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Tirisporellaceae, a new family in the order Diaporthales (Sordariomycetes, Ascomycota)

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Abstract – *Tirisporella beccariana,* a monotypic marine ascomycete, commonly occurs on the lower base of fronds and rhizomes of the brackish water palm, *Nypa fruticans*. This fungus has globose to subglobose ascomata, immersed to superficial, ostiolate and periphysate, three layered peridium. It has cylindrical bitunicate-like asci, short peduncle, thick-walled, brown ascospores, 4-7 septate, falcate to lunate, verrucose, apical cell appendaged, hyaline basal cell pointed. Taxonomically it was originally assigned to the Dothideomycetes *incertae sedis* based on the bitunicate-like asci. During on-going investigations of mangrove fungi in Thailand, the fungus was found in intertidal regions in Trang and Trat provinces, Thailand. Maximum parsimony, likelihood and Bayesian analyses using partial SSU and LSU rDNA sequences place *T. beccariana* in the Diaporthales, Sordariomycetidae, Sordariomycetes; however, the familial level relationship was unidentified. This genus forms a distinct clade with *Thailandiomyces bisetulosus* isolated from senescent trunks of the palm *Licuala longicalycata* in Sirindhorn peat swamp forest, with a high statistical support, although they share few morphological features in common.

Ascomycetes / Diaporthales / *Nypa* palm / LSU rDNA / SSU rDNA/ taxonomy

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INTRODUCTION

Nypa palm (*Nypa fruticans*) is an interesting host for colonization by fungi as it occurs along shorelines from fully saline habitats to brackish estuarine locations in the tropics, and is a common mangrove species in South East Asia (Tomlinson, 1986). A number of studies of fungi associated with *Nypa* palm have been undertaken: Pilantanapak *et al*. (2005) listed 81 fungal taxa, while Loilong *et al*. (2012) reported 54 ascomycetes, 33 asexual fungi, 14 basidiomycetes and 1 myxomycete from collections made in Thailand. *Tirisporella beccariana* was first reported from *N. fruticans* collected in Sarawak (Cesati, 1880) as *Sphaeria beccariana* and then referred to *Melanomma cesatianum* by Saccardo (1883). Hennings (1908) collected an identical taxon *Gibberidea nipae* from Philippines. Cesati (1880) described ascospores as cylindric-lunate, brown, and 3-septate (although illustrated as 5-septate), while Saccardo (1883) described them as 5-septate. This fungus has historically been classified in the Loculoascomycetes *incertae sedis*, Ascomycota, because of its bitunicate-like asci (Jones *et al*., 1996), while Kirk *et al*. (2008) assigned it to the Dothideomycetes. Thus its phylogenetic position is not clear. The objective of this study is to re-appraise the systematic status of *Tirisporella beccariana* based on molecular sequence data.

MATERIALS AND METHODS

***Collection and growth of fungi*:** Collections of decaying fronds and stems of the brackish water palm *Nypa fruticans* were made in Tambon Bang Pao, Trang Province and Ao Salak Phet, Mu Ko Chang National Park, Trat Province, Thailand, and returned to the laboratory. Samples were washed of mud and incubated in plastic boxes with sterile tissue. Single spore isolates were established with cultures and dried specimens deposited in BCC (BIOTEC Culture Collection) and BBH (BIOTEC Bangkok Herbarium), respectively. Five isolates were selected for phylogenetic analyses.

***DNA extraction, amplification and sequencing*:** Isolates were grown in potato dextrose seawater broth (PDB) at 25°C for 14 days or until enough mycelium for DNA extraction was obtained. Genomic DNA extraction was as outlined in Suetrong *et al*. (2011a). Sequence data were generated from two loci: partial nuclear SSU and LSU regions of the rRNA gene using primer combinations NS1, NS3, NS4 and NS6 for SSU (White *et al*., 1990) and LROR, LR7 for LSU (Bunyard *et al*., 1994; Landvik, 1996) respectively. Protocols and primer sequences for amplification and sequencing have been described (White *et al*., 1990; Bunyard *et al*., 1994; Landvik, 1996). The PCR products were purified using a NucleoSpin® Plant DNA purification kit (MACHEREY-NAGEL, Catalogue No. 740 570. 50). The purified PCR products were sequenced using the above mentioned primers in an Applied Biosystem 3730XL DNA Analyzer at Macrogen, Inc., South Korea.

***Sequence alignment and phylogenetic analyses*:** Each sequence was checked for ambiguous bases and assembled using BioEdit 6.0.7 (Hall, 2004). Sequence homologies were also analysed using BLAST search engine at the National Centre for Biotechnology Information (NCBI). Alignments were checked and manually optimised along with other sequences obtained from GenBank nucleotide

database. The consensus sequences for each DNA region were initially aligned with ClustalW v. 1.6 (Thompson *et al*., 1994) and improved in MUSCLE (Edgar, 2004). Sequences generated in this study are deposited in GenBank (Table 1).

Manual gap adjustments were made to improve the alignment with ambiguously aligned regions excluded. Missing data at the 5'- and 3'-end of partial sequences were coded by "?". The tree construction procedure was performed in PAUP* 4.0b10 (Swofford, 2002). Phylogenetic trees were visualized using the program Treeview (Page, 1996). The final alignment was again optimised by eye and manually corrected using Se-Al v. 2.0a8 (Rambaut, 1996). The phylogenetic analyses of different datasets were performed using maximum parsimony, maximum likelihood and Bayesian methods (Suetrong *et al*., 2011b).

i) Maximum parsimony analyses were performed using PAUP v. 4.0b10 (Swofford, 2002), with gaps treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters given equal weight. Tree topologies from parsimony analyses were tested with the Kashino-Hasegawa (K-H) maximum likelihood test (Kishino & Hasegawa, 1989) to find the most likely tree. Branch support for all parsimony analyses was estimated by performing 1,000 bootstrap replicates (Felsenstein, 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The consistency indices (CI; Kluge & Farris, 1969), retention indices (RI; Farris, 1989) and rescaled consistency indices (RC; Farris, 1989) were calculated for each tree generated. Confident branch support is defined as bootstrap values (BSMP) equal or more than 70%.

ii) Maximum likelihood analyses (ML) were performed using RAxML v. 7.2.2 (Stamatakis, 2006). A general time reversible (GTR+I+G model) plus invariant sites plus gamma distributed model A tree was obtained by simultaneously running a fast bootstrap search of 1,000 pseudoreplicates followed by a search for the most likely tree under functional setting "a". Maximum likelihood bootstrap value (BSML) equal or greater than 70% are given above each node.

iii) Bayesian analyses: The model of nucleotide substitution for Bayesian analysis was determined using the program Mrmodeltest 2.2 (Nylander, 2004). Independent Bayesian phylogenetic analysis was performed in MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001) using a uniform [GTR+I+G] Model, Iset nst = 6 rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Four Markov chains were run from random starting tree for 2,000,000 generations and sampled every 100 generations. The first 2,000 trees, which represented the burn-in phase of the analysis, were discarded, with 18,000 trees used for calculating posterior probabilities (BYPP) in the consensus tree. Posterior probabilities were obtained for each clade.

Table 1. Fungal sequences generated from this study

Taxon	*Voucher/culture*	*SSU*	*LSU*
Tirisporella beccariana	SAT1345/BCC38300	JQ655456	JQ655448
Tirisporella beccariana	SAT1365/BCC38312	JQ655457	JQ655449
Tirisporella beccariana	SAT1273/BCC36737	JQ655454	JQ655450
Tirisporella beccariana	SAT1274/BCC36738	JQ655453	JQ655451
Tirisporella beccariana	NFTR86/N/A	JQ655455	JQ655452

BCC = BIOTEC Culture Collection

Confident branch support is defined as Bayesian posterior probabilities equal or more than 0.95. Maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap values greater than 70% are given above the node. Bayesian posterior probabilities greater than 0.95 are given below each node (BYPP). Scale bar indicates 10 character state changes. The internodes that are highly supported by all bootstrap (100%) and posterior probabilities (1.00) are shown as a thicker bold line.

RESULTS

SSU and LSU rDNA sequences of *T. beccariana* obtained from this study were 1022 and 1144 nucleotides, respectively. BLAST search revealed the closest matches (taxa, GenBank accession number and % similarity) for different loci: SSU rDNA (*Phruensis brunneispora*, AY581944 with 93%; *Diaporthe eres*, DQ471015 with 93%; *Diaporthe phaseolorum*, FJ392654 with 93%; *Diaporthe amygdale*, AB454228 with 93%; *Chrysoporthe cubensis*, DQ862047 with 93%; *Valsa ambiens*, DQ862056 with 93%; *Leucostoma niveum*, NG013203 with 93% and *Melanconis alni*, DQ862052 with 93%). LSU rDNA (*Thailandiomyces bisetulosus,* EF622231, EF622230 with 95%, 95%; *Melanconis desmazieri*, AF408372 with 89%; *Valsa mali*, AF362559 with 89%; *Diaporthe pustulata*, AF408358 with 88%; *Valsa fabianae*. DQ923539 with 88%; *Harknessia gibbosa,* EF110615 with 88%; *Diaporthe phaseolorum*, AY346279 with 88%, and *Valsella salicis*, AF408389 with 88%).

Selected taxa from Sordariomycetes, Sordariomycetidae from the GenBank were included in the combined SSU and LSU analyses. Sequences were aligned and analysed separately by maximum parsimony, maximum likelihood and Bayesian inference, and the resulting trees compared. The data set consisted of 69 taxa, with *Xylaria acuta* and *X. hypoxylon* as the out-group. The complete combined SSU and LSU rDNA dataset had 2167 characters, of which 1585 were constant, 425 variables and 157 parsimony informative. A maximum parsimony analysis of the dataset resulted in 15 most parsimonious trees (MPTs) with a length of 14234 steps (CI = 0.536, RI = 0.833 and RC = 0.447). The difference between these MPT is in the position of *Jobellisia luteola, J. guangdongensis, J. fraterna*, *Lollipopaia minuta, Phruensis brunneispora,* harknessiaceous genera, and melanoconiaceous genera (trees not shown). The major branches were stable and varied only with minor differences in the position of *Phaeoacremonium aleophilum* and some taxa of diaporthaceous genera. However, these differences did not affect the overall topology of the tree (trees not shown). One hundred successive searches using a rapid hill-climbing algorithm from distinct randomised starting trees in RAxML yielded a best scoring likely tree (data not shown) with log likelihood -10801.001011, alpha: 0.615619, invar: 0.489236, Tree-Length: 2.044571, rate A <–> C: 1.039899, rate A <–> G: 2.756897, rate A <–> T: 1.473665, rate C <–> G: 0.766163, rate C <–> T: 8.038239, rate G <–> T: 1.000000, freq pi(A): 0.258056, freq pi(C): 0.220690, freq pi(G): 0.289393, freq pi(T): 0.23186. The trees obtained from maximum likelihood and Bayesian analyses were topologically similar to the maximum parsimony tree. One of the 15 MPTs inferred with the best topology from K-H test is shown in Fig. 1. Maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap values greater than 70% are given above the node. Bayesian posterior probabilities greater than 0.95, are given below each node (BYPP). The internodes that are highly supported by all bootstrap proportions (100%) and posterior

probabilities (1.00) are shown as a thicker line. We introduce a new family based on agreement in support for all three computational methods as well as morphological distinction.

Nine clades representing the families Cryphonectriaceae, Diaporthaceae, Gnomoniaceae, Harknessiaceae, Jobellisiaceae, Melanoconidaceae, Schizoparmaceae, Togniniaceae and Valsaceae are indicated in Fig. 1. The five *T. beccariana* isolates formed a monophyletic group with 100% BSMP, 100% BSML and 1.00 BYPP support, with *Thailandiomyces bisetulosus* as a sister group with high support (100% BSMP, 100% BSML and 1.00 BYPP), and with the *Jobellisiaceae* as a sister clade. Consequently a new family is introduced to accommodate this taxon.

TAXONOMY

Figure 1 shows the phylogenetic relationships of ten families: Tirisporellaceae, Jobellisiaceae, Diaporthaceae, Cryphonectriaceae, Gnomoniaceae, Melanoconiaceae, Harknessiaceae, Schizoparmaceae, Valsaceae and Togniniaceae. The Tirisporellaceae clade comprises five strains of *T. beccariana*, a common species on the brackish water palm, *Nypa fruticans*, with two strains of *Th. bisetulosus* as a sister group.

Tirisporellaceae Suetrong, E.B.G. Jones and K.L. Pang, **fam. nov.** **Fig. 2**

MycoBank: MB812184.

Ascomata partially immersed to superficial, globose to subglobose, black, coriaceous, ostiolate, scattered to gregarious, with necks and periphysate. ***Peridium*** thick-walled. ***Paraphyses*** present. ***Asci*** cylindrical to clavate. ***Ascospores*** 2-3 seriate, 1-7-septate, fusoid, straight to falcate to lunate, hyaline to verrucose with appendages.

Type genus: *Tirisporella* E.B.G. Jones, K.D. Hyde & Alias.

Tirisporella beccariana (Ces.) E.B.G. Jones, K.D. Hyde & Alias, Can. J. Bot. 74: 1490, 1996 **Fig. 2**

≡ *Sphaeria beccariana* Ces., Atti Accad. Sci. Fis. Mat. Napoli 8: 20 (1879).

≡ *Melanomma cesatianum* Sacc., Syll. Fung. (Abellini) 2: 113 (1883).

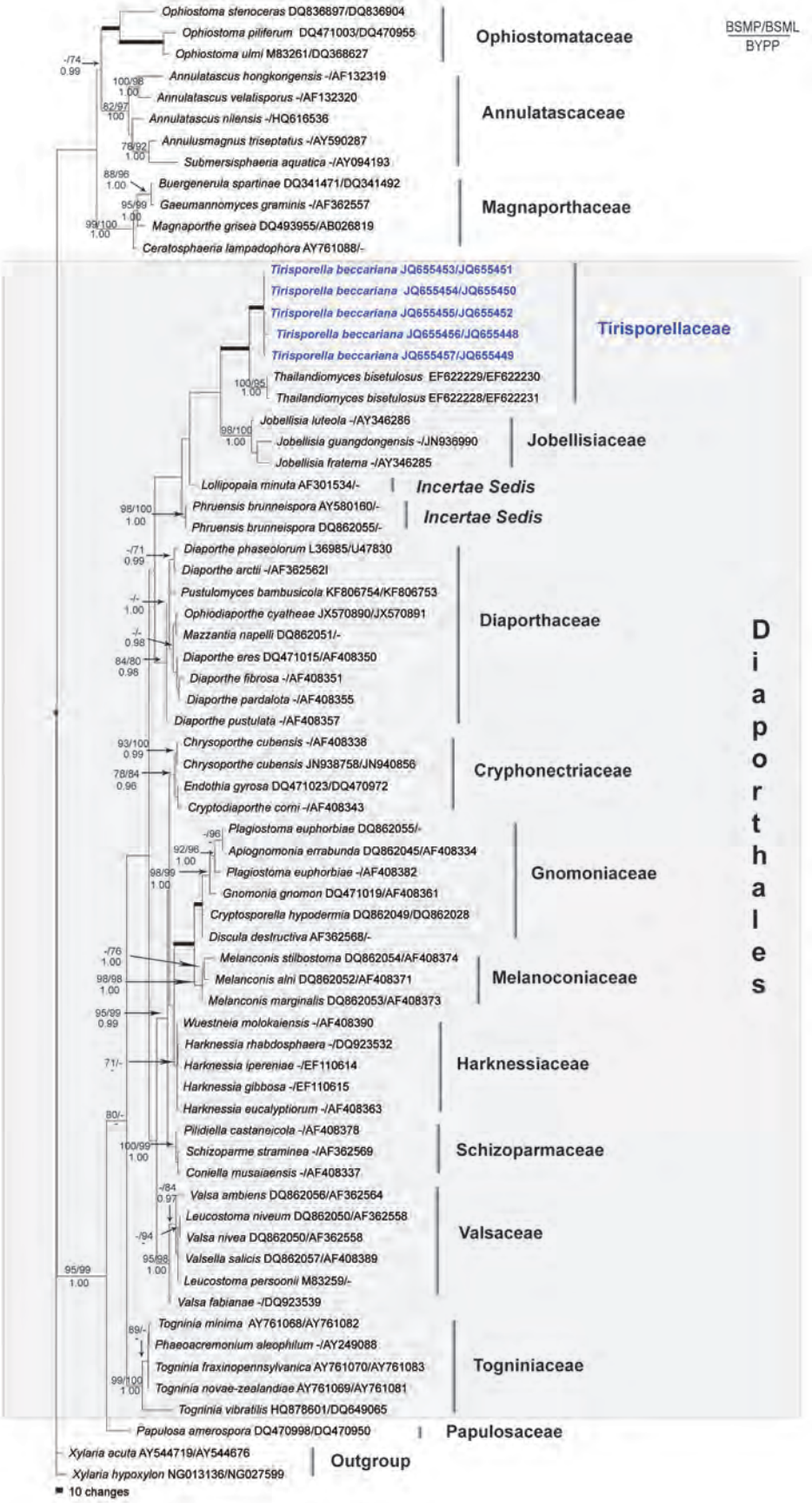
≡ *Gibberidea nipae* Henn., Hedwigia 47: 257 (1908).

Ascomata 800-1,350 µm in diameter, 1400-2,100 µm high, globose to subglobose, crater-like at maturity, single to mainly gregarious, subglobose becoming superficial as ascomata break through the epidermal layer of the host, ostiolate, coriaceous, becoming carbonaceous, necks short, periphysate (Fig. 2 a-b). ***Peridium*** thick-walled and three-layered (Fig. 2 c-d). ***Hamathecium*** comprised of single, unbranched hyaline, septate paraphyses 3.75-5.0 µm wide (Fig. 2 k). ***Asci*** 152.50-205 × 17.50-25 µm, 8-spored, cylindrical, short peduncle, thick-walled, apex flattened (Fig. 2h), with an apical apparatus (Fig. 2 g-j). ***Ascospores*** from Tambon Bang Pao, Trang Province: 37.5-55 × 8.75-11.25 µm and Mu Ko Chang National Park collection 32.5-72.5 × 8.75-11.25 µm, brown, 4-7-septate, falcate to lunate, verrucose, apical cell appendaged, basal cell pointed and hyaline (Fig. 2 l-o).

Asexual stage: *Phialophora* cf. *olivacea* W. Gams.

Colonies: Effuse on potato dextrose seawater agar (PDA/SW) brown.

Cultures: BCC 36305-36307, 36735-36738 Tambon Bang Pao, Trang Province; BCC 32339-32340, 38300, 38312-38313 Ao Salak Phet, Mu Ko Chang National Park, Trat Province.


BSMP/BSML
BYPP
Ophiostoma stenoceras DQ836897/DQ836904
Ophiostoma piliferum DQ471003/DQ470955
Ophiostoma ulmi M83261/DQ368627
Ophiostomataceae
-/74
0.99
Annulatascus hongkongensis -/AF132319
100/98
1.00
Annulatascus velatisporus -/AF132320
82/97
100
Annulatascus nilensis -/HQ616536
Annulusmagnus triseptatus -/AY590287
78/92
1.00
Submersisphaeria aquatica -/AY094193
Annulatascaceae
88/96
1.00
Buergenerula spartinae DQ341471/DQ341492
95/99
1.00
Gaeumannomyces graminis -/AF362557
99/100
1.00
Magnaporthe grisea DQ493955/AB026819
Ceratosphaeria lampadophora AY761088/-
Magnaporthaceae
Tirisporella beccariana JQ655453/JQ655451
Tirisporella beccariana JQ655454/JQ655450
Tirisporella beccariana JQ655455/JQ655452
Tirisporella beccariana JQ655456/JQ655448
Tirisporella beccariana JQ655457/JQ655449
Tirisporellaceae
100/95
1.00
Thailandiomyces bisetulosus EF622229/EF622230
Thailandiomyces bisetulosus EF622228/EF622231
98/100
1.00
Jobellisia luteola -/AY346286
Jobellisia guangdongensis -/JN936990
Jobellisia fraterna -/AY346285
Jobellisiaceae
Lollipopaia minuta AF301534/-
Incertae Sedis
98/100
1.00
Phruensis brunneispora AY580160/-
Phruensis brunneispora DQ862055/-
Incertae Sedis
-/71
0.99
Diaporthe phaseolorum L36985/U47830
Diaporthe arctii -/AF362562I
-/-
1.00
Pustulomyces bambusicola KF806754/KF806753
Ophiodiaporthe cyatheae JX570890/JX570891
-/-
0.98
Mazzantia napelli DQ862051/-
84/80
0.96
Diaporthe eres DQ471015/AF408350
Diaporthe fibrosa -/AF408351
Diaporthe pardalota -/AF408355
Diaporthe pustulata -/AF408357
Diaporthaceae
93/100
0.99
Chrysoporthe cubensis -/AF408338
Chrysoporthe cubensis JN938758/JN940856
78/84
0.96
Endothia gyrosa DQ471023/DQ470972
Cryptodiaporthe corni -/AF408343
Cryphonectriaceae
-/96
Plagiostoma euphorbiae DQ862055/-
92/96
1.00
Apiognomonia errabunda DQ862045/AF408334
98/99
1.00
Plagiostoma euphorbiae -/AF408382
Gnomonia gnomon DQ471019/AF408361
Cryptosporella hypodermia DQ862049/DQ862028
Discula destructiva AF362568/-
Gnomoniaceae
-/76
1.00
Melanconis stilbostoma DQ862054/AF408374
98/98
1.00
Melanconis alni DQ862052/AF408371
Melanconis marginalis DQ862053/AF408373
Melanoconiaceae
95/99
0.99
Wuestneia molokaiensis -/AF408390
Harknessia rhabdosphaera -/DQ923532
71/-
Harknessia ipereniae -/EF110614
Harknessia gibbosa -/EF110615
Harknessia eucalyptiorum -/AF408363
Harknessiaceae
80/-
Pilidiella castaneicola -/AF408378
100/99
1.00
Schizoparme straminea -/AF362569
Coniella musaiaensis -/AF408337
Schizoparmaceae
-/84
0.97
Valsa ambiens DQ862056/AF362564
Leucostoma niveum DQ862050/AF362558
-/94
Valsa nivea DQ862050/AF362558
95/98
1.00
Valsella salicis DQ862057/AF408389
Leucostoma persoonii M83259/-
95/99
1.00
Valsa fabianae -/DQ923539
Valsaceae
89/-
Togninia minima AY761068/AY761082
Phaeoacremonium aleophilum -/AY249088
Togninia fraxinopennsylvanica AY761070/AY761083
99/100
1.00
Togninia novae-zealandiae AY761069/AY761081
Togninia vibratilis HQ878601/DQ649065
Togniniaceae
Papulosa amerospora DQ470998/DQ470950
Papulosaceae
Xylaria acuta AY544719/AY544676
Xylaria hypoxylon NG013136/NG027599
Outgroup
10 changes
Diaporthales

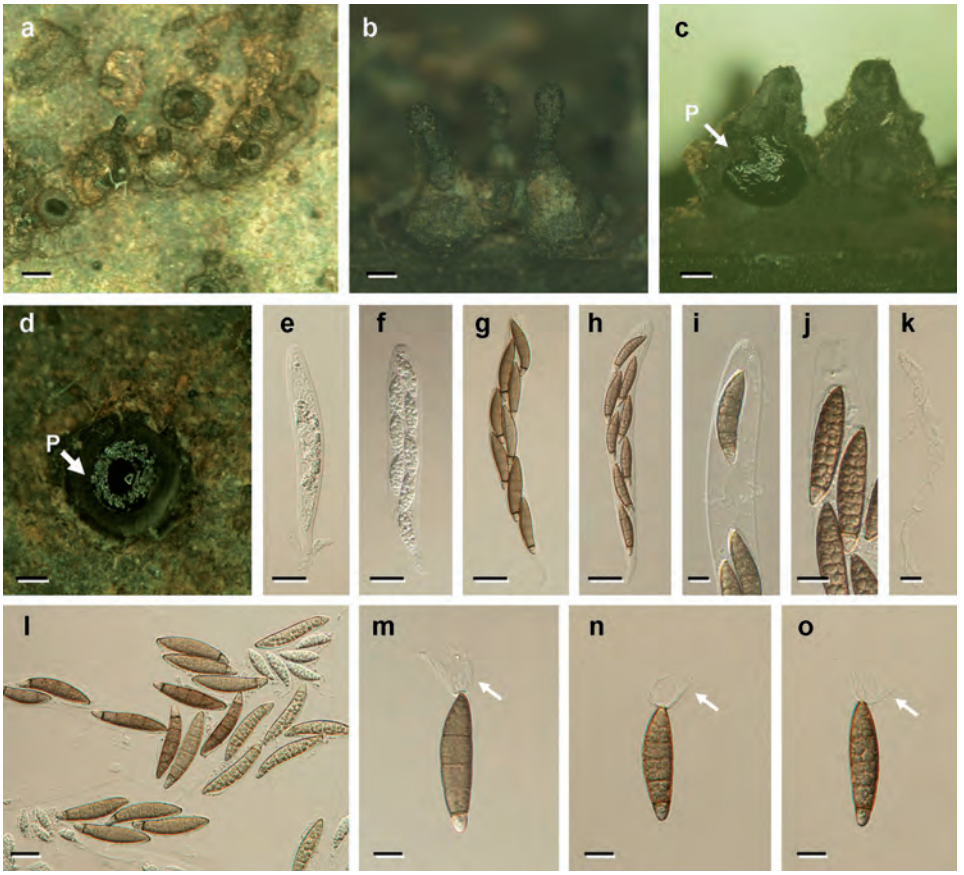


Fig. 2. Morphological characteristics of the marine ascomycete *T. beccariana* from *Nypa fruticans*; **a, b.** Superficial ascomata, with ostiole. **c.** Longitudinal section through ascomata showing thick-walled peridium (P). **d.** Tangential section through ascomata. **e, f.** Immature thick-walled asci. **g, h.** Mature asci with ascospores. **i, j.** Tip of mature asci with ascospores. **k.** Septate paraphyses. **l, m, n, o.** Ascospores with apical appendage (arrowed). Scale bars: a = 1000 µm, b-d = 500 µm, e-h = 25 µm, i-j =10 µm, k = 5 µm, l-o = 10 µm.

◀ Fig. 1. One of 15 MPTs inferred from combined SSU + LSU rRNA sequences of *Tirisporella beccariana* with other taxa of the Diaporthales, generated with maximum parsimony. Maximum parsimony (BSMP, left) and likelihood (BSML, right) bootstrap values greater than 70% are given above the node. Bayesian posterior probabilities greater than 0.95 are given below each node (BYPP). The internodes that are highly supported by all bootstrap proportions (100%) and posterior probabilities (1.00) are shown as a thicker line.

Material examined: Thailand, Trang Province: Tambon Bang Pao, (N 7.405, E 99.515) on frond base of *N. fruticans*, 13 March 2009, A. Loilong, E.B.G. Jones, BBH 25981; Thailand, Trat Province, Ao Salak Phet, Mu Ko Chang National Park (N12.103, E 100.987), on frond base of *N. fruticans*, 29 June 2009, S. Suetrong, J. Sakayaroj, A. Loilong, E.B.G. Jones, S. Preedanon, BBH 31840.

Sequence data: JQ655452-JQ655457.

Habitat and host range: Superficial on leaf petiole (or rachis) bases of brackish water palm *N. fruticans*.

Known distribution: Pacific Ocean, Malaysia, Philippines and Thailand.

Note: Tirisporella was formally established by Jones *et al.* (1996) as a monotypic genus and was assigned to the Dothideomycetes *incertae sedis* based on the bitunicate-like asci with an apical ring and the presence of interascal tissue.

Thailandiomyces Pinruan, Sakay., K.D. Hyde & E.B.G. Jones, Fungal Diversity 29: 91 (2008)

Ascomata partially immersed to superficial, globose, black, coriaceous, ostiolate, scattered to gregarious, with long cylindrical necks, periphysate with short hyaline cells. ***Peridium*** composed of one stratum of compressed cells, (*textura angularis*), black to the outside, brown inwardly. ***Paraphyses*** present but deliquescent, irregular in width, rarely septate, tapering towards the apices, embedded in a mucilaginous matrix. ***Asci*** cylindrical to clavate, unitunicate, apedicellate, free-floating, apically truncate, with a J- subapical ring. ***Ascospores*** overlapping 2-seriate, fusoid, straight or curved, hyaline, 1-septate, smooth walled, with bipolar appendages.

Anamorph: *Craspedodidymum* Hol.-Jech.

Type species: *Thailandiomyces bisetulosus* Pinruan.

Mode of life: saprobic.

Substrata: senescent trunks of the palm *Licuala longicalycata* in a peat swamp in Thailand.

Thailandiomyces bisetulosus Pinruan, Sakay., K.D. Hyde & E.B.G. Jones, Fungal Diversity 29: 91 (2008)

Ascomata 275-325 μm diameter, partially immersed to superficial, globose, black, coriaceous, ostiolate, scattered to gregarious. Neck up to 1 mm long, 100 μm diam., periphyses with short hyaline cells, central, cylindrical, black. ***Peridium*** up to 45 μm thick, composed of one stratum of compressed cells of *textura angularis*, black to the outside, brown inwardly. ***Paraphyses*** present but deliquescent, irregular in width, up to 5-6.5 μm wide, rarely septate, tapering towards apices, embedded in a mucilaginous matrix. ***Asci*** 65-75 × 6-7.5 μm, 8-spored, cylindrical to clavate, unitunicate, apedicellate, free-floating, apically truncate, with a J- subapical ring, 2 × 2 μm. ***Ascospores*** 20-25 × 3.5-5 μm, overlapping 2-seriate, fusoid, straight or curved, hyaline, 1-septate, smooth walled, with 4-5 large guttules, with bipolar spine-like appendages, usually bent laterally, 5-7.5 μm long, 1-1.5 μm diam.

Asexual stage: *Craspedodidymum licualae* Pinruan.

Colonies: on natural substratum effuse, black. Mycelium superficial. *Conidiophores* macronematous, mononemataus, erect, brown, paler toward the apex, straight or flexuous, smooth, but rough at the apex. *Conidiogenous* cells integrated, terminal, 20-27.5 × 6.2-7.5 μm, enteroblastic and monophialidic. *Conidia* 13.7-17.5 × 7.5-10 μm, obovoid or ellipsoid, broadly rounded at both ends, brown, papillate at the basal end.

Cultures: BCC00018, BCC00020 Sirindhorn Peat Swamp Forest, Narathiwat province.

Material examined: THAILAND, Narathiwat, Sirindhorn Peat Swamp Forest, 12 May 2001, U. Pinruan.

Sequence data: EF622228-EF622231.

Habitat and host range: on submerged trunk of *Licuala longicalycata*.

Known distribution: Thailand.

Note: *Th. bisetulosus* shares a number of features with other members of the Diaporthales: its saprobic habitat on decaying plant material, partially-immersed ascomata, long periphysate necks, unbranched paraphyses that deliquesce early in development, unitunicate asci that float free within the centrum and asci with a refractive, apical J-ring (Barr, 1991; Samuels & Blackwell, 2001). However, morphological and molecular results show that *Th. bisetulosus* is not closely related to species of *Diaporthe* (Diaporthaceae), the genus it morphologically most resembles. Sequence data presented in Fig. 1 confirms that it is a new lineage in the Diaporthales (Diaporthomycetidae) and warrants the introduction of a new family to accommodate it and the genus *Tirisporella*.

DISCUSSION

Many new freshwater ascomycetes have recently been described: *Lollipapaia* (Inderbitzin & Berbee, 2001), *Phruensis* (Pinruan *et al.*, 2004), *Thailandiomyces* (Pinruan *et al.*, 2008), all referable to the Diaporthales, but not grouping in any known family. All have been collected on wood or palms in freshwater streams or peat swamps in Thailand. *Tirisporella beccariana*, a brackish water species, also belongs to this group of aquatic ascomycetes, and share morphological characteristics in common, with superficial ascomata, that are globose, black, ostiolate, scattered to gregarious, neck short; thick-walled peridium; cylindrical asci; and septate ascospores with appendages.

Our results clearly show that *T. beccariana* does not have any phylogenetic affinity with the Dothideomycetes (Fig. 2) although it shares some morphological similarities to the class in the possession of bitunicate-like asci, ascospores with appendages and pseudoparaphyses-like hamathecial tissue. Phylogenetic analyses showed that *T. beccariana* is well positioned in the order Diaporthales (Sordariomycetes, Diaporthomycetidae) (Zhang *et al.*, 2006; Hibbett *et al.*, 2007; Schoch *et al.*, 2007, 2009; Sajeewa *et al.*, 2015), with high bootstrap and posterior probabilities. Members of the Diaporthales are characterized by brown to black perithecial ascomata immersed in stromata or substrata, lack true paraphyses at maturity and have a refractive ring in the ascus apex (Barr, 1978; Samuels & Blackwell, 2001), and molecular data supports their inclusion as a distinct order within the Sordariomycetes (Farr *et al.*, 2001; Zhang & Blackwell, 2001; Sajeewa *et al.*, 2015). Jones *et al*. (1996) opinioned, that *T. beccariana* should be referred to the Loculoascomycetes *incertae sedis* based on morphological evidence, but our phylogenetic data does not support this hypothesis. Common features of *T. beccariana* with the *Diaporthales* include: black perithecial ascomata, ostiolate neck; thick-walled peridium, cylindrical asci and brown ascospores.

Tirisporella beccariana and *Tha. bisetulosus* form a sister group to two *Jobellisia* species, a genus with six species, with *J. viridifusca* known from freshwater, and assigned to the family *Jobellisiaceae* (Huhndorf *et al.*, 1999). *Jobellisia* species are characterized by an ascus with a well-developed refractive,

J- apical ring, brown ascospores and ascomata with a thick-peridial wall, lacking ascospore appendages (Cai *et al.*, 2006). *Jobellisia* spp., *T. beccariana* and *Tha. bisetulosus* are saprobes on wood or palm fronds, have a well-developed periphysate central necks, thick ascomal walls, asci J- apical ring and ascospores with variable morphology. Many of these taxa have anamorphs: *Th. bisetulosus* (*Craspedodidymum*), *T. beccariana* (*Phialophora* cf. *olivacea*) (Jones *et al.*, 1996; Pinruan *et al.*, 2008).

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