Cryptogamie, Mycologie, 2011, 32 (1): 3-12

Studies on *Microthyriaceae*: placement of *Actinomyxa*, *Asteritea*, *Cirsosina*, *Polystomellina* and *Stegothyrium*

Haixia WU[a], Kevin D. HYDE[a, b, c] & Hang CHEN[a]

[a]International Fungal Research and Development Centre, the Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming 650224, China, e-mail: haixiawu1983@126.com

[b]School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand

[c]Botany and Microbiology Department, College of Science, King Saud University, Riyadh 11442, Saudi Arabia

Abstract – *Actinomyxa*, *Asteritea*, *Cirsosina*, *Polystomellina* and *Stegothyrium* are genera presently included in *Microthyriaceae*. We have examined the generic types and consider that their characters differ from those of *Microthyriaceae*. In *Actinomyxa* ascoma dissolve as a slimy mass in water and ostioles are star-like, thus the genus would be better placed in *Stictidaceae*. *Asteritea* has flattened ascomata with a star-like opening and superficial mycelium with hyphopodia and is better placed in *Asterinaceae*. *Cirsosin*a has superficial hyphae without hyphopodia and elongate ascomata opening by a longitudinal split and is better placed in *Aulographaceae*. In *Polystomellina* ascomata also open by a longitudinal slit and have superficial hyphae and is better placed in *Asterinaceae*. *Stegothyrium* is a very unusual genus as it has thick-walled asci that appear to be bitunicate, but there is a J+ apical ring which is indicative of unitunicate asci. We suggest that *Stegothyrium* is excluded from *Microthyriaceae* and placed in *ascomycetes incertae sedis* until fresh collections can be made and molecular sequence data obtained and only then can we solve the problem of a suitable placement for this genus.

***Asterinaceae* / *Aulographaceae* / *Dothideomycetes* / *Stictidaceae* / taxonomy**

INTRODUCTION

We are studying genera in the *Dothideomycetes* to provide detailed descriptions and illustrate the generic types with a view to providing a natural classification (Zhang et al. 2008, 2009; Wu et al. 2010; Li et al. 2010).

The present paper deals with the generic types of *Actinomyxa* Syd. & P. Syd, *Asteritea* Bat. & R. Garnier, *Cirsosina* Bat. & J.L. Bezerra, *Polystomellina* Bat. & A.F. Vital and *Stegothyrium* Höhn., genera presently included in *Microthyriaceae* (Sydow, H & Sydow, P., 1917; Batista, A. C. & R. Garnier, Maia, H. da S., 1961; Batista. & J.L. Bezerra, 1960; Batista. & A.F. Vital, 1958; Höhnel, F. von, 1918). All genera are excluded from the *Microthyriaceae* and alternative placements suggested. The type species of each genus is fully described and illustrated.

MATERIALS AND METHODS

Type specimens were obtained from S, URM and K. Ascomata were rehydrated in 3% KOH prior to examination and sectioning. Specimens were examined under a stereo microscope (Leica MZ16A) and fine forceps were used to remove one or two ascomata, which were mounted in water. Melzer's or cotton blue reagents were used to stain microstructures. Observations and micrographs were made under light microscopes (Nikon E800 and Leica DM3000). For some hyaline structures differential interference contrast microscopy was used. Hand sections were cut with a sharp razor blade and thin (8 μm) sections were cut using a Leica CM1100 freezing microtome. The sections were transferred to a drop of water or lactic acid and a drop of cotton blue for examination and photography.

TAXONOMY

Actinomyxa Syd. & P. Syd., Annls mycol. 15(1/2): 146 (1917).

Ascomata superficial, scattered, brown to black, soft, rounded, ostioles opening in an irregular stellate manner, when placed in water ostiole dissolving and fruit body becoming a slimy mass. Asci 8-spored, cylindrical to clavate, paraphyses present. Ascospores clavate, hyaline, 3-4-septate.

Type species:

Actinomyxa australiensis Syd. & P. Syd., Annals mycol. 15(1/2): 146 (1917). Fig.1A-I.

Parasitic or saprobic on the lower surface of hairy leaves forming small black spots, sparse, spots emerging with hairs (Figs. A, B). Superficial mycelium not present. Ascomata 310-330 μm diam. × 105-160 μm high, scattered, superficial, brown to black, soft, rounded and dome-like in section (Figs. C, D), ostioles opening in an irregular stellate manner, when placed in water ostiole dissolving and fruit body becomes a slimy mass. Peridium 19-25 μm thick, one-layered, composed of brown-black, parallel, pseudoparenchymatous cells of compact *textura prismatica*. Hamathecium of dense, long paraphyses, 1.5 μm wide, asci in parallel arrangement (Figs. E, F, G). Asci 60-75 × 7-12 μm (mean = 68.6 × 9.5 μm), 8-spored, fissitunicate dehiscence not seen, cylindro-clavate, with a long pedicel 24-32 × 2-3.5 μm (Figs. J, K, L). Ascospores 13-15 × 2-3 μm (mean = 14.6 × 2.5 μm), overlapping uniseriate, clavate to fusiform with tapering ends, apex rounded and base tapering when clavate, hyaline, 3-septate, smooth walled (FIGS. H, I).

Material examined: AUSTRALIA, on *Lasiopetala ferruginea* var. *cordata,* October 1899, Maiden (comm. J. Bornmüller), (S F12590, holotype).

Remarks: Actinomyxa was formally established by Sydow. & P. Sydow (1917) as a monotypic genus represented by *Actinomyxa australiensis* placed in *Microthyriaceae*. No other species have been included in the genus. Luttrell (1973) followed Sydow's family placement as do Lumbsch and Huhndorf (2007). Morphological characters of *Actinomyxa australiensis* are typical of genera in *Stictidaceae* where it is most similar to *Stictis* (Sherwood, 1977). Fresh collections are needed so that DNA sequence data can be used to clarify the taxonomic placement of the genus.

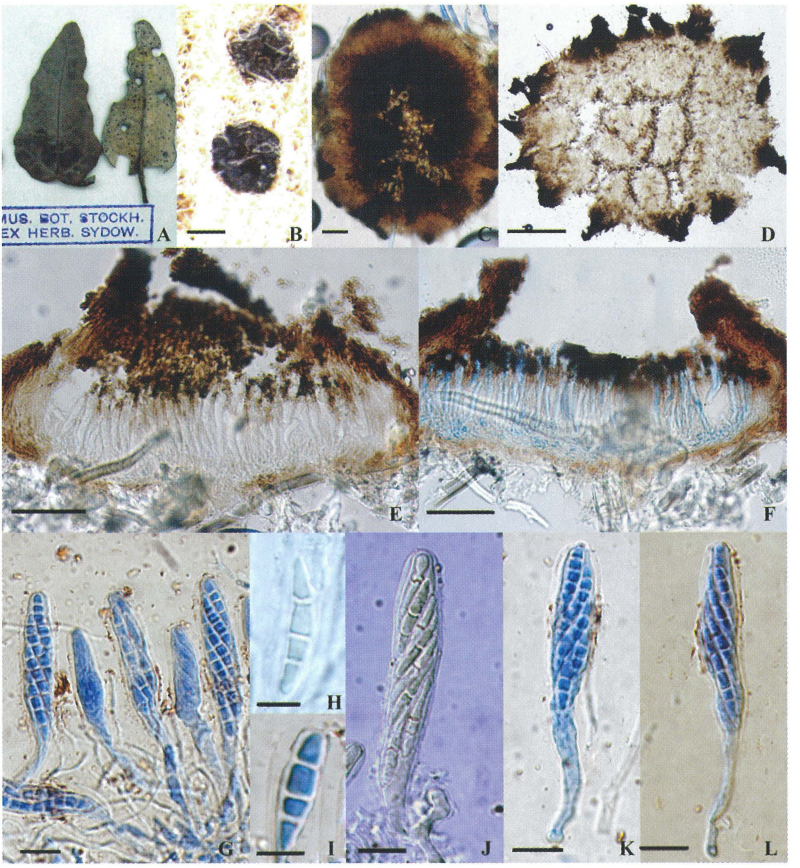

Fig. 1. ***Actinomyxa australiensis*** **(holotype). A, B.** Appearance of fungi body on lower surface of leaf. Note the ascomata in B. **C, D.** Squash mount of ascoma. Note the stellate ostiolar opening. **E, F.** Section of ascoma. Note the peridium which comprises one layer of parellel cells and hamathecium. **G, J-L.** Asci. Note the long paraphyses in G. G, K, L stained with in Lactophenol Cotton Blue. **H, I.** Ascospores. I stained in Lactophenol Cotton Blue. Scale bars: **B** = 500 µm, **D** = 20 µm, **H, I** = 5 µm, **C, G, J-I** = 10 µm.

Asteritea Bat. & R. Garnier, in Batista, Garnier, Maia & Silva, Brotéria, sér. bot. 30(1-2): 41 (1961).

Ascomata, superficial, flattened, gregarious, with superficial mycelium present, round in outline, opening by stellate ostiole. Asci saccate and sessile. Ascospores ellipsoidal and hyaline becoming brown when mature, 2-celled, constricted at the septum, verruculose.

Type species:

Asteritea roureae Bat. & R. Garnier, in Batista, Garnier, Maia & Silva, Brotéria, sér. bot. 30(1-2): 42 (1961). Figs. 2A-K.

Parasitic or saprobic on the lower surface of leaves forming as black speckles (Figs. A, B), superficial hyphae present and hyphae with hyphopodia.

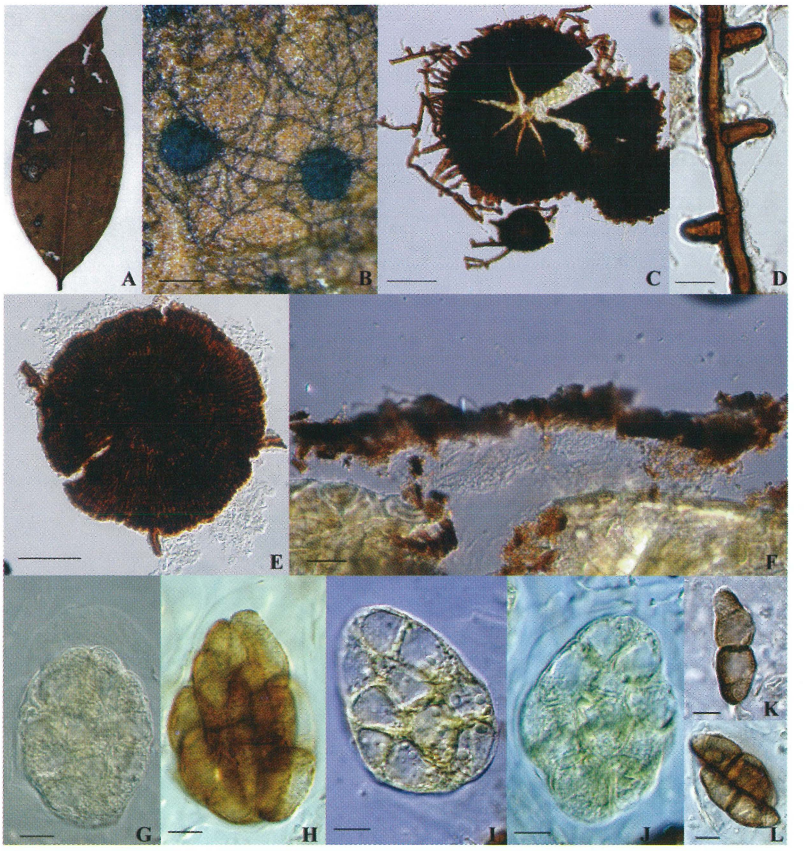

Fig. 2. ***Asteritea roureae*** **(holotype). A, B.** Appearance of ascomata and mycelium on lower surface of leaf. **C, E.** Squash mount of ascoma. Note the radially arranged cells and stellate opening following squashing. D. Superficial hyphae. Note with hyphopodia. **F.** Section of ascoma. Note the peridium which comprises one layer of cells; **G-J.** Asci. Mature in H with sessile base and bitunicate. **K, L.** Ascospores. Note brown at maturity and verruculose. Scale bars: **B** = 500 μm, **C, F** = 20 μm, **D, E, G-L** = 10 μm.

Hyphopodia 10-14 × 6-8 μm short-ellipsoidal (Fig. D). Ascomata 160-270 μm diam., 43-55.5 μm high, gregarious, superficial, flattened, in section dome-shape, round or conical in outline, with a stellate opening, brown to black, membranaceous (Figs. C, E). Peridium 5-10 μm wide, black-brown, comprised of one cell type, of radiating isodiametric cells (Fig. F). Hamathecium not observed. Asci 80-98 × 32-50 μm (mean = 78.5-36 μm), 8-spored, bitunicate, fissitunicate, saccate, sessile (Figs. G-J). Ascospores 41-48 × 15-17 μm (mean = 44-15.6 μm), almost fasciculate, hyaline when young, becoming brown when mature, 2-celled, constricted at the septum, ellipsoidal, verruculose (Figs K-L).

Material examined: BRAZIL, on *Rourea glabra* (*Connaraceae*), collection time unknown, Batista, A.C. (URM 20314, holotype).

Remarks: *Asteritea* was established by Batista et al. (1961) as a monotypic genus represented by *Asteritea roureae* and placed in *Asterinaceae*. Lumbsch and Huhndorf (2007) placed *Asteritea* in *Microthyriaceae*. This genus is parasitic on

leaves having superficial hyphae with hyphopodia, the peridium is comprised of radiating isodiametric cells and asci are bitunicate and globose, while ascospores are two-celled with verruculose walls and brown when mature. Taxa of *Asterinaceae* and *Microthyriaceae* are similar in appearance, having superficial hyphae and shield-shaped ascomata with a radial structure. However, Arx and Müller (1975) consider that *Asterinaceae* are not related to *Microthyriaceae*, but are often confused. In our study, we also agree that *Asterinaceae* and *Microthyriaceae* differ, the ascomata of *Microthyriaceae* have a central ostiole, sometimes surrounded by thickened tissue, and interascal tissue is present. In *Asterinaceae* ascomata lack an ostiolate, but open by star-like or longitudinal splits and interascal tissue is absent (Kirk et al. 2001; Hofmann et al. 2010). In *Asteritea* ascoma open by star-like splits and interascal tissue was not observed and thus we consider the taxon to be asterinaceous. We suggest that *Asteritea* is placed into *Asterinaceae*.

Cirsosina Bat. & J.L. Bezerra, Revta Biol., Lisb. 2(2): 132 (1960).

Superficial mycelium present but hyphopodia absent. Ascomata elongate and oblong, opening with a longitudinal slit. Asci 8-spored, spherical, with a pedicel. Ascospores elliptic, hyaline, two-celled, with guttules and with smooth walled.

Type species:

Cirsosina rhododendri Bat. & J.L. Bezerra, Revta Biol., Lisb. 2: 134 (1960). Figs. 3A-I; Figs. 4 A-B.

Parasitic on the surface of leaves, superficial mycelium present, hyphopodia absent, Ascomata 30-45 µm high × 170-400 µm diameter, scattered, or some gregarious, superficial, black-brown, membranaceous, elongate or oblong and dome-like in section, wall cells parallel, opening with a longitudinal slit (Figs. A, B, C). Peridium 6-9 µm wide at the top and 15-25 µm wide at the base, one-layered, composed of black-brown pseudoparenchymatous cells of compact *textura angularis* (Figs. D, E). Hamathecium of sparse, asci immersed in dense mucilage (Fig. F). Asci 20-40 × 18-30 µm (mean = 26.5 × 22.5 µm), 8-spored, bitunicate, fissitunicate dehiscence not seen, obovoid or nearly spherical, with a pedicel 3 µm wide × 5 µm long or sessile at maturity (Fig. H). Ascospores 16-22 × 5.5-8 µm (mean = 18.3 × 6.6 µm), almost fasciculate, elliptic, rounded at the ends, hyaline, two-celled, slightly constricted at septa, upper cell slightly smaller than lower cell, with one large guttule and many smaller guttules per cell and smooth walled (Figs. G, I).

Material examined: NETHERLANDS, on leaves of *Rhododendron ponticum*, March 4 1959, A.C. Batista & J.L. Bezerra, (URM 11421, holotype).

Remarks: Cirsosina was formally established by Batista et al. (1960), with *Cirsosina rhododendri* as the type species and a second species *Cirsosina calami* Bat. and was placed in *Asterinaceae*, while Lumbsch and Huhndorf (2007) place this genus in *Microthyriaceae*. *Cirsosina rhododendri* has superficial hyphae without hyphopodia and elongate ascomata, opening by a longitudinal slit. Because *Cirsosina* has superficial hyphae without hyphopodia it should not be placed into *Asterinaceae*, and placement in *Aulographaceae* is better. *Aulographaceae* are characterized by brown superficial mycelium which lack hyphopodia and flattened elongate ascomata opening by slit (Kirk et al, 2008; Luttrell, 1973). *Cirsosina* is similar to the *Cirsosia* and only the superficial hyphae

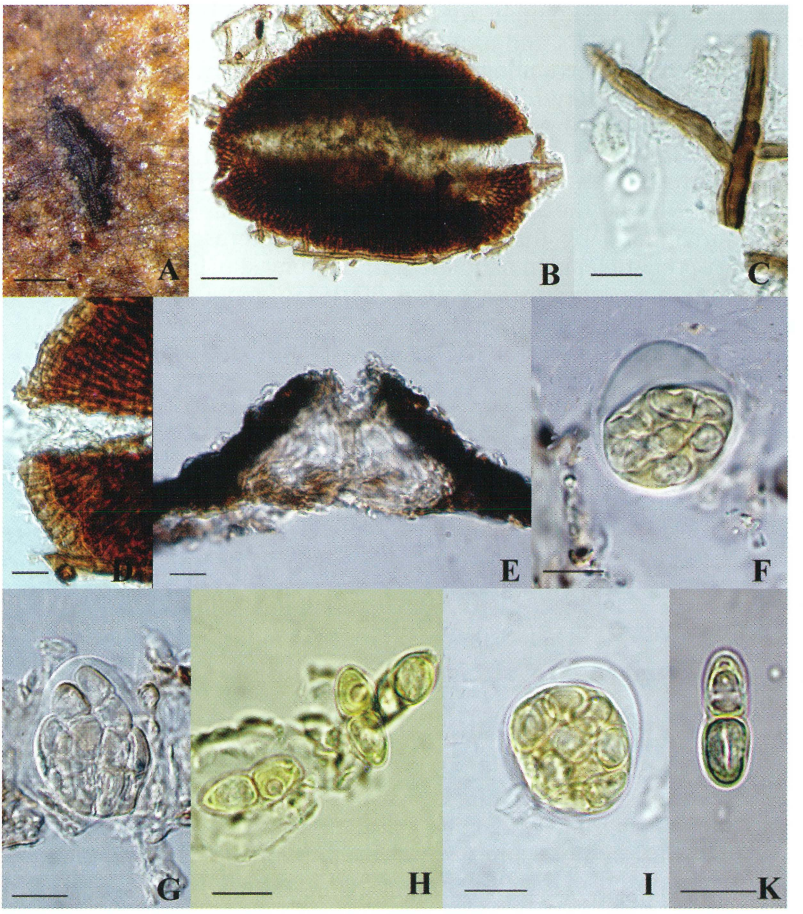

Fig. 3. ***Cirsosina rhododendri*** **(holotype). A.** Appearance of colony and ascomata on the host surface. Note the superficial hyphae. **B, D.** Squash mount of ascoma. **C.** Superficial hyphae without hyphopodia. **E.** Section of ascoma. Note the peridium. **F, I.** Asci, mounted in Melzer's reagent. **G.** Hamathecium. Note asci embeddedwith dense of mucilage. **H, K.** Ascospores. Scale bars: **A** = 500 µm, **B** = 50 µm, **C-K** = 10 µm.

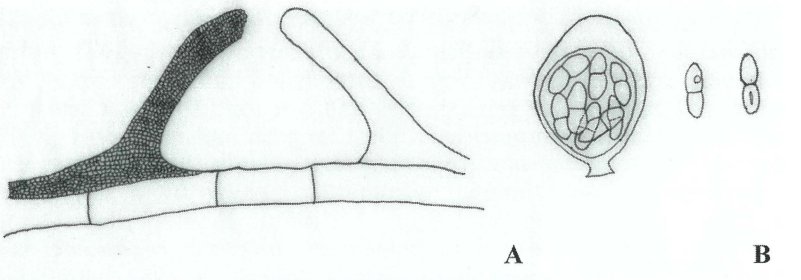

Fig. 4. ***Cirsosina rhododendri*** (line drawing from holotype). **A**. Ascomata. **B.** Asci and Ascospores.

with intercalary hyphopodia in *Cirsosina* can distinguish these genera (von Arx and Müller, 1975). Fresh collections are needed so that DNA sequence data can be used to clarify the taxonomic placement of these genera and also establish the taxonomic importance of characters such as presence or absence of hyphopodia, type of ostiolar opening and presence of pseudoparaphyses.

Polystomellina Bat. & A.F. Vital, Revta Biol., Lisb. 1(3-4): 280 (1958).

Ascomata on the surface of leaves, elongate, sparse, small and not easily seen, superficial, Y-shaped, with longitudinal slit. Asci 8-spored, oblong or nearly spherical. Ascospores two-celled, elliptic, slightly constricted at septa, with guttules and smooth walled.

Type species:

Polystomellina didymopanacis Bat. & A.F. Vital [as "didymopanecis"], Revta Biol., Lisb. 1(3-4): 281 (1958). Figs. 5A-M.

Parasitic or saprobic on the surface of leaves, superficial mycelium not observed, elongate ascomata on the surface of leaves, sparse, small and not easily seen. Ascomata 30-50 μm high × 310-460 μm long and 90-170 μm wide, gregarious, superficial, red-brown, submembranaceous, Y-shaped, irregularly lobed and dome-like in section, with a longitudinal slit-like opening (Figs. A, B, C, D). Peridium 8-11 μm wide, one-layered, composed of black-brown pseudoparenchymatous cells of compact *textura angularis* and cells thick-walled. Hamathecium of sparse asci embedded in mucilage (Figs. E, F). Asci 22-45 × 15-25 μm (mean = 32.5 × 20 μm), 8-spored, bitunicate, fissitunicate dehiscence not seen, obovoid, oblong or nearly spherical, with a short pedicel 3 μm wide × 4 μm long (Figs. G-J). Ascospores 12-18 × 5-6 μm (mean = 15.4 × 5.3 μm), overlapping, elliptic, hyaline, two-celled, slightly constricted at septa with the upper cell smaller than lower cell, every cell with one guttule and smooth walled (Figs. K-M).

Material examined: BRAZIL, on leaves of *Didymopanax morototoni*, collection date unknown, A.C. Batista & A.F. Vital (URM 2699B, holotype).

Remarks: Polystomellina was formally established by Batista et al. (1958), with *Polystomellina didymopanacis* as the type species and was placed in the family *Microthyriaceae* and this was followed by Lumbsch and Huhndorf (2007). *Polystomellina* has elongate ascomata opening by a longitudinal slit, bitunicate and spherical asci, two-celled hyaline ascospores with guttules. These morphological characters are different from the *Microthyriaceae*. We suggest that *Polystomellina* is excluded from *Microthyriaceae*. There has been no molecular work on *Polystomellina*. *Polystomellina* may have a close relationship with *Morenoina Asterinaceae* (Ellis, 1980). We suggest that *Polystomellina* is placed in *Asterinaceae* which includes taxa with ascomata that are flattened and elongate ascomata comprising radiating cells and opening with star-like or longitudinal splitting (Kirk et al, 2008). Fresh collections are needed and DNA sequence data can be used to clarify the taxonomic placement of the genus.

Stegothyrium Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 127: 382 [54 repr.] (1918).

Ascomata superficial and orbiculate, only comprises the upper part, base absent, with a central rounded ostiole. Asci immerged in long paraphyses. Asci unitunicate, with an apical stopper-shaped J+ ring. Ascospores one-celled, elongate-fusoid, hyaline, with one or two guttules, smooth-walled.

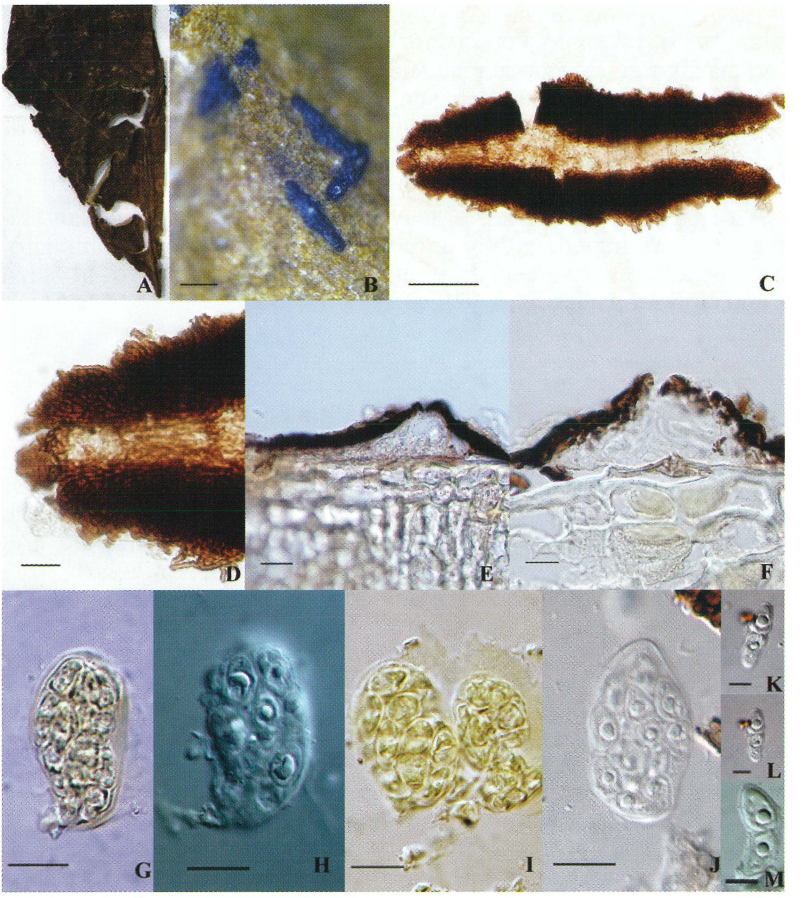

Fig. 5. ***Polystomellina didymopanacis*** **(holotype). A, B.** Appearance of colony and ascomata on the host surface. **C, D.** Squash mount of ascoma. **E, F.** Section of ascoma. Note the peridium. **G-J.** Asci. Note I mounted in Melzer's reagent. **K-M.** Ascospores. Note the two-cells with guttules Scale bars: **B** = 200 µm, **C** = 50 µm, D-F = 20 µm, **G-J** = 10 µm, **K-M** = 5 µm.

Type species:

Stegothyrium denudans (Rehm) Höhn. Sitzber. K. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. I 127: 382 (1918). Figs. 6A-M.

≡ *Muyocopron denudans* Rehm, Hedwigia 42: 292 (1903).

Parasitic or saprobic on leaves, not causing leaf spots, not easily observed. Superficial hyphae not observed. Ascomata 100-140 µm diameter, 17-42 µm high, mostly solitary or less often gregarious, superficial, orbiculate, black, subcarbonaceous; hemispherical or dome-like in section, only comprises the upper part, base absent, with a central rounded ostiole (Figs. A, B, C). Peridium 5-13 µm thick, brown to brown- black, thick at the apex and base, comprising two types of cells, outer cells pseudoparenchymatous, small light yellow-brown, of thin-walled of *textura epidermoidea*, inner cells prosenchymatic, brown thick-walled of t*extura prismatica* (Figs. D, E). Hamathecium of sparse, long paraphyses 2 µm wide

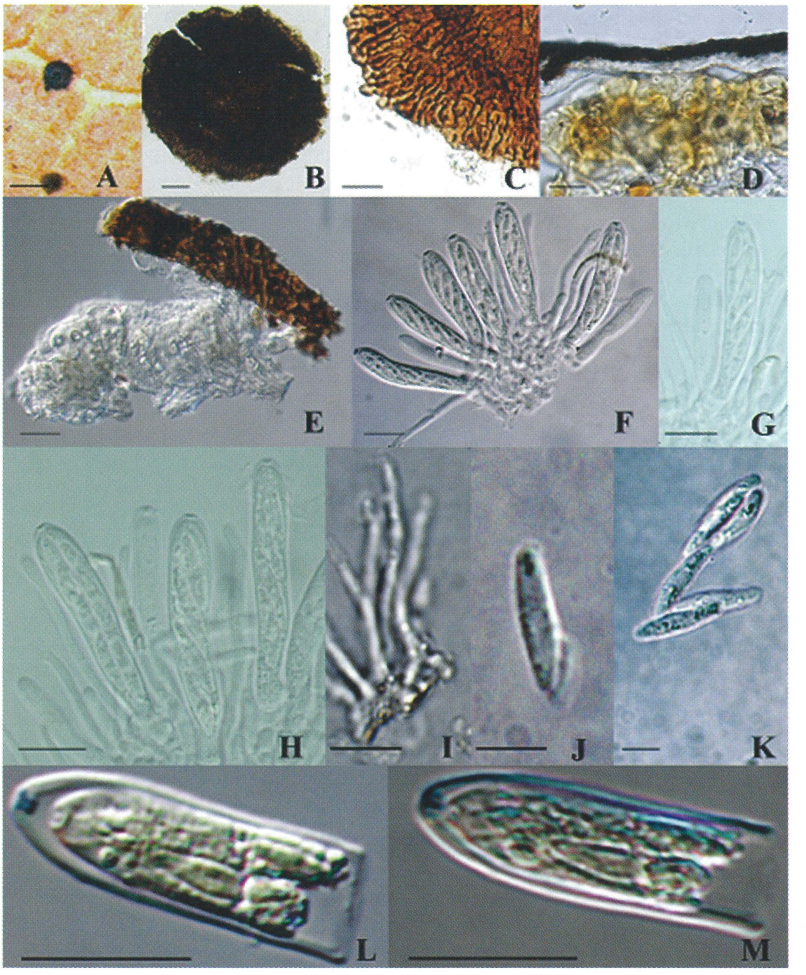

Fig. 6. ***Stegothyrium denudans* (syntype). A.** Ascomata appearance on the host surface. **B, C.** Squash mount of *ascoma*. **D, E.** Section of ascoma. Note the peridium. **F, H, I.** Hamathecium Note the asci in paraphyses, I note paraphyses. **G, L, M.** Asci stained in Melzer's reagent. Note the apical J+ ring. **J, K.** Ascospores. Scale bars: **A** = 200 µm, **B** = 20 µm, **C-M** = 10 µm.

(Figs. F, H, I). Asci 33-45 µm × 5-8 µm (mean = 36.5 × 6.2 µm), 8-spored, unitunicate, clavate, sessile, with 1 apical stopper-shaped J+ ring (Figs. G, L, M). Ascospores 10-14 µm × 3-4 µm ($\bar{x}$ = 12.8 × 3.1 µm), one-celled, overlapping uniserate to biseriate, elongate-fusoid, hyaline, with one or two guttules, smooth-walled.

Material examined: GERMANY, Saxony, on leaves of *Fagus sylvatica* (*Fagaceae*), October, 1902, Rehm (K 158706, syntype).

Remarks: Stegothyrium was first established by Höhnel (1918) as a monotypic genus represented by *Stegothyrium denudans*. It appears to have not been studied since its introduction by Höhnel (1918). Rehm (1903) has originally described this species in the genus *Muyocopron* but it was reassigned to

Stegothyrium by Höhnel (1918). The genus is placed in *Microthyriaceae* by Lumbsch and Huhndorf (2007). This is a very unusual genus as it has thick-walled asci that appear to be bitunicate, but there is a J£´ apical ring, which is indicative of unitunicate asci. Ascospore are elongate-fusoid and one-celled. Paraphyses are present. According to these morphological characters, *Stegothyrium* should be excluded from *Microthyriaceae* and placed in ascomycetae incertae sedis until the species can be recollected and subjected to sequence analysis.

Acknowledgements. The Research Institute of Resource Insects, Chinese Academy of Forestry provided financial support to Wu Haixia Ph.D.'s study. Funds for research were provided by the Grant for Essential Scientific Research of National Non-profit Institute (no. CAFYBB2007002). The curators of the following herbaria are thanked for providing materials on loan for this study: S, URM and K. We also thank Professor Xiaoming Chen for much help.BIOTEC Special Program for Biodiversity Research and Training Program Grant BRTR251181 and R253012 is thanked for partially funding this research.

REFERENCES

ARX J.A. & MULLER E.A., 1975 — A re-evaluation of the bitunicate ascomycetes with keys to families and genera. *Studies in Mycology* 9: 40-46.

BATISTA A.C. & VITAL A.F., 1958 — Dois novos gêneros de fungos Polystomellaceae *Revista de Biologia Lisbõa.* 1(3-4): 280-286.

BATISTA A.C. & BEZERRA J.L., 1960 — *Revista de Biologia Lisbõa.* 2: 134-143.

BATISTA A.C., GARNIER R & MAIA H. DA SILVA., 1961 — *Asteritea* n.gen. e outros Asterinaceae. *Brotéria Sér. Botânica* 30 (1-2): 41-48.

ELLIS J.P., 1980 — The genus *Morenoina* in Britain. *Transactions of the British Mycological Society* 74(2): 297-307.

HOHNEL F.X.R. VON., 1918 — Fragmente zur Mykologie. Sitzungsberichten der Kaiserliche Akademie der Wissenschaften in Wien Mathematische-Naturwissenschaftliche Klasse, Abt. 1 127 (4): 329-393.

KIRK P.M., CANNON P.F., DAVID J.C. & STALPERS J.A., 2001 — Ainsworth & Bisby´s dictionary of the fungi. 9th edn. *CAB International, Wallingford, UK.* 667 p.

KIRK P.M., CANNON P.F., DAVID J.C. & STALPERS J.A., 2008 — Ainsworth & Bisby´s dictionary of the fungi. 10th edn. *CAB International, Wallingford, UK.*

LUTTRELL E. S., 1973 — A Taxonomic Review with Keys: Ascomycetes and Fungi Imperfecti. *The Fungi-an advanced Treatise.* Volume IVA. 7: 135-141.

LUMBSCH H.T. & HUHNDORF S.M. (ed.), 2007 — Outline of Ascomycota – 2007. *Myconet* 13: 1-58.

REHM H.J., 1903 — Beiträge zur Pilzflora von Südamerika. *Hedwigia* 42: 292.

SYDOW H. & SYDOW P., 1917 — Novae fungorum species – XV. *Annales Mycologici* 15: 143-148.

SHERWOOD M.A., 1977 — The ostropalean fungi. *Mycotaxon* 5(1): 1-277.

TINA A. HOFMANN., KIRSCHNER R. & PIEPENBRING M., 2010 — Phylogenetic relationships and new records of *Asterinaceae* (*Dothideomycetes*) from Panama. *Fungal Diversity* 43: 39-53.

HAIXIA WU., YANMEI LI., HANG CHEN & KEVIN D. HYDE., 2010 — Studies on *Microthyriaceae*: some excluded genera. *Mycotaxon* 113: 147-156.

YANMEI LI., HAIXIA WU., HANG CHEN & KEVIN D. HYDE., 2010 — Morphological studies in *Dothideomycetes*: *Elsinoe* (*Elsinoaceae*), *Butleria* and three excluded genera. *Mycotaxon* (in press).

ZHANG YING., FOURNIER J., POINTING SB. & HYDE KD., 2008 — Are *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa congeneric*? *Fungal Diversity* 33: 47-60.

ZHANG YING., FOURNIER J., Crous P.W., JEEWON R., POINTING SB. & HYDE K.D., 2009 —Towards a phylogenetic clarification of *Lophiostoma/Massarina* and morphologically similar genera in the *Pleosporales*. *Fungal Diversity* 38: 225-251.