Research Article

A Molecular and Morphological Reassessment of Diademaceae

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We revisit the family Diademaceae based on available sequence data and morphology. Diademaceae is characterized by ascomata opening with a flat circular lid and fissitunicate, short orbicular frequently cylindrical, pedicellate asci. Ascospores are frequently circular in section but narrowing to one end with three or more transverse septa, without longitudinal septa, and mostly with a thick sheath. In recent treatments Clathrospora, Comoclathris, Diadema, Diademosa, and Graphyllium were placed in the family. Following molecular and morphological study, Clathrospora, Comoclathris, and Diademosa, are excluded from the family and referred to Pleosporaceae. Graphyllium is excluded from Diademaceae, based on hysterothecium-like ascomata with a longitudinal opening, and tentatively placed in Hysteriaceae with uncertainty; species with hysterothecia have now been accommodated in at least five families. The study accepts only Diadema in the family. The status of Diademaceae as a distinct family, based on the ascomata opening by a flat circular lid, is thought to be doubtful. Fresh collections of Diadema are needed for epitypification and to obtain sequence data to establish if this is a well-resolved family.

1. Introduction

Based on the ascomata opening by a flat circular lid, Shoemaker and Babcock [1] introduced Diademaceae, which they considered to be a unique family in the order Pleosporales. Initially five genera, that is, Clathrospora, Comoclathris, Diadema, Diademosa, and Macrospora, were included in the family [1]. Other than the ascomata opening by a flat circular lid, the family was characterized by bitunicate and fissitunicate, clavate or ellipsoidal, short pedicellate asci, and apllanate or rarely cylindrical ascospores with three or more transverse septa with or without longitudinal septa and usually with a thick sheath and frequently circular in section but narrowing to one end [1, 2].

Species of the order Pleosporales with apllanate ascospores can be found in three families (Diademaceae, Hysteriaceae, and Pleosporaceae), which differ in the way the ascomata open [1]. Ascomata openings by a flat circular lid are characteristic of Diademaceae. In species of Hysteriaceae ascomata open via a long narrow slit and species of Pleosporaceae open by a central pore [1]. Various authors have included and excluded different genera in Diademaceae by giving priority to different morphological characters [1, 3, 4]. Platyspora had been referred to this family by various authors [4] or was considered a synonym of Graphyllium [2, 4] or Comoclathris [5]. Lumbsch and Huhndorf [6] assigned Macrospora to Pleosporaceae, as the genus was considered to be a synonym of Pyrenophora and this treatment was followed by Zhang et al. [3, 4]. In the same study, Lumbsch and Huhndorf [6] had also referred Graphyllium to Diademaceae. Sequence data is now available for some of these genera thus the importance of their morphological characters and overall relationships can now be tested based on molecular phylogeny.

We have been studying the families of Pleosporales in order to provide a natural classification via morphological characterization together with molecular analysis.
[2–4, 7]. The family Diademaceae has been poorly studied and presently comprises five genera [6], but this has not changed since the family was introduced by Shoemaker and Babcock [1]. Given the considerable taxonomic confusion we revisited this family based on phylogenetic analyses of rDNA sequence data coupled with morphological characters. The aims of the study are to (i) discuss the familial placement of the genera in Diademaceae and assess whether they represent natural groups, (ii) determine which morphological characters are useful for generic delineation by observing the typespecies of groups, (iii) illustrate the genera to stimulate fresh collections being made so that molecular data can be used to resolve the systematic relationships of the family.

2. Materials and Methods

2.1. Specimen Examination. The basic methodology used in this study was the same as Ariyawansa et al. [7]. The type specimens were loaned from the US National Fungus Collections (BPI), Agriculture and Agri-Food Canada (DAOM), and New York Botanical Garden (NY). Ascomata were rehydrated in 5% KOH prior to examination and sectioning. Hand sections of the fruiting structures were mounted in water for microscopic studies and photomicrography. The fungus was examined in a Nikon ECLIPSE 80i compound microscope and photographed by a Cannon 450D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems Inc., USA).

2.2. Phylogenetic Analysis. The large and small subunits of the nuclear ribosomal RNA genes (LSU, SSU) were included in the analysis. All sequences obtained from GenBank were used in Schoch et al. [8] and Zhang et al. [3] and are listed in Table 1. Sequences were aligned using Bioedit v7.2.0 version [9] and ClustalX v. 1.83 [10]. The alignments were checked visually and improved manually where necessary. Maximum Likelihood analysis was performed in RAxML [11] implemented in raxmlGUIv.0.9b2 [12]. The search strategy was set to rapid bootstrapping and the analysis was carried out using the GTRGAMMA1 model of nucleotide substitution. The number of replicates was automatically inferred using the stopping criterion [13]. Maximum Likelihood bootstrap values equal or greater than 50% are given below or above each node (Figure 1). Phylogenetic trees were drawn using Treeview v.1.6.6 [Page 2001].

3. Results

3.1. Molecular Phylogeny Based on Combined nrSSU and nrLSU. The combined 18S and 28S nrDNA data set comprised 52 taxa including strains of Clathrospora elynae (CBS 196.54 and CBS 161.51), Comoclathris magna (CBS 174.52), Clathrospora heterospora (CBS 175.52), and Comoclathris compressa (CBS 157.53 and CBS 156.53) with Dothidea sambuci as the out-group taxon. The 52 taxa analyzed in the cladogram formed 13 familial clades. Maximum Likelihood analysis used 1000 bootstrap replicates and yielded a tree with the likelihood value of ln:-9930.28726 and the following model parameters: alpha: 0.512987 and invar: 0.499567; Π(A): 0.259512, Π(C): 0.207265, Π(G): 0.277826, and Π(T): 0.255397. Phylogenetic trees obtained from maximum likelihood analyses yielded trees with similar overall topology at family and generic relationship in agreement with previous work [3, 4, 8].

3.2. Molecular Phylogeny of Diademaceae. Two putative strains of Clathrospora elynae (CBS 196.54 and CBS 161.51) which had been previously referred to Diademaceae by Lumbsch and Huhndorf [6] and Shoemaker and Babcock [1] were clustered in the family Pleosporaceae but separated from other genera of the family with a relatively high bootstrap value (55%). The type species of Comoclathris, C. lanata, was not available for study, but the two Comoclathris compressa strains cluster in a well supported clade within the Pleosporaceae, outside the Alternaria complex. Therefore we confer with Zhang et al. [4] and Woudenberg et al. [14] in transferring these two genera to Pleosporaceae. Two putative strains of Comoclathris magna (CBS 174.52) and Clathrospora heterospora (CBS 175.52) were clustered within the Alternaria complex as in Woudenberg et al. [14]. Woudenberg et al. [14] have tentatively considered Comoclathris magna (CBS 174.52) and Clathrospora heterospora (CBS 175.52) as Alternaria species. There is, however, confusion concerning the CBS 175.52 strain, because Dong et al. [15] used the name Comoclathris baccata in their paper for strain CBS 175.52 but submitted sequences to GenBank under the name Clathrospora diplospora [14]. In their study, Woudenberg et al. [14] have synonymised Comoclathris baccata with C. heterospora. We could not locate the type species of Diadema, Diadema tetramerum, and Diademosa, Diademosa californiana, for phylogenetic analysis due to the unavailability of sequence data. Therefore recollection, epitypification, and sequence data of Diadema, Diadema tetramerum, and Diademosa, Diademosa californiana, are necessary to validate Diademaceae genera and species relationships.

3.3. Taxonomy

Diademaceae. Shoemaker & C.E. Babc., Can. J. Bot. 70(8): 1618 (1992), MycoBank: MB 81955.

Parasitic or saprobic in stems and leaves. Sexual state: Ascomata subepidermal or subcuticular and later become superficial, globose, opening via flat circular lid, dark brown to black. Peridium thin, consisting of small pigmented thick-walled cells of textura angularis. Hamathecium of dense cellulosic pseudoparaphyses. Asci 8-spored, bitunicate, fissitunicate, clavate or ellipsoidal, short orbicular pedicel, without an ocellar chamber. Ascospores partially overlapping to biseriate, fusiform, brown, with three or more transverse septa without longitudinal septa, mostly terete (cylindrical; frequently circular in section but narrowing to one end), mostly with a thick sheath. Asexual state: Unknown.

Type: Diadema. Shoemaker & C.E. Babc.
Table 1: Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. Culture and voucher abbreviations are indicated where available.

| Taxon                                  | Culture | SSU          | LSU          |
|----------------------------------------|---------|--------------|--------------|
| Aigialus grandis                       | JK5244A | GU296131     | GU301793     |
| Aigialus parvus                        | BCC18403| GU479743     | GU479777     |
| Alternaria alternata                   | CBS916.96| KC584507     | DQ678082     |
| Anmisculicola immersa                  | CBS123083| GU456295     | FJ795498     |
| Ammiculicola parva                     | CBS123092| GU296134     | FJ795497     |
| Asocoratera manglicola                 | JK5262C | GU296136     | GU301799     |
| Bimuria novae-zelandiae                | CBS107.79| AY016338     | AY016356     |
| Boeremia exigua                        | CBS431.74| EU754084     | EU754183     |
| Byssothecium circinans                 | CBS675.92| AY016339     | AY016357     |
| Clathrospora elynae                    | CBS161.51| KC584628     | KC584370     |
| Clathrospora elynae                    | CBS196.54| KC584629     | KC584371     |
| Clathrospora heterospora (Alternaria sp.) | CBS175.52| KC584577     | KC584320     |
| Cochliobolus heterostrophus            | CBS134.39| AY544727     | AY544645     |
| Comoclathris compressa                 | CBS156.53| KC584630     | KC584372     |
| Comoclathris compressa                 | CBS157.53| KC584631     | KC584373     |
| Comoclathris magna (Alternaria sp.)    | CBS174.52| KC584578     | DQ678068     |
| Didymella exigua                       | CBS183.55| EU754056     | EU754155     |
| Dithidea sambuci                       | DAOM231303| AY544722     | AY544681     |
| Dithidiothia aspera                    | CPC12933 | EU673228     | EU673276     |
| Dithidiothia symphoricarpi             | CPC12929 | EU673224     | EU673273     |
| Halojulella avicenniae                 | BCC18422 | GU371831     | GU371823     |
| Halojulella avicenniae                 | BCC20173 | GU371830     | GU371822     |
| Helicascus nypae                       | BCC36752 | GU479755     | GU479789     |
| Katamotoa bambusicola                  | MAFF239641| AB524454     | AB524595     |
| Lentithecium aquaticum                 | CBS123099| GU296156     | GU301823     |
| Lentithecium fluviatile                | CBS122367| GU296158     | GU301825     |
| Leptosphaeria doliiolom                | CBS505.75| GU296159     | GU301827     |
| Leptosphaeria dryadis                  | CBS643.86| DQ678068     | GU301828     |
| Leptosphaeria maculans                 | DAOM229267| DQ470993     | DQ470946     |
| Leptosphaerula unisolaris              | CBS317.83| GU296160     | GU301830     |
| Massarina eburnea                      | CBS473.64| GU296170     | GU301840     |
| Montagnula opulenta                    | CBS168.34| AF164370     | DG678086     |
| Morosphaeria ramunculicola             | BCC18405| GQ925839     | GQ925854     |
| Morosphaeria ramunculicola             | JK5304B | GU479760     | GU479794     |
| Neophaeosphaeria filamentosa           | CBS102202| GQ387516     | GQ387577     |
| Neottioporia paspali                   | CBS331.37| EU754073     | EU754172     |
| Ophiophysaeria herpotricha             | CBS240.31| DQ678010     | DG678062     |
| Pheosphaeria eustoma                   | CBS573.86| DQ678011     | DG678063     |
| Phoma radicina                         | CBS111.79| EU754092     | EU754191     |
| Pleosmassaria siparia                  | CBS279.74| DQ678027     | DQ678078     |
| Pleospora betae                        | CBS10940| EU754079     | EU754178     |
| Pleospora calveacens                   | CBS246.79| EU754032     | EU754131     |
| Pleospora chenopodi                    | CBS206.80| JF740095     | JF740266     |
| Pleospora herbarum                     | CBS191.86| DQ247812     | DQ247804     |
| Pleospora incompta                     | CBS467.76| GU238220     | GU238087     |
| Pleospora typhicola                    | CBS132.69| JF740105     | JF740325     |
Table 1: Continued.

| Taxon                  | Culture       | SSU           | LSU           |
|------------------------|---------------|---------------|---------------|
| Preussia terricola     | DAOM 230091   | AY544726      | AY544686      |
| Prosthemium betulinum  | CBS 127468    | AB553644      | AB53754       |
| Prosthemium canba      | JCM 16966     | AB553646      | AB53760       |
| Pyrenophora phaeocomes | DAOM 222769   | DQ499595      | DQ499596      |
| Sporormiella minima    | CBS 524.50    | DQ678003      | DQ678056      |
| Sporormiella minima    | CBS 524.50    | DQ678003      | DQ678056      |

Figure 1: RAxML tree based on a combined dataset of SSU and LSU. Bootstrap support values >50% are shown above or below the branch. The putative strains of Clathrospora elynae (CBS 196.54 and CBS 161.51) and Comoclathris compressa (CBS 157.53 and CBS 156.53) are indicated in red. Dothidea sambuci is the out-group taxon. The original isolate numbers are noted after the species names.
Shoemaker and Babcock [1] introduced Diademaceae which they considered to be a distinctive family comprising Clathrospora, Comoclathris, Diadema, Diademosa, and Macrospora whose species have ascomata opening by a flat circular lid [1]. The feature of ascomata opening via a flat circular lid was considered to be an adaptation to the alpine habitat [16]. Ascospores are fusiform, brown, with three or more transverse septa, with or without longitudinal septa, and frequently terete, usually with a thick sheath [1, 2, 4].

Lumbsch and Huhndorf [6] excluded Macrospora from Diademaceae and assigned it to Pleosporaceae, as it was considered to be a synonym for Pyrenophora. We have seen type material of Macrospora scirpicola and it is neither diademaceous nor pleosporaceous and therefore will be considered as subject of a future paper. Lumbsch and Huhndorf [6] also included Graphyllium in the family Diademaceae, but this classification has not been followed by many authors. Shoemaker and Babcock [1] and Zhang et al. [4] referred Graphyllium to the family Hysteriaceae based on its hysterothecium-like ascomata forming a longitudinal, slit-like opening. Shoemaker and Babcock [1] assigned Clathrospora to Diademaceae based on ascomata opening with an intraepidermal discoid lid and muriform appanlate ascospores with more than one row of longitudinal septa. Ascomata, however, have slightly papillate ostioles and Alternaria-like asexual morphs, and recent molecular data shows that Clathrospora has an affinity with the family Pleosporaceae [4, 14]. Platyospora has been referred to Diademaceae [4] and was considered a synonym of Graphyllium [2, 4] or as a synonym of Comoclathris [5].

Species of the order Pleosporales with applanate ascospores were previously separated into three families (Diademaceae, Hysteriaceae, and Pleosporaceae) which differ in the way the ascomata open [2]. Ascomata openings by a flat circular lid were characteristic of the family Diademaceae, while species of Hysteriaceae open via a long narrow slit and species of Pleosporaceae open by a central pore [1]. Based on the above discussion we exclude Clathrospora, Comoclathris, Diademosa, and Graphyllium from the Diademaceae. Based on morphology and/or molecular data and at this time, we accept only Diadema, which has mostly terete ascospores (except *D. obtusa* which has flattened ascospores), in the family. Diademaceae is, however, not supported by molecular data; however, no sequence data is available for the generic type *Diadema*. Further studies are required to resolve the phylogenetic relationship in the Pleosporales. In the light of all of the above, we retain the Diademaceae to include a single genus *Diadema* which has immersed, intraepidermal ascomata, opening via a flat circular lid, and asci with a short orbicular pedicle without an ocular chamber and ascospores are reddish-brown, usually cylindrical, and frequently circular in section but narrowing to one end with a distinct, mucilaginous sheath.

3.4. Accepted Genus in Diademaceae

*Diadema*. Shoemaker & C.E. Babc., Can. J. Bot. 67(5): 1349 (1989), MycoBank: MB 25293.

*Saprobic* on culms of grasses (*Poaceae*). *Sexual state*: Ascomata scattered, immersed, intra-epidermal, globose to subglobose, black to brown, smooth-walled and opening via a flat circular lid. *Peridium* 1-layered, composed of small pigmented thick walled compressed cells, base composed of small pigmented thick-walled cells of *textura angularis*. *Hamathecium* of dense, numerous, septate, hyaline, cellu-larpsuedoparaphyses. Asci 8-spored, numerous, bitunicate, fissitunicate, broadly-clavate, with a short orbicular pedicle, without an ocular chamber. Ascospores obliquely biseriate, broadly fusiform, usually cylindrical; frequently circular in section but narrowing to one end, brown to reddish-brown, without longitudinal septa, guttulate, smooth-walled or finely punctate, with wide, distinct mucilaginosus sheath. *Asexual state*: Unknown.

Type Species: *Diadema tetrramerum*. Shoemaker & C.E. Babc. [as "tetramerum"], Can. J. Bot. 67(5): 1354 (1989), MycoBank: MB 136222 (see Figure 2).

*Saprobic* on culms of grasses (*Poaceae*). *Sexual state*: Ascomata 170–200 × 150–270 μm (*X* = 190 × 250 μm, *n* = 10), scattered, immersed, intra-epidermal, globose to subglobose, black to brown, smooth-walled and opening via a flat circular lid. *Peridium* 10–22 μm (*X* = 16, *n* = 20), 1-layered, composed of small, pigmented, thick-walled, compressed cells, base composed of small, pigmented, thick-walled cells of *textura angularis*. *Hamathecium* of dense, 2–3 μm diam (*X* = 2, *n* = 20), numerous, septate, hyaline, cellu- larpsuedoparaphyses. Asci 100–150 × 20–25 μm (*X* = 110 × 22 μm, *n* = 20), 8-spored, numerous, bitunicate, fissitunicate, broadly-clavate, with a short orbicular pedicle, rounded at apex without an ocular chamber. Ascospores 30–48 × 14–20 μm (*X* = 44 × 13 μm, *n* = 40), obliquely biseriate, broadly fusiform, brown to reddish-brown, 3-transseptate, without longitudinal septa, guttulate, smooth-walled or finely punctate, with a distinct, 4–5 μm wide, mucilaginosus sheath. *Asexual state*: Unknown.

Material Examined. USA, California, Mt. Shasta, ridge south of Horse Camp, elevation 8250 ft, on culms of *Trisetum spicatum* (L.) Richter, 2 July 1947 W.B. Cooke 20223 (DAOM, holotype).

Shoemaker and Babcock [16] introduced *Diadema* and characterized the genus by large ascospores without longitudinal septa with a distinct mucilaginosus sheath and ascomata with a circular lid-like opening. Currently eight species of *Diadema* are listed in Index Fungorum [5]. Six species were included when the genus was introduced and another two species (*Diadema ahmadii*, Kaz. Tanaka & S.H. Iqbal, and *Diadema sieversiae* (Peck) Huhndorf) were later added [4, 16]. The nature of the ascomata appears to be an important character of this genus and family. Except *D. obtusa* all other species of *Diadema* have terete; that is, ascospores are cylindrical, frequently circular in section but narrowing to one end. We observed *D. tetramerum*, the generic type of *Diadema* and besides ascomata opening via a circular lid, asci with the short orbicular pedicle without an ocular chamber and trans-septate, ascospores, lacking longitudinal septa, and surrounded by a very broad sheath narrowed to a waist near
the middle septum are considered to be significant for the genus.

No molecular data is available for the type or other species of Diadema. Therefore recollection, epitypification, and sequence data is essential to establish family and species relationships.

3.5. Excluded Genera

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

Saprobic on wood and stems. Sexual state: Ascomata semi-immersed, scattered on putrid host stems and foliage, brown to blackish brown, subglobose or nearly globose, with a central sunken ostiole open via a circular lid, asci and pseudoparaphyses forming at the base of the peridium. 

Peridium composed of 3–5 layers of brown, relatively thick-walled cells of textura angularis, inner cells flattened, thin-walled and lighter. Hamathecium composed of dense, hyaline, filiform, pseudoparaphyses which are longer than the asci. Asci 8-spored, bitunicate, fissitunicate, thick-walled, cylindrical to clavate, with a short pedicle and shallow ocular chamber. Ascospores biseriate, fusiform 7-transseptate, two or many rows of longitudinal septa, muriform, constricted only at the central septum, dark brown to brown, surrounded by a thin, hyaline mucilaginous sheath. Asexual State: Alternaria-like.

Type Species: Clathrospora elynae. Rabenh., Hedwigia 1:116 (1857) (see Figure 3).

Saprobic on wood and stems. Sexual State: Ascomata 140 × 220–145 × 175 µm (X = 170 × 150 µm, n = 10), semi-immersed, scattered on the putrid host stems and foliage, subglobose or nearly globose, brown to blackish brown, with a central sunken ostiole open via a circular lid, asci and pseudoparaphyses forming on the base of the peridium. Peridium 20–55 µm (X = 38, n = 20), composed of 3–5 layers of brown, relatively thick-walled cells of textura angularis, inner cells flattened, thin-walled and lighter. Hamathecium composed of dense, 2-3 µm diam (X = 2, n = 20), hyaline, filiform, pseudoparaphyses, longer than the asci. Asci 160–230 × 24–48 µm (X = 190 × 35 µm, n = 20), 8-spored, bitunicate, fissitunicate, thick-walled, cylindrical to clavate, with a short pedicle and ocular chamber. Ascospores 40–65 × 18–27 µm (X = 53 × 23 µm, n = 40), biseriate, fusiform, 7-transseptate, two or many rows of longitudinal septa, muriform, constricted only at the central septum, dark brown to brown, surrounded by a thin, hyaline mucilaginous sheath. Asexual State: Alternaria-like.
**Figure 3:** *Clathrospora elynae* (isotype). (a) Herbarium material. (b) Closeup of ascomata. (c) Section of the ascomata. (d) Closeup of the peridium (e) Hyaline, filiform, and pseudoparaphyses. ((f)–(h)) Cylindrical to clavate asci with a short pedicle and ocular chamber. ((i)–(k)) Dark brown to brown muriform ascospores surrounded by a thin, hyaline mucilaginous sheath. Scale bars: (b) = 100 μm, (c) = 10 μm, ((d)–(g)) = 60 μm, and ((h)–(j)) = 30 μm.

Brown to brown, surrounded by a thin, hyaline mucilaginous sheath. **Asexual State:** Alternaria-like.

**Material Examined.** Switzerland, on the stem of *Carex curvula*, September 1898, Winter (BPI 627748, isotype).

Shoemaker and Babcock [1] assigned *Clathrospora* to *Diademaceae* and included an additional nine species and provided a key to the genus based on the number of septa and length of ascospores. *Clathrospora* was characterized by circular lid-like opening and applanate, muriform ascospores. Currently, 50 *Clathrospora* species are listed in the genus in Index Fungorum [5]. Molecular studies based on combine gene analysis showed that two putative strains of *Clathrospora, C. elynae* (CBS 196.54) and *C. diplospora* (IMI 68086), were clustered in *Pleosporaceae* [4, 8]. We obtained similar results in the phylogenetic tree produced from combined nrLSU and nrSSU sequence analysis (Figure 1). *Clathrospora elynae* the type of *Clathrospora* formed a separate clade with relatively high bootstrap support (55%) within *Pleosporaceae*. Based on the phylogenetic result together with the morphological characters (slightly papillate ostiole and *Alternaria*-like asexual morph) we refer *Clathrospora* to *Pleosporaceae*.

*Comoclathris.* Clem., Gen. fung. (Minneapolis): 37, 173 (1909) \equiv Platyspora Wehm., World Monograph of the Genus *Pleospora* and its Segregates: 254 (1961).
Habitat saprobitic on dead wood or stems. Sexual state: Ascomata semi-immersed to superficial, scattered or aggre- gated, subglobose or nearly globose, brown to blackish brown coriaceous, ascomata opening via a large circular aperture or lid. Peridium comprising 3-4 layers of brown, relatively thick- walled cells of textura angularis. Hamathecium composed of dense, hyaline, filiform, septate pseudoparaphyses. Asci 8-spored, bitunicate, fissitunicate, cylindrical to cylindro- clavate, with an ocular chamber. Ascospores uniseriate or partially overlapping, fusiform, muriform, brown to reddish- brown, surrounded by a thin, hyaline, mucilaginous sheath. Asexual state: Alternaria-like.

Type Species: Comoclathris lanata. Clem. [as “Comoclathris”], Gen. fung. (Minneapolis): 1-227 (1909). MycoBank: MB 209341.

Comoclathris, typified by Comoclathris lanata, was intro- duced by Clements (1909). The genus is characterized by ascomata with circular lid-like openings and applanate reddish-brown to dark reddish-brown, muriform ascospores, with single longitudinal septa [1]. Zhang et al. [4] tentatively placed Comoclathris in the Pleosporaceae based on Alternaria-like asexual morphs and this was followed by Woudenberg et al. [14]. Comoclathris shares common char- acters with Pleospora herbarum, the type of Pleospora, in having cylindrical to cylindroclavate asci with an ocular chamber and muriform, brown or pale brown, with or without sheath ascospores. Comoclathris and Pleospora differ in the opening of ascomata (opening via a large circular aperture or lid versus open by a central pore). Comoclathris and Pleosptom share similar characters in having globose, black, ascomata, and cylindrical to cylindroclavate ascii with muriform, yellowish to dark brown ascospores. Comoclathris differs from Pleospora in having superficial ascomata with circular lid-like openings composed of comparatively thin peridium and applanate and fusiform ascospores surrounded by a distinct hyaline, mucilaginous thick sheath [3, 4]. In Pleosptom ascomata are immersed, usually with a papillate apex, with a relatively broad peridium and ovoid to fusoid ascospores [2, 3]. 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 [3]. Shoemaker and Babcock [1] provided a key to 21 species of Comoclathris. Presently 32 epites are listed for Comoclathris in Index Fungorum [5]. Molecular data for Comoclathris lanata, the type species of Comoclathris, is not available. Two strains of Comoclathris compressa (CBS 157.53 and CBS 156.53), however, cluster together in a well- supported clade within the family Pleosporaceae [14]. Based on the phylogenetic result coupled with the morphological characteristics (Alternaria-like asexual morph) we agreed with Zhang et al. [4] and Woudenberg et al. [14] to place Como- clathris in Pleosporaceae. This is, however, based on a species and recollection of the type species is essential to establish the correct placement of the genus.

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

Saprobic on stems and wood. Sexual state: Ascomata immersed, initially erumpent becoming superficial, scattered, depressed-globose, some flattened at the base, opening a disc- like lid of brown prismatic cells with setae. Peridium com- posed of brown pseudoparenchyma cells of textura angularis. Hamathecium of numerous, dense, septate, hyaline, cellu- lar pseudoparaphyses. Asci 8-spored, bitunicate, fissitunicate, clavate with short narrow pedicel and minute ocular chamber. Ascospores biseriate, partially overlapping, fusiform, straight, frequently circular in section but narrowing to one end, with transverse and vertical septa, pale brown to dark brown, smooth walled. Asexual state: Unknown.

Type Species: Diademosa californiana. (M.E. Barr) Shoemaker & C.E. Babc. [as “californianum”], Can. J. Bot. 70(8): 1641 (1992) ≡ Graphyllium californianum M.E. Barr, Mem. N. Y. bot. Gdn 62: 40 (1990) (see Figure 4).

Diademosa was established by Shoemaker and Babcock [1] and typified by D. californiana, based on the ascoma opening via a circular lid and ascopores being frequently circular in section, but narrowing to one end. Diademosa californiana was initially introduced as Graphyllium californianum by Barr [17] and referred to Hysteriaceae based on the pore or slit like opening. Reexamination of the type specimens by Shoemaker and Babcock [1] concluded that Diademosa opened by a flat lid similar to Diadem and assigned it into Diademaceae. The lid is hard to observe in sections unless they are mounted directly in lactic acid because excessive swelling occurs in water [1]. Diademosa differs from Comoclathris in having cylindrical, frequently circular in section, but narrowing to one end ascospores compared with flattened ascospores of Comoclathris. Diademosa and the generic type of Pleospo- racæ, Pleospora share common characters. Both Diademosa and Pleospora comprise narrowly oblong ascomata with cellular pseudoparaphyses and cylindrical to clavate asci with muriform, brown or pale brown ascospores. However, Diademosa differs from Pleospora in having an ascomata

Material Examined. USA, Bump-Cold Boiling Lake Trail, Lassen Volcanic National Park, Shasta, California, on branch of Wyethia, 12 July 1966, W.B. Cooke & D.L. Hawksworth. (NY, holotype).
opening via a circular lid, covered with setae and asci with short narrow pedicel, while *Pleospora* species have ascomata opening by a central pore without setae and asci with a short, thick, furcated pedicel. Except the ascomata opening via disc-like lid, *Diademosa* resembles some characters of *Pyrenophora*. That is, both *Diademosa* and *Pyrenophora* have superficial ascomata with setae and muriform, smooth-walled, light brown to dark brown ascospores. Currently four species of *Diademosa* are listed in Index Fungorum [5], but no molecular data is available for the genus. We place *Diademosa* in *Pleosporaceae* because of its similarities with other genera in this family, but confirmation of the phylogenetic status of this genus depends on recollecting the fungus and epitypification with molecular sequences.

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

*Habitat* saprobic on woody stems. *Sexual state: Ascomata* semi-immersed, hysteriform, black to brown, subglobose to ovoid. *Peridium* comprising 2-3 layers of brown, relatively thick cells of *textura angularis*, inner cells flattened, thin-walled and lighter. *Asci* 8 spored, bitunicate, fissitunicate, clavate. *Ascospores* biseritate overlapping, muriform, applanate, obpyriform, straight, with 3-4 transverse septa, 1-2 longitudinal septa or no longitudinal septa, brown to olive green. *Asexual state: Unknown.*

*Type Species: Graphyllium chloës*. Clem., Bot. Surv. Nebraska 5: 6 (1901) ≡ *Pleospora chloës* (Clem.) Petr., Sydowia 6(5-6): 337 (1952).

Initially *Graphyllium* was placed in the *Hypodermiaceae* by Clémenet (1901) and described as “Hysterothecium innate, then erumpent, linear, simple, membranaceous-plectenchymatous, black; asci ovoid or cylindrical-clavate, 8-spored; spores brown, elliptical to oblong, with transverse- and longitudinal septa, but not muriform; pseudoparaphyses simple or branched, septate, forming an epithecium.” Later
Barr [17] transferred the genus to order Pleosporales and referred to Phaeosphaeriaceae. Platyspora was considered as a synonym of Graphyllium [4]. Shoemaker and Babcock [1] assigned Graphyllium to Hysterotheciae considering the ascomatal characters along with apllanate ascospores that are at least 3-septate in side view and have some longitudinal septa in front view. Lumbsch and Huhndorf [6] included Graphyllium in the family Diademaceae, but Zhang et al. [4] referred to Hysterotheciae. We examined the generic type of Graphyllium, G. chloës we also agreed to refer Graphyllium tentatively in Hysterotheciae because of its hysterothecium-like ascomata forming a longitudinal opening which is clearly deviated from the lid-like opening in Diademaceae. However the correct placement of this taxon still depends on epitypification with molecular data.

4. Concluding Remarks

The importance of molecular data in determining the importance of morphological characters and relationship of microfungi cannot be overstressed and has proved significant at establishing genus and species relationships [18, 19] and resolving cryptic species in important plant pathogenic genera, for example, Diaporthe [20] and Pestalotiopsis [21]. Shoemaker and Babcock [1] introduced Diademaceae which they considered to be a distinctive family based on the ascomata opening via a flat, circular lid and comprising Clathrospora, Comoclathris, Diadema, Diademosa, and Macrospora [1]. Recent studies based on molecular phylogeny [4, 14], including this study, conclude that Clathrospora and Comoclathris clustered within Pleosporales. Molecular data, however, is not available for Diadema and Diademosa. Graphyllium is placed in Hysterotheciae because of its hysterothecium-like ascomata with a slit like opening; this clearly diverges from the lid like opening in Diademaceae. The characteristic feature of ascomata opening via a flat circular lid is considered as an adaptation to the alpine habitat [16]. It is, however, doubtful if this character is significant and whether Diademaceae is a separate family in the order Pleosporales. Until further molecular data becomes available we maintain Diademaceae with a single genus Diadema based on its large transseptate ascospores surrounded by a distinct mucilage sheath and ascomata with a circular, lid-like opening [16].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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