

Towards a monograph of *Dothideomycetes*: Studies on *Diademaceae*

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Abstract – The family *Diademaceae* presently comprises the genera *Clathrospora*, *Comoclathris*, *Diadema*, *Diademosa* and *Graphyllum* and is characterized by ascomata opening as a flat circular lid, bitunicate asci and ascospores with three or more transverse septa, with or without longitudinal septa. The ascospores are fusiform, brown, mostly applanate and usually have a mucilaginous sheath. The type species of *Clathrospora*, *Comoclathris* and *Graphyllum* are revisited in this paper and re-described and illustrated with light micrographs based on examination of holotype specimens. The status of the family is discussed based on morphology and sequence data. *Comoclathris* is excluded from the family based on morphology and phylogenetic analysis, and should be accommodated in *Pleosporaceae*. *Clathrospora* is tentatively placed in *Pleosporaceae* pending molecular studies. *Graphyllum* has hysterothecial ascomata with a slit-like ostiole and should be excluded from *Diademaceae*. It can be accommodated in *Hysteriaceae*, but with uncertainty as species with hysterothecia have now been accommodated in at least five families. Molecular work is needed on *Graphyllum* to establish its familial placement. The status of *Diadema* and *Diademosa* as a distinct family *Diademaceae* based on ascomata opening as a flat circular lid is thought to be doubtful, but awaits molecular study.

Clathrospora / *Comoclathris* / *Graphyllum* / *Pleosporales*

INTRODUCTION

We are in the process of carrying out studies on *Dothideomycetes* in order to provide a natural classification (Zhang *et al.*, 2008, 2009a, b; Wu *et al.*, 2010, 2011; Li *et al.*, 2011). The study involves examining type material of generic types and making fresh collections with isolations where possible so that molecular analysis can be carried out; and reporting the findings in publications.

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As part of this work, we are restudying the type species of genera placed in the *Diademaceae*. This family was introduced by Shoemaker & Babcock (1992) and is characterized by ascomata opening as a flat circular lid and bitunicate asci. Ascospores are fusiform, brown, some being applanate, and having three or more transverse septate and with or lacking longitudinal septa and usually having a sheath. The family presently includes five genera, comprising *Clathrospora* Rabenh., *Comoclathris* Clem., *Diadema* Shoemaker & C.E. Babc., *Diademosia* Shoemaker & C.E. Babc. and *Graphyllum* Clem. (Lumbsch & Huhndorf, 2010).

The objective of the present paper is to revisit genera in the *Diademaceae* by examining type material where available or in cases where this is lost, not in good condition, or cannot be loaned we refer to descriptions, illustrations and figures in the original publications.

MATERIALS AND METHODS

Type or specimens of *Clathrospora*, *Comoclathris* and *Graphyllum* were obtained from BPI, COLO, E, and NEB and studied. Type material of *Diadema* and *Diademosia* could not be obtained from DAOM as they are presently not loaning material for study. Ascomata were rehydrated in 5% KOH prior to examination and sectioning. Specimens were examined under a Leica MZ16A stereo microscope and fine forceps were used to move one or two ascomata from the herbarium specimen, which were mounted in water and lactic acid or stained with cotton blue. Observations and photographs were made under the light microscope (Nikon Ei800 and Leica DM3000), for some hyaline structures differential interference contrast microscopy was used. Hand sections were cut with a sharp razor blade and thin (4-10 μm) sections were cut using a Leica CM1100 freezing microtome. The sections were transferred to a drop of water, a drop of lactic acid or a drop of cotton blue for examination and photographs.

Phylogenetic analysis

Among the five genera of *Diademaceae*, only *Clathrospora* has sequences in GenBank. The 28S and 18S ribosomal RNA sequences of *Clathrospora* and some other taxa of *Pleosporales* were downloaded from GenBank and separately aligned using Clustal X. In order to obtain maximum sequence similarity, sequences were manually aligned with Bioedit software 5.0.9 (Hall, 1999). The aligned dataset was analyzed using PAUP* 4.0b10 (Swofford, 2002). Gaps were treated as missing data, and the ambiguously aligned regions were excluded from analyses. Parsimonious trees were generated using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Branches of zero length were collapsed and all parsimonious trees were saved.

RESULTS

Phylogeny

The aligned dataset for each region (SSU and LSU) as well as the combined data sets for the two regions were analyzed separately. The phylogenetic position of *Clathrospora* in the datasets of each gene and the combined genes showed similar topology. As a consequence, only one of the trees that were generated from the combined genes datasets is represented here. The SSU and LSU combined alignment consisted of 41 taxa including *Schismatomma decolorans* as the outgroup taxa. The maximum parsimony dataset used in the phylogenetic analysis consists of 1308 total characters, 238 were informative, 488 were variable and parsimony-uninformative and 582 were constant. Of six equally most parsimonious trees obtained from a heuristic search, a best scoring tree with a length of 1437 steps (CI = 0.644, RI = 0.626, RC = 0.403, HI = 0.356) is shown in Figure 4.

Taxonomy

Diademaceae Shoemaker & C.E. Babcock, Can. J. Bot. 70(8): 1618 (1992)

Type genus: Diadema Shoemaker & C.E. Babcock, Can. J. Bot. 67(5): 1349 (1989).

Ascomata subepidermal or subcuticular, finally superficial, globose, depressed or patelliform, dark brown, often tomentose, opening with a central flat to papilliform lid of a few layers of small very dark brown cells. Hamathecium of pseudoparaphyses. Asci 8-spored, bitunicate. Ascospores biseriate to tetraseriate, brown, with 3 or more transverse septa, applanate or rarely terete, with or without longitudinal septa, usually with a thick sheath (modified from Shoemaker & Babcock, 1992).

Remarks: The family *Diademaceae* was introduced by Shoemaker & Babcock (1992) and incorporated five genera (*Clathrospora*, *Comoclathris*, *Diadema*, *Diademosia* and *Macrospora*). *Macrospora* was however, considered as a synonym of *Pyrenophora* by Eriksson & Hawksworth (1991) which is placed in *Pleosporaceae*. More recently *Macrospora* has been accepted as a good genus and is included in *Pleosporaceae* (Lumbsch & Huhndorf, 2010).

Shoemaker & Babcock (1992) considered *Diademaceae* to be distinct in being characterized by ascomata opening by a flat circular lid and generally applanate ascospores with numerous transverse septa and in some genera longitudinal septa. *Diademosia* however, differs in having terete as compared to applanate ascospores (Shoemaker & Babcock, 1992). *Platyspora* was at various times included in the family either as a standalone genus or as a synonym of *Graphyllum*, while it is now considered as a synonym of *Comoclathris* (www.indexfungorum.org).

The family appears to have been poorly studied and presently comprises five genera (Lumbsch & Huhndorf, 2010) and this has not changed since its introduction by Shoemaker & Babcock (1992). Although, *Graphyllum* Clem. was placed in *Hysteriaceae* by Shoemaker & Babcock (1992) in the same paper in which they introduced *Diademaceae* it has generally been placed in *Diademaceae* in other classification systems, but not Index Fungorum. No asexual state has been linked with most members of this family, however *Clathrospora* and *Comoclathris*

have been linked with an *Alternaria*-like anamorphs (Hyde *et al.*, 2011) which suggests a relationship with *Pleosporaceae*. We have studied the type material of *Clathrospora*, *Comoclathris* and *Graphyllum*, however DAOM is presently not in a position to loan type specimens and therefore we have used the adequate descriptions and illustrations in the literature to characterize these species.

The pivotal characters for the family appear to be the yellow to brown, phragmosporous or muriform ascospores which may be applanate, bitunicate asci and ascomata opening by a circular lid. The circular lid-like opening was thought to be an adaptation to the alpine habitat (Shoemaker & Babcock, 1989), but whether this feature is family specific or has evolved many times has not been tested. The applanate nature of the spores is only found in *Diadema obtusa* (of *Diadema* species) and yet the *Diademaceae* is thought to comprise species with applanate ascospores. *Diadema obtusa* is not the type species of *Diadema* and the genus is characterized by mostly terete ascospores. The circular lid-like opening and brown transeptate to muriform ascospores are therefore the only character that seems to link the genera presently included in *Diademaceae* and we would suggest the family as it stands is doubtful. Molecular data is needed to analysis the phylogenetic significance of the circular lid-like opening and establish if the family has validity.

Clathrospora Rabenh., *Hedwigia* 1(18): 116 (1857)

Generic description: Ascomata subglobose to globose, immersed, becoming semi-immersed to erumpent, scattered, brown to blackish brown, coriaceous, with a central sunken ostiole and opening by a circular lid, asci and pseudoparaphyses forming from the base of the peridium. Peridium comprising several layers of brown, relatively thick-walled cells of *textura angularis*, inner cells flattened, thin-walled and lighter. Pseudoparaphyses hyaline, filiform, longer than the asci. Asci 8-spored, bitunicate, fissitunicate, thick-walled, cylindrical to clavate, with a short pedicle and shallow ocular chamber. Ascospores irregularly fusiform, applanate, muriform, constricted only at the ascospore centre, brown to dark-brown, with a thin, hyaline mucilaginous sheath.

Anamorphs reported for genus: *Alternaria*-like (Hyde *et al.*, 2011)

Type species:

Clathrospora elyanae Rabenh., *Hedwigia* 1: 116 (1857)

Figs 1A-I

≡ *Pleospora elyanae* (Rabenh.) Ces. & De Not., *Comm. Soc. Critt. Ital.* 1: 218. 1863

Ascomata 100-300 µm in diameter, immersed, becoming semi-immersed to erumpent, scattered on the putrid host stems and foliage, brown to blackish brown, coriaceous; in vertical section 143-222 × 145-175 µm, subglobose or nearly globose, with a slightly sunken central ostiole, asci and pseudoparaphyses forming from the base of the peridium (Figs 1A, B). Peridium 23-55 µm wide, comprising 3-5 layers of brown, relatively thick-walled cells of *textura angularis*, inner cells flattened, thin-walled and lighter (Fig. 1C). Pseudoparaphyses hyaline, filiform, longer than the asci (Figs 1C-E). Asci 163-231 × 28-48 µm (mean = 194 × 37 µm, n = 20), 8-spored, bitunicate, fissitunicate, thick-walled, cylindrical to clavate, with a short pedicle and shallow ocular chamber (Figs 1D-G). Ascospores 40-65 × 18-27 µm (mean = 53 × 23 µm, n = 20), biseriate to overlapping trisetiate, applanate, irregularly broadly fusiform, muriform, with 7-8 transverse septa and 6-8 longitudinal septa, constricted only at the ascospore centre, brown to dark-brown, surrounded by a thin, hyaline mucilaginous sheath 2-3 µm wide (Figs 1H, I).

Anamorph: *Alternaria*-like.

Cultures and DNA sequences: CBS 196.54: SSU (GU296142), LSU (GU323214)

Material examined: Italy, Riva-Valdobbia (Novara), on rotting stem of *Carex curvula*, September 1898, A. Carestia (BPI 627747); Switzerland, Albulapass, on stem of *Carex curvula*, G. Winter, Rehm Ascomyceten, No. 1590 (BPI 627748); Switzerland, Furkapasshöhe, 2540 m alt, on stem of *Carex curvula*, 4 August 1905, O. Jaap, Otto Jaap, Fungi selecti exsiccate No. 156 (BPI 627749); Austria, Salzburg, Lungau, 47°05'N / 13° 24'E, 2000 m alt, on stem of *Carex curvula*, 20 August 1981, Ch. Scheuer (BPI 748847); Switzerland, Berner Alpen, Aletschgebiet, Eggishorn, on stem of *Carex curvula*, July 1905, K. Ronniger (BPI, 1111777).

Remarks: Although there are 50 epithets for this genus in Index Fungorum, only ten species were accepted in the paper of Shoemaker & Babcock (1992) that provided a key to species, descriptions and illustrations. Species were characterized by a circular lid-like opening and muriform appanate ascospores. With the exception of a new combination by Vassiljeva (1998) the genus has not been studied since Shoemaker & Babcock (1992). We could not locate the type of this species but looked at several representative collections.

Phylogenetic study: The 18s and 28s ribosomal RNA gene has been partially sequenced for putative strains of *Clathrospora elyinae* and *C. diplospora* (Dong *et al.*, 1998; Schoch *et al.*, 2009). Dong *et al.* submitted sequences U43464 and U43481 to GenBank with the name *C. diplospora* (strain IMI 68086) but they used the name *Comoclathris baccata* in their paper (Dong *et al.*, 1998). The reconstructed phylogenetic tree containing *Clathrospora* and the members of other *Pleosporales* show that the two *Clathrospora* (*C. elyinae* and *C. diplospora*?) species nested within *Pleosporaceae* with 67% bootstrap support value (Fig. 4). The results of phylogeny here are consistent with the previous phylogenetic study of Schoch *et al.* (2009), and supports exclusion of the genus from *Diademaceae* and placement in *Pleosporaceae*. The nature of the ascomata with a slightly papillate ostiole and *Alternaria*-like anamorph also support this conclusion.

Concluding remarks: The 50 epithets for this genus listed in Index Fungorum have not been adequately dealt with as Shoemaker & Babcock (1992) did not discuss many of these species and it is likely they did not consider them in their acceptance of ten species. Molecular and morphological data suggest generic placement in *Pleosporaceae*. This genus is in need of revision.

Comoclathris Clem., Gen. fung. (Minneapolis): 37, 173 (1909)

Synonyms: ?*Platyspora* Wehm., World Monograph of the Genus *Pleospora* and its Segregates: 254 (1961).

Generic description: Ascomata subglobose or nearly globose, superficial, scattered or aggregated on the host stem, coriaceous, brown to blackish brown, covered with short, brown radial septate hyphae, opening by a large circular aperture of lid. Peridium comprising 3-4 layers of brown, relatively thick-walled cells of *textura angularis*, inner cells flattened, thin-walled and lighter. Pseudoparaphyses and asci forming from the base of the ascomata. Pseudoparaphyses hyaline, filiform, septate. Asci 8-spored, bitunicate, fissitunicate, thick-walled, cylindrical to cylindro-clavate, with a short knob-like pedicel, and indistinct shallow ocular chamber. Ascospores fusiform, muriform, with 4-5-transverse septa and 1-2-longitudinal septa, not constricted at the septa, brown to reddish brown, surrounded by a distinct, hyaline, mucilaginous thick sheath.

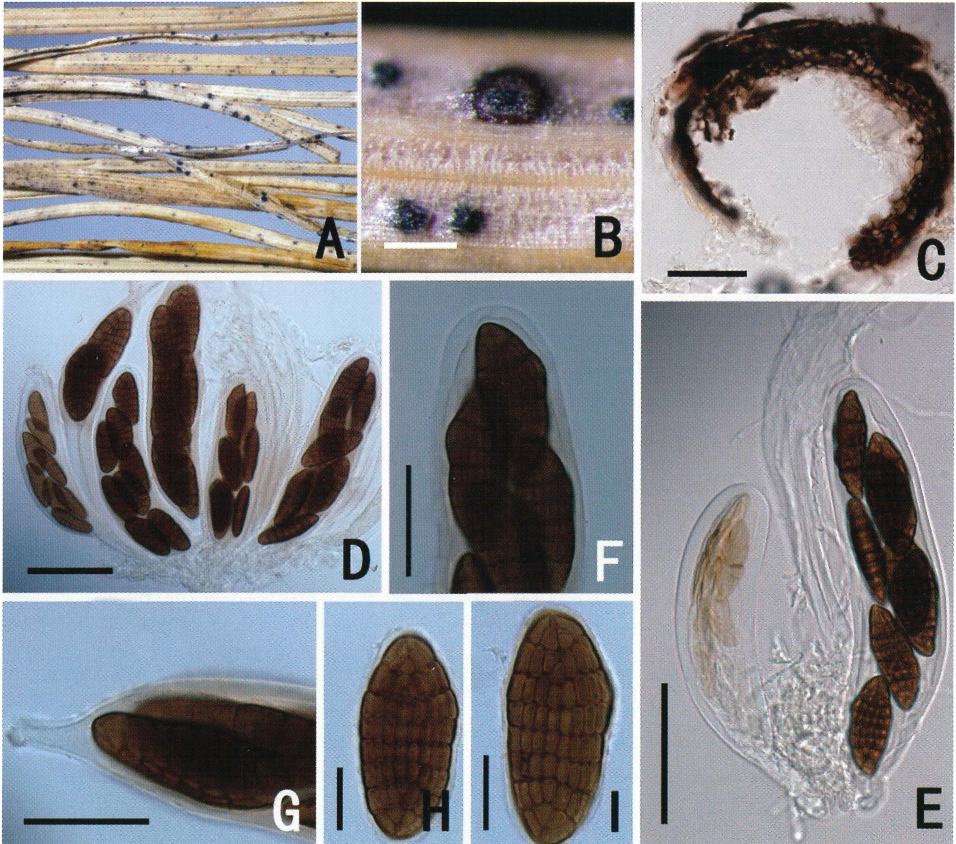


Fig. 1. *Clathrospora elynae* (BPI 627747). **A.** Herbarium material. **B.** Habit, illustrating ascomata on the host stems. **C.** Section of ascoma. **D-G.** Immature and mature 8-spored asci and hyaline pseudoparaphyses. The asci are bitunicate, short-stalked and a shallow ocular chamber. **H, I.** Muriform, applanate ascospores with a narrow mucilaginous sheath. Scale bars: B = 300 μm , C-E = 40 μm , F, G = 20 μm , H, I = 10 μm .

Known anamorphs: *Alternaria*-like (Hyde *et al.*, 2011).

Type species:

***Comoclathris lanata* Clem. [as “*Comochlathris*”],**

Gen. fung. (Minneapolis): 1-227 (1909)

Figs 2A-F

= *Comoclathris planispora* (Ellis) J. Harr, *Nova Hedwigia* 20(3-4): 868. 1970(1971)

Ascomata 190-300 μm diameter, scattered or aggregated on the host stem, subglobose or nearly globose, sunken in the centre upper when dry, superficial, coriaceous, brown to blackish brown; in vertical section 175-245 μm diameter \times 142-197 μm high (mean = 205 \times 159 μm , n = 20), covered with short, brown, radial septate hyphae (Fig. 2B), with a central opening (Figs 2A-B). Peridium 10-22 μm wide, comprising 3-4 layers of brown, relatively thick-walled cells of *textura angularis*, inner cells flattened, thin-walled and lighter (Fig. 2D).

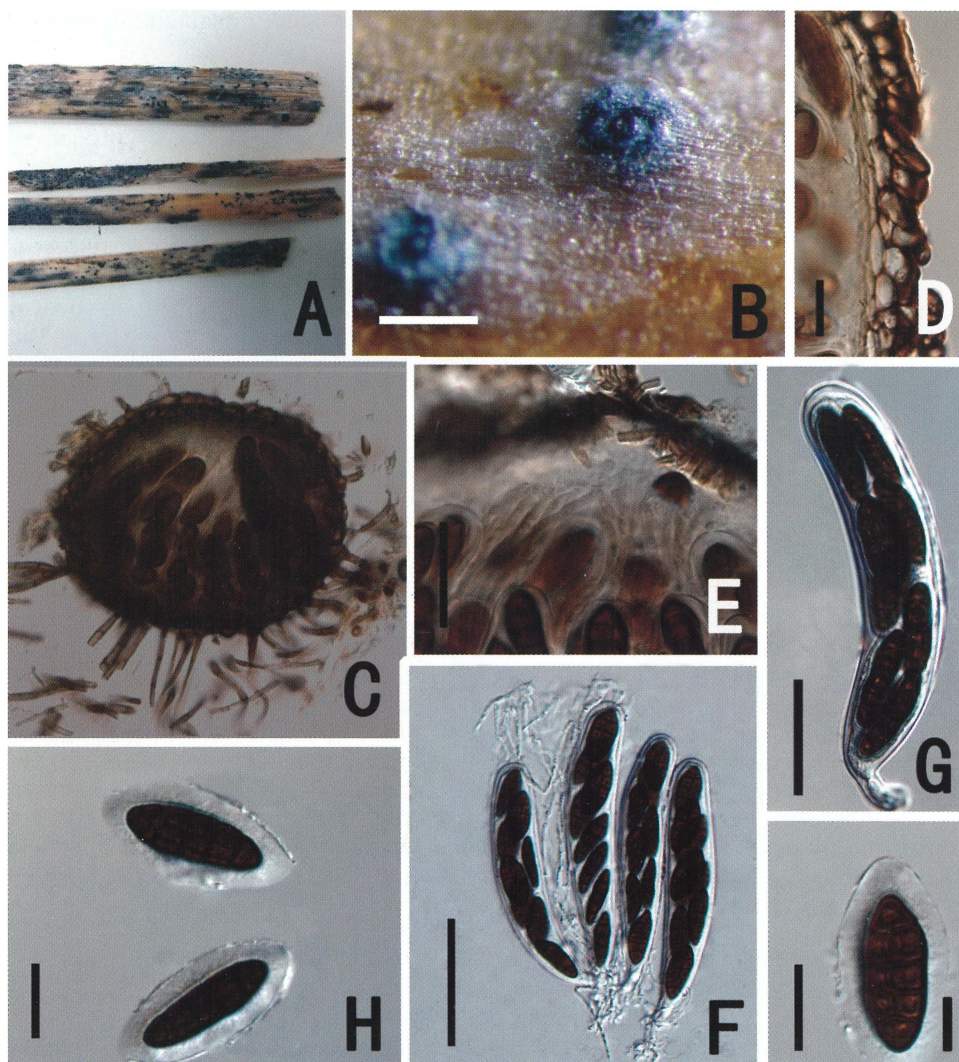


Fig. 2. *Comoclathris lanata* (COLO 62872, lectotype). **A.** Herbarium material showing habit of fungus on host stem. **B.** Erumpent ascomata. **C.** Thick section through ascoma. Note the arrangement of asci and external setae. **D.** Section showing peridial cells of ascoma. **E.** Section of ascoma showing apex of ascoma. **F, G.** Asci with short knob-like pedicels and shallow ocular chamber. **H, I.** Muriform ascospores with thick sheath. Scale Bars: B = 200 μm , C = 40 μm , E, H, I = 10 μm , F, G = 20 μm .

Asci and pseudoparaphyses forming from the base of the ascomata. Pseudoparaphyses 2–4 μm wide, septate, hyaline, filiform, longer than the asci (Figs 2C, E, F). Asci 108–149 \times 20–30 μm (mean = 125 \times 24 μm , n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to cylindro-clavate, with a short knob-like pedicel, and indistinct shallow ocular chamber (Figs 2F–G). Ascospores 20–32 \times 8–13 μm (mean = 28 \times 12 μm , n = 20), 1–2 overlapping seriate, fusiform, muriform, with 4–5–transverse septa and 1–2–longitudinal septa, not constricted at the septa,

brown to reddish brown, surrounded by a distinct, hyaline, mucilaginous sheath, 3-8 µm wide (Figs 2H-I).

Anamorph: *Alternaria*-like.

Material examined: USA, Colorado, on stem of *Leptotaenia multifida* (*Umbelliferae*), Silverton, 2800 m, 8 July 1907, F.E. & E.S. Clements, Cryptogamae Formationum Coloradensium 444 (COLO 62872, lectotype designated here); *ibid.* (E00297884, syntype).

Remarks: Although there are 32 epithets for this genus in Index Fungorum, 21 species were accepted in the paper of Shoemaker & Babcock (1992) that provided a key to species, descriptions and illustrations. Species were characterized by a circular lid-like opening and muriform applanate brown ascospores. *Comoclathris* was considered to differ from *Clathrospora* as in the latter genus species have two or more rows of longitudinal septa as compared with a single row in *Comoclathris* (Shoemaker and Babcock, 1992). With the exception of a new combination by Checa (1998) the genus has not been studied since Shoemaker & Babcock (1992). Material loaned from NEB (Cryptogamae Formationum Coloradensium 1212, NEB 311094) and BPI (Cryptogamae Formationum Coloradensium 444, BPI 718242) was not in good condition. The nature of the ascomata opening is hard to observe in the dried specimens but appears to be an unusually large ostiolar opening which occurs through breakdown of the upper ascomata wall to produce a large aperture or circular lid as mentioned by Shoemaker & Babcock (1992).

Phylogenetic study: There are no sequences available for this genus in GenBank.

Concluding remarks: The 32 epithets for this genus listed in Index Fungorum have not been adequately dealt with as Shoemaker & Babcock (1992) did not discuss many of these species and it is likely they did not consider them in their acceptance of 21 species. The pivotal characters for this genus appear to be the circular lid-like opening and applanate mid to dark reddish-brown muriform ascospores with single longitudinal septa. It is not clear whether these characters would hold up to molecular phylogenetic analysis and the genus and its placement is in need of revision.

Diadema Shoemaker & C.E. Babc., Can. J. Bot. 67(5): 1349 (1989)

Type species: *Diadema tetramerum* Shoemaker & C.E. Babc. [as “*tetramera*”], Can. J. Bot. 67(5): 1354 (1989).

Remarks: This genus was introduced by Shoemaker & Babcock (1989) and comprises eight species, six introduced at the same time as the genus, a new combination proposed by Huhndorf (1992) and a new species introduced by Tanaka *et al.* (2010). This is the type of *Diademaceae* and one species has applanate ascospores and all have ascomata opening by a circular lid (Shoemaker and Babcock, 1992). It differs from other genera in the family in having ascospores which lack longitudinal septa.

Phylogenetic study: There are no sequences available for this genus in GenBank.

Concluding remarks: This is the generic type of *Diademaceae* and its uniqueness from *Pleosporaceae* needs establishing by molecular analysis. The salient characters that define this genus include the ascomata with a circular lid-like opening and phragmosporous reddish-brown ascospores (i.e. lacking longitudinal septa), the latter distinguishing it from the muriform ascospores found in *Clathrospora* and *Comoclathris*. It is not clear whether these characters

would hold up to molecular phylogenetic analysis and the genus and its placement is in need of revision.

Diademosia Shoemaker & C.E. Babc., *Can. J. Bot.* 70(8): 1641 (1992)

Type species: Diademosia californiana (M.E. Barr) Shoemaker & C.E. Babc. [as "*californianum*"], *Can. J. Bot.* 70(8): 1641 (1992).

≡ *Graphyllum californianum* M.E. Barr, *Mem. N. Y. bot. Gdn* 62: 40 (1990).

Remarks: This genus was introduced by Shoemaker & Babcock (1992) and comprises four species, the generic type introduced by Shoemaker & Babcock (1992), a new combination proposed by Ahn & Shearer (1998) and two new species introduced by Barr & Rogerson (1999) and Checa & Barr (1999). *Diademosia* has ascomata opening by a circular lid (Shoemaker & Babcock, 1992). Its species differ from other genera in the family in having terete (cylindrical, circular in cross section) ascospores. The nature of the ascomata which open by a circular lid is a unique characteristic.

Phylogenetic study: There are no sequences available for this genus in GenBank.

Concluding remarks: The salient characters that define this genus include the ascomata with circular lid-like opening and terete muriform dark-brown ascospores, the terete character distinguishing it from the applanate ascospores found in *Clathrospora*, and *Comoclathris*. It is not clear whether these characters would hold up to molecular phylogenetic analysis and the genus and its placement is in need of revision.

Graphyllum Clem., *Botanical Survey of Nebraska* 5: 6 (1901)

Generic description: Ascumata in linear rows on woody stems, semi-immersed, elongate, hysteroform, black, coriaceous, glabrous, with a central long ostiolar opening. Peridium comprising 2-3 layers of brown, relatively thick cells of *textura angularis*, inner cells flattened, thin-walled and lighter. Asci 8-spored, bitunicate, clavate to cylindrical, short-stalked, numerous in a basal cluster from thick, hyaline basal pseudoparenchyma layer. Pseudoparaphyses lacking. Ascospores applanate, muriform, with 3-4 transverse septa, (0)1-2 longitudinal septa, brown to dark brown.

Type species:

Graphyllum chloes Clem., *Botanical Survey of Nebraska* 5: 6 (1901) Figs 3A-G

≡ *Pleospora chloes* (Clem.) Petr., *Sydowia* 6(5-6): 337 (1952)

Ascumata 210-330 μm long \times 40-80 μm wide, a hysterothecium, in linear rows on woody stems, semi-immersed, elongate, black, coriaceous, glabrous, with a central long ostiole 100-190 μm long \times 10-20 μm wide μm (Figs 3A-C). In vertical section 70-124 μm high \times 64-110 μm diameter (mean = 95.2 \times 93 μm , n = 10), subglobose to ovoid (Figs 3D-E). Peridium 14-25 μm wide, comprising 2-3 layers of brown, relatively thick-walled cells of *textura angularis*, inner cells flattened, thin-walled and lighter (Fig. 3F). Asci 70-82 \times 22-29 μm (mean = 55 \times 19 μm , n = 10), 8-spored, bitunicate, clavate to cylindrical, short-stalked, in a basal cluster (Figs 3G-I, K). Ascospores 18-22 \times 7-10 μm (mean = 19.4 \times 8.4, n = 20), biserial to overlapping triseriate, muriform, constricted at the septa, applanate, obpyriform, straight, with 3(-4) transverse septa, (0)1-2 longitudinal septa, brown to dark brown when mature (Figs 3J-K).

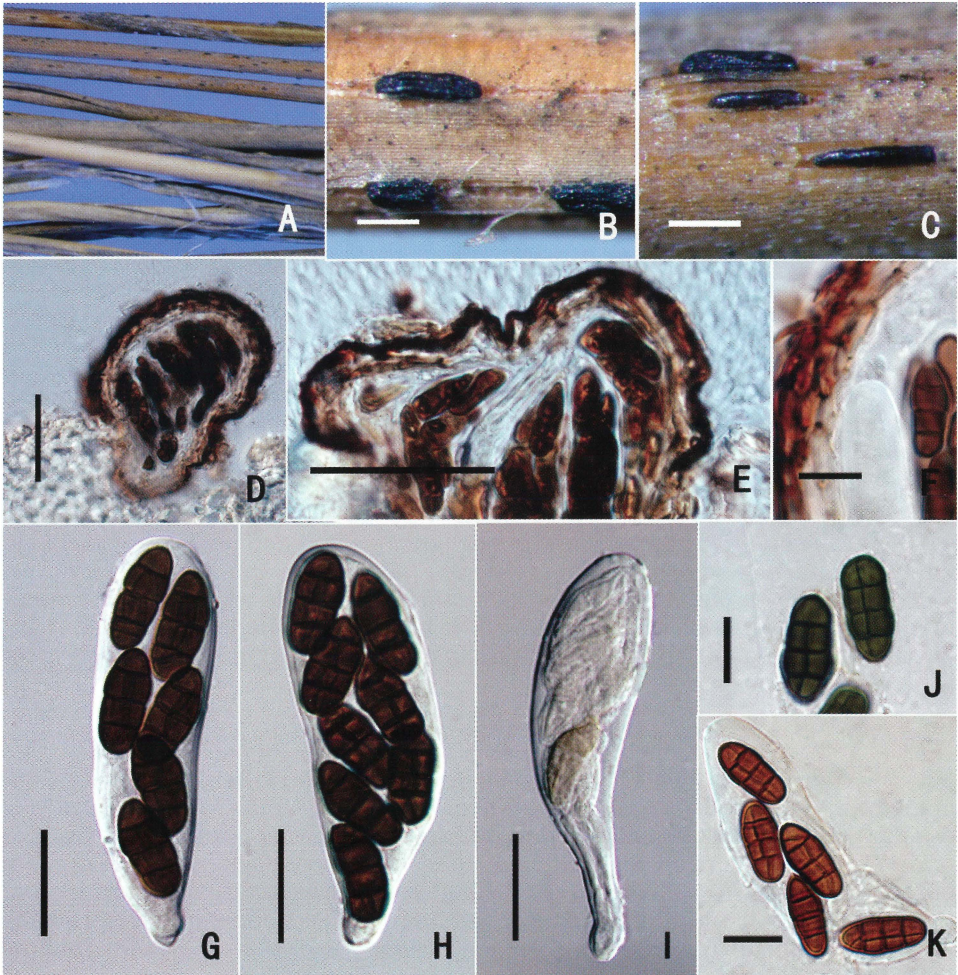


Fig. 3. *Graphyllum chloes* (NEB 158190, lectotype). **A.** Ascomata on stems. **B.** Ascomata in rows with a central slit. **C.** Side elevation of ascomata. **D, E.** Vertical section of ascomata, note the slit. **F.** 2-3 layers peridium. **G, H, I.** Mature and immature asci; asci are 8-spored with biseriate to overlapping triseriate ascospores. **J, K.** Immature and mature muriform ascospores, distinctly constricted at the septa. Scale bars: B, C = 200 μm , D = 50 μm , E = 40 μm . F, G, H, I, J, K = 20 μm .

Material examined: USA, Nebraska, Brown County, Long Pine, on the woody stem of *Aristida longiseta*, 3 January 1901, J.M. Bates (NEB 158190, lectotype designated here); *ibid.*, on dead stems of *Bouteloua oligostachya*, J.M. Bates (NEB 158189, syntype).

Remarks: The genus *Graphyllum* was placed in the family *Hysteriaceae* because of its hysterothecia with a slit like opening, which differs from *Diademaceae* characterized by asoma opening by a flat circular lid (Shoemaker & Babcock, 1992). When described this species was introduced from two hosts and the type material is in two packets from two hosts. We therefore choose one as the lectotype and the other as the syntype.

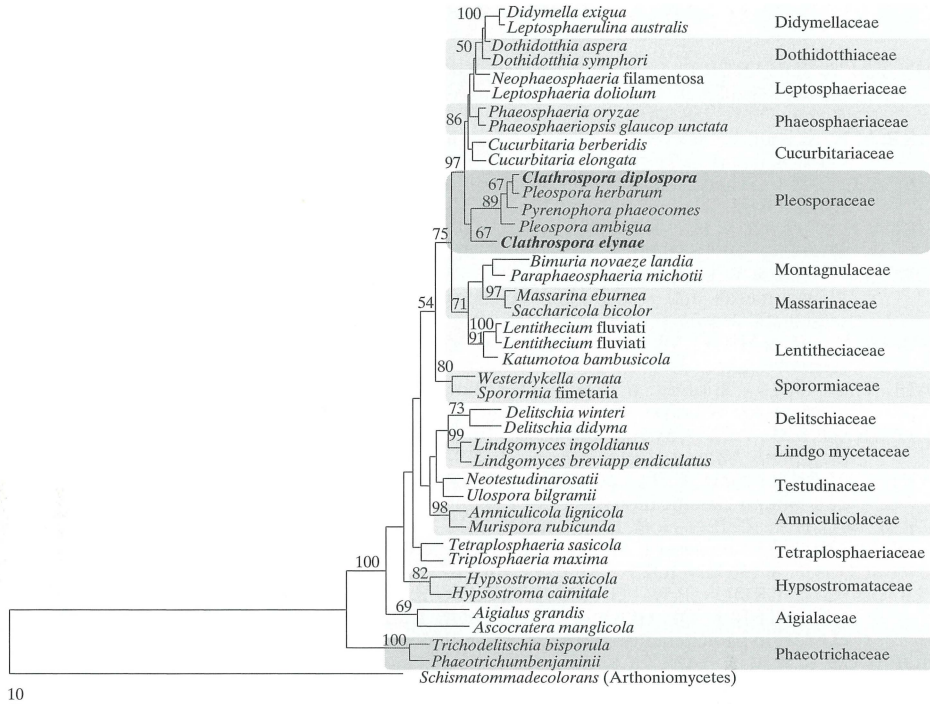


Fig. 4. Maximum parsimony tree based on the combined small subunit and large subunit rRNA sequences of *Clathrospora* and selected species in *Pleosporales*. Bootstrap support values >50 % are shown at the nodes.

Phylogenetic study: There are no sequences available for this genus in GenBank.

Concluding remarks: This genus is placed in *Diademaceae* in Lumbsch and Huhndorf (2010) and in *Hysteriaceae* in Index Fungorum (www.indexfungorum.org). The longitudinal opening and hysterothecium-like ascomata certainly indicate that the genus should be included in the *Hysteriaceae* which is suggested here. Hysteriaceous fungi share a similar morphology (i.e., the hysterothecium) and have now be classified in no fewer than five different groups (É. Boehm, pers. comm.) and therefore the final placement of *Graphyllum* must depend on molecular data.

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REFERENCES

- AHN Y.M. & SHEARER C.A., 1998 — Reexamination of taxa in *Leptosphaeria* originally described on host species in Ranunculaceae, Papaveraceae, and Magnoliaceae. *Canada Journal of Botany* 76(2): 258-280.
- BARR M.E. & ROGERSON C.T., 1999 — Some loculoascomycete species from the Great Basin, USA. *Mycotaxon* 71: 473-480.
- CHECA J., 1998 — Annotated list of the Pleosporacean fungi and related genera reported from the Iberian Peninsula and Balearic Islands, *Mycotaxon* 68: 205-249.
- CHECA J. & BARR M.E., 1999 — Pyrenomycetes sensu lato from Almería (Spain). *Cryptogamie, Mycologie* 20(2): 79-90.
- DONG J.W., CHEN W.D. & CRANE J.L., 1998 — Phylogenetic studies of the Leptosphaeriaceae, Pleosporaceae and some other Loculoascomycetes based on nuclear ribosomal DNA sequences. *Mycological Research* 102(2): 151-156.
- ERIKSSON O.E. & HAWKSWORTH D.L. (ed.), 1991 — Outline of the ascomycetes – 1990, *Systema Ascomycetum* 8: 39-271.
- HALL T.A., 1999 — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symp Ser* 41: 95-98.
- HUHNDORF S.M., 1992 — Systematics of *Leptosphaeria* species found on the Rosaceae. *Bulletin of the Illinois Natural History Survey* 34: 479-533.
- HYDE K.D., MCKENZIE E.H.C. & KOKO T.W., 2011 — Towards incorporating anamorphic fungi in a natural classification – checklist and notes for 2010. *Mycosphere* 2(1): 1-88.
- LI Y.M., WU H.X., CHEN H. & HYDE K.D., 2011 — Morphological studies in Dothideomycetes: *Elsinoe* (Elsinoaceae), *Butleria* and three excluded genera. *Mycotaxon* (in press).
- LUMBSCH H.T. & HUHNDORF S.M. (ed.), 2010 — Outline of Ascomata – 2009. *Mycotax* 14: 1-64.
- SCHOCH C.L., CROUS P.W., GROENEWALD J.Z., BOEHM E.W.A., BURGESS T.I., DE GRUYTER J., DE HOOGS G.S., DIXON L.J., GRUBE M., GUEIDAN C., 2009 — A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1-15.
- SHOEMAKER R.A. & BABCOCK C.E., 1992 — Applanodicyosporous Pleosporales: *Clathrospora*, *Comoclathris*, *Graphyllum*, *Macrospra*, and *Platysporoides*. *Canadian Journal of Botany* 70(8): 1617-1658.
- SWOFFORD D.L., 2002 — PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4b10. Sunderland, Massachusetts: Sinauer Associates.
- TANAKA K., HIRAYAMA K. & IQBAL S.H., 2010 — *Diadema ahmadii* (Pleosporales), a new ascomycetous species from Pakistan. *Mycotaxon* 113, 337-342.
- VASSILJEVA L.N., 1998 — Pyrenomycetidae et Loculoascomycetidae. *Plantae non Vasculares, Fungi et Bryopsidae. Orientis extremi Rossica. Fungi* 4:1-418. (in Russian).
- WU H.X., LI Y.M., CHEN H. & HYDE K.D., 2010 — Studies on Microthyriaceae: some excluded genera. *Mycotaxon* 113: 147-156.
- WU H.X., HYDE K.D. & CHEN H., 2011 — Studies on Microthyriaceae: Placement of *Actinomyxa*, *Asteritea*, *Crisosina*, *Polystomellina* and *Stegothyrium*. *Cryptogamie, Mycologie* 32 (1): 1-10.
- ZHANG Y., FOURNIER J., POINTING S.B. & HYDE K. D., 2008 — Are *Melanomma pulvis-pyrius* and *Trematosphaeria pertusa* congeneric. *Fungal Diversity* 33: 47-60.
- ZHANG Y., FOURNIER J., CROUS P.W., JEEWON R., POINTING S.B. & HYDE K.D., 2009a — Towards a phylogenetic clarification of *Lophiostoma/Massarina* and morphologically similar genera in the Pleosporales. *Fungal Diversity* 38: 225-251.
- ZHANG Y., SCHOCH C.L., FOURNIER J., CROUS P.W., DE GRUYTER J., WOUDEBERG J.H.C., HIRAYAMA K., TANAKA K., POINTING S.B., SPATAFORA J.W. & HYDE K.D., 2009b — Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. *Studies in Mycology* 64: 85-102.