**Sedecimiella taiwanensis** gen. et sp. nov., a marine mangrove fungus in the Hypocreales (Hypocreomycetidae, Ascomycota)

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**Abstract**

In an ongoing survey of marine mangrove fungi in Taiwan, a fungus with light-coloured ascomata, resembling members of the Hypocreales, was found on twigs of *Kandelia obovata*. Based on morphological characteristics and partial nuclear small and large subunit ribosomal DNA data, the fungus is described as a new genus and species, *Sedecimiella taiwanensis*, and placed in the Hypocreales (Hypocreomycetidae, Ascomycota). Phylogenetic analyses based on LSU/SSU sequences did not resolve the familial placement of *S. taiwanensis* within the Hypocreales, but it may be related to the Niessliaceae. The characteristic features of *S. taiwanensis* are orange to dark-brown globose, subglobose, erumpent ascomata with the inner wall layer of the neck extending into the upper part of the centrum; cylindrical, 16-spored, unitunicate asci without an apical pore; and hyaline, smooth-walled and globose ascospores.

**Keywords:** aquatic fungi; *Kandelia obovata*; Sordariomycetes.

**Introduction**

During ongoing studies of mangrove fungi in coastal waters of Taiwan, a new unitunicate ascomycete with 16-spored asci was found on twigs of *Kandelia obovata* Sheue, Liu et Yong (Rhizophoraceae). Morphological characters of this fungus, such as orange to dark-brown globose, subglobose, erumpent ascomata, periphysate, cylindrical asci, and hyaline, thin-walled, round, unicellular ascospores are also features of the Hypocreales. The goals of this study were to: (1) characterise and describe this novel fungus from a mangrove in Taiwan, and (2) analyse partial nuclear small and large subunit ribosomal DNA (SSU, LSU rDNA) data to determine the phylogeny of the new taxon.

**Materials and methods**

**Morphological study**

Drift and attached mangrove wood subject to seawater immersion was collected from Chunan, Taiwan, on 12 August 2008 (holotype), from Futian Nature Reserve, Shenzhen, China on 14 March 2006 (isotype) and from Kukup Island, Malaysia on 5 August 2009. Wood samples were transferred to the laboratory and incubated on a tray lined with moist tissue paper for up to one month. Fruiting bodies were observed using an Olympus SZ61 stereomicroscope (Tokyo, Japan), sectioned with a razor blade, grasped with fine forceps, and mounted on a slide in sterile seawater. Morphology of the asci and ascospores was observed using an Olympus BX51 microscope (Tokyo, Japan). Photographs were taken with an Olympus DP20 Microscope Camera (Tokyo, Japan).

Two wood pieces (2×1×1 cm³) with ascomata, cut out from a larger piece of collected wood, were fixed by immersion in FAA (50% ethanol, 5% glacial acetic acid and 4% paraformaldehyde) overnight at 4°C. The fixed samples were rinsed three times in the same buffer, followed by three rinses in distilled water. The samples were then dehydrated in a graduated ethanol and 1-butanol series, infiltrated gradually and embedded in paraffin (Paraplast X-tra, Kendall, Mansfield, USA). Paraffin sections (8–10 μm) were cut on RM2125RT rotary microtome (Leica, Nussloch, Germany), floated over a water-bath at 42°C to relax sections, and then mounted on Superfrost Plus microscope slides (Menzel-Gläser, Germany). Dried sections were deparaffinised and rehydrated through a graded series of ethanol. Sections were stained with 0.1% safranin O in 50% ethanol and 0.5% methyl green (each for 30 min). After washing and dehydration, each stained section was permanently mounted with a cover slip and Permount (Fisher, Fair Lawn, USA). Specimens were observed on an Axioplan 2 Imaging microscope (Carl Zeiss, Göttingen, Germany) and light micrographs were acquired using a ColorView 12 CCD camera (Soft Imaging System, Münster, Germany) using analySIS (version 3.2) software.
Molecular analysis

A mycelial culture (CY5100) from materials collected at Futian, Shenzhen, China (see Figure 7) was used for a molecular study as ascospores from Chunan did not germinate. The isolate was grown in GYPS (glucose 4 g l⁻¹, yeast extract 4 g l⁻¹ and peptone 2 g l⁻¹ in full strength natural sea water). Mycelial pellets were washed with sterile water, blotted dry and ground into powder in a mortar and pestle, pre-cooled in a -80°C freezer overnight. Fungi Genomic DNA Purification Kit (Catalogue no. E070, BioMi, Taichung, Taiwan) was used for 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/LR7 Banyard et al. 1994), 0.2 mM of each dNTP, 1.5 mM MgCl₂ and 1 U of Taq Polymerase (Catalogue no. F530-S, FINNZYMES, Espoo, Finland). 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 purified using a PCR Purification Kit (Catalogue no. K310001, Invitrogen, Carlsbad, USA) according to the manufacturer’s instructions. Purified PCR product was shipped to Tri-I Biotech (Taipei, Taiwan) for direct sequencing with the primers described above.

Returned sequences were checked for ambiguity, assembled and deposited in GenBank (accession numbers in Figures 9 and 10). Sequences were compared with available sequences using nucleotide blast search in GenBank. These and GenBank sequences were manually adjusted in Se-Al v1.0a1 (Rambaut 1999). Both nuclear SSU and LSU rDNA datasets were entered into PAUP* 4.0b10 for maximum parsimony analyses (Swofford 2002). Heuristic searches were run for both datasets with the following settings: gaps treated as missing data, starting tree(s) obtained via stepwise addition, random sequence addition of 10,000 replicas, a tree-bisection-reconnection (TBR) branch-swapping algorithm, MULTREES on. A thousand parsimony bootstrap analyses were used to reflect the support of the clades with the same settings, except that 10 replicates of random sequence addition were used.
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Results and discussion

Sedecimiella K.L. Pang, Alias et E.B.G. Jones, gen. nov.


Typus generis  Sedecimiella taiwanensis K.L. Pang, Alias et E.B.G. Jones, sp. nov. (Figures 1–8)

(Mycobank MB518531). Ascomata 63-(110)-145×65-(100)-140 μm (n=20), pyriforma, solitaria, flavida, brunnea vel atra, globosa vel subgloboza, immersa, subimmersa vel exposita, coriacea, ostiolata, teres. Colla 30-(63)-101×42-(66)-93 μm (n=20), cum periphysibus. Peridium 14-(17)-25 μm (n=20), flavidum, brunneum vel atrum, bistratum, exterior stratum ex cellularum ex textura angularis, interior stratum ex cellularum elongatum. Asci 25.8-(30.3)-35.3×3.5-(4.3)-4.7 μm (n=31), unitunicati, cylindrici, pedicellati, leptodermi, sedecim ascospori, persistentes, ex pulvino cellularum pseudoparenchymatarum ad basim ascomati orientes. Paraphyses praesens, 13.5-(25.3)-37.1×1.8-(3.4)-7.6 μm (n=10). Ascosporae 2.3-(2.8)-3.5 μm (n=50), globosae, sine septatae, hyalinae, leptodermae, sine appendiculatae.

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Ascomata 63-(110)-145×65-(100)-140 μm (n=20), pyriform with globose to subglobose venter, orange to dark brown, globose to subglobose, immersed, erumpent or exposed, coriaceous, ostiolate, smooth, not collapsing upon drying, KOH-, lactic acid-. Neck 30-(63)-101×42-(66)-93 μm (n=20), thick, inner wall layer extends into upper part of centrum, periphyses present. Peridium 14-(17)-25 μm in diameter (n=20), orange to dark brown, two-layered, outer stratum with 3–6 rows of thick-walled cells of textura angularis, 2.5-(3.5)-4.5×2.0-(2.5)-3.0 μm; inner stratum with 3–6 rows of elongated, hyaline thin-walled cells, 5.5-(7.0)-9.0×0.5-(1.0)-2.0 μm. Asci 26.0-(30.0)-35.5×3.5-(4.0)-4.5 μm (n=31), unitunicate, cylindric, short pedunculate, thin-walled, no apical pore, 16-spored, persistent, developing from inner wall of ascoma base. Paraphyses 13.5-(25.3)-37.0×2.0-(3.5)-7.5 μm (n=10), irregular, often tapering towards apex, branched, septate. Ascospores 2.5-(3.0)-3.5 μm (n=50), globose, one-celled, hyaline, smooth, thin-walled, without appendage or sheath.

Etymology  “Sedecim” meaning 16 in Latin.

Anamorph  Unknown.

Sedecimiella taiwanensis K.L. Pang, Alias et E.B.G. Jones, sp. nov. (Figures 1–8)

Ascomata orange to dark brown, pyriform with globose to subglobose venter, immersed, erumpent or exposed, coria-

Isotype  China: Shenzhen: Futian Nature Reserve. On a twig of unidentified mangrove wood, 14 March 2006, K.L. Pang, CY5100-CY5101 (City University of Hong Kong, China), fungal culture, HM451495(SSU)-HM451496(LSU), GenBank sequences.


Known geographical distribution  Chunan, Taiwan; Shenzhen, China; Kukup Island, Malaysia.

Substrata  Drift and dead intertidal branches of mangrove wood.

Decaying mangrove substrates (wood, leaves and fruits) support the growth of many marine mangrove fungi. Taiwan has 22 mangrove forests and four true mangrove species including *Avicennia marina* (Forssk.) Vierh., *Kandelia obovata*, *Lumnitzera racemosa* Willd. and *Rhizophora stylosa* Griff. The only report of marine fungi for Taiwan is by Hsieh et al. (2002) who documented 59 species, of which 32 were from mangroves. Thus our knowledge of mangrove fungi for Taiwan is fragmentary.

Thirty-one species of marine mangrove fungi were identified in the current study from 111 pieces of wood collected at Chunan (results not shown). The dominant fungal species were *Saagaromyces abonnis* (Kohlm.) K.L. Pang et E.B.G. Jones (37 collections), *Lignincola laevis* Höhnk (28 collections) and *Halosarpheia fibrosa* Kohlm. et E. Kohlm. (26 collections). *Sedecimiella taiwanensis* was discovered on only two pieces of mangrove wood, indicating that it is not a common species in that area. Abundant ascomata, however, were produced on the wood (Figure 1).
Sedecimiella taiwanensis was tentatively identified as a member of the Hypocreales based on its light-coloured, soft-textured, perithecial ascomata, and unitunicate asci (Rossman et al. 1999). However, its affinity within the Hypocreales is unknown. Seven marine genera of the Hypocreales have been reported: Emericellopsis, Halonectria, Heleococcum, Kallichroma, Neocosmospora, Payosphaeria and Pronectria (Jones et al. 2009). Sedecimiella taiwanensis most closely resembles Neocosmospora (Nectriaceae) and Payosphaeria (Hypocreales incertae sedis) (Leong et al. 1990, Rossman et al. 1999). In Neocosmospora, the ascomatal wall is composed of two regions, asci are cylindrical, ascospores are yellow to yellow-brown or reddish brown, unicellular, globose and ornamented, but lack paraphyses (Rossman et al. 1999). Sedecimiella taiwanensis differs from Neocosmospora in having branched paraphyses, asci with 16 hyaline, and round ascospores without any ornamentation. Both Sedecimiella and Payosphaeria have light-coloured ascomata, branched paraphyses, cylindrical asci and hyaline, globose ascospores, but the ascomatal wall of Sedecimiella is composed of two regions instead of one as in Payosphaeria. Only eight ascospores are present in Payosphaeria. Sedecimiella differs from Neocosmospora and Payosphaeria in that the inner wall layer of the neck extends into the upper part of the centrum (Figures 2 and 3), an anatomical feature that appears to be previously unknown in the Hypocreales. A similar structure has been observed in Rostrapiella danica J.K. Koch, K.L. Pang & E.B.G. Jones (Lulworthiales), a Lulworthia-like marine fungus (Koch et al. 2007). Asci with 16 ascospores are also reported for many Hypocrea species, but generally are the result of disarticulation of 1-septate ascospores (Rossman et al. 1999). Globose ascospores have also been reported in Roumegueriella Speg. and Trichosphaerella E. Bommer, M. Rousseau et Sacc. (Samuels and Barr 1997,
Rossman et al. 1999). *Roumegueriella* differs from *Sedecimiella* in its saccate ascii and ornamented ascospores (Rossman et al. 1999), while ascomata of *Trichosphaerella* bear short, simple, dark setae that are absent in *Sedecimiella*, and the 16 ascospores in the asci are the result of disarticulation from oblong, uniseptate ascospores (Samuels and Barr 1997).

Ascomspores from the holotype did not germinate, but those from the isotype collected in China did, and these were used for the molecular study. The isotype was morphologically identical to the holotype and the asci and ascospores are illustrated in Figure 7. However, no herbarium material was kept for the isotype. In view of this, a piece of wood with abundant ascomata from the collection in Taiwan was deposited as the holotype.

Maximum parsimony analysis of the partial nuclear SSU rRNA gene confirmed the placement of *Sedecimiella taiwanensis* in the Hypocreales, Hypocreomycetidae (Figure 9). In order to determine the familial placement of *S. taiwanensis*, the partial nuclear LSU rRNA gene was sequenced and analysed with maximum parsimony (Figure 10). *S. taiwanensis* consistently grouped with *Niesslia exilis* (Alb. et Schwein.) G. Winter (Niessliaceae), although without bootstrap support. This result is in agreement with the blast search results of the LSU gene (results not shown). Only three taxa from the Niessliaceae [*Melanopsamma pomiformis* (Pers.:Fr.) Sacc., *N. exilis* and *Valetoniellopsis laxa* Samuels et M.E. Barr] are available in the GenBank for comparison, and they were not monophyletic in the LSU analysis in this study. Members of the Niessliaceae are saprotrophic and characteristic of marine habitats, members of the group are present and may truly belong in this family. Although generally not well documented in marine habitats, members of the group are present and may occupy specific habitats [e.g., *Cosmospora cf. diminuta* found on the petiole base of the brackish water palm *Nypa fruticans* (Thun.) Wurmb.] (E.B.G. Jones and A. Loilong, unpublished data). Further studies may therefore yield a wider range of hypocreaceous marine fungi from such habitats.

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**References**


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