

Article



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The status of Myriangiaceae (Dothideomycetes)

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Abstract

The family Myriangiaceae is relatively poorly known amongst the Dothideomycetes and includes genera which are saprobic, epiphytic and parasitic on the bark, leaves and branches of various plants. The family has not undergone any recent revision, however, molecular data has shown it to be a well-resolved family closely linked to Elsinoaceae in Myriangiales. Both morphological and molecular characters indicate that Elsinoaceae differs from Myriangiaceae. In Elsinoaceae, small numbers of asci form in locules in light coloured pseudostromata, which form typical scab-like blemishes on leaf or fruit surfaces. The coelomycetous, "Sphaceloma"-like asexual state of Elsinoaceae, form more frequently than the sexual state; conidiogenesis is phialidic and conidia are 1-celled and hyaline. In Myriangiaceae, locules with single asci are scattered in a superficial, coriaceous to sub-carbonaceous, black ascostromata and do not form scab-like blemishes. No asexual state is known. In this study, we revisit the family Myriangiaceae, and accept ten genera, providing descriptions and discussion on the generic types of Anhellia, Ascostratum, Butleria, Dictyocyclus, Diplotheca, Eurytheca, Hemimyriangium, Micularia, Myriangium and Zukaliopsis. The genera of Myriangiaceae are compared and contrasted. Myriangium duriaei is the type species of the family, while Diplotheca is similar and may possibly be congeneric. The placement of Anhellia in Myriangiaceae is supported by morphological and molecular data. Because of similarities with Myriangium, Ascostratum (A. insigne), Butleria (B. inaghatahani), Dictyocyclus (D. hydrangea), Eurytheca (E. trinitensis), Hemimyriangium (H. betulae), Micularia (M. merremiae) and Zukaliopsis (Z. amazonica) are placed in Myriangiaceae. Molecular sequence data from fresh collections is required to confirm the relationships and placement of the genera in this family.

Key words: Anhellia, Ascostratum, Butleria, Dictyocyclus, Diplotheca, Eurytheca, Hemimyriangium, Micularia, morphology, Myriangium, saprobes, Zukaliopsis

Introduction

The family Myriangiaceae is a relatively poorly known, but interesting family of Dothideomycetes (Ascomycota) comprising the genera *Anhellia*, *Diplotheca*, *Eurytheca* and *Myriangium* (Lumbsch & Huhndorf 2007, 2010). The species are generally saprobic in nature, occurring on dead leaves, stems or bark of various host plants (Gäumann 1928). Distribution of Myriangiaceae is chiefly tropical or sub-tropical (Barr 1979). The consistency of the ascostromata is often suggestive of the Hypocreales, although in *Myriangium* itself they are more cartilaginous to gelatinous or generally brittle (Gäumann 1928). The family is characterized by only one ascus in each locule, superficial, erumpent, scattered, solitary or gregarious, generally dark ascostromata, apedicellate or short pedicellate asci and smooth to verruculose ascospores with 1–9-transverse septa, 0–5-longitudinal septa, rarely having a mucilaginous sheath (only in *Diplotheca*). The asexual states of the family are unknown. We are

monographing the genera of all families of Dothideomycetes. By providing modern descriptions with comprehensive micrographs we hope to stimulate recollection of genera and species so that sequence data from fresh specimens can be generated and analyzed to provide a natural classification. In this paper we have added six genera (*Ascostratum, Butleria, Dictyocyclus, Hemimyriangium, Micularia* and *Zukaliopsis*) to the Myriangiaceae, as they have similar morphological characters.

Historical overview of Myriangiaceae

Myriangiaceae is a family of saprobes, but with some genera being parasitic and epiphytic on leaves. The family was introduced by Nylander (1854) and included two species, *Myriangium duriaei* Mont. & Berk. (1845) and *M. curtisii* Berk. & Mont. (1845). Millardet (1870) placed the family near the Tuberaceae. Many classical treatments have placed Elsinoaceae in synonymy with Myriangiaceae, but Höhnel (1909) was convinced that the former constituted a separate family. Petch (1924) provided a thorough review of *Myriangium*. Gäumann *et al.* (1928) divided Myriangiaceae into two groups, based on the formation of the asci. In one group, the asci are scattered irregularly over the whole ascostromata, while in the other group they are localized in definite regions of the ascostromata, which are differentiated into sterile and fertile parts. No other authors have followed this grouping. Clements & Shear (1931) placed the family Myriangiaceae in the order Dothideales, due to the presence of ascostromata containing numerous locules, while von Arx (1963) treated Myriangiaceae in Myriangiales. von Arx & Müller (1975) treated Myriangiaceae as a parasitic family on higher plants or on other fungi, especially ascomycetes. According to Petch (1924), *Myriangium duriaei*, *M. curtisii*, *M. montagnei* Berk. (1845) and *M. thwaitesii* Petch (1924) are entomogenous, but this needs confirming. Barr (1979) and Eriksson (1981) were of the opinion that two separate families should be maintained as Elsinoaceae and Myriangiaceae with the latter predominantly occurring on branches, while Elsinoaceae is restricted to foliar pathogens.

The order Myriangiales was introduced by Stärback (1899) to accommodate species having crustose ascostromata and muriform ascospores, and several apparently unrelated forms, with the distinguishing characters of angiocarpous fruiting bodies with asci arranged irregularly in colourless locules and released by the breaking away of the covering layer. The families Myriangiaceae and Saccardiaceae (Höhnel 1909) were treated by von Arx (1963) as comprising the Myriangiales. Luttrell (1973) distinguished Atichiaceae (Raciborski 1900) and Saccardiaceae in Myriangiales indicating that the asci are borne in discoid ascostromata in a hymenium-like layer and are separated from each other by paraphyses-like filaments often forming an epithecium. von Arx & Müller (1975) gave a new description for the order with asci borne singly, often at different levels in small loculi immersed in a stromatic body, which opens by rupture or dehiscence. Barr (1987) distinguished the family Myriangiaceae and included taxa that formed a pseudoparenchymatous or plectenchymatous ascostromata with interascal tissue persisting between mono-ascal cavities. Kirk *et al.* (2008) included three families, Cookellaceae, Elsinoaceae and Myriangiaceae in Myriangiales, while Lumbsch & Huhndorf (2010) accepted only Elsinoaceae and Myriangiaceae in the order based on phylogenetic results.

Various treatments have been proposed for the genera in family Myriangiaceae (Table 1). Nylander (1854) placed a single genus Myriangium, with two species under this family, but von Arx & Müller (1975) placed 16 genera in the family. After differentiating Elsinoaceae as a separate family from Myriangiaceae based on molecular evidence, Schoch et al. (2006) maintained only Anhellia, Diplotheca, Eurytheca and Myriangium in Myriangiaceae. This classification was followed in Lumbsch & Huhndorf (2007, 2010). The familial position of Anhellia was, however, considered uncertain by Lumbsch & Huhndorf (2010), but in the phylogenetic analyses by Pinho et al. (2012) its inclusion in this family was supported. The placement of Eurytheca is also confused. Theissen & Sydow (1917) placed Eurytheca in the family Saccardiaceae (parts of the Saccardiaceae were classified by Müller & von Arx (1965) within the family Schizothyriaceae, while Lumbsch & Huhndorf (2010) referred the genus to the Myriangiaceae). Jayawardena et al. (2014) separated Myriangiaceae from Elsinoaceae in their phylogenetic analysis with high bootstrap support value (82%). They analyzed the internal transcribe spacer (ITS), large and small subunits of the nuclear ribosomal RNA genes (LSU, SSU) and two protein coding genes, second largest subunit of RNA polymerase II (RPB2), and translation elongation factor-1 alpha (TEF1) genes from 16 taxa of Myrangiales and the resulting tree supported Elsinoaceae and Myriangiaceae as distinct families. In this study we re-examine Anhellia, Diplotheca, Eurytheca and Myriangium and provide full descriptions, photographic plates and notes suggesting where these genera should be placed. Ascostratum, Butleria, Dictyocyclus, Hemimyriangium, Micularia and Zukaliopsis were also studied and are considered to also belong in Myriangiaceae. Further molecular sequence data from fresh collections is required to confirm the relationships and placement of the genera in Myriangiaceae.

TABLE 1. Genera included in *Myriangiaceae* by various authors.

Nylander (1854)	von Arx & Müller (1975)	Barr (1987)	Lumbsch & Huhndorf (2007, 2010)	This paper (2014)
Myriangium	Anhellia	Angatia	?Anhellia	Anhellia
	Beelia	Myriangium	Diplotheca	Ascostratum
	Butleria		?Eurytheca	Butleria
	Cookella		Myriangium	Dictyocyclus
	Diplotheca			Diplotheca
	Elsinoe			Eurytheca
	Hyalotheles			Hemimyriangium
	Micularia			Micularia
	Molleriella			Myriangium
	Myriangium			Zukaliopsis
	Pycnoderma			-
	Pycnodermina			
	Saccardinula			
	Stephanotheca			
	Uleomyces			
	Xenodium			

Materials and methods

Type specimens of Anhellia tristis, Ascostratum insigne, Butleria inaghatahani, Dictyocyclus hydrangea, Diplotheca tunae, Eurytheca trinitensis, Hemimyriangium betulae, Micularia merremiae, Myriangium duriaei and Zukaliopsis amazonica were obtained from L, S, PC, BPI and IMI. Fruiting bodies were rehydrated in 5% KOH prior to examination and sectioning. Specimens were examined under a stereo microscope and fine forceps were used to remove one or two ascostromata, which were mounted in water or cotton blue. Observations and photographs were made with a Nikon Eclipse 80i, and a Discovery v-8 microscope. Hand sections were cut with a sharp razor blade. The sections were transferred to a drop of water or a drop of cotton blue for examination and photography. Cross section of Myriangium duriaei was redrawn from Gäumann (1928).

Results

Taxonomy

Myriangiaceae Nylander, Mémoires de la Société Impériale des Sciences Naturelles de Cherbourg 2: 9 (1854) MycoBank: MB81866

Synonym: Phymatosphaeriaceae Speg., Anal. Soc. cient. argent. 26(1): 57 (1888)

Saprobic on bark, leaves and branches, some genera epiphytic or parasitic on leaves. Sexual state: Ascostromata superficial, scattered, solitary or aggregated, coriaceous to sub-carbonaceous, semi-immersed or immersed to erumpent, generally dark, globose to oval, sometimes surrounded by the remains of the ruptured epidermis, multi-loculate, locules generally in the upper layer or scattered throughout the ascostromata. Loculues with single ascus, ostiolate. Ostiole minute or asci pushed out through pseudoparenchymatous cells. Paraphyses absent. Cells of ascostromata comprising pseudo-parenchymatous cells of pale yellow to brown pigmented textura angularis, textura globusa or textura intricata. Asci 8-spored, bitunicate, fissitunicate, globose to subglobose, apedicellate or with a minute pedicel, apically rounded with indistinct ocular chamber. Ascospores hyaline to sub-hyaline or brown, oblong or fusiform with slightly acute ends, with 1–9-transverse septa, with 0–5-longitudinal septa, muriform, smooth-walled to verruculose, rarely having a sheath. Asexual state: Unknown.

Key to genera of Myriangiaceae

1.	Locules located only at the upper layer of the ascostromata	
	Locules located only at the upper layer of the ascostromata	
2.	Ascospores with only transverse septa.	Hemimvriangiun
	Ascospores muriform	
3.	Ascospores muriform Saprobic on bark	Myriangiun
	Parasitic on leaves	4
4.	Ascostromata on upper surface of the leaf	Zukaliopsis
	Ascostromata on lower surface of the leaf	Anhellia
5.	Ascospores hyaline	6
	Ascospores brown	Butleric
6.	Ascospores with only transverse septa	
	Ascospores muriform	9
7.	Ascospores with only one transverse septum	Micularia
	Ascospores with 3–9-transverse septa	
8.	Ascostromata connected to the host with a prominent stalk	
	Ascostromata embedded in the host, erumpent through ruptured epidermis	Eurytheco
9.	Ascospores covered with a mucilaginous sheath	Diplotheco
	Ascospores without a mucilaginous sheath	Dictyocyclus

Type genus:—*Myriangium* Montagne & Berkeley, London Journal of Botany 4: 72 (1845) Possible synonyms

Phymatodiscus Speg., Boln Acad. nac. Cienc. Córdoba 23: 484 (1919) Phymatosphaeria Pass., Nuovo G. bot. ital. 7: 188 (1875) Pyrenotheca Pat., Bull. Soc. bot. Fr. 33: 155 (1886)

Saprobic on bark of Acer, Citrus, Fraxinus, and other plants. Sexual state: Ascostromata superficial, scattered, solitary or aggregated, coriaceous to sub-carbonaceous, erumpent, dark brown to black, globose to oval, multiloculate, locules scattered throughout the ascostromata but most are concentrated at the upper region, containing only one ascus in each locule, ostiolate. Ostioles minute. Paraphyses absent, Cells of ascostromata comprising thick-walled pigmented cells of textura angularis. Asci 8-spored, bitunicate, fissitunicate, globose to subglobose, apedicellate, apically rounded with indistinct ocular chamber. Ascospores hyaline to subhyaline, elliptical to oblong, fasciculate, muriform, usually with 4–7-transverse septa and one or more longitudinal septa, smoothwalled, lacking a sheath. Asexual state: Unknown.

Notes:—The genus *Myriangium* was erected by Montage & Berkeley (1845), with *M. duriaei* as the type species. Montage & Berkeley (1845) added M. curtisii and placed both species in the lichen family, Collemaceae. Nylander (1854) erected the family Myriangeaceae (Miller 1940). The generic concept of Myriangium included multi-loculate apothecia with asci forming in single locules (Miller 1938). Wolf & Wolf (1947) considered that M. duriaei, M. curtisii, M. montagnei and M. thwaitesii, which are included in Myriangium, are parasitic on scale insects. Miller (1938) studied the morphology and cytology of M. duriaei and M. curtsii and found that both occur as parasites on scale insects attacking Nyssa sylvatica, Carya illinoensis and other broad-leaved trees in southeastern United States. In this study we have examined the type material of Myriangium and it does not appear to be associated with any scale insects, although this should be confirmed in fresh collections. There are 50 species epithets for Myriangium in Index Fungorum (2013) and the genus requires a restudy. There have been few molecular studies on this genus. Lumbsch & Lindemuth (2001) used LSU and SSU gene data to analyze the major lineages of Dothideomycetes in which the Myriangium species were included in the order Myriangiales. Schoch et al. (2006) used M. duriaei and four Elsinoe spp. in their phylogenetic analyses and found that these Elsinoe species belong to a different family, Elsinoaceae. This concept was confirmed by Schoch et al. (2009). Jayawardena et al. (2014) used M. duriarei and M. hispanicum J.B. Martínez (1931) in their phylogenetic analysis to differentiate Myriangiaceae from Elsinoaceae with a high bootstrap value (82%). Fresh collections and molecular studies are needed to substantiate these results.

Type species:—*Myriangium duriaei* Montagne & Berkeley, London Journal of Botany. 4: 73 (1845) MycoBank: 218324 (Fig. 1)

Saprobic on bark. Sexual state: Ascostromata 1.5–1.8 \times 1.1–1.4 mm (\overline{x} = 1.6 \times 1.3 mm, n = 5), superficial, scattered, solitary or aggregated, coriaceous to sub-carbonaceous, erumpent, dark brown to black, globose to oval, multi-loculate, locules scattered throughout the ascostromata but concentrated mainly at the upper region, locules with one ascus, ostiolate. Ostioles minute. Cells of the ascostromata comprising several layers of thick-walled pale yellow pigmented cells of textura angularis. Paraphyses not observed. Asci 28–35 \times 23–28 µm (\overline{x} = 30.4 \times 25.2 µm, n = 13), 8-spored, bitunicate, fissitunicate, globose to subglobose, apedicellate, apically rounded with indistinct ocular chamber. Ascospores 20–25 \times 7–10 µm (\overline{x} = 22 \times 9 µm, n = 12), hyaline, oblong or fusiform with slightly acute ends, muriform, with 4–7-transverse septa, 1–5-longitudinal septa, smooth-walled, lacking a sheath. Asexual state: Unknown.

Material examined:—ALGERIA. MC Durieu de Maisonneuve, on *Lentiscos vivos* (= *Pistacia lentiscus*, Anacardiaceae) (PC, NL 8414!, holotype).

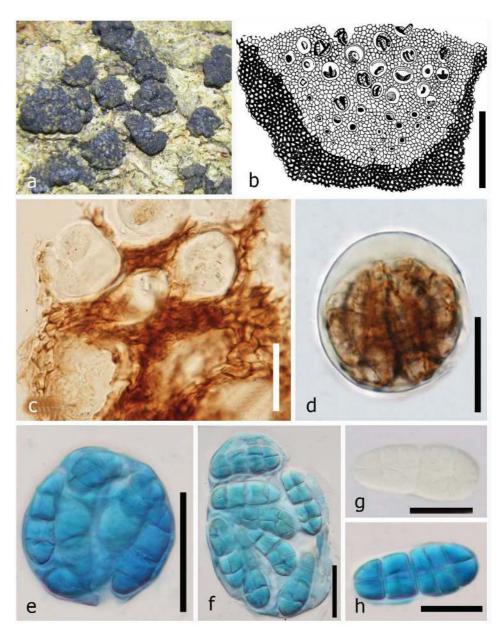


FIGURE 1. *Myriangium duriaei* (holotype). a. Ascostromata on host surface. b–c. Locules in cross-section of the ascostromata (b redrawn from Gäumann 1928). d. Apedicellate ascus with 8 ascospores. e–f. Asci stained in cotton blue. g. Muriform, hyaline ascospore. h. Ascospore stained in cotton blue. Scale bars: $b = 500 \mu m$, $c-e = 30 \mu m$, $f = 20 \mu m$, $g-h = 10 \mu m$.

Parasitic on leaves. Sexual state: Ascostromata superficial, scattered, solitary or gregarious, carbonaceous, immersed, dark brown to black, hemispherical or globose to oval, connected to the host by erumpent hypostroma, multi-loculate, locules scattered in the upper layer of the ascostromata, one ascus in each locule, ostiolate. Ostioles minute, asci pushed out through pseudoparenchymatous cells. Paraphyses absent. Cells of the ascostromata comprising pale yellow to brown textura angularis. Asci 8-spored, bitunicate, fissitunicate, globose to saccate, pedicellate, apically rounded with indistinct ocular chamber. Ascospores hyaline or yellowish brown, elliptical to oblong, muriform, with 4–5-transverse septa, 1–2-longitudinal septa, constricted at the septa, smooth-walled, lacking a sheath. Asexual state: Unknown.

Notes:—Anhellia was introduced by Raciborski (1900) in the "Myriangiearum" and comprised a single species, A. tristis. This genus remained monotypic until von Arx (1963) added A. calami (Racib.) Arx, A. escharoides (Syd.) Arx, A. purpurascens (Rehm.) Arx, A. lantanae (Henn.) Arx and A. nigra (Viégas) Arx. Luttrell (1973) placed this genus in Myriangiaceae based on its discoid to hemiglobose ascostromata, with stalk erumpent through epidermis from an intraepidermal hypostroma. Anhellia tristis, A. escharoides and A. tetracerae (Hansf.) Arx, with obviously larger dimensions than A. tabebuiae Inácio & Dianese have been found on Vaccinium teysmannianum and Tetracera alnifolia (Boedijn 1961, von Arx 1963). Anhellia calami has been reported on Calamus sp. (von Arx 1963). Anhellia nectandrae differs from A. escharoides by having paler ascospores and narrower asci, while A. tetracerae and A. tristis are different in having hyaline ascospores (Raciborski 1900, von Arx 1963). Anhellia nectandrae was the first Anhellia species reported parasitizing a member of Lauraceae and differs morphologically and phylogenetically from other previously described species (Pinho et al. 2012). Anhellia tabebuiae is a first record of Anhellia on Tabebuia species (Pinho et al. 2012). The genus currently comprises nine species, which are mainly tropical in distribution (von Arx & Müller 1975, Kirk et al. 2008) and is characterized by its dark ascostromata, bearing many-celled ascospores inside bitunicate, fissitunicate asci mostly in the upper layer, connected with the host by an erumpent, discoid hypostroma (von Arx & Müller 1975, Pereira 2003). Pinho et al. (2012) investigated the phylogenetic position of Anhellia (Myriangiales) by analysis of nucleotide sequences of the ribosomal large subunit (LSU) and ITS regions. Twenty-eight aligned sequences were obtained from GenBank and this was the first attempt to resolve the placement of the genus Anhellia within Myriangiales. The genus Anhellia is strongly supported in Myriangiaceae in this phylogenetic analysis.

Type species:—Anhellia tristis Raciborski, Parasitische Algen und Pilze Javas 2: 10 (1900) MycoBank: 219955 (Fig. 2)

Parasitic on leaves. Sexual state: *Ascostromata* 0.8–1.1 × 0.2–0.3 mm (\overline{x} = 1 × 0.25 mm, n=5), superficial on the lower surface, scattered, solitary, carbonaceous, immersed, flattened, brown to black, globose to oval, connected to the host by an erumpent hypostroma, multi-loculate, locules scattered in the upper layer of the ascostromata, one ascus in each locule, ostiolate. *Ostioles* minute, asci pushed out through pseudo-parenchymatous cells. *Cells of the ascostromata* comprising several layers of thin-walled pale yellow to brown *textura angularis* cells. *Paraphyses* absent. *Asci* 34–89 × 18–33 µm (\overline{x} = 57 × 28 µm, n = 15), 8-spored, bitunicate, fissitunicate, globose to saccate, pedicel short and broad, indistinct ocular chamber. *Ascospores* 20–32 × 6–12 µm (\overline{x} = 27 × 10 µm, n = 33), hyaline, elliptical to oblong, muriform, 4–5-transverse septa, 1–2-longitudinal septa, constricted mainly at the first forming septum, with slightly acute or tapered ends, smooth-walled, lacking a sheath. Asexual state: Unknown.

Material examined:—INDONESIA. Java, Gedeh, on leaves of *Vaccinium teysmannia* (Ericaceae), *M. Raciborski* (S, F47931!, isotype).

Ascostratum Sydow & P. Sydow, Annales Mycologici 10(1): 41 (1912)

Saprobic on bark in terrestrial habitats. Sexual state: Ascostromata superficial, scattered, solitary or aggregated, carbonaceous, semi-immersed to erumpent, black, globose to sub-globose, ruptured epidermis, multi-loculate, locules scattered in the middle region of the ascostromata, ostiolate. Ostioles minute, ascospores release via breakdown of the cells of ascostromata. Hamathecium lacking paraphyses. Cells of the ascostromata comprising flattened textura intricate. Asci 8-spored, bitunicate, fissitunicate, oval to globose, short pedicellate or apedicellate, apically rounded with indistinct ocular chamber. Ascospores hyaline, ellipsoid to fusiform, obtuse at both ends, 7–9-transverse septa, longitudinal septa absent, smooth-walled, lacking a sheath. Asexual state: Unknown.

Notes:—Ascostratum was introduced by Sydow & Sydow (1912) and was typified by Ascostratum insigne Syd. & P. Syd. The genus was placed in Dothideomycetes, genera incertae sedis by Lumbsch & Huhndorf (2010). Ascostratum is characterized by ascostromata with numerous locules scattered in the middle region of the ascostromata, semi-immersed or erumpent, asci oval to globose, and ascospores hyaline with 7–9 transverse septa. Ascostratum shows similarities with Anhellia by releasing ascospores via breakdown of the cells of ascostromata.

Ascostratum includes only two species (Index Fungorum 2013) and phylogenetic analysis is lacking for either species and is needed to establish the natural classification of this genus. Here we provide detailed description and illustrations as a contribution for future work. We refer Ascostratum to the family Myriangiaceae based on its morphological similarity.



FIGURE 2. *Anhellia tristis* (isotype). a–b. Herbarium material. c–d. Ascostromata on host substrate. e–f. Cross section of the ascostromata showing locules mostly at the periphery. g. Immature ascus stained in cotton blue. h. Mature ascus. i–j. Mature asci stained in cotton blue. k. Dehiscence of mature ascus. l. Smooth-walled ascospore. m–n. Ascospores stained in cotton blue. Scale bars: $c = 200 \ \mu m$, $d-f = 100 \ \mu m$, $g-k = 10 \ \mu m$, $l-n = 5 \ \mu m$.

Type species:—Ascostratum insigne Sydow & P. Sydow, Annales Mycologici 10(1): 41 (1912) MycoBank: 214383 (Fig. 3)

Saprobic on bark of Euphorbia. Sexual state: Ascostromata $0.65-1.2 \times 0.55-0.85$ mm ($\overline{x}=0.95 \times 0.70$ mm, n = 10), superficial, scattered, solitary or aggregated, carbonaceous, semi-immersed to erumpent, black, oval to globose, ruptured epidermis, multi-loculate, locules spread around the middle region of the ascostromata, ostiolate. Ostiole minute, ascospores release via breakdown of the cells of ascostromata. Hamathecium lacking paraphyses. Cells of the ascostromata composed of dark brown to black thick-walled flattened textura intricate cells. Asci 70–96 × 52–70 µm ($\overline{x}=81 \times 60$ µm, n = 10), 8-spored, bitunicate, fissitunicate, oval to globose, short pedicellate or apedicellate, apically rounded with indistinct ocular chamber. Ascospores 53–68 × 19–24 µm ($\overline{x}=60 \times 21$ µm, n = 10), hyaline, ellipsoid to fusiform, obtuse at each end, 7–9-transverse septa, longitudinal septa absent, constricted at each septum, smooth-walled, lacking a sheath. Asexual state: Unknown.

Material examined:—SOUTH AFRICA. KwaZulu-Natal, Natal, Amanzintote, on *Euphorbia* bark (Euphorbiaceae), *E.M. Doidge*, 10 July 1911 (S, F7426!, holotype).

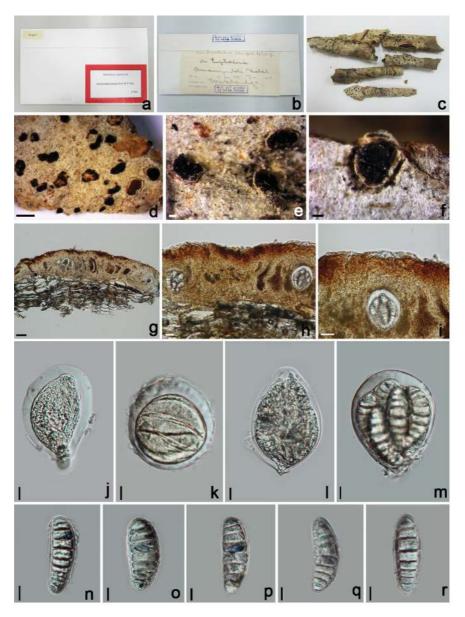


FIGURE 3. Ascostratum insigne (holotype). a–c. Herbarium material. d–f. Ascostromata on host surface. g–i. Cross section of ascostromata. j–l. Immature asci with distinct, short pedicel in j. m. Asci with ascospores. n–r. Ascospores. Scale bars: $d = 1000 \mu m$, $e-f = 200 \mu m$, $g = 50 \mu m$, $h-i = 20 \mu m$, $j-r = 10 \mu m$.

Parasitic on leaves forming leaf spots. Sexual state: Ascostromata superficial, scattered, solitary or aggregated, erumpent, black, globose to oblong, multi-loculate, numerous locules distributed at different levels inside ascostromata, one ascus inside each locule, ostiolate. Ostiole minute. Paraphyses absent. Cells of ascostromata are textura angularis. Asci 8-spored, bitunicate, fissitunicate, globose to sub-globose, apedicellate, apically rounded with indistinct ocular chamber. Ascospores brown, oblong to ovoid, 1-transverse septum, longitudinal septa absent, smooth-walled, constricted at the septum, lacking a sheath. Asexual state: Unknown.

Notes:—*Butleria* is a monotypic genus established by Saccardo (1914). von Arx & Müller (1975) referred this genus to Myriangiaceae, while Barr (1979) placed it under Elsinoaceae. Kirk *et al.* (2001), Lumbsch & Huhndorf (2007, 2010), Li *et al.* (2011) and Hyde *et al.* (2013) have retained this genus under family Elsinoaceae. *Butleria* has similarities with Myriangiaceae in having a single ascus in each locule. Therefore the genus can be better placed under the family Myriangiaceae. New collections of this genus and molecular analysis are needed to clarify the familial placement.

Type species:—*Butleria inaghatahani* Saccardo, Annales Mycologici 12: 302 (1914) MycoBank: 156750 **Notes:**—This species is described and illustrated in Li *et al.* (2011)

Dictyocyclus Sivan., W.H. Hsieh & Chi Y. Chen, Botanical Journal of the Linnean Society 126(4): 324 (1998)

Foliar epiphyte on the lower surface of leaves. Sexual state: Ascostromata superficial, scattered, solitary, immersed, black, discoid, multi-loculate, opening by irregular fissures; locules arranged around a raised, sterile, central column of ascostromata, ostiolate. Ostiole minute. Paraphyses absent. Cells of the ascostromata comprising a single stratum of more or less thick-walled textura angularis brown cells. Asci 8-spored, bitunicate, fissitunicate, obovoid, thick-walled, apedicellate, apically rounded. Ascospores hyaline, obovoid, partially overlapping, with broadly to narrowly rounded ends, muriform, 3–5-transverse septa, one longitudinal or oblique septum in each of the end cells, smooth-walled, lacking a mucilaginous sheath. Asexual state: Unknown.

Notes:—*Dictyocyclus* was introduced by Sivanesan *et al.* (1998) as a monotypic genus of the family Parmulariaceae. *Dictyocyclus* shares most of the characteristics similar to *Myriangium*, including a single ascus inside each locule and muriform ascospores.

Type species:—*Dictyocyclus hydrangeae* Sivan., W.H. Hsieh & Chi Y. Chen, Botanical Journal of the Linnean Society 126(4): 324 (1998) MycoBank No: 444846 (Fig. 4)

Foliar epiphyte on the lower surface of Hydrangea integrifolia leaves, appearing as small black spots. Sexual state: Ascostromata 0.4– 0.5×0.5 –0.6 mm ($\overline{x} = 0.45 \times 0.55$ mm, n = 5), superficial, scattered, solitary, immersed, black, discoid, multi-loculate, opening by irregular fissures; locules arranged around a raised, sterile, central column of ascostromata, ostiolate, Ostiole minute. Paraphyses absent. Cells of the ascostromata comprising thick-walled lightbrown textura angularis. Asci 28– 50×22 – $30 \, \mu m$ ($\overline{x} = 37 \times 26 \, \mu m$, n = 20), 8-spored, bitunicate, fissitunicate, obovoid, thick-walled, apedicellate, apically rounded with indistinct ocular chamber. Ascospores 13– 20×5 – $9 \, \mu m$ ($\overline{x} = 17 \times 6 \, \mu m$, n = 30), hyaline, obovoid, partially overlapping, thin-walled, broadly to narrowly rounded ends, muriform, 3–5-transverse septa, one longitudinal or oblique septum in each of the end cells, smooth-walled, lacking a sheath. Asexual state: Unknown.

Material examined:—TAIWAN. Taitung Hsien, on leaves of *Hydrangea integrifolia* (Hydrangeaceae), *C.Y. Chen* 1995 (IMI, 371179!, holotype).

Diplotheca Starbäck, Botaniska Notiser: 30 (1893)

Parasitic on bark. Sexual state: Ascostromata superficial, scattered, aggregated, carbonaceous, immersed to erumpent, black, globose to sub-globose, multi-loculate, locules generally located at the lower part of the ascostromata, ostiolate, Ostioles minute. Paraphyses absent. Cells of the ascostromata comprising several layers of thick-walled pale brown cells of textura globosa. Asci 8-spored, bitunicate, fissitunicate, globose or ellipsoid,

minute pedicel, apically rounded with indistinct ocular chamber. *Ascospores* hyaline or pale gray, fusiform, both ends bluntly tapered, muriform, 3–6-transverse septa, 1–3-longitudinal septa, constricted at septum, smoothwalled, surrounded by a mucilaginous sheath. Asexual state: Unknown.

Notes:—*Diplotheca* was introduced by Starbäck (1893). *Diplotheca tunae* was transferred to *Myriangium* by Petrak (1929), however, due to its parasitic nature, Miller (1940) suggested retaining the genus name *Diplotheca*. Petrak (1951) compared the size of ascostromata and accepted the separation of *D. tunae* from *Myriangium*. Lumbsch & Huhndorf (2007, 2010) placed *Diplotheca* in the family Myriangiaceae. *Diplotheca* is parasitic on various plant species in Cactaceae, especially on *Epiphyllum phyllanthus* an epiphytic plant, forming scab-like symptoms on most stems (Pereira & Barreto 2006).



FIGURE 4. *Dictyocyclus hydrangea* (holotype). a. Herbarium material. b. Ascostroma on host surface. c–d. Squash mount of ascostromata. e. Cross section through ascostroma. f. Immature ascus. g–i. Mature asci with 8 ascospores. j–m. Ascospores. Scale bars: $c = 200 \, \mu m$, $d-e = 50 \, \mu m$, $f = 10 \, \mu m$, $g-i = 20 \, \mu m$, $j-m = 10 \, \mu m$.

Type species:—Diplotheca tunae (Spreng.) Starbäck, Botaniska Notiser: 30 (1893) MycoBank No: 247593 (Fig. 5)

Parasitic on bark. Sexual state: Ascostromata $0.2-0.3 \times 0.25-0.3$ mm ($\overline{x} = 0.25 \times 0.3$ mm, n = 5), superficial, scattered, aggregated, carbonaceous, immersed to erumpent, black, globose to sub-globose, multi-loculate, locules located at the lower part of the ascostromata, ostiolate. Ostioles minute. Paraphyses absent. Cells of the ascostromata brown to dark brown, comprising several layers of thick-walled textura globosa. Asci 46–52 × 40–46

 μ m ($\overline{x} = 48 \times 41 \ \mu$ m, n = 20), 8-spored, bitunicate, fissitunicate, globose or slightly ellipsoid, minute pedicel, apically rounded with indistinct ocular chamber. *Ascospores* 25–30 × 12–14 μ m ($\overline{x} = 27 \times 12 \ \mu$ m, n = 20), hyaline or pale grey, oblong-oval to elliptical, both ends bluntly tapered, muriform, with 3-transverse septa, 1–3-longitudinal septa, slightly constricted at the septa, smooth-walled, covered with a mucilaginous sheath. Asexual state: Unknown.

Material examined:—PUERTO RICO. Mayaguez, on *Opuntia* sp. (Cactaceae), *F.L. Stevens* 1913 (BPI, 684149!, isotype).

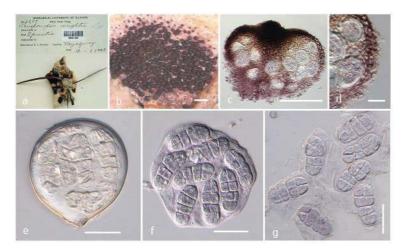


FIGURE 5. *Diplotheca tunae* (isotype). a. Herbarium material. b. Ascostromata occurring on substrate. c. Partial section through ascostroma. d. Peridium wall. e–f. Asci. g. Ascospores. Note colour change in 3% KOH, becoming pinkish or purple in c, f and g. Scale bars: $b = 500 \mu m$, $c = 100 \mu m$, $d-g = 20 \mu m$.

Eurytheca De Seynes, Bulletin de la Société botanique de France 25: 88 (1878)

Saprobic on bark. Sexual state: Ascostromata superficial, scattered, solitary or aggregated, carbonaceous, erumpent through the ruptured epidermis, black, orbicular to irregular, multi-loculate, locules scattered in the outer region of the ascostromata, one ascus in each locule, ostiolate. Ostiole minute. Paraphyses absent. Cells of the ascostromata comprising several layers of thick-walled textura globosa pigmented cells. Asci 8-spored, bitunicate, fissitunicate, globose to saccate, short pedicellate, with indistinct ocular chamber. Ascospores hyaline, fusiform, 3—6-transverse septa, longitudinal septa absent, not constricted at the septa, base or both base and apical end bluntly tapered, smooth-walled, lacking a sheath. Asexual state: Unknown.

Notes:—The genus *Eurytheca* was erected by De Seynes (1878), and placed under family Saccardiaceae by Höhnel (1917). Wolf & Wolf (1947) remarked that this genus belongs to "Myriangiaceen". Lumbsch & Huhndorf (2007) placed this genus under family *Myriangeaceae*. In the original description of the type species *E. trinitensis* (Sydow & Sydow 1914), the ascospores are described as 10–14-septate while Theissen & Sydow (1917) describe them as 4–5-septate. Lumbsch & Huhndorf (2007, 2010) placed *Eurytheca* in family Myriangiaceae. The spores examined in the current study showed only 3–6-transverse septa. There are only three species epithets for this genus (Index Fungorum 2013). Fresh collections and phylogenetic analysis are needed to confirm the familial placement of this genus in Myriangiaceae.

Type species:—*Eurytheca trinitensis* Sydow & P. Sydow, Annales Mycologici 13(1): 40 (1915) MycoBank No: 221235 (Fig. 6)

Saprobic on bark. Sexual state: Ascostromata $1-2 \times 0.6-1$ mm ($\overline{x}=1.5 \times 0.9$ mm, n=5), superficial, scattered, solitary or aggregated, carbonaceous, erumpent through the ruptured epidermis, black, orbicular to irregular, multiloculate, locules scattered in the outer region of the ascostromata, one ascus in each locule, ostiolate. Ostioles minute. Paraphyses absent. Cells of the ascostromata comprising several layers of thick-walled textura globosa pigmented cells. Asci $10-16 \times 8-15$ µm ($\overline{x}=13 \times 11$ µm, n=10), 8-spored, bitunicate, fissitunicate, globose to

saccate, short pedicellate, with indistinct ocular chamber. *Ascospores* 8–23 × 2–7 μ m (\overline{x} = 14 × 5 μ m, n = 10), hyaline, fusiform, 3–6-transverse septa, longitudinal septa absent, not constricted at the septa, base or both base and apical end bluntly tapered, smooth-walled, lacking a sheath. Asexual state: Unknown.

Material examined:—TRINIDAD. Tobago, Balanchisseuse, on *Truncum arboris viventis* (Bromeliaceae), *W.E. Broadway*, 15 May 1908 (S, F11411!, holotype).



FIGURE 6. *Eurytheca trinitensis* (holotype). a. Herbarium material. b. Ascostromata on host substrate. c. Section of ascostroma. d–e. Asci arrangement within hamathecium. f. Immature ascus. g–h. Mature asci with short pedicel bearing eight irregularly arranged ascospores. i–k. Smooth-walled, hyaline ascospores. l. Ascospore stained with cotton blue. Scale bars: c-f = 100 mm, g-l = 10 mm.

Hemimyriangium Reid & Pirozynski, Canadian Journal of Botany 44: 650 (1966)

Saprobic on twigs forming spots. Sexual state: Ascostromata superficial, scattered, solitary or gregarious, carbonaceous, semi-immersed to erumpent, black, globose to oval, multi-loculate, locules arrange in two layers in the outer layer of the ascostromata, one ascus in each locule, ostiolate. Ostioles minute. Paraphyses absent. Cells of the ascostromata comprised thick-walled textura angularis. Asci 8-spored, bitunicate, fissitunicate, globose, apedicellate, apically rounded with indistinct ocular chamber. Ascospores hyaline, oblong to oval, 1–4- transverse

septa, longitudinal septa absent, constricted at the septa, smooth-walled, lacking a sheath. Asexual State: Unknown.

Notes:—The hemispherical shape of the ascostromata of *Hemimyriangium* has been assumed due to the sporogenous layer, which has formed a hemispherical resin droplet. As the resin diffuses the ascostromata seems flat in shape.

This genus was placed in the family Myriangiaceae by Reid & Pirozynski (1966). Though Lumbsch & Huhndorf (2007) placed *Hemimyriangium* in the family Elsinoaceae it shows similarity to type genus *Myriangium* having one ascus in each locule and the arrangement of locules in the outer layer of the ascostromata. *Hemimyriangium* also bears some superficial resemblance to ascostromata of genus *Anhellia* (Reid & Pirozynski 1966). Piley & Larsen (1968) also were able to show that *Hemimyriangium* has a close phylogenetic relationship with *Myriangium* and *Diplotheca*. This taxon does not appear to be a candidate for Elsinoaceae as it has a single ascus in locules scattered throughout the outer layer of ascostromata. We suggest *Hemimyriangium* is better referred to the family Myriangiaceae than in Elsinoaceae. Fresh collections and molecular analyses are needed to clarify the familial position of this genus.

Type species:—*Hemimyriangium betulae* Reid & Pirozynski, Canadian Journal of Botany 44: 651 (1966) MycoBank No: 331874 (Fig. 7)

Saprobic on twigs forming black superficial spots on drops of transparent, yellowish resinous secretions produced by peltate glandular hairs of the host. Sexual state: Ascostromata 2–4 × 3–4 mm (\overline{x} = 3.2 × 3.6 mm, n = 12), superficial, scattered, solitary or gregarious, carbonaceous, semi-immersed to erumpent, black, hemispherical or globose to oval, multi-loculate, locules arrange in two layers in the outer layer of the ascostromata, one ascus in each locule, ostiolate. Ostioles minute. Paraphyses absent. Cells of the ascostromata comprising thick-walled textura angularis. Asci 17–47 × 15–3 µm (\overline{x} = 33.0 × 24 µm, n = 12), 8-spored, bitunicate, fissitunicate, subglobose to globose, apedicellate, apically rounded with indistinct ocular chamber. Ascospores 15–29 × 4–8 µm (\overline{x} = 19 × 7 µm, n = 34), hyaline, oblong to oval, 1–3-transverse septa, longitudinal septa absent, constricted at the septa, smooth-walled, lacking a sheath. Asexual state: Unknown.

Material examined:—SWEDEN. Österlövsta parish, Skyttskär, on the resinous warts of young *Betula pendula* (= *B. verrucosa*, Betulaceae), *K. & L. Holm*, 1981 (S, F30030!, isotype).

Micularia Boedijn, Persoonia 2(1): 67 (1961)

Parasitic on the upper surface of leaves forming darkened areas. Sexual state: Ascostromata superficial, scattered, solitary or gregarious, coriaceous, semi-immersed, dark brown to black, globose to sub-globose, multi-loculate, locules scattered throughout the ascostromata, containing a single ascus in each locule, ostiolate. Ostioles minute, with dark brown hairs around apex. Paraphyses absent. Cells of ascostromata comprising dark brown cells of textura angularis. Asci 8-spored, bitunicate, fissitunicate, sub-globose to pyriform, short pedicellate, apically rounded with small ocular chamber. Ascospores hyaline, oblong to ellipsoid, 1-transverse septum, longitudinal septa absent, constricted at the septum, smooth-walled to verruculose, lacking a sheath. Asexual state: Unknown.

Notes:—*Micularia* was introduced by Boedijn (1961) and remained monotypic until Peres & Bezerra (1981) added *Micularia tabebuiae* to the genus. *Micularia* is characterized by having bundles of small spreading hairs at the apex (Boedijn 1961). Boedijn (1961) placed this genus in the family Saccardiaceae, while Lumbsch & Huhndorf (2007, 2010) placed *Micularia* in the family Elsinoaceae. This placement was followed by Hyde *et al.* (2013). Even though it is a parasite on leaves, the inclusion of this genus in Elsinoaceae causes confusion, and as it has only one ascus in each locule we suggest to place it in Myriangiaceae.

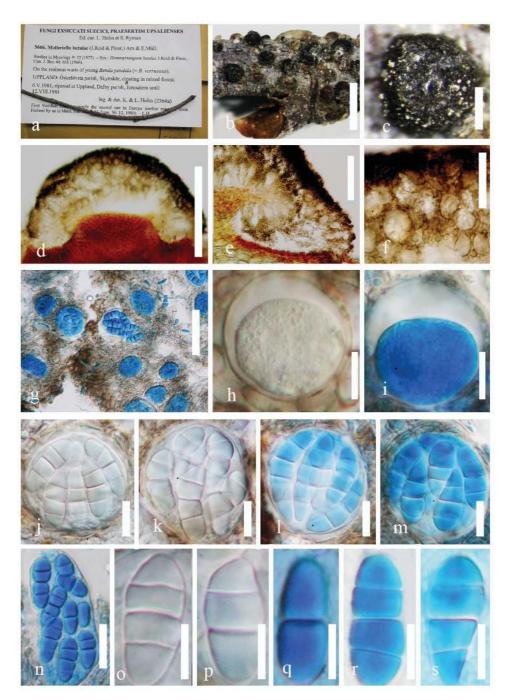


FIGURE 7. Hemimyriangium betulae (isotype). a. Herbarium material. b–c. Ascostromata on host substrate. d. Cross section of ascostroma. e–f. Peridium of locules. g. Immature and mature asci stained with cotton blue. h. Immature ascus. i. Immature ascus stained with cotton blue. j–k. Mature asci. l–n. Mature asci stained with cotton blue. o–p. Smooth to verrculose walled ascospores. q–s. Ascospore stained with cotton blue. Scale bars: b = 2 mm, $c = 200 \text{ }\mu\text{m}$, $d = 400 \text{ }\mu\text{m}$, $e = 100 \text{ }\mu\text{m}$, $f = 400 \text{ }\mu\text{m}$, $e = 100 \text{ }\mu\text{m}$.

Type species:—Micularia merremiae Boedijn, Persoonia 2(1): 67 (1961) MycoBank: 334354 (Fig. 8)

Parasitic on the upper surface of leaves forming black spots. Sexual state: *Ascostromata* 0.5– 0.65×0.63 – $0.\overline{X}$ 75 mm (= 0.6×0.7 mm, n = 5), superficial, scattered, solitary or gregarious, coriaceous, immersed, dark brown to black, globose to sub-globose, multi-loculate, locules generally scattered throughout the ascostromata, comprising a single ascus in each locule, ostiolate. *Ostioles* minute; dark brown hairs frequently set around the blunt or acute apex of the ascostromata, 20– 40×3 – $6 \, \mu m$ ($\overline{x} = 28 \times 4 \, \mu m$, n = 10). *Paraphyses* absent. *Cells of ascostromata* comprising dark brown cells of *textura angularis*. *Asci* 20– 30×19 – $23 \, \mu m$ ($\overline{x} = 26 \times 21 \, \mu m$, n = 10), 8-spored, bitunicate, fissitunicate, sub-globose to pyriform, short pedicellate, apically rounded with small ocular chamber. *Ascospores*

 $11-15 \times 5-7 \ \mu m \ (\overline{x} = 13 \times 6 \ \mu m, n = 20)$, hyaline, oblong to ellipsoid, 1-transverse septum, longitudinal septa absent, constricted at the septum, smooth-walled or verruculose, lacking a sheath. Asexual state: Unknown.

Material examined:—INDONESIA. Java, Hortus Bogor, on leaves of *Merremia* sp. (Convolvulaceae), *K.B. Boedijn*, 17 May 1954, (L, 0793023!, holotype).



FIGURE 8. *Micularia merremiae* (holotype). a. Herbarium specimen. b–d. Superficial ascostromata on substrate. e. Section through ascostroma. f. Dark brown hairs around apex of ascostroma. g. Cells of ascostroma. h–j. Immature and mature asci. k. Mature ascus stained with cotton blue. l–p. Ascospores. Scale bars: b, c = 1 mm, d = 50 μ m, e–f = 10 μ m, g = 5 μ m, h–p = 10 μ m.

Zukaliopsis Hennings, Hedwigia 43: 367 (1904)

Parasitic on leaves. Sexual state: Ascostromata superficial, scattered, solitary, coriaceous to sub-carbonaceous, semi-immersed to erumpent, black to brown, sub-globose, multi-loculate, locules arranged in the outer margin of the ascostromata, one ascus in each locule, ostiolate. Ostioles minute. Paraphyses absent. Cells of the ascostromata comprising dark brown pseudoparenchymatous textura angularis. Asci 8-spored, bitunicate, fissitunicate, ovoid, short pedicellate, narrowly pointed at the base, rounded at the apex with indistinct ocular chamber. Ascospores hyaline, oblong to cylindro-clavate, muriform, with 4–6-transverse septa, 0–1-longitudinal septum, slightly constricted at the septa, smooth-walled to veruculose, lacking a sheath. Asexual state: Unknown.

Notes:—*Zukaliopsis* was introduced by Hennings (1904) with *Z. amazonica* as type species and placed in the family Perisporiaceae. Höhnel (1909) proposed that *Zukaliopsis* is morphologically related to *Molleriella*. von Arx

(1963) synonymized *Zukaliopsis* with *Molleriella* and suggested that this genus resembles a transition to *Saccardinula* Speg. (1885) more than *Molleriella*. Sydow & Theissen (1917) placed this genus into the family Myriangiaceae providing a detailed description. We observed that *Zukaliopsis* has one ascus in each locule and, therefore, suggest placing it in the family Myriangiaceae. New collections and molecular analysis are needed to clarify the familial position of this genus.

Type species:—Zukaliopsis amazonica Hennings, Hedwigia 43(6): 367 (1904) MycoBank No: 213216 (Fig. 9)

Parasitic on leaves of Sapindaceae. Sexual state: *Ascostromata* 0.3– 0.4×2 –3 mm ($\overline{x} = 0.38 \times 2.3$ mm, n = 10), superficial, scattered, solitary or aggregated, coriaceous to sub-carbonaceous, semi-immersed to erumpent, black to brownish grey, ovoid to sub-globose, multi-loculate, numerous locules distributed throughout the upper part of the ascostromata, one ascus in each locule, ostiolate. *Ostioles* minute. *Paraphyses* not observed. *Cells of the ascostromata* comprising dark brown pseudoparenchymatous *textura angularis*. *Asci* 7–8 × 6–7 µm ($\overline{x} = 7.5 \times 7$ µm, n = 20), 8-spored, bitunicate, fissitunicate, ovoid to clavate, short pedicellate, narrowly pointed at the base, apically rounded with indistinct ocular chamber. *Ascospores* 5–6 × 1–2 µm ($\overline{x} = 6 \times 2$ µm, n = 40), hyaline, oblong to cylindro-clavate, muriform, with 4–6-transverse septa, 0–1-longitudinal septum, slightly constricted at the septa, smooth-walled to verruculose, lacking a sheath. Asexual state: Unknown.

Material examined:—PERU. Iquitos, Rio Amazon, on *Paullinia* sp. (Sapindaceae), 1902, (S, F5604!, isotype).

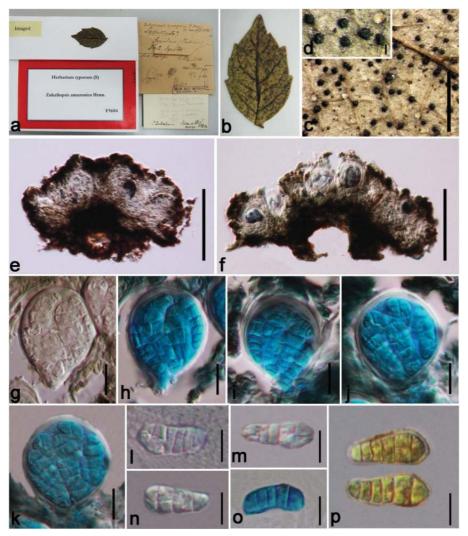


FIGURE 9. *Zukaliopsis amazonica* (isotype). a–b. Herbarium specimen. c–d. Ascostromata on natural substrate. e–f. Cross section of ascostromata. g. Ascus in water. h–k. Asci in cotton blue. l–n. Ascospores in water. o. Ascospore in cotton blue. p. Ascospores in Melzer's. Scale bars: c = 1 mm, $d = 200 \mu\text{m}$, $e-f = 50 \mu\text{m}$, $g-p = 10 \mu\text{m}$.

Discussion

Starbäck (1899) introduced the order Myriangiales for the species characterized by crustose ascostromata and muriform ascospores, similar to the type species *Myriangium duriaei* (Miller 1938). Phylogenetic studies based on multigene analysis have indicated that Myriangiales always clusters with Dothideomycetes (Schoch *et al.* 2009, Zhang *et al.* 2011). Kirk *et al.* (2008) included three families, Cookellaceae, Elsinoaceae and Myriangiaceae in Myriangiales, while Lumbsch & Huhndorf (2010) accepted only Elsinoaceae and Myriangiaceae in the order based on phylogenetic results. The order is characterized by pulvinate, irregular ascostromata in which the asci are irregularly arranged in one or more layers in locules. Locules have single or multiple asci within each locule. Asci are globose, with minute pedicels and indistinct ocular chambers. Ascospores which are irregularly arranged are liberated only by the breakup of the stromatal layers above them. Asexual states are unknown.

Elsinoaceae is a family of plant pathogens that cause anthracnose and scab. Many classical treatments have placed Elsinoaceae in synonymy with Myriangiaceae. However, Höhnel (1909) was convinced that the former constituted a separate family. Eriksson (1981) agreed and maintained two separate families with Myriangiaceae predominantly saprobic on branches and Elsinoaceae restricted to foliar pathogens. In Elsinoaceae the pseudoascostromata form whitish, crust-like patches on leaves with a small number of asci in each locule. Asci are globose and mostly 4–5 asci are embedded in one locule. Species of Schizothyriaceae Höhn. have similar characters in the shape of the asci to those in Myriangiaceae, but differ as asci form in a single hymenial layer and not in locules scattered in an ascostromata or pseudostromata (Hyde *et al.* 2013).

In this paper the generic types of the existing Myriangiaceae members, Anhellia tristis, Diplotheca tunae, Eurytheca trinitensis and Myriangium duriaei, were re-examined and their placement in the family confirmed. The generic types of Ascostratum insigne, Butleria inaghatahani, Dictyocyclus hydrangea, Hemimyriangium betulae, Micularia merremiae and Zukaliopsis amazonica were also examined and we recommend placement in the family Myriangiaceae because locules with single asci form in a well-developed dark ascostromata.

The position of *Anhellia* was uncertain within Myriangiaceae (Lumbsch & Huhndorf 2007, Krik *et al.* 2008). Pinho *et al.* (2012) analysed nucleotide sequences of ribosomal large subunit (LSU) and ITS region gene data from *Anhellia nectandrae* Pinho & Pereira (2012) showed it to cluster in Myriangiaceae. This and other genera that lack sequence data need recollection so that molecular studies can confirm and resolve their relationships in Myriangiales.

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