Mycoscience 39: 307–311, 1998

Lignicolous freshwater Ascomycota from Thailand: 1. *Ascotaiwania sawada* and its anamorph state *Monotosporella*

Somsak Sivichai[1], Nigel Hywel-Jones[1] and E. B. Gareth Jones[2]

[1] National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, 73/1 Rama VI Road, Rajdhevee, Bangkok 10400, Thailand
[2] Department of Biology and Chemistry, City University, Tat Chee Avenue, Kowloon, Hong Kong

Accepted for publication 4 July 1998

Single ascospore isolates of *Ascotaiwania sawada* gave rise to conidia of a *Monotosporella* species. Repeated isolations confirmed that the *Monotosporella* was the anamorph of *A. sawada*. These findings are discussed and compared with the other known anamorph-teleomorph stages of freshwater fungi. This species of *Monotosporella* is new to science but because it has not been found in nature it is referred to as the *Monotosporella* state of *A. sawada*.

Key Words——anamorph-teleomorph connections; freshwater Ascomycota; lignicolous fungi; systematics.

The genus *Ascotaiwania* Sivanesan & H. S. Chang, with *A. lignicola* Sivanesan & H. S. Chang as the type species, was described from dead wood collected at Wulae, Taipei, Taiwan (Sivanesan and Chang, 1992). A further four species have since been described (Hyde, 1995; Chang et al., 1998). No anamorphic state has been reported for *Ascotaiwania*. Furthermore, although anamorph-teleomorph connections have been made for several freshwater discomycetes and hyphomycetes, few anamorphs have been reported for freshwater lignicolous Pyrenomycetes (Shearer, 1993; Goh, 1997; Hyde et al., 1997).

One of the species described by Chang et al. (1998) is *Ascotaiwania sawada* H. S. Chang & S.-Y. Hsieh collected on dead wood in a stream at Hsutoupu, Puli, Nantou, Taiwan. As part of a survey of the freshwater fungi of Thailand, *A. sawada* was collected on an undetermined well-rotted hardwood in a stream at Khao Yai National Park. In culture, an anamorph developed in association with the teleomorph and these observations are reported here.

Materials and Methods

Dead, decorticated and barked twigs and wood were collected in Khao Yai National Park. On return to the laboratory samples were washed and incubated in sterile plastic boxes on a layer of moist, sterile, tissue paper. The wood was periodically examined for fungi. Single spore isolates were made on corn meal agar (CMA). Axenic freshwater cultures are maintained on CMA at room temperature (ca. 25°C) and in the light.

Results

Ascotaiwania sawada was identified and isolated. All the 10 single spore isolates which established yielded an anamorph and teleomorph. The following description is based on material from the field and from cultures on potato dextrose agar (PDA).

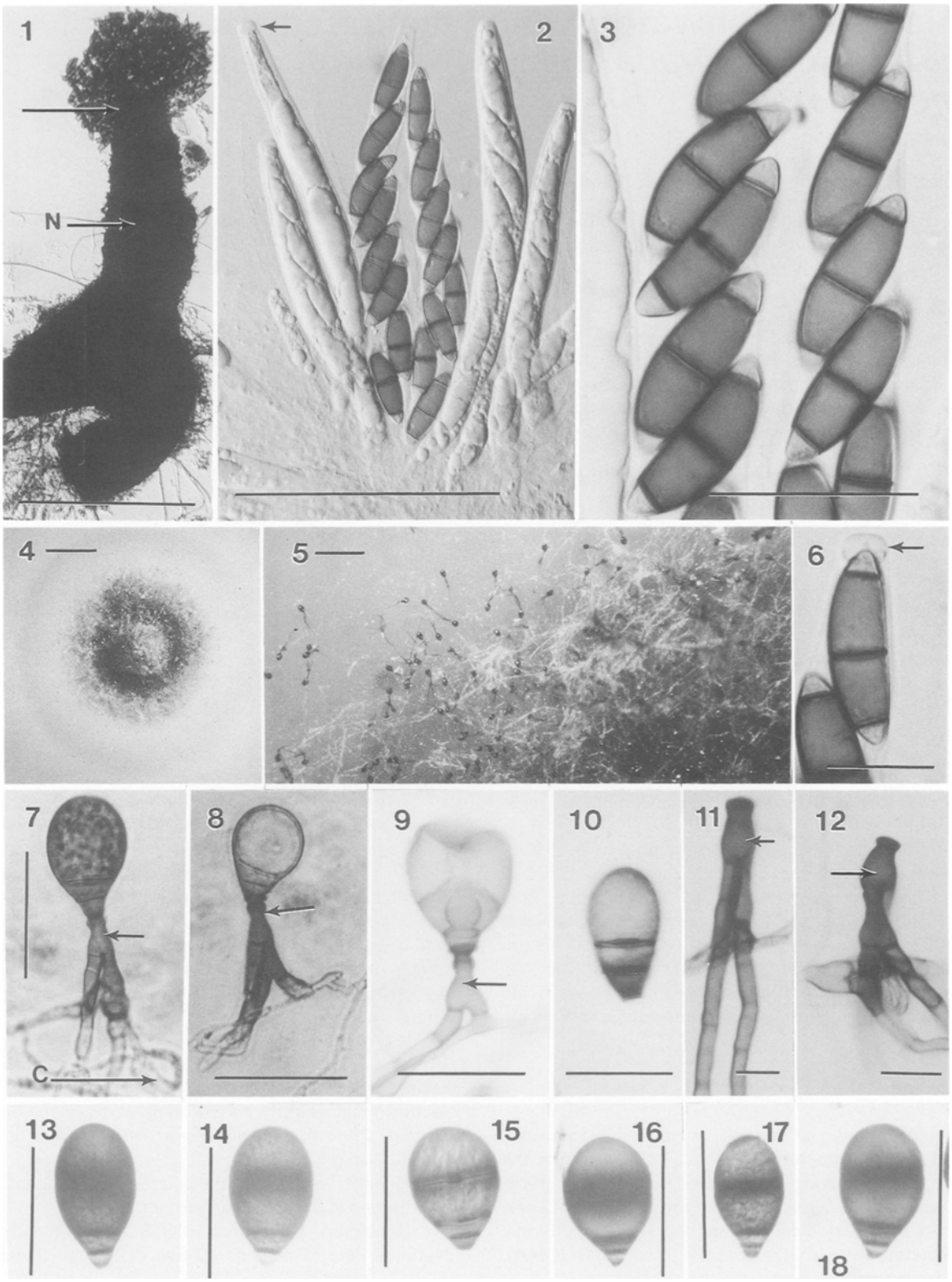
Ascotaiwania sawada H. S. Chang & S.-Y. Hsieh, Mycol. Res. **102**: 713. 1998.

Ascomata 240–300 μm high, 200–240 μm (n=4) in diam, semi-immersed or superficial, globose, brown to dark brown, ostiolate, periphysate, solitary (Fig. 1). Neck 140–250×60–100 μm (n=4), cylindrical, bending perpendicularly to become erect. Peridium thick-walled, brown, pseudoparenchymatous cells on the outer layers and compressed. Paraphyses hyaline, septate, filiform, simple to rarely branched, up to 3.8 μm wide. Asci 145–185×12.5–17.5 μm (n=14), cylindrical, 8-spored, short pedicellate, unitunicate with a distinct, wedge-shaped, non-amyloid apical ring (Figs. 2, 6). Ascospores 25–30×7.5–10 μm (n=33), uniseriate to overlapping biseriate in the ascus, fusoid, 3-septate, not constricted (occasionally slightly constricted), straight to somewhat curved, smooth, central cells brown, end cells hyaline to pale brown (Figs. 3, 6).

Habitat: on well-rotted, decorticated hard wood, submerged in a freshwater stream.

Specimen examined: BIOTEC, SS51, Khao Yai National Park, Thailand, 25 Sep. 1996, S. Sivichai and E. B. G. Jones.

Isolation: The culture is deposited at the BIOTEC Culture Collection as SS51. Ascospores germinated within 24–36 h on CMA. Appressorial-like pegs deve-


1
N
2
3
4
5
6
7
C
8
9
10
11
12
13
14
15
16
17
18

loped from each terminal cell and usually in synchrony. They then elongated as normal germ tubes.

Colony morphology: colony diffuse, no aerial mycelium, hyaline, becoming dark brown with sporulation of the fungus (Fig. 4). Cultures grew moderately well reaching 16 mm in 24 d at 25–28°C in normal daylight conditions. Mycelium hyaline to pale brown, septate and branching, up to 4 μm. Anamorph formed after 4 wk on CMA (Fig. 5) while ascomata were produced after 6–8 wk. Sporulation on CMA at room temperature (ca. 25–28°C) and in the light.

This is only the second collection of *A. sawada* and is a new record for Thailand. No anamorph was recorded in the original description or for any other species assigned to *Ascotaiwania* (Chang et al., 1998). The following description is based on the isolates made from ascospores of *A. sawada*.

Anamorph: Conidiogenous cells erect, 7.5–12 × 5–10 μm (10.7 ± 1.38 × 7.3 ± 0.98, n = 32), stiff, pale to dark brown, septate, smooth and thick-walled, cylindrical, flask-shaped, arising from brown 2 or 3-septate rhizoids (Figs. 7–9, 11, 12). A branch grows out from the conidiogenous cell and elongates downward, the secondary lobe (Rao and de Hoog, 1986) which may be regarded as a rhizoid. Rhizoid cells 30–100 μm long by 2.5–3.8 μm across (Figs. 11, 12). A coiled hyaline to pale brown cell, 5–10 × 3.2–7.5 μm, is often associated with the rhizoids (Fig. 7). Conidia solitary, dry, smooth and thick-walled, obovate to oval, 25–35 × 17.5–25 μm (29.4 ± 1.94 × 20.3 ± 1.92, n = 96), with 1–4 septa, slightly constricted at the septa (Figs. 7–10, 13–18). The basal cells are mid-brown while the upper cell is blackish brown to black. Basal cells 5–12.5 μm; apical cell before third septum is formed 17.5–27.5 × 17.5–25 μm; apical cell only 7.5–15 × 17.5–25 μm. There is a prominent scar on the conidium at the point of session from the conidiogenous cell. The size of the conidia did not influence the number of septa present 68% had four septa, 24% had three septa while 8% had one septum.

We examined cultures maintained at 22°C for more than 6 mo. In all such cultures we could only locate a single conidium associated with each conidiophore. There was no evidence of percurrent proliferation which would have resulted in two or more discharged conidia on the agar near the base of the conidiophore. We conclude that under normal circumstances there is no percurrent proliferation leading to the production of two or more conidia associated with each conidiophore.

Discussion

Five species are now accepted in the genus *Ascotaiwania*. Three species are either 7-septate (*A. lignicola* and *A. wulai* H. S. Chang & S.-Y. Hsieh) or 5-septate (*A. hisilio* H. S. Chang & S.-Y. Hsieh). The Thai specimen appears intermediate between the Taiwanese *A. sawada* and *A. palmicola* K. D. Hyde. Comparison of the Thai material suggests this is referable to *A. sawada*. The most obvious point of similarity is the 3-septate ascospores. The Thai specimen, however, does not match exactly with the Taiwanese *A. sawada* material – the latter having larger ascomata, asci and ascospores (Table 1). Ascoma size compares with *A. palmicola* (210–350 × 200–250 μm) while the asci were narrower being 12.5–17.5 μm compared with 7.5–8 μm. The ascospores were smaller and narrower in *A. palmicola*. Paraphyses of the Thai *A. sawada* were persistent and present in ascomata with fully developed asci containing mature ascospores. This is in contrast to that reported for the type species and for the genus in general.

As *A. sawada* is known only from a single specimen in Taiwan, we do not believe the differences are significant enough to warrant the naming of a new species. We consider the Thai material closer to *A. sawada* than to *A. palmicola* especially with respect to its host (dicotyledonous woody) tissue and habitat (freshwater). However, Hyde and Goh (1997) reported *A. palmicola* from submerged wood in a small stream on Mt. Lewis,

Table 1. Comparison of teleomorphs of *Ascotaiwania palmicola* with *A. sawada*.

Fungus	Ascomata[a]	Asci[a]	Ascospores[a]	Reference
A. palmicola	210–350 × 200–250	150–175 × 7.5–8	17.5–20 × 5–6.5	Hyde (1995)
A. sawada	541–564 × 242–278; neck 106–155 × 58–93	180–187 × 13.3–15.5	25.2–44.6 × 7.1–10.3	Chang et al. (1998)
Thai collection of *A. sawada*	240–300 × 200–240; neck 140–240 × 60–100	145–185 × 12.5–17.5	25–30 × 7.5–10	This paper

a) All measurements: μm.

Figs. 1–18. *Ascotaiwania sawada*. Light microscope micrographs.
1: Ascoma with long neck (arrowed N) and discharged ascospores (arrowed) at the tip of the ostiole. 2: Asci at various stages of development; apical ring arrowed. 3: Ascospores uni- to bi-seriate within ascus, 3-septate, end cells hyaline, central cells brown. 4: Colony on CMA. 5: Sporulating culture of the *Monotosporella* stage of *A. sawada* with glistening conidia. 6: Well-developed, non-amyloid ring to the ascus (arrowed). 7–9: Conidiogenous cell (arrowed), with downward projecting rhizoidal hyphae. Coiled hypha (arrowed C in Fig. 7). 10: Released 2-septate conidium. 11, 12: Conidiogenous cells (arrowed) with dark brown, septate, branched rhizoids. 13–18: Conidia 2–3 septate, obovate or oval, the apical cell with a septum (the 3rd septum) in Figs. 15 and 17, sometimes appearing as a dark band (Figs. 13, 14, 16, 18). Scale bars: 1 = 250 μm; 2 = 100 μm; 3 = 25 μm; 4 = 2 cm; 5 = 100 μm; 6 = 15 μm; 7–10 = 30 μm; 11, 12 = 10 μm; 13–18 = 30 μm.

North Queensland but gave no data.

The genera *Savoryella* and *Ascotaiwania* have many common features and both have been tentatively assigned to the Amphisphaeriaceae (Eriksson and Hawksworth, 1987; Sivanesan and Chang, 1992). However, Chang et al. (1998) after an ultrastructural study of *A. lignicola*, and compared with *Savoryella* species (Read et al., 1992) concluded that both genera be retained. With respect to placement in a higher taxon, Wong et al. (1998) propose to erect a new family – the Annulatascaceae, to accommodate several freshwater genera with a well-developed apical apparatus. *Ascotaiwania* with its well-developed, non-amyloid apical ring (Fig. 6), with pendant would fit well in this proposed family.

There is interest in linking so-called freshwater ascomycetes with deuteromycetous counterparts. Sivanesan and Chang (1992) recorded that ascospores of *A. sawada* germinated from one or both ends but that "attempts to grow the ascospores in culture were unsuccessful." The Thai material of *A. sawada* was easily isolated onto CMA.

Without any special treatment an anamorph developed in single spore isolates of *A. sawada*. This resembles several dematiaceous hyphomycetes: *Monotosporella* S. J. Hughes, *Brachysporiella* M. B. Ellis, *Acrogenospora* M. B. Ellis and *Monotospora* Sacc. (Table 2). Our species most closely resembles *Monotosporella rhizoidea* Vasant Rao & de Hoog (Rao and de Hoog, 1986). *Monotosporella* S. J. Hughes (1958) – with *M. setosa* (Berk. & Curt.) S. J. Hughes as the type – was erected to replace *Monosporella* S. J. Hughes (Hughes, 1958) which was a later homonym of *Monosporella* Keilin (Hughes, 1958). Hughes (1958) erected *Monotosporella* for species with unbranched, percurrent conidiophores. *Monotosporella setosa* was later transferred to *Brachysporiella* Batista by Ellis (1959) with the species *B. gayana* Batista, *B. setosa* (Berk. & Curt.) M. B. Ellis and *B. turbinata* (Cooke & Harkn.) M. B. Ellis. However, *Brachysporiella* spp. generally have erect conidiophores with short branches. Later, Ellis (1976) transferred *Endophragmia laxa* Hudson to *Brachysporiella*. Ellis (1959, 1976) clearly did not accept the genus *Monotosporella* as it was considered by Hughes (1958). He preferred to distribute *Monotosporella* spp. between *Brachysporiella* and *Acrogenospora*.

Another similar genus is *Acrogenospora* M. B. Ellis (established to contain *Monotospora sphaerocephala* Berk. & Broome) which had previously been transferred to *Monotosporella* by Hughes (1958). *Acrogenospora*, however, is recognized as having percurrent conidiogenous cells and black aseptate conidia. According to these features the Thai material could not be accommodated in this genus. *Monotospora* Corda is considered a nomen dubium while *Monotospora* Sacc. has been treated as a synonym of *Acrogenospora* by Hawksworth et al. (1995). Ellis (1976) assigned *Monotosporella setiformis* (Wallr.) S. J. Hughes to it. Hughes (1979), however, considered *Acrogenospora* and *Monotosporella* to be closely related if not congeneric-especially when based on their teleomorph connections which are placed in the genus *Farlowiella* Sacc. (Hysteriaceae: Dothideales). All these fungi grow on well-decayed wood.

We have examined the authenticated material of various *Brachysporiella* spp. at Herb IMI. There are several significant differences between the Thai fungus and species assigned to *Brachysporiella*. In the latter genus conidiophores are long (>250 μm) and branched, at their tips. They form bud-like conidiogenous cells from which solitary conidia are produced. Released conidia often have one or two of these bud-like conidiogenous cells attached. *Brachydesmiella* spp. lack the melanised rhizoids reported for *M. rhizoidea*.

Although Hawksworth et al. (1995) follow Ellis in considering *Monotosporella* as a synonym of *Brachysporiella* we disagree. We prefer to follow Hughes

Table 2. Comparison of species assigned to *Monotosporella*, *Brachysporiella* and *Acrogenospora*.

Species	Conidiophores[a)]	Conidia[a)]	Teleomorph	Substratum
Acrogenospora sphaerocephala	3–6-septate, up to 380×9–11	spherical to sub-spherical, 0-septate, 15–33×14–33	*Farlowiella carmichaeliana*	Rotten wood
Brachysporiella gayana	multiseptate, up to 250×8–12	obovate, 2–4-septate, 20–38×12–20, bulbous base to the conidium (type: 12.5–15×20–28)	unknown	date and oil palm
Brachysporiella setosa	3-septate, up to 450×9–13	obovate, 2-septate, 20–40×15–25	unknown	rotten wood
Brachysporiella turbinata	3–4-septate, up to 45×6–8	obovate to obpyriform, 3–5-septate, 20–35×10–16	unknown	*Sequoia* wood
Monotosporella rhizoidea	2-septate, 30–40×4–5	turbinate to obovoidal, 2–3-septate, 35–40×27–35	unknown	rotten bark of *Tectona grandis*
Monotosporella state of *A. sawada*	0–1-septate, 7.5–13.5×5–10	obovate to ovate, 2–3-septate, 25–35×17.5–25	*Ascotaiwania sawada*	rotten wood

a) All measurements: μm.

(1958) and Rao and de Hoog (1986) in treating *Monotosporella* as distinct from *Brachysporiella*. This is based on examination of a range of herbarium material and the cultural work with *A. sawada*. However, there is a problem in that *Monotosporella* is considered to have percurrent proliferation. The Thai collection did not show any evidence of such proliferation. If proliferation is considered important at the generic level then it may be necessary to separate the Thai *Monotosporella* at least into a separate genus.

The Thai fungus most closely resembles *M. rhizoidea* which has nearly black, turbinate, septate conidia. In contrast, *Brachysporiella* species are much lighter in colour, yellowish, pale brown to brown, rarely black. Rao and de Hoog (1986) further noted, however, that the conidia of *M. rhizoidea* are "mostly formed singly at the tips of usually short conidiophores." They did note the occasional percurrent growth which is typical of *Endophragmia* Duvornoy & Maire. They also noted that this phenomenon was observed in earlier collections of the species. They noted recurvature of conidial proliferation in *Kramabeeja shrungashakha* Vasant Rao & K. A. N. Reddy and in *Sporidesmium arengae* Mats. However, Hawksworth et al. (1995) consider *Kramabeeja* to be a synonym of *Brachysporiella*.

After examining several likely genera we conclude that *Monotosporella* is the most appropriate genus for the anamorphic stage of *A. sawada*. Our species differs from *M. rhizoidea* in several respects: the conidiophores are longer in the latter species, the terminal cell of the conidia never becomes septate and the conidia are larger than those of the Thai species (Table 2). Also Rao and de Hoog (1986) note the presence of percurrent proliferation in *M. rhizoidea* which we failed to demonstrate in the Thai collection. Consequently we recognize the Thai collection as a new species. However, as this is only known from culture and is linked already to a described teleomorph it may be known as the *Monotosporella* state of *A. sawada*.

Brachysporiella and *Monotosporella* species are common on decaying wood with many collections in Herb. IMI from Malaysia and deposited by Mr. Sulaiman, Professor Nawawi and Professor Kuthubutheen. Additionally, Hyde and Goh (1997) have collected *M. setosa* on decaying wood in a freshwater stream on Mt. Lewis, Queensland, Australia. Table 2 summarises the differences between species that are similar to the Thai *Monotosporella*.

Acknowledgements——Professor Gareth Jones is grateful to the British Council and BIOTEC for financial support for this collaborative research programme and to the Royal Society and the City University of Hong Kong for the award of a Kan Tang Po Visiting Professorship. We are grateful to the Director, Khao Yai National Park for permission to collect and for their support; to Dr. Kevin Hyde for his comments on this manuscript and permission to refer to unpublished data; to the Director, IMI, for the use of the herbarium.

Literature cited

Chang, H. S., Hsieh, S.-Y., Jones, E. B. G., Read, S. J. and Moss, S. T. 1998. Aquatic ascomycota: New freshwater species of *Ascotaiwania* and *Savoryella* from Taiwan. Mycol. Res. **102**: 709–718.

Ellis, M. B. 1959. *Clasterosporium* and some allied Dematiaceae-Phragmosporae. II. Mycol. Pap. **72**: 1–75.

Ellis, M. B. 1976. More dematiaceous Hyphomycetes. CMI, Kew, Surrey, UK.

Eriksson, O. and Hawksworth, D. L. 1987. Notes on ascomycete systematics. Nos. 225–463. Syst. Ascomycet. **9**: 11–165.

Goh, T. K. 1997. Tropical freshwater hyphomycetes. In: Biodiversity of tropical microfungi, (ed. by Hyde, K. D.), pp. 189–228. Hong Kong University Press, Hong Kong.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. 1995. Ainsworth and Bisby's Dictionary of the fungi, 8th ed. CAB International, Wallingford, Oxon, UK.

Hughes, S. J. 1958. Revisiones Hyphomycetum aliquot cum appendice de nominibus rejiviendis. Can. J. Bot. **35**: 727–836.

Hughes, S. J. 1979. Relocation of species of *Endophragmia* with notes on relevant generic names. New Zealand J. Bot. **17**: 139–188.

Hyde, K. D. 1995. Fungi on palms XXV. A new species of *Ascotaiwania*. Sydowia **47**: 31–37.

Hyde, K. D. and Goh, T. K. 1997. Fungi on submerged wood in a small stream on Mt. Lewis, North Queensland, Australia. Muelleria **10**: 145–151.

Hyde, K. D., Wong, S. W. and Jones, E. B. G. 1997. Freshwater ascomycetes. In: Biodiversity of tropical microfungi, (ed. by Hyde, K. D.), pp. 179–188. Hong Kong Univ. Press, Hong Kong.

Rao, V. and de Hoog, G. S. 1986. New or critical hyphomycetes from India. Stud. Mycol. **28**: 1–84.

Read, S. J., Jones, E. B. G. and Moss, S. T. 1992. Taxonomic studies of marine Ascomycotina: ultrastructure of the asci, ascospores and appendages of *Savoryella* species. Can. J. Bot. **71**: 273–283.

Shearer, C. A. 1993. The freshwater ascomycetes. Nova Hedwigia **56**: 1–33.

Sivanesan, A. and Chang, H. S. 1992. *Ascotaiwania*, a new amphisphaeriaceous ascomycete genus on wood from Taiwan. Mycol. Res. **96**: 481–484.

Wong, S. W., Hyde, K. D. and Jones, E. B. G. 1998. Annulatascaeae, a new ascomycete family from the tropics. Syst. Ascomycet. **16**: 17–25.