# *Tamsiniella labiosa* gen. et sp.nov., a new freshwater ascomycete from submerged wood

**Sze-Wing Wong, Kevin D. Hyde, Wai-Hong Ho, and Susan J. Stanley**

**Abstract**: Investigations into the fungi occurring on wood submerged in freshwater ecosystems have revealed a unique, but characteristic group of fungi. In this paper a new pyrenomycete, *Tamsiniella labiosa* gen. et sp.nov., is described and illustrated with light, scanning, and transmission electron micrographs. The genus has remarkable short stipitate cylindrical asci with an internal refractive apical ring that are apically truncate and have an external thickening. Ascospores are ellipsoidal–fusiform and surrounded by a mucilaginous sheath. At the transmission electron microscope level, the annulus part of the ascus apical apparatus is differentiated from the inner ascus wall layer and is composed of horizontally oriented, electron-dense fibrillar material. A narrow plug is present in the centre of the apical ring. An electron-dense amorphous region occurs between the outer ascus wall layer and the annulus part of the apical apparatus. The outer ascus wall layer is lacking at the apex. The ultrastructure of the ascus apex differs from those described in the Lasiosphaeriaceae, Sordariaceae, and Xylariaceae.

*Key words*: aquatic fungi, *Myelosperma*, new genus, transmission electron microscope.

**Résumé** : Des recherches effectuées sur les champignons qui poussent sur le bois submergé, en eau douce, a permis d'observer un groupe unique et caractéristique de champignons. Dans ce travail, les auteurs décrivent et illustrent le *Tamsiniella labiosa* gen. et sp.nov., à l'aide de micrographies obtenues en microscopie photonique, et électronique par balayage et par transmission. Le genre montre des asques remarquables, courts, stipités et cylindriques ayant un anneau apical interne réfringent, tronqués apicalement et munis d'un épaississement externe. Les ascospores sont ellipsoïdes–fusiformes et entourées d'une enveloppe mucilagineuse. La microscopie électronique par transmission permet de distinguer la partie annulaire de l'appareil apical de l'asque, de la couche pariétale interne de l'asque, qui est composée de matériel fibrillaire dense aux électrons et orienté horizontalement. On trouve un bouchon étroit dans le centre de l'anneau apical et une région amorphe, dense aux électrons, entre la couche pariétale externe de l'asque et la partie annulaire de l'appareil apical. La couche pariétale externe de l'asque est absente à l'apex. L'ultrastructure de l'apex de l'asque diffère de celles déjà décrites chez les Lasiosphaeriaceae, Sordariaceae et Xylariaceae.

*Mots clés* : champignons aquatiques, *Myelosperma*, genre nouveau, microscopie électronique par transmission.

[Traduit par la rédaction]

## Introduction

Several fungi from wood submerged in tropical rivers in northern Queensland, Australia, were described by Hyde (1992, 1995, 1996) and Hyde et al. (1997). These aquatic fungi have proved to be a common group and many have a pantropical distribution (Goh and Hyde 1996). Most of the unitunicate ascomycete species have cylindrical asci with refractive apical rings, and the ascospores have a sheath or appendages, e.g., *Annulatascus* K.D. Hyde (Hyde 1992, 1995), *Submersisphaeria* K.D. Hyde (Hyde 1996), and *Rivulicola* K.D. Hyde (Hyde et al. 1997). In this paper a new taxon, *Tamsiniella labiosa* gen. et sp.nov., is described. It has short-stalked cylindrical asci with truncate apices, with an internal refractive apical ring and unusual external thickenings. Ascospores are ellipsoidal–fusiform and surrounded by a mucilaginous sheath.

There are no similar species occurring in marine or freshwater habitats, although the asci in *Tamsiniella* are reminiscent of *Myelosperma* Syd. & P. Syd. In *Myelosperma* species, asci have rounded apical ends and ascospores are constricted at the centre (Hyde 1993). *Myelosperma* is a genus presently only known from palms and in the type species, *Myelosperma tumidum* Syd. & P. Syd., ascomata cluster around a common central pore (Hyde 1993). *Myelosperma* is presently placed in the Lasiosphaeriaceae (sensu Hawksworth et al. 1995), and *Tamsiniella* is placed among the genera incertae cedis based on its unique ascus morphology. However, both genera are unlike species in the type genus *Lasiosphaeria* Ces. & De Not. that have superficial ascomata and long cylindrical asci with an apical ring. It may be that a new family or families will eventually be required for *Myelosperma*, *Tamsiniella*, and related genera.

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**S.W. Wong,[1] K.D. Hyde, W.H. Ho, and S.J. Stanley.**
Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong.

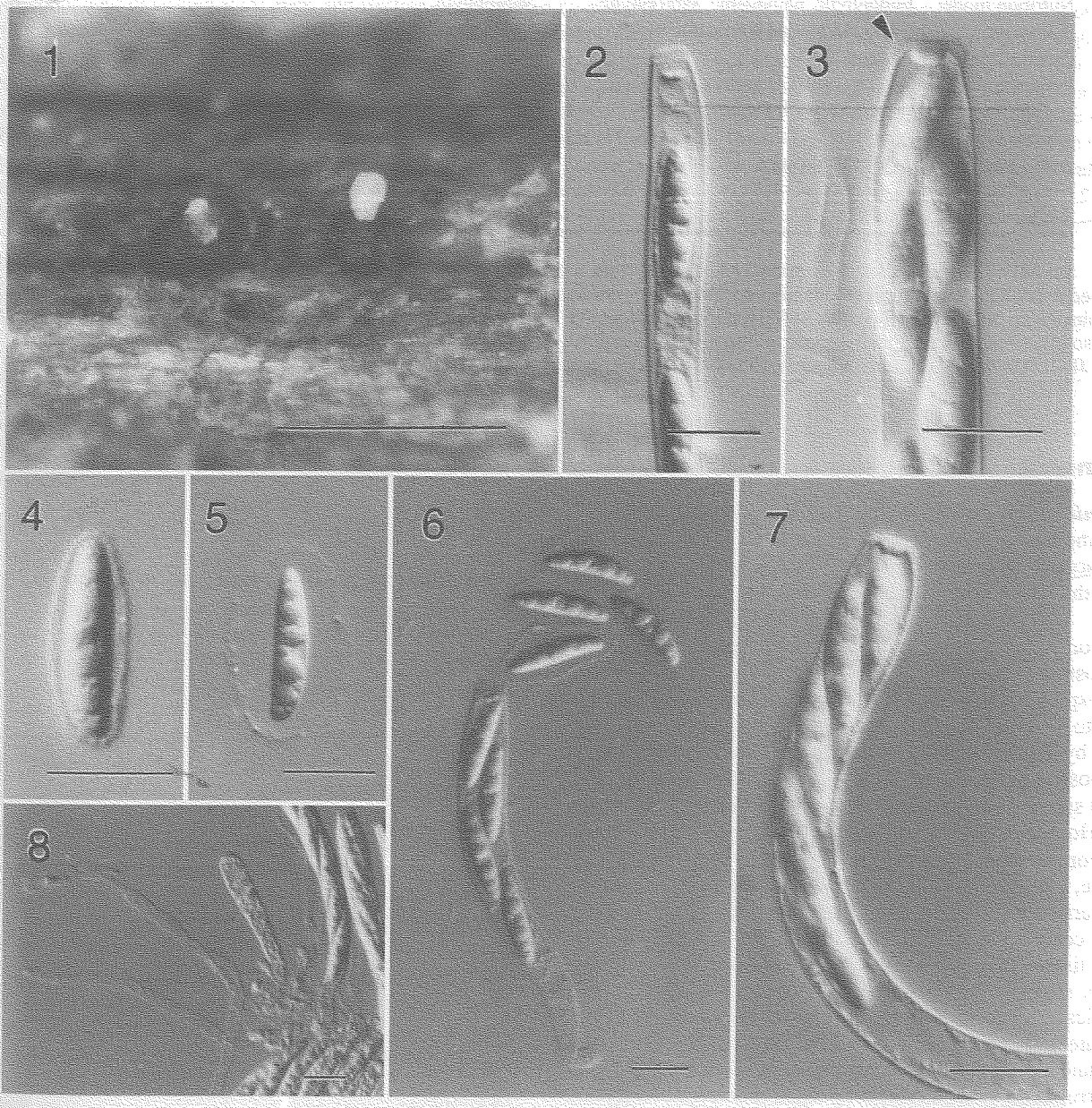
[1] Author to whom all correspondence should be addressed.
e-mail: wswwongb@hkucc.hku.hk

## Materials and methods

### Light microscopy

Semi-immersed ascomata on wood were selected and photographed

**Figs. 1–8.** Light micrographs of *Tamsiniella labiosa* (from holotype). Fig. 1. Ascomata on host surface. Scale bar = 0.5 mm. Fig. 2. Swollen thickening at the ascus apex and annulus, which is located in the subapical region. Fig. 3. Ascus apical ring and external thickening (arrowhead). Fig. 4. Ascospore with a mucilaginous sheath. Fig. 5. As in Fig. 4, but sheath is swollen in water. Fig. 6. Ascus containing ascospores, with a further four released ascospores. Fig. 7. The ascus swells and elongates prior to the release of ascospores and the ascospores move to the apex of the ascus. Fig. 8. Paraphyses. Scale bars = 10 μm for Figs. 2–8.




using an Olympus SZH10 stereomicroscope. Immersed ascomata were identified by cutting the surface tissue of the wood using blades. Squash mounts of ascomata in water were prepared on glass slides; paraphyses, asci, and ascospores were examined using a Leitz Dialux 22EB interference microscope. The apical rings of the asci were stained by Melzer's reagent.

### Scanning electron microscopy

An ascospore suspension was settled onto a polycarbonate membrane (Nucleopore) with a pore size of 5 μm. Subsequently, the membranes with settled ascospores were fixed in 2% (w/v) aqueous osmium tetroxide at 4°C overnight. Fixed material was dehydrated through a graded ethanol series from 10 to 90% (in 10% steps), then 95%, and followed by three changes of absolute ethanol. Each of the above changes was for 15 min. After critical point drying in carbon dioxide and sputter coating with gold–palladium, the material was examined in a Leica Cambridge Stereoscan 440 scanning electron microscope operated at 20 kV.

### Transmission electron microscopy

A suspension of fresh asci and ascospores was embedded in 2% (w/v) Ion agar and subsequently fixed in 4% (v/v) glutaraldehyde with added ruthenium red in 0.1 M sodium cacodylate buffer at pH 7.2 for 4 h at room temperature, and postfixed in 2% (w/v) osmium tetroxide with added ruthenium red in 0.1 M sodium cacodylate buffer at pH 7.2 overnight at 4°C. The dehydration process was the same as that described for scanning electron microscopy, but the material was finally transferred to absolute acetone (three times, 15 min each), and

**Table 1.** Comparision of selected characters in *Myelosperma tumidum* and *Tamsiniella labiosa*.

| | *Myelosperma tumidum* (from Hyde 1993) | *Tamsiniella labiosa* |
|---|---|---|
| Host | Palm leaf | Wood |
| Habitat | Terrestrial | Fresh water |
| Nutrition mode | Endophytic, pathogenic, saprophytic | Saprophytic |
| Ascomata | Clustered around a common central pore | Solitary or gregarious |
| Pseudostromata | Present | Absent |
| Peridium | Brown-walled elongate cells fusing outward with the host | One to two layers of brown, elongated, angular cells, lined with a pale brown and disintegrated layer, which is discontinuous at the base |
| Asci | J- subapical ring | J- apical ring, annulus part appears to be located at subapical region when the upper thickened material swells |
| Ascospores | Reniform | Ellipsoidal–fusiform |

embedded in Möllenhauer's resin (Möllenhauer 1964). The sections were stained with lead citrate (Reynolds 1963) for 15 min and uranyl acetate solution for 30 min. Finally, the specimens were examined using a JEOL 100SX transmission electron microscope operated at 80 kV.

## Taxonomy

***Tamsiniella*** S.W. Wong, K.D. Hyde, W.H. Ho & S.J. Stanley, gen.nov.

ETYMOLOGY: Named in honor of Tamsin M. Hyde, who collected this species, for putting up with her father, Kevin D. Hyde.

Ascomata semi-immersa vel immersa, atrobrunnea, subglobosa, ostiolata, papillata, periphysata, solitaria vel gregaria. Asci 8-spori, cylindrici, breve pedicellati, unitunicati, truncati, apparato apicale praediti. Ascosporae uniseriatae vel biseriatae, hyalinae, ellipsoideo–fusiformes, unicellularis, tunica gelatinosa praeditae.

TYPE SPECIES: *Tamsiniella labiosa* S.W. Wong, K.D. Hyde, W.H. Ho & S.J. Stanley.

Ascomata semi-immersed to immersed, dark brown, subglobose, ostiolate, papillate, periphysate, solitary or gregarious. Peridium comprising 1–2 layers of elongated angular, brown cells, lined with a layer of very pale brown, disintegrated tissue, discontinuous at the base. Paraphyses cellular, septate, sparse. Asci 8-spored, cylindrical, short pedicellate, unitunicate, apically truncate, with a nonamyloid, refractive, bifurcate apical ring and an external thickening. Ascospores uniseriate to biseriate, hyaline, ellipsoidal–fusiform, unicellular, surrounded by a narrow, roughened mucilaginous sheath.

***Tamsiniella labiosa*** S.W. Wong, K.D. Hyde, W.H. Ho & S.J. Stanley, sp.nov.

Figs. 1–10

ETYMOLOGY: From the Latin labiosa, meaning conspicuously lipped.

Ascomata semi-immersa vel immersa, atrobrunnea, subglobosa, ostiolata, papillata, periphysata, solitaria vel gregaria, 130–225 μm alta, 180–250 μm diam. Asci 8-spori, cylindrici, breve pedicellati, unitunicati, truncati, apparato apicale praediti, 80–102 × 8–10.5 μm. Ascosporae imbricatus 1–2 seriate, hyalinae, ellipsoideo–fusiformes, unicellularis, tunica gelatinosa praeditae, (12–)15–21 × 3.8–4.5(–5) μm.

HOLOTYPUS: Australia, north Queensland, Mount Lewis, ad lignum submersum, July 1993, T.M. et K.D. Hyde ML9 (HKU (M) 2276).

Ascomata semi-immersed to immersed, dark brown, subglobose, ostiolate, papillate, solitary or gregarious, 130–225 μm high, 180–250 μm diam. (Fig. 1). Neck conical, comprising dark brown pseudoparenchymatous cells and melanised host tissue, periphysate, ca. 20 μm high, 46 μm wide (Figs. 9, 10). Periphyses simple, filamentous, hyaline, aseptate, ca. 1 μm diam. Peridium 5–11 μm thick, thicker near the ostiole (to 14 μm), comprising 1–2 layers of elongated angular, brown cells, 5–9 × 3–5 μm (Fig. 10); lined with a layer of very pale brown, disintegrated tissue, up to 14 μm thick, discontinuous at the base. Paraphyses 4–5 μm wide at the base, cellular, septate, sparse (Fig. 8). Asci 8-spored, cylindrical, short pedicellate, unitunicate, apically truncate, with a nonamyloid, refractive bifurcate apical ring and an external thickening, 80–102 × 8–10.5 μm ($\bar{x}$ = 89 × 8.7 μm, $n$ = 30) (Figs. 2, 3, 6, 7). Ascospores overlapping 1–2 seriate (Fig. 7), hyaline, ellipsoidal–fusiform, unicellular, surrounded by a narrow roughened mucilaginous sheath, (12–)15–21 × 3.8–4.5(–5) μm ($\bar{x}$ = 17.7 × 4 μm, $n$ = 50) (Figs. 4–6).

HABITAT: Saprobic on submerged wood in fresh water.

KNOWN DISTRIBUTION: Australia, Hong Kong.

HOLOTYPE: Australia, north Queensland, Mount Lewis, on wood submerged in a small stream, July 1993, T.M. and K.D. Hyde ML9 (HKU (M) 2276).

Other material examined: Australia, north Queensland, Mount Lewis, on submerged wood, 15 July 1993, J. Fröhlich and K. Halfpap (HKU (M) 1546); Hong Kong, New Territories, Tai Po Kau Forest Stream, on submerged wood, 10 Dec. 1995, W.H. Ho WH190 (HKU (M) 2949); ibid. (HKU (M) 2959).

## Ultrastructure

### Scanning electron microscopy

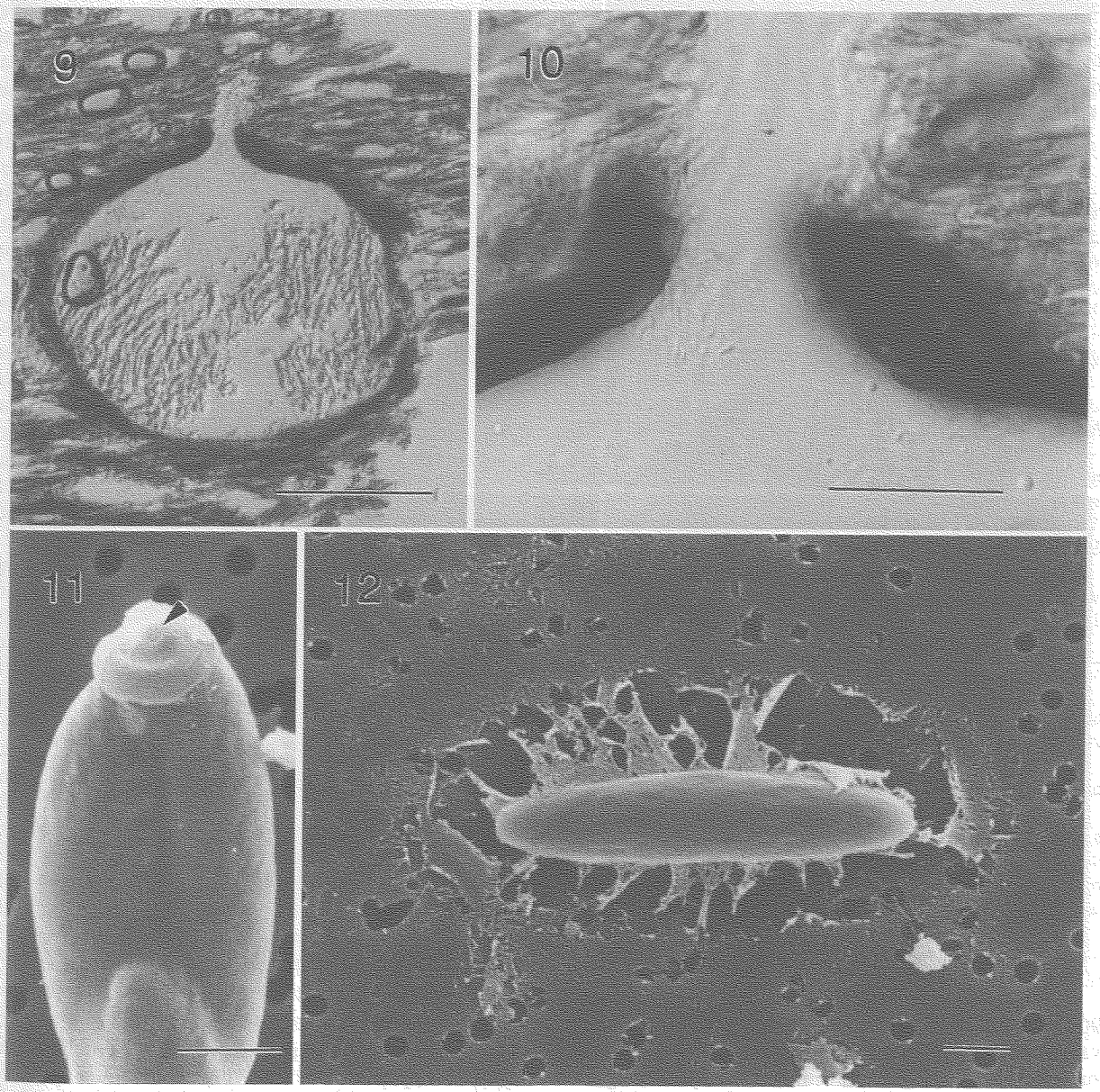
The scanning electron micrographs illustrate the external apical thickening forming a large, ring-like structure at the apex of the ascus (Fig. 11). A pore occurs at the middle of the apical ring (Fig. 11). Ascospores are surrounded by a wide sheath (Fig. 12).

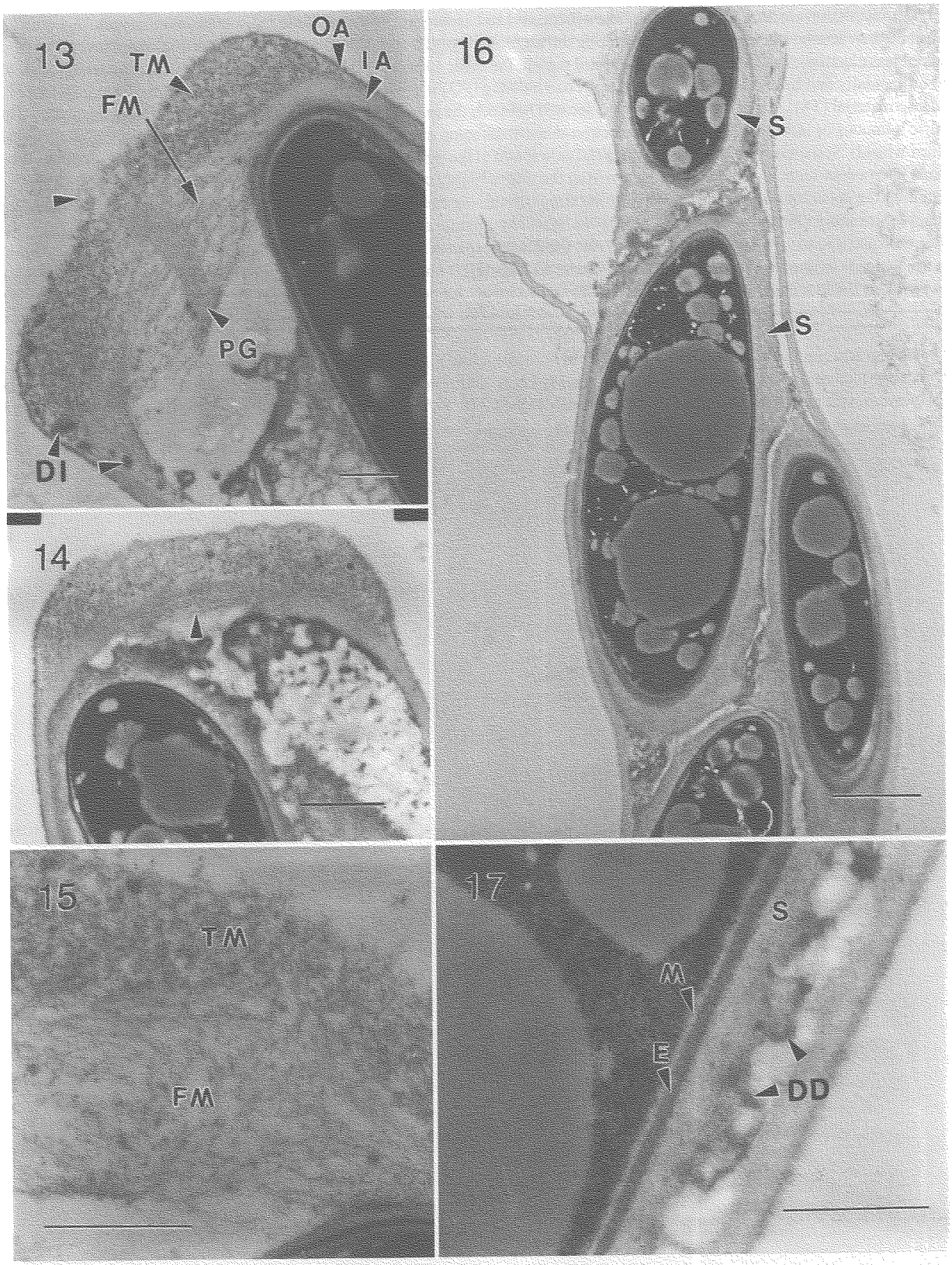
### Transmission electron microscopy

The ascus wall consists of two layers, an outer, thin, electron-dense layer (ca. 30 nm), and an inner, thick, less electron-dense

**Figs. 13–17.** Transmission electron micrographs of the mature asci and ascospores of *Tamsiniella labiosa* (from holotype). Fig. 13. Longitudinal median section of the ascus apex illustrating the ascus wall, which comprises an outer, thin, electron-dense layer (OA) and an inner, thicker, less electron-dense layer (IA). At the apical region, the inner ascus wall (IA) differentiates into fibrillar material (FM), which is orientated horizontally and forms the lower apical ring. A narrow plug (PG) is present at the middle of the apical ring. A thickening of electron-dense material (TM) (i.e., upper apical ring) is located between the inner (IA) and outer (OA) ascus walls. Note the outer ascus wall, which is discontinuous or lacking at the apex, and electron-dense thickening material (TM), which appears to be dissolving at the region above the plug (arrowhead). Some electron-dense inclusions (DI) are found at the peripheral region of the apical ring. Scale bar = 0.5 µm. Fig. 14. Longitudinal nonmedian section of the apical ring showing the ring is highly compressed (arrowhead). Scale bar = 1 µm. Fig. 15. High magnification of the apical ring illustrating a lower apical ring, comprising fibrillar material (FM); and an upper apical ring, comprising an electron-dense thickening (TM). Scale bar = 5 µm. Fig. 16. Asci with mature ascospores surrounded by a mucilaginous sheath (S). The sheath is thin when the spores remain in the ascus. Subsequently, the sheath expands in water. Scale bar = 5 µm. Fig. 17. Mucilaginous sheath (S) of an ascospore, which is amorphous with some electron-dense deposits (DD) lining the periphery of the sheath. The mature ascospore wall comprises an outer, thicker, electron-dense episporium (E) and an inner, thinner, electron-transparent mesosporium (M). Scale bar = 0.5 µm.

**Figs. 9, 10.** Light micrographs of *Tamsiniella labiosa* (from holotype). Fig. 9. Ascoma, median transverse section. Scale bar = 100 µm. Fig. 10. Detail of ascomatal neck. Scale bar = 20 µm. **Figs. 11, 12.** Scanning electron micrographs. Fig. 11. A ring-like structure is found at the apex of ascus with a pore in the middle of the ring (arrowhead). Fig. 12. Ascospore surrounded by a wide sheath. Scale bars = 2.5 µm.






layer (90–150 nm) (Fig. 13). At the apical region, the inner ascus wall differentiates into electron-dense fibrillar material that is oriented horizontally to form the lower apical ring. A thickening of more electron-dense material is present between the outer and the inner ascus wall layers and forms the upper apical ring. The outer ascus wall layer, however, is discontinuous or absent at the apex. In addition, some electron-dense material appears to be dissolving at the region above the plug. Some electron-dense inclusions are found within the peripheral regions of the lower and upper apical ring. Prior to the

release of the ascospores, the apical ring is highly compressed, which may be a result of the pressure building up inside the ascus (Fig. 14).

Mature ascospores are fusiform or ellipsoidal, overlapping uniseriate within the ascus (Fig. 16), and surrounded by a mucilaginous sheath (Figs. 16, 17). The mucilaginous sheath is thin when the ascospores remain in the ascus (Fig. 17). Once in water, the mucilaginous material absorbs water and swells to form a wide sheath (Fig. 16). Some electron-dense deposits line the periphery of the sheath (Fig. 17). The wall of mature ascospores comprises an outer electron-dense episporium (ca. 40 nm) and an inner electron-transparent mesosporium (20–30 nm) (Fig. 17). It is unusual that the episporium is thicker than the mesosporium.

## Discussion

The general morphology of the ascus apical ring in *T. labiosa* appears to be unique when compared with members of other families having refractive apical rings, e.g., the Amphisphaeriaceae, Lasiosphaeriaceae, Sordariaceae, and Xylariaceae (sensu Barr 1990). At the ultrastructural level, the morphology and ontogeny of the apical ring differ because the lower ring is directly differentiated from the inner ascus wall layer and has some thickening material (i.e., upper ring) between the outer and inner ascus wall layers. In *Lasiosphaeria*, for example, the apical ring is not directly differentiated from the ascus wall (Griffiths 1973); in *Sordaria*, no partition of the ascus wall is observed (Griffiths 1973); in *Xylaria*, electron-dense material is deposited within the ring (Beckett et al. 1974); and in *Nectria*, the ring is amorphous throughout, rather than comprising horizontally oriented electron-dense fibrillar material (Beckett et al. 1974).

Although the characteristics of asci and ascospores of *T. labiosa* are superficially similar to those of *M. tumidium* at light-microscope level (Hyde 1993), they have different host specificity, nutrition mode, ascomata arrangement, peridium composition, and ascospore shape (Table 1), and their ascus apical rings also differ at the ultrastructural level. In *Myelosperma*, electron-dense deposits occur within the ring, and the outer ascus wall is continuous over the ascus apex (K.D. Hyde and S.W. Wong, personal observation). Ascus morphology, including the ascus wall and apical ring, is considered to be taxonomically significant at the family level (Verkley 1992, 1993). This would indicate that *T. labiosa* is unique and requires placement in its own family. However, it is premature to assign it to a new family and it is presently best considered a genus incertae cedis (sensu Hawksworth et al. 1995). The ascus apical ring of *T. labiosa* is also similar to that in other common tropical freshwater ascomycetes, such as *Annulatascus* species (Hyde 1992, 1995) at the light-microscopic level. However, the apical ring of *Annulatascus* species differs in lacking an external thickening and in its ontogeny at the ultrastructural level (Wong 1996). In *Annulatascus* species, the apical ring is bipartite, with the upper part differentiated from the ascus wall, and the lower part elongating downwards during maturation (S.W. Wong, W.H. Ho, K.D. Hyde, and E.B.G. Jones, personal observation).

A large and refractive ascus apical ring is a common character in freshwater ascomycetes, e.g., *Annulatascus* K.D. Hyde (1992), *Ascotaiwania* Sivanesan and Chang (1992), *Rivulicola* K.D. Hyde (Hyde et al. 1997), and *Submersisphaeria* K.D. Hyde (1996). The forcible ejection of ascospores in freshwater ascomycetes is considered an important mechanism in maintaining their population in fast-flowing rivers (K.D. Hyde, T.K. Goh, and S.W. Wong, unpublished data). However, in *T. labiosa*, the function of the external thickening located above the lower apical ring is unknown. The ultrastructural data indicate that the external thickening is thinner towards the region above the plug, and some electron-dense material dissolves in this region. These characteristics indicate that the apical ring is possibly used for active ejection of the ascospores (see Fig. 6), and the external thickening may have evolved to increase the strength of the apical ring, and thus discharge.

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