



Towards a natural classification of Dothideomycetes: 8. The genera *Cocconia*, *Dianesea*, *Endococcus* and *Lineostroma*

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Abstract

The generic types of four genera with unclear placement in Dothideomycetes were re-examined. These genera were hitherto poorly illustrated or described. The type specimens of *Cocconia* (*C. palmae*), *Dianesea* (*D. palmae*), *Endococcus* (*E. rugulosus*) and *Lineostroma* (*L. banksiae*), were re-examined in order to determine their familial and higher level placements, according to their morphology and based on modern taxonomic concepts. A resume of the history along with descriptions and illustrations of these genera are provided. *Endococcus* and *Lineostroma* are placed in families Lichenotheliaceae and Didymosphaeriaceae, respectively, based on morphology. The placement of *Cocconia* within Parmulariaceae is confirmed. *Dianesea* is retained in Dothideomycetes genera *incertae sedis* as it is not typical of any existing family of Dothideomycetes. Fresh collections of these genera are needed for further study, so that they can be epitypified and molecular data is needed to validate their phylogeny and natural classification.

Key words: Didymosphaeriaceae, *Discothecium*, Lichenotheliaceae, Parmulariaceae

Introduction

The class Dothideomycetes is one of the largest and most significant classes in the Ascomycota (Kirk *et al.* 2008, Schoch *et al.* 2009, Hyde *et al.* 2013). It has approximately 20,000 species (Hane *et al.* 2011). The most important morphological characters used to define major groups in the phylum Ascomycota, are the type of ascus, septation of ascospores, the morphology and development of the ascoma, as well as the structure and organization of the centrum (Schoch *et al.* 2009). A Dothideomycetes class-wide morphological assessment was carried out by Barr (1979, 1987). Her subclasses were determined based on the morphological characteristics of the centrum, including the absence, presence and types of hamathecial tissues (Schoch *et al.* 2009). Recent studies using multigene analysis, coupled with morphology, have provided the basis for classification of the Dothideomycetes (Liew *et al.* 2000, Lumbsch & Lindemuth 2001, Spatafora *et al.* 2006, Schoch *et al.* 2009, Zhang *et al.* 2012, Hyde *et al.* 2013). Several recent publications on the genera of Dothideomycetes (Wu *et al.* 2011, Liu *et al.* 2012, Zhang *et al.* 2012, Hyde *et al.* 2013, Ariyawansa *et al.* 2013, 2014, Dai *et al.* 2014, Li *et al.* 2014, Thambugala *et al.* 2014a, b, Tian *et al.* 2014) have aimed to provide a natural classification of this large class. Some studies have been based solely on morphological characterization (Ariyawansa *et al.* 2013, 2014b, Dai *et al.* 2014, Li *et al.* 2014, Thambugala *et al.* 2014 a, b, Tian *et al.* 2014) and some have included molecular analysis. The aim of the present study, the eighth paper in this series, is to re-visit the generic types of genera that have been categorized in Dothideomycetes *incertae sedis* and are poorly understood. In this paper we re-describe *Cocconia*, *Dianesea*, *Endococcus* and *Lineostroma* from type material, provide illustrations and discuss suitable placements at the family and ordinal levels.

Materials and Methods

The type specimens of *Cocconia* (*C. palmae* (Berk. & Broome) Sacc.), *Dianesea* (*D. palmae* (F. Stevens) Inácio & P.F. Cannon), *Endococcus* (*E. rugulosus* Nyl.) and *Lineostroma* (*L. banksiae* (Cooke) H.J. Swart) were loaned from Kew, US. Ascomata were rehydrated in 5% KOH prior to examination and sectioning. Specimens were examined using a stereo microscope (Motic SMZ 168) and fine forceps were used to remove one or two ascomata, which were mounted in water. Hand sections were made with a sharp razor blade. The sections were transferred to a drop of water or a drop of lactophenol cotton blue for examination. Observations and photomicrographs were made from using a Nikon ECLIPSE 80i light microscope with a Cannon 450D digital camera. Measurements were made with Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Results and Discussion

In this study genera *Endococcus* and *Lineostroma* are placed in families Lichenotheliaceae and Didymosphaeriaceae, respectively, based on morphology. The placement of *Cocconia* within Parmulariaceae is confirmed. *Dianesea* is retained in Dothideomycetes genera *incertae sedis* as it is not typical of any existing family of Dothideomycetes. Morphological details are discussed below.

Taxonomy

Lichenotheliaceae Henssen, Syst. Ascom. 5: 137 (1986)

The family Lichenotheliaceae was introduced by Henssen (in Eriksson & Hawksworth 1986). This is a monotypic family and the type genus, *Lichenothelia* was described by Hawksworth (1981), for two rock-colonizing species. Lichenotheliaceae is saprobic and loosely associated with algae or lichens (Hyde *et al.* 2013).

Endococcus Nyl., Mém. Soc. Sci. Nat. Cherbourg 3: 193 (1855)

Facesoffungi Number: FoF 00558

Lichenicolous, parasymbiotic or parasitic on the thalli of lichen-forming fungi. **Sexual morph:** *Ascomata* arising singly, perithecioid, black, subglobose, immersed to erumpent, ostiolate, ostiole internally lined with distinct periphyses. *Peridium* carbonaceous, comprising 3–6 layers of radially compressed, thick-walled, dark brown pseudoparenchymatous cells. *Hamathecium* lacking paraphysoids and pseudoparaphyses, asci originating in a gelatinized matrix. *Asci* 4–8 spored, bitunicate, elongate-clavate to sub-cylindrical, with short furcate, broad pedicel, thickened and rounded at apex, with an ocular chamber. *Ascospores* uniseriate or 2-seriate, dark brown, ellipsoidal, with rounded or somewhat pointed apices, 1-septate, sometimes markedly constricted at the septum, smooth-walled. Lacking a sheath or appendages.

Asexual morph: Undetermined.

Type species: Endococcus rugulosus Nyl.

Endococcus rugulosus Nyl., Mém. Soc. Sci. Nat. Cherbourg 3: 193 (1855)

Facesoffungi number: FoF 00559 (Fig. 1)

≡ *Verrucaria rugulosa* Borrer ex Leight., The British species of angiocarpous lichens: 47 (1851)

≡ *Microthelia rugulosa* (Borrer ex Leight.) Mudd, A manual of British lichens: 306 (1861)

Habitat on lichen thallus on old walls. **Sexual morph:** *Ascomata* 180–260 μm high \times 165–250 μm diam., solitary or scattered, wall dark brown to black, semi-immersed, globose to subglobose, smooth, coriaceous, ostiolate. *Ostiole* usually widely porate, shortly papillate or epapillate, ostiolar canal filled with periphyses. *Peridium* 30–45 μm diam., comprising several layers of thick-walled, heavily pigmented, small, dark brown to black cells of *textura angularis*. *Hamathecium* without pseudoparaphyses. *Asci* 67–75 \times 14–18 μm (\bar{x} = 72.6 \times 16 μm), 8-spored, bitunicate, fissitunicate, clavate to sub-cylindrical, with short furcate, broad pedicel, thickened and rounded at apex, with an ocular chamber. *Ascospores* 14–16 \times 7–9 μm (\bar{x} = 15.6 \times 7.6 μm), uniseriate or biseriate, partially overlapping, brown

to chestnut brown, ellipsoidal with broadly rounded ends, 1-septate, slightly constricted at the septum, verruculose, thick-walled, lacking a sheath. **Asexual morph:** Undetermined.

Material examined: —UK. East Sussex: on lichen thallus on old walls, W. Borrer (K(M): 155758, **isotype**).

Notes:—Fungi formerly referred to *Tichothecium*, fall into two groups, based on ascus structure (Hawksworth 1979). Those with 4- or 8-spored, bitunicate asci are placed in *Endococcus* (syn. *Discothecium*), while those with multi-spored asci, discharging by a bursting of the apex (Hawksworth 1979), are considered to belong to *Muellerella*. There are two groups of *Endococcus*; one type with spores that have rounded ends and are hardly constricted at the septum, and another with spores that have somewhat pointed apices and are very markedly constricted at the septum (Hawksworth 1979). Sérusiaux *et al.* (1999) and Kocourková (2000) suggested that *Endococcus* species are generally host-specific, and the name *E. rugulosus* Nyl. *Endococcus rugulosus* (\equiv *Verrucaria rugulosa*) was referred to the genus *Verrucaria*, family Verrucariaceae by Borrer ex Leight (1815) (Hawksworth 1979). *Endococcus* was placed in the order Dothideales by Hawksworth (1979).

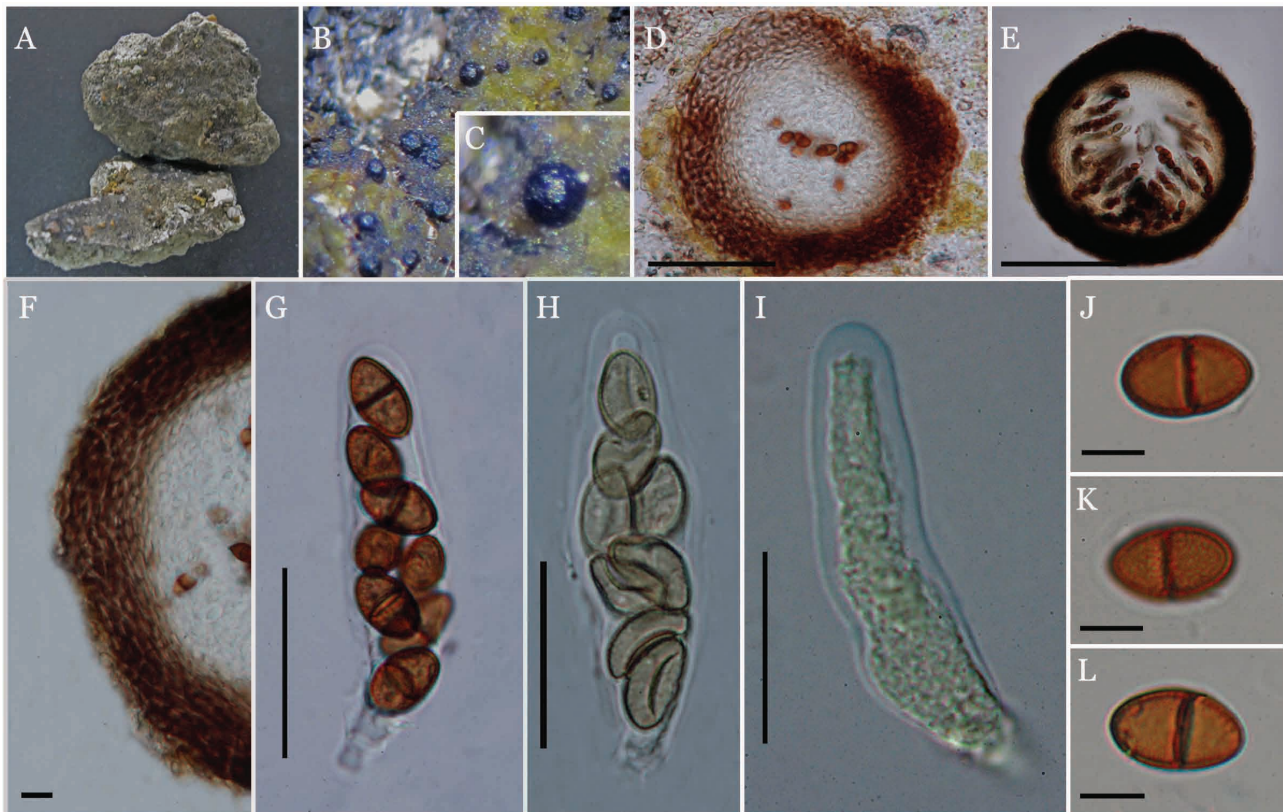


FIGURE 1. *Endococcus rugulosus* (isotype). **A** Herbarium specimen. **B** Ascomata on host substrate. **C** Close up of ascoma. **D, E** Sections of ascomata. **F** Close up of the peridium. **G–I** Asci with short, broad pedicel, containing 8 ascospores. **J–L** Verruculose ascospores with rounded ends. Scale bars: D–E=60 µm, F=10 µm, G–H=20 µm, J–L=10 µm.

Lumbsch & Huhndorf (2010) and Hyde *et al.* (2013) placed *Endococcus* in Dothideomycetes genera *incertae sedis*. In the present study we refer it to the family Lichenotheliaceae based on morphological similarity with the type species of *Lichenothelia* (*Lichenothelia scopularia* (Nyl.) D. Hawksw.), which colonizes diverse types of rock substrates and is sometimes found on lichens. Due to the perithecioid ascomata with widely porate ostioles, globose to broadly clavate asci and hyaline or brown, 1-septate ascospores, we regard *Endococcus* as a distinct genus in the family Lichenotheliaceae. Index Fugorum lists 79 species in this genus but none have had their placement confirmed by molecular data. From studies of morphology they have been put into different families, hence this group is obviously polyphyletic. In this study we only re-examined the type species, *Endococcus rugulosus*.

Didymosphaeriaceae Munk, Dansk Bot. Ark. 15(no. 2): 128 (1953)

Munk (1953) introduced the family Didymosphaeriaceae, typified by the genus *Didymosphaeria*. The family was characterized by 1-septate ascospores and trabeculate pseudoparaphyses, which anastomosed mostly above the asci (Aptroot 1995, Ariyawansa *et al.* 2014a). Based on its trabeculate pseudoparaphyses, Barr (1990) referred Didymosphaeriaceae to the order Melanommatales. However, the importance of trabeculate pseudoparaphyses was later

disputed (Liew *et al.* 2000). The confusion surrounding Didymosphaeriaceae and *Didymosphaeria* was discussed in detail by Ariyawansa *et al.* (2014a). They explained that considering the morphological data only (peridium comprising flattened or irregular cells, 1-septate ascospores and trabeculate pseudoparaphyses mostly anastomosing above the asci) the family Didymosphaeriaceae appears to be distinct from Pleosporales. Molecular and morphological data showed *Didymosphaeria* species clustered within the family Montagnulaceae, which is the oldest name (Ariyawansa *et al.* 2014 a,b). In this study we determined the placement of *Lineostroma* to be within the family Didymosphaeriaceae because of its peridium, comprising flattened or irregular cells, 1-septate ascospores and trabeculate pseudoparaphyses, anastomosing above the asci (Hyde *et al.* 2013, Ariyawansa *et al.* 2014a). As *Lineostroma* is monotypic we provide only a single description.

Lineostroma H.J. Swart, Trans. Br. Mycol. Soc. 91(3): 464 (1988)

Type species: *Lineostroma banksiae* (Cooke) H.J. Swart, Trans. Br. Mycol. Soc. 91(3): 464 (1988)

≡ *Didymosphaeria banksiae* Cooke, Grevillea 19(no. 92): 90 (1891)

Facesoffungi number: FoF 01293 (Fig. 2)

Pathogen on leaves of *Banksia*, ascomata in necrotic spots on upper surface of living leaves. **Sexual morph:** *Ascostromata* 75–125 μm high, 70–95 μm diam., linear, intraepidermal, at maturity breaking through the upper leaf surface, locules small, containing a few asci between paraphysoid threads, anastomosing, at maturity forming a small apical ostiole. *Hamathecium* of dense, septate, narrow, trabeculate pseudoparaphyses in a gelatinous matrix. *Peridium* 15–20 μm wide, inner layer hyaline, outer layer brown, comprising cells of *textura angularis* to *textura globularis*. *Asci* 45–70 \times 8–10 μm (\bar{x} = 53 \times 10 μm), 8-spored, bitunicate, cylindro-clavate, short pedicellate, apically rounded, with a minute ocular chamber. *Ascospores* 21–26 \times 3–5 μm (\bar{x} = 23 \times 4 μm), uniseriate to biseriate, hyaline, becoming brown when mature, 1-septate, constricted at the septum. **Asexual morph:** Undetermined.

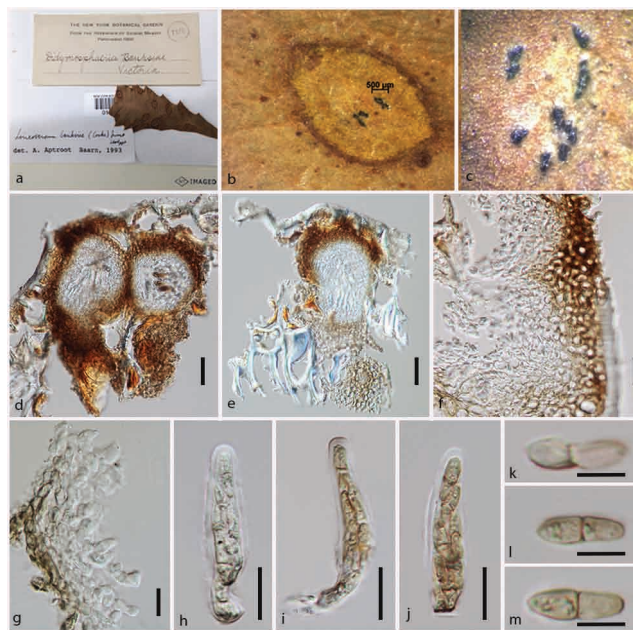


FIGURE 2. *Didymosphaeria banksiae* (holotype). **A** Herbarium material. **B** Ascostromata on host surface. **C** Close up of ascostromata. **D, E** Section of the ascostromata. **F** Close up of the peridium. **G** Peridium. **H–J** Cylindrical to clavate asci with a small pedicel and ocular chamber. **K–M** Clavate-fusiform ascospores. Scale bars: D=100 μm , E=50 μm , F=5 μm , G=30 μm , I–L=10 μm .

Material examined:—AUSTRALIA. Victoria: on leaves of *Banksia serrata* (Proteaceae), 1892, Cooke (K(M) 143926, holotype).

Notes:—The family Didymosphaeriaceae contains 13 sexual genera and two asexual genera (Ariyawansa *et al.* 2014a). *Lineostroma banksiae* has morphological characters that are distinct to other genera in the family, and hence we place it as a separate genus in the family Didymosphaeriaceae. *Lineostroma banksiae* is most similar to *Phaeodothis* in having linear, intra-epidermal ascostromata, trabeculate pseudoparaphyses, asci with a short pedicel and 1-septate ascospores. *Phaeodothis tricuspis* differs in ascus arrangement and the peridium comprising hyaline, compressed cells (Ariyawansa *et al.* 2014a).

Parmulariaceae E. Müll. & Arx ex M.E. Barr, Mycologia 71(5): 944 (1979)

The family *Parmulariaceae* was introduced by Müller and von Arx (1962) for taxa with colonies growing on the surface of living plant tissues, mostly leaves, and the family was validated by Barr (1979). The family is clearly polyphyletic and contains a range of ascostromata types, ascostromata and even thyrtothecia, ascus and ascospore forms (Inácio & Cannon 2008). Lumbsch & Huhndorf (2010) included 34 genera in the family and Inácio & Cannon (2008) included 59 genera (Guatimosim *et al.* 2014).

Cocconia Sacc., Syll. Fung. (Abellini) 8: 738 (1889)

Saprobic on leaves. **Sexual morph:** *Ascostromata* solitary to gregarious, or in groups, superficial, shield-like, dark brown to black, carbonaceous, flattened, connected to internal stroma by hyphal tubes or clusters, ascostromata margin smooth, Internal stroma easily distinguished, well-developed, with ridges radiating from the centre to the outer rim, ridges containing elongate locules, which open by a longitudinal slit and contain numerous asci, cells of ascostromata thick-walled, elongate and composed of black, amorphous tissues in the stromatic crust. *Hamathecium* of dark brown to hyaline, septate pseudoparaphyses, swollen and brown at their apices. *Asci* 8-spored, thick-walled, bitunicate, cylindrical, short-pedicellate, with a distinct ocular chamber. *Ascospores* 2–3-seriate, ellipsoidal, with rounded ends, hyaline to dark brown, 1-septate, constricted at the septum, smooth walled (Hyde *et al.* 2013).

Notes:—Sections of fruit-bodies of the type specimens of *Cocconia palmae* F. Stevens (Stevens 1927), current name *Dianesea palmae* revealed two different taxa and Imicio & Cannon (2002) stated it was clear that the original description was a composite of both species. Hence, in this study we re-examined the morphology of both *Cocconia placenta* under *Cocconia* and *Cocconia palmae* under *Dianesea*. We have provided only a single description as this genus contains a single species with two varieties.

Cocconia placenta (Berk. & Broome) Sacc., Syll. Fung. (Abellini) 8: 738 (1889)

Facesoffungi number: FoF 00563 (Fig. 3)

≡ *Rhytisma placenta* Berk. & Broome, J. Linn. Soc., Bot. 14: 131 (1873)

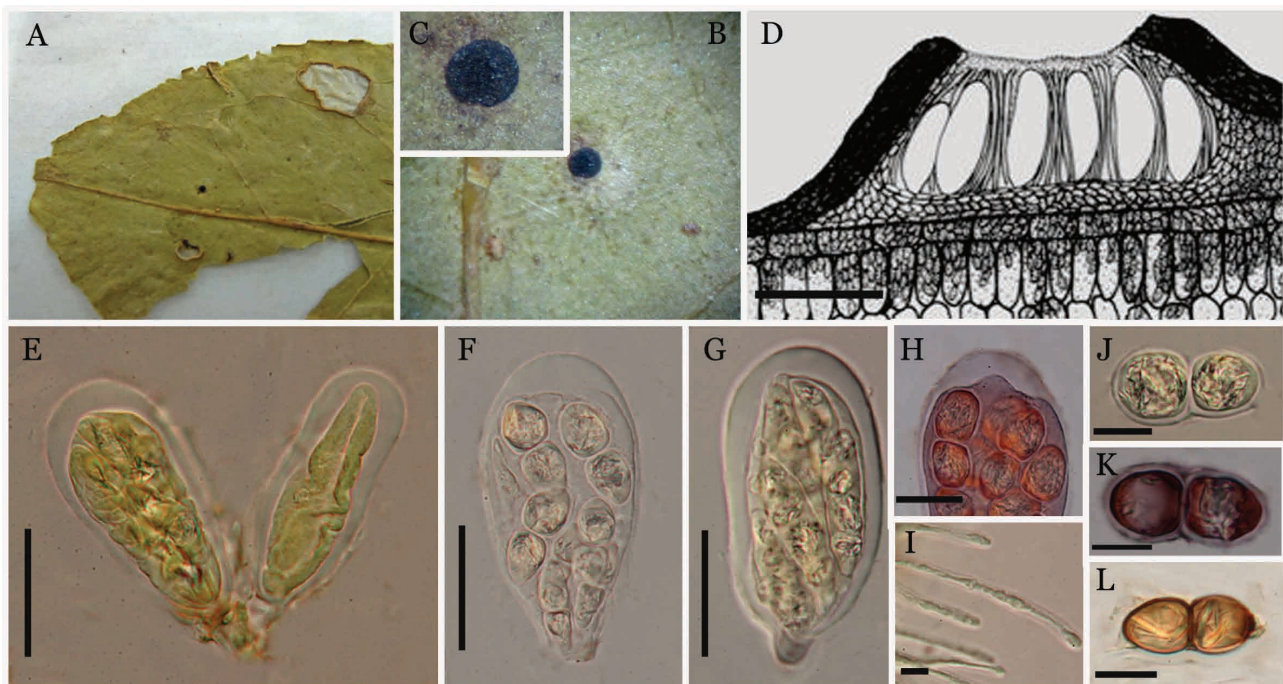


FIGURE 3. *Cocconia placenta* (holotype). **A, B** Ascostromata on host substrate. **C** Close up of ascostroma. **D** Section of ascostroma. **E–G** Asci with short, broad pedicel, containing four ascospores. **H** Apical part of the ascus having ocular chamber stained in Congo red. **I** Pseudoparaphyses. **J** Smooth-walled immature spore. **K** Ascospore stained with Congo red. **L** Mature dark brown ascospore. Scale bars: D=100 µm, E–G=40 µm, H=15 µm, I=5 µm, J–L=15 µm.

Habitat on living leaves of *Symplocos spicata* Roxb. Dark brown coloured hyphae penetrate the plant tissue intracellularly. **Sexual morph:** *Ascostromata* solitary, superficial, globose to subglobose, wall dark brown to black with single locules, smooth, coriaceous, ostiolate. *Ostirole* usually wide porate, broadly papillate. *Peridium* comprising

several layers of thick-walled *textura angularis*, comprising wide cells. *Hamathecium* of numerous, 1–2 μm diam., long, septate, cellular pseudoparaphyses. *Asci* 84–108 \times 28–40 μm (\bar{x} =96 \times 32 μm), 8-spored, bitunicate, clavate or broadly clavate, with short, furcate pedicel rounded and thickened at the apex, with a large ocular chamber. *Ascospores* 30–38 \times 10–16 μm (\bar{x} =34 \times 14 μm), uniseriate to partially overlapping, hyaline to pale olivaceous when immature, dark brown when mature, ellipsoid or cylindrical to clavate, thick-walled, 1-septate, distinctly constricted at septum, smooth-walled. **Asexual morph:** Undetermined.

Material examined:—SRI LANKA. Peradeniya: on leaves of *Symplocos spicata* (Symplocaceae), November 1867, G.H.K. Thwaites (K (M) 409, **holotype**).

Notes:—*Cocconia* is morphologically similar to genera within Parmulariaceae and Asterinaceae. The family Parmulariaceae differs from Asterinaceae in having ascostromata formed by pigmented cells in several layers, pseudoparaphyses, broadly ellipsoidal to obclavate asci, and lacking appressoria (Inácio & Cannon 2008, Inácio *et al.* 2012). Species of Asterinaceae have superficial hyphae mostly with appressoria, thyriothecia with a thin upper wall layer and poorly developed base and subglobose asci (Pirozynski & Shoemaker 1970, Inácio *et al.* 2012, Hongsanan *et al.* 2014). In this study we accept the placement of *Cocconia* within the family Parmulariaceae because of the ascostromata with several layers of pigmented cells, pseudoparaphyses and broadly ellipsoidal to obclavate asci. *Cocconia* differs from other genera in the way the ascostromata connect to host surface, surface hyphae, absence or present of haustoria, ascospore shape and septation (Hyde *et al.* 2013).

Dothideomycetes genera *incertae sedis*

Dianesea Inácio & P.F. Cannon, Fungal Divers 9: 72 (2002)

Type species: Dianesea palmae (F. Stevens) Inácio & P.F. Cannon, Fungal Divers 9: 72 (2002)

≡ *Cocconia palmae* F. Stevens, Illinois Biol. Monogr. 11(2): 175 (1927)

Facesoffungi number: FoF 00561 (Fig. 4)



FIGURE 4. *Cocconia palmae* (lectotype). **A, B** Appearance of ascostromata on host substrate. **C** Close up of ascostroma. **D** Section of ascostroma. **E, F** Peridium. **G** Pseudoparaphyses. **H–K** Asci with short, broad pedicel, containing 8 ascospores. **L–N** Ascospores. Scale bars: A=500 μm , B–E=100 μm , G=10 μm , H–K=20 μm , I=5 μm , L–N=15 μm .

Habitat on unidentified palm. *Hemibiotrophic* as small leaf spots 4.5 mm long \times 2 mm wide, scattered, rarely confluent, amphigenous, as small, \pm flat, black, stromatic structures, variable in shape, mostly elliptical, but sometimes circular or irregular, within dark brown to greyish leaf spots, dark brown intracellular hyphae penetrate the plant tissues. **Sexual morph:** *Ascostromata* 300–232 μm high \times 315–258 μm diam., solitary, wall dark brown to black, superficial, globose to subglobose, with single locule, smooth, coriaceous, ostiolate. *Ostiole* usually widely porate, broadly papillate. *Peridium* 46–52 μm , comprising several layers of thick-walled, cells of *textura angularis*. *Hamathecium* of numerous,

2–3 μm diam., long, septate, cellular pseudoparaphyses. *Asci* 90–120 \times 18–29 μm (\bar{x} = 112 \times 25 μm), 8-spored, bitunicate, clavate to subcylindrical, with a short, narrow pedicel, thickened and rounded at the apex, with an ocular chamber. *Ascospores* 21–26 \times 5–6 μm (\bar{x} = 23 \times 5 μm), uniseriate to partially overlapping, hyaline to pale olivaceous when immature, dark brown when mature, ellipsoid or cylindrical to clavate, 1-septate, distinctly constricted at the septum, smooth-walled, thick-walled, surrounded by hyaline mucous sheath. **Asexual morph:** Undetermined.

Material examined:—COSTA RICA. Peralta: on unidentified palm, 13 July 1923, F.L. Stevens 432 (K, **isotype**).

Notes:—Genera within the family Phaeosphaeriaceae have thin-walled ascomata that are occasionally aggregated into multi-ocular stromata, but are saprobes or necrotrophs and have quite distinct asexual morphs (Imicio & Cannon 2002, Phookamsak *et al.* 2014); hence *Dianesea* could not be placed in this family. In this study, we suggest that *Dianesea* is morphologically similar to *Metameris* (family Botryosphaeriaceae), except spore morphology especially the narrow ascospores with hyaline mucous sheath (Phookamsak *et al.* 2014). Therefore, the genus might be better accommodated in Botryosphaeriales regarding the similar characters to *Metameris*. However, we retain this genus in Dothideomycetes, genera *incertae sedis* until morphology coupled with molecular data can resolve the correct placement of this unusual genus.

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