited at Institute of Marine Biology, National Taiwan Ocean University and School of Science, Mae Fah Luang University.

Section of ascomata: Wood pieces with ascomata were cut out and fixed in FAA solution (5% formaldehyde and 5% glacial acetic acid in 50% ethanol) overnight at 4°C. The fixed samples were washed three times in 50% ethanol. Samples were then dehydrated in a graduated t-butanol/ethanol/water series (10/40/50, 20/50/30, 35/50/15, 55/45/0, 75/25/0, 100/0/0, 100/0/0, in percentage), and infiltrated gradually and embedded in paraffin. Paraffin sections (7 µm) were cut on a FRM-200P rotary microtome (Japan), floated on 42°C water-bath to relax compression and mounted on microscope slides. Dried sections were deparaffinised and rehydrated through a graded series of ethanol. The sections were then stained with 1% safranin O in 50% ethanol (10 sec) and 0.5% Orange G in 95% ethanol (30 sec). After washing and dehydration, each stained section was permanently mounted with a cover slip and Permout (Fisher, USA). Specimens were observed on the Olympus BX51 microscope with light micrographs taken.

Molecular analysis: The isolates were grown on potato dextrose seawater agar plates (Difco) for 2 weeks at 25°C. Mycelium was scrapped off from the agar surface and ground into powder in a mortar and pestle in liquid nitrogen. DNeasy Plant Mini Kit (Qiagen, California, USA) was used for genomic DNA extraction according to the manufacturer's instructions. Extracted DNA was used directly for PCR reactions with the following ingredients: 0.2 µM of each primer (NS1/NS4: White *et al.*, 1990, LROR/LR6: Bunyard *et al.*, 1994, TEF1-983F/ TEF1-2218R: Rehner and Buckley 2005), 0.2 mM of each dNTP, 1.5 mM MgCl₂ and 1 U of Taq Polymerase (Invitrogen). The amplification cycle consisted of an initial denaturation step of 94°C for 5 min followed by 35 cycles of (i) denaturation (94°C for 0.5 min), (ii) annealing (55°C for 0.5 min) and (iii) elongation (72°C for 0.5 min) and a final 11 min elongation step at 72°C. The PCR products were analysed by agarose gel electrophoresis and PCR product shipped to Genomics BioSci. & Tech., Taiwan, for purification and direct sequencing with the same primers.

Returned sequences were checked for ambiguity, assembled and deposited in GenBank. Sequences used in the phylogenetic analysis are listed in Table 1, which include sequences from the Dothideomycetes, other major classes and closest sequence matches after BLAST search in NCBI. Alignment was performed on the sequences in the program MUSCLE (Edgar 2004). The alignments of the partial nuclear SSU and LSU and TEF1 genes were entered into BEAUti v1.7.2 for prior settings and generation of XML files for Bayesian analysis in BEASTv.1.7.2 and analyzed simultaneously (Drummond and Rambaut 2007) with the following analytical settings: GTR, gamma+invariant sites, number of gamma categories set at 4, a strict clock, coalescent tree prior for populations of constant size as the speciation model, running 15 million generations with parameters and trees sampled every 1000 generations. The first 10% of the trees were treated as the burn-in and discarded based on the effective sample size (ESS) of the parameter statistics in Tracer v1.5 (Drummond and Rambaut 2007). A summary tree was produced using TreeAnnotator v1.7.2 (Drummond and Rambaut 2007) and viewed and edited in FigTree v1.3.1 (Rambaut 2009).

Table 1. Taxa used in the phylogenetic analysis and their GenBank accession numbers

Taxa	GenBank accession numbers		
	18S rDNA	28S rDNA	Tef1
<i>Acrospermum adeanum</i>	EU940031	EU940104	–
<i>Acrospermum compressum</i>	EU940012	EU940084	–
<i>Acrospermum gramineum</i>	EU940013	EU940085	–
<i>Aigialus grandis</i>	GU479738	GU479774	GU479838
<i>Anisomeridium phaeospermum</i>	JN887374	JN887394	JN887418
<i>Astrosphaeriella bakeriana</i>	–	GU349015	GU349015
<i>Botryosphaeria dothidea</i>	DQ677998	DQ678051	DQ676637
<i>Botryosphaeria stevenii</i>	DQ678012	DQ678064	DQ677907
<i>Capnodium coffeae</i>	DQ247808	DQ247800	DQ471089
<i>Davidiella tassiana</i>	DQ678022	DQ678074	DQ677918
<i>Delitschia winteri</i>	DQ678026	DQ678077	DQ677922
<i>Dendryphiella arenaria</i>	DQ471022	DQ470971	DQ677890
<i>Dothidea inculpta</i>	DQ247810	DQ247802	DQ471081
<i>Dyfrolomyces (Saccardoella) rhizophorae</i>	GU479766	GU479799	GU479860
<i>Dyfrolomyces (Saccardoella) rhizophorae</i> BCC15481	KF160009	–	–
<i>Dyfrolomyces tiomanensis</i>	KC692155	KC692156	KC692157
<i>Elsinoe veneta</i>	DQ767651	DQ767658	DQ767641
<i>Eupenicillium limosum</i>	EF411061	EF411064	EF411070
<i>Falciformispora lignatilis</i>	GU371835	GU371827	GU371820
<i>Geoglossum nigratum</i>	AY544694	DQ471044	DQ471044
<i>Gloniopsis praelonga</i>	FJ161134	FJ161173	FJ161090
<i>Guignardia citricarpa</i>	GU296151	GU301815	GU349053
<i>Helicascus nypae</i>	GU479754	GU479788	GU479854
<i>Herpotrichia juniperi</i>	DQ678029	DQ678080	DQ677925
<i>Hysterobrevium mori</i>	–	GU301819	GU397338
<i>Hysterographium fraxini</i>	FJ161132	FJ161171	FJ161088
<i>Keissleriella cladophila</i>	GU296155	GU301822	GU349043
<i>Lecanora hybocarpa</i>	DQ782883	DQ782910	DQ782901
<i>Leptosphaeria maculans</i>	DQ470993	DQ470946	DQ471062
<i>Lophium mytilinum</i>	DQ678030	DQ678081	DQ677926
<i>Mycopezon smithii</i>	AF279399	AF279400	–
<i>Mycosphaerella euryptami</i>	GU479761	GU301852	GU371722
<i>Passalora ageratinae</i>	JN938702	GU214453	–
<i>Patellaria atrata</i>	GU296181	GU301855	GU349038
<i>Petriella setifera</i>	DQ471020	DQ470969	DQ836911
<i>Platystomum scabridisporum</i>	GQ925832	GQ925845	GU479856
<i>Rhizocarpon oederi</i>	DQ983486	DQ986804	FJ772239
<i>Roccellographa cretacea</i>	DQ883705	DQ883696	DQ883733
<i>Schismatomma decolorans</i>	NG_013155	NG_027622	DQ883725
<i>Spiromastix warcupii</i>	DQ782882	DQ782909	DQ782900
<i>Trichoglossum hirsutum</i>	AY544697	AY544653	DQ471049
<i>Trypetheliopsis kalbii</i>	JN887391	JN887406	JN887435
<i>Tubeufia paludosa</i>	GU296203	GU301877	GU349024
<i>Verruculina enalia</i>	GU479770	GU479802	GU479863
<i>Westerdykella cylindrica</i>	AY016355	AY004343	DQ497610
<i>Xylaria acuta</i>	AY544719	AY544676	DQ471048

TAXONOMY

Dyfolromycetaceae K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones, fam. nov.

Mycobank MB 804662

Saprobic on wood in aquatic environments. **Ascomata** relatively large, solitary to gregarious, immersed, globose or subglobose, coriaceous, clypeate, ostiole rounded, papillate. **Peridium** broadest at the sides, comprising two layers, an outer layer of cells of *textura intricata* composed of host cells interspersed with fungal hyphae and an inner layer of thick-walled cells of *textura angularis*. **Hamathecium** comprising numerous, relatively narrow (up to 2 µm, wide), septate pseudoparaphyses embedded in a gelatinous matrix. **Asci** 8-spored, bitunicate, fissitunicate, cylindrical, with a relatively short pedicel and apically rounded or flattened with a distinct ocular chamber and ring-like subapical apparatus. **Ascospores** overlapping uniseriate, broadly fusiform, symmetrical, hyaline, multi-septate with wide septa (distoseptate?), smooth-walled, with/without a sheath.

Asexual state: Unknown.

Family type: *Dyfolromyces* K.D. Hyde *et al.*

Dyfolromyces K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones, gen. nov.

Mycobank MB 804660

Saprobic on wood in aquatic or terrestrial environments. **Ascomata** relatively large, solitary to gregarious, immersed, globose or subglobose, coriaceous, clypeate, ostiole rounded, papillate. **Peridium** broadest at the sides, comprising two layers, an outer layer of cells of *textura intricata* composed of host cells inter-

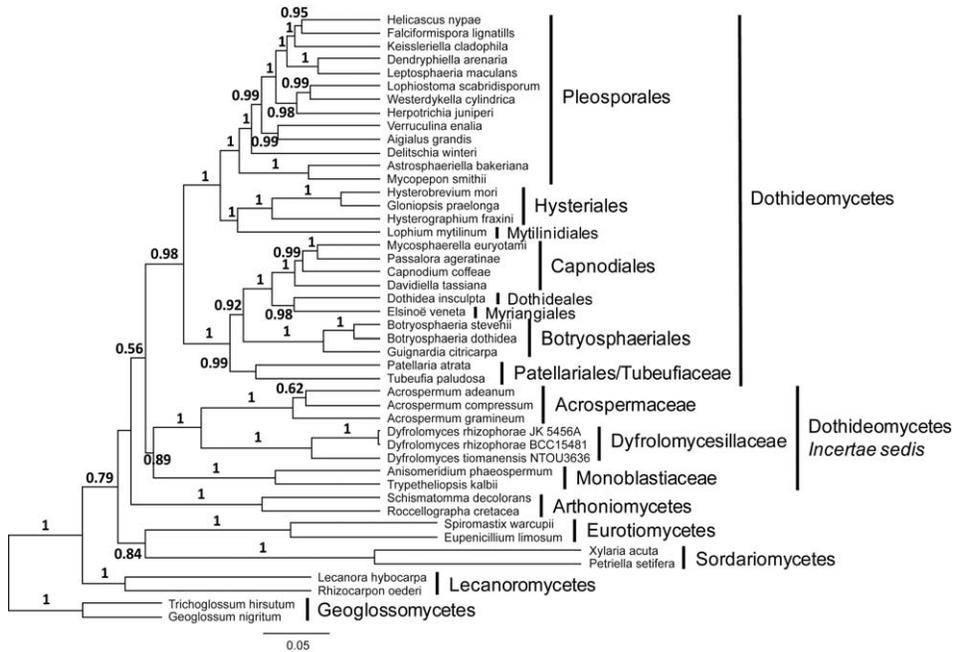


Fig. 1. A Bayesian likelihood tree based on a combined analysis of the partial 18S and 28S rDNA and TEF1 genes using BEASTv.1.7.2. Posterior probability (PP) is shown on the branches.

persed with fungal hyphae and an inner layer of thick-walled cells of *textura angularis*. **Hamathecium** comprising numerous, relatively narrow (up to 2 µm, wide), septate pseudoparaphyses embedded in a gelatinous matrix. **Asci** 8-spored, bitunicate, fissitunicate, cylindrical, with a relatively short pedicel and apically rounded or flattened with a distinct ocular chamber and ring-like subapical apparatus. **Ascospores** overlapping uniseriate, broadly fusiform, symmetrical, hyaline, transseptate with wide septa (distoseptate?), smooth-walled, with/without a sheath.

Etymology: From the Welsh word 'dyfol' meaning 'aquatic' and the Greek word 'myces' meaning 'fungus'.

Asexual state: Unknown.

Generic type: *Dyfrolomyces tiomanensis* K.L. Pang *et al.*

Dyfrolomyces tiomanensis K.L. Pang, S.A. Alias, K.D. Hyde, Suetrong & E.B.G. Jones, **sp. nov.**

Figs 2-8

Mycobank: MB 804661

Ascomata 565–(615)–667 × 283–(374)–446 µm, pyriform, some with a broad flattened base, immersed, clypeate, ostiolate, papillate, coriaceous, dark-coloured, solitary or gregarious. **Clypeus** 326–(414)–500 × 152–(163)–179 µm, extending outwards around the papilla. **Papilla** 251 × 55 µm, black, conical, without periphyses. **Peridium** 16–(27)–36 µm, two-layered, an outer layer of cells of *textura intricata* composed of host cells interspersed with fungal hyphae and an inner layer of thick-walled cells of *textura angularis*, cells 2–(6)–9 × 1–(3)–7 µm. **Hamathecium** comprising numerous, hypha-like pseudoparaphyses, attached at the base and top of the ascoma, branching and anastomosing above the asci, and in a gelatinous matrix. **Asci** 316–(323)–333 × 12–(16)–17 µm, 8-spored, cylindrical, thin-walled, fissitunicate, short-pedicellate, apically rounded, with a faint ring-like subapical apparatus, asci forming at the base of the ascoma. **Ascospores** 69–(74)–82 × 9–(10)–11 µm, overlapping uniseriate, hyaline at maturity, spindle-shaped, 20–24-septate, slightly constricted at the septa.

Etymology: In reference to the place of discovery of the holotype, Tioman Island, Malaysia.

Holotype: MALAYSIA: Tioman Island, on a piece of unidentified mangrove wood, 13 July 2010, K.L. Pang, MFLU13-00063, sections of the ascomata; MFLUCC13-0440, an ex-type culture.

Known geographical distribution: Tioman Island, Malaysia.

Substrata: Decaying mangrove wood.

New combinations:

Dyfrolomyces rhizophorae (K.D. Hyde) K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones, **comb. nov.**

≡ *Saccardoella rhizophorae* K.D. Hyde, Mycologia 84(5): 806 (1992)

Mycobank: MB 804663

Dyfrolomyces mangrovei (K.D. Hyde) K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones, **comb. nov.**

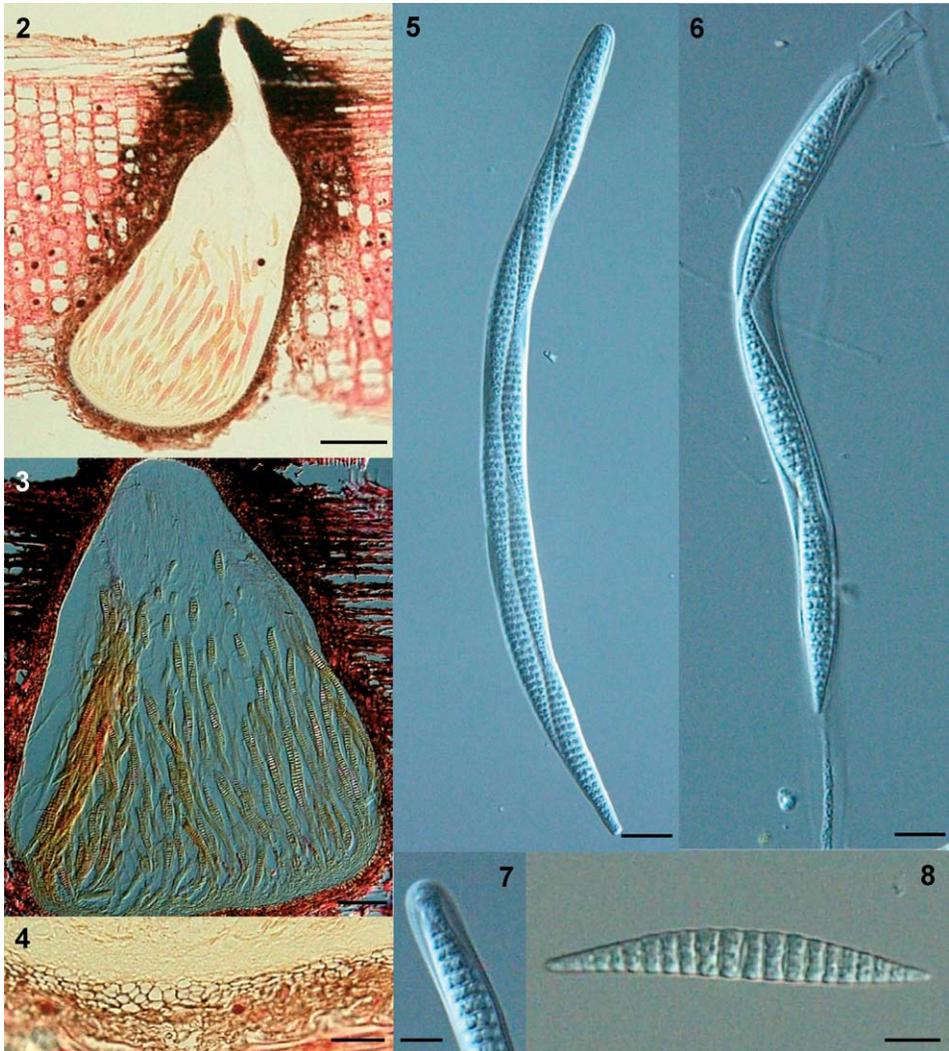
≡ *Saccardoella mangrovei* K.D. Hyde, Mycologia 84(5): 803 (1992)

Mycobank: MB 804665

Dyfrolomyces marinospora (K.D. Hyde) K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones, **comb. nov.**

≡ *Saccardoella marinospora* K.D. Hyde, Mycologia 84(5): 806 (1992)

Mycobank: MB 804664



Figs 2-8. *Dyfrolomyces tiomanensis* (holotype). **2.** Immersed, pyriform ascoma with blackened clypeus. **3.** Asci forming at the base of ascoma with pseudoparaphyses attached at the base and top of ascoma. **4.** Peridium comprising two layers, an outer layer of cells of *textura intricata* composed of host cells interspersed with fungal hyphae and an inner layer of thick-walled cells of *textura angularis*. **5.** Cylindrical ascus. **6.** Dehiscence of the ascus. **7.** Faint ring-like subapical apparatus. **8.** Spindle-shaped ascospore with 20 septa. Scale bars: **2** = 100 μm , **3** = 50 μm , **4-6** = 20 μm , **7-8** = 10 μm .

Key to *Dyfrolomyces* species:

- 1a. Ascospores spindle-shaped, lacking a sheath..... *D. tiomanensis*
- 1b. Ascospores ellipsoidal to fusiform, with a sheath..... 2
- 2a. Ascospores with 7 to 9 septa..... *D. mangrovei*
- 2b. Ascospores with fewer than 7 septa..... 3
- 3a. Ascospores with 4 to 6 septa..... *D. rhizophorae*
- 3b. Ascospores consistently with 3 septa..... *D. marinospora*

RESULTS AND DISCUSSION

A combined analysis of the 18S and 28S regions of the rRNA and the partial TEF1 genes was run and the phylogenetic tree resulting from the Bayesian analysis is shown in Figure 1 with posterior probabilities. The mean likelihood is -43733.7774. The initial BLAST search for sequences of *Dyfrolomyces tiomanensis* suggested that it was a member of the *Dothideomycetes* (results not shown) and the closest sequence matches were included in the phylogenetic analysis. Two isolates of *Dyfrolomyces* (*Saccardoella*) *rhizophorae* and the new fungus *D. tiomanensis* form a monophyletic group but they do not group with the core taxa of the *Dothideomycetes*. *Dyfrolomyces* rather clusters with taxa of the *Acrospermaceae* (*Dothideomycetes incertae sedis*) with a strong posterior probability, while taxa of the *Monoblastiaceae* form a sister clade to the *Acrospermaceae* and *Dyfrolomyces* spp.

Saccardoella has been variously assigned to the *Clypeosphaeriaceae* (Barr 1990), and unitunicate *Ascomycetes incertae sedis* (Jones *et al.*, 2009). Suetrong *et al.* (2009) sequenced a strain of *Saccardoella rhizophorae* isolated by Dr. J. Kohlmeyer from collections made in Hawaii. This sequence did not group with any known taxon in the *Dothideomycetes*, but formed a unique clade that could not be referred to any family or order. In this study, the monophyly of *S. rhizophorae* and the new marine fungus, *S. tiomanensis*, confirms the placement of the former species in the *Dothideomycetes* (Suetrong *et al.*, 2009) and suggests that marine *Saccardoella* constitutes a disparate group from *S. montellica*, the type species of the genus in the *Sordariomycetes*. *Saccardoella montellica* is a sordariomycetous taxon with unitunicate asci, while *D. rhizophorae* and *D. tiomanensis* have bitunicate asci. Ascospores of *S. montellica* are partly immersed in wood; asci are 4-spored, long cylindrical and “iodine fluorescent”; and ascospores are 100-130 µm long and 20-30 septate, with apical spines (Spegazzini’s drawing: http://www.cybertruffle.org.uk/spegazzini/eng/002103a_.htm). The iodine positive reaction of the asci was not mentioned in the protologue (Spegazzini 1879) or subsequent papers (Petraik 1962; Barr 1990; Hyde 1992; Tsui *et al.*, 2006) and this has caused considerable confusion. The iodine positive ring may indicate that *Saccardoella montellica* belongs to the *Xylariales*. We cannot confirm this, as LPS will no longer loan Spegazzini’s type material, but deduce from the drawing provided by Spegazzini (1879). The asci and ascospores of the *Dyfrolomyces* species are significantly different to those of *S. montellica*. Asci of *S. montellica* are 4-spored while those of *Dyfrolomyces* species are 8-spored. One needle-like appendage is present at polar position in ascospores of *S. montellica* while this is lacking in those of *Dyfrolomyces* species but a sheath may be present. Therefore, any dothideomycetous elements in *Saccardoella* should be transferred to one or more new genera.

Consequently, a new genus, *Dyfrolomyces*, is introduced to accommodate *D. tiomanensis* while the three marine *Saccardoella* species are transferred to the new genus. Ascospore structure of *D. tiomanensis* is similar to other three described marine species of *Dyfrolomyces*, while the species can be differentiated based on ascospore characteristics, in particular ascospore septation. Ascospores of *D. tiomanensis* are spindle-shaped with 20-24 septa, those of *D. mangrovei*, *D. marinospora* and *D. rhizophorae* have 7-9, 3 and 4-6 septa, respectively.

A new family *Dyfrolomycetaceae* is established for *Dyfrolomyces* in the *Dothideomycetes*. Phylogenetically, *Dyfrolomyces* forms a well-supported monophyletic clade with *Acrospermum* spp. but ecologically and morphologically, they are very different. *Acrospermum* is a terrestrial genus growing on grass, while

Dyfrolomyces spp. are marine taxa growing on decaying mangrove wood. Ascomata of *Acrospermum* are stalked and ascospores are filiform with some species having length over 1000 µm (Minter *et al.*, 2007; Hyde *et al.*, 2013). Ascomata of *Dyfrolomyces* are immersed forming a clypeus on the wood surface and ascospore shape is broadly ellipsoidal. However, functional dehiscence of the bitunicate asci in *Acrospermum* species has not been observed (Minter *et al.*, 2007), an observation previously found in the marine *Saccardoella* species. In this study, a clear dehiscence of an ascus was observed for *D. tiomanensis* (Fig. 6), confirming the bitunicate nature of the asci. The freshwater species of *Saccardoella* will need to be recollected and sequenced to establish their taxonomy affinities.

Acknowledgements. K.L. Pang would like to thank National Science Council of Taiwan for financial support (NSC101-2621-B-019-001-MY3). K.D Hyde thanks Mae Fah Luang University grant number 56101020032 to study Dothideomycetes. Satinee Suetrong thanks Anupong Klaysuban for laboratory assistance. This work was supported by the TRF/BIOTEC program Biodiversity Research and Training Grants BRT R_251006 and R_325015. For their continued interest and support, we also thank BIOTEC including Prof Morakot Tanticharoen, Dr Kanyawim Kirtikara and Dr Lily Eurwilaichitr. We would like to thank Ministry of Science, Technology & Innovation for Science Fund (02-01-03-SF0675), Ministry of Higher Education (MOHE) for Fundamental Research Grant Scheme (FRGS - FP087) and University of Malaya (UM) for University Malaya Research Grant (RG057-09SUS) to undertake this research. We also would like to thank Marine Park Malaysia for the permit to undertake this research at the protected area, National park, Tioman Island.

REFERENCES

- BARR M.E., 1989 — The genus *Dothidothia* (Botryosphaeriaceae) in North America. *Mycotaxon* 34: 517-526.
- BARR M.E., 1990 — Prodrumus to nonlichenized, pyrenomycetous members of Class Hymenascomycetes. *Mycotaxon* 34: 43-184.
- BARR M.E., 1994 — Notes on the Amphisphaeriaceae and related families. *Mycotaxon* 51: 191-224.
- 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.
- CAI L., LUMYONG P., ZHANG K.Q. & HYDE K.D., 2002 — New species of *Annulatacus* and *Saccardoella* from the Philippines. *Mycotaxon* 84: 255-263.
- CAI L., ZHANG K., MCKENZIE E.H.C. & HYDE K.D., 2003 — Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. *Fungal Diversity* 13: 1-12.
- DRUMMOND A.J. & RAMBAUT A., 2007 — BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.
- EDGAR R.C., 2004 — MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797.
- FALLAH P.M. & SHEARER C.A., 2001 — Freshwater ascomycetes: new or noteworthy species from north temperate lakes in Wisconsin. *Mycologia* 93: 566-602.
- HYDE K.D., 1992 — The genus *Saccardoella* from intertidal mangrove wood. *Mycologia* 84: 803-810.
- HYDE K.D. *et al.*, 2013 — Families of Dothideomycetes. *Fungal Diversity* [In Press]
- INDEX FUNGORUM, 2013 — <http://www.indexfungorum.org/names/Names.asp>
- JONES E.B.G., SAKAYAROJ J., SUETRONG S., SOMRITHIPOL S. & PANG K.L., 2009 — Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. *Fungal Diversity* 35: 1-187.
- LUO J., YIN J.F., CAI L., ZHANG K.Q. & HYDE, K.D., 2004 — Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. *Fungal Diversity* 16: 93-112.
- MATHIASSEN G., 1989 — Some corticolous and lignicolous Pyrenomycetes s. lat. (Ascomycetes) on *Salix* in Troms, N Norway. *Sommerfeltia* 9: 1-100.
- MINTER D.W., PEREDO H.L. & WATSON A.T., 2007 — *Acrospermum chilense* sp. nov. from Chile and the Acrospermales ord. nov. *Bulletin of the Botanical Society of Argentina* 42: 107-112.

- PETRAK F., 1962 — Über die Gattung *Saccardoella* Speg. *Sydowia* 15: 233-236.
- RAMBAUT A., 2009 — FigTree v1.3.1. [online]. <http://tree.bio.ed.ac.uk/software/figtree/>
- REHNER S.A. & BUCKLEY E., 2005 — A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84-98.
- SPEGAZZINI C., 1879 — Nova addenda ad mycologiam venetam. *Michelia* 1: 453-487.
- SUETRONG S., SCHOCH C.L., SPATAFORA J.W., KOHLMAYER J., VOLKMANN-KOHLMEYER B., SAKAYAROJ J., PHONGPAICHT S., TANAKA K., HIRAYAMA K. & JONES E.B.G., 2009 — Molecular systematics of the marine Dothideomycetes. *Studies in Mycology* 64: 155-173.
- TSUI K.M., HYDE K.D., HODGKISS I.J. & GOH T.K., 1998 — A new freshwater species of *Saccardoella* from Hong Kong and South Africa. *Mycologia* 90: 701-704.
- WHITE T.J., BRUNS T., LEE S. & TAYLOR J.W., 1990 — Amplification and 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 application*. San Diego, Academic Press, pp. 315-322.