A PHYLOGENETIC RE-APPRAISAL OF THE FAMILY LIAGORACEAE SENSU LATO (NEMALIALES, RHODOPHYTA) BASED ON SEQUENCE ANALYSES OF TWO PLASTID GENES AND POSTFERTILIZATION DEVELOPMENT

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The marine red algal family Liagoraceae sensu lato is shown to be polyphyletic based on analyses of a combined rbcL and psaA data set and the pattern of carposporophyte development. Fifteen of eighteen genera analyzed formed a monophyletic lineage that included the genus Liagora. Nemalion did not cluster with Liagoraceae sensu stricto, and Nemaliaceae is reinstated, characterized morphologically by the formation of the primary gonimolobes by longitudinal divisions of the gonimoblast initial. Yamadaella and Liagoropsis, previously placed in the Dermoneptaceae, are shown to be independent lineages and are recognized as two new families Yamadaellaceae and Liagoropsidaceae. Yamadaellaceae is characterized by two gonimoblast initials cut off bilaterally from the fertilized carpogonium and diffusely spreading gonimoblast filaments. Liagoropsidaceae is characterized by at least three gonimoblast initials cut off by longitudinal septa from the fertilized carpogonium. In contrast, Liagoraceae sensu stricto is characterized by a single gonimoblast initial cut off transversely or diagonally from the fertilized carpogonium. Reproductive features, such as diffuse gonimoblasts and unfused carpogonial branches following postfertilization, appear to have evolved on more than one occasion in the Nemaliales and are therefore not taxonomically diagnostic at the family level, although they may be useful in recognizing genera.

Key index words: carposporophyte development; Liagoraceae sensu stricto; Liagoropsidaceae fam. nov.; molecular phylogeny; Nemaliaceae; psaA; rbcL; Yamadaellaceae fam. nov

Abbreviations: bp, base pairs; BP, Bootstrap proportion values; ML, maximum likelihood; MP, maximum parsimony; PP, posterior probabilities

The Liagoraceae Kützing (1843) sensu lato is the largest family of the marine red algal order Nemaliales F. Schmitz (1892 in Engler, as “Reihe Nemalinales,” Kylin 1956, as “Nemaliane”) and contains some 25 genera, including several that have been previously placed in the families Nemaliaceae (Farlow) De Toni and Levi (1886), Helminthocladiaceae

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J. Agardh (1851), and Dermonemataceae (Schmitz & Hauptfleisch) I.A. Abbott (Abbott 1976, 1985, Kraft 1988, 1989, Tseng 2005, Huisman 2006, Lin et al. 2011a,b). The life history of members of the Liagoraceae has been shown to have an alternation of microscopic, filamentous tetrasporophytes with macroscopic gametophytes. The filamentous phase produces either monosporangia or tetrasporangia, or both, the tetraspores generally germinating to form monocious or dioecious gametophytes (Guiry 2019). The taxonomic features used for separating the previously established families Nemaliaceae, Helminthocladiaceae (=Liagoraceae sensu stricto), and Dermonemataceae were based largely on differences in postfertilization stages, such as the orientation of the division plane of gonimoblast initials (= cells directly arising from the zygote), the morphology of gonimoblast filaments, and the absence or presence and sites of origin of involucral filaments associated with the carposporophyte (Abbott 1976, Huisman 2006 for a review). Doty and Abbott (1964) revised the taxonomic concepts of the Nemaliaceae and the Helminthocladiaceae, and subsequently, Abbott (1976) proposed elevating the helminthocladiaceous tribe Dermonemataceae Schmitz and Hauptfleisch (1896) to family level as the Dermonemataceae, to include the genera *Dermonema* Harvey ex Heydrich, *Yamadaella* (Decaisne) I.A. Abbott, and the new genus *Dotyophycus* I.A. Abbott. She considered that in the manner in which the gonimoblast initials were cut off from the fertilized carpogonial branches, e.g., *Helminthocladia* J. Agardh and the Dermonemataceae (with diffuse gonimoblast filaments, e.g., *Yamadaella*) remained obscure and largely unexplored.

A further reproductive feature used to distinguish genera of the Liagoraceae sensu lato is the presence and nature of involucral filaments that densely or laxly surround developing carpogamophytes. In the present study, we examined genera that lack such structures altogether (viz. Nemalion, *Yamadaella*, *Liagoropsis*, and *Dermonema*) and focused on postfertilization events, providing additional information on *Helminthocladias*, the type genus of the previously recognized family Helminthocladiaceae. The significance of the differences in carpogamophyte development among genera in the Liagoraceae sensu lato is further assessed, as well as their phylogenetic relationships as inferred from rbcL and psaA sequence analyses.
RESULTS

Molecular analyses. DNA sequences were newly generated for 18 genera in the Liagoraceae sensu lato (Liagora, Izziella Doty, Stemephitis Itono & Yoshi-zaki, Neoizziella Showe M. Lin, S. Y. Yang, & Huisman, Macarocarpus Showe M. Lin, S. Y. Yang, & Huisman, Titanophyllum Huisman, G.W. Saunders, & A.R. Sherwood, Akalaphycus Huisman, I.A. Abbott, & A.R. Sherwood, Helminthora, Threchoglaoeopsis I.A. Abbott & Doty, Hommersandiophyceus Showe M. Lin & Huisman, Dotyophyllum, Gannemona K. C. Fan & Y. C. Wang, Helminthocladia, Camagloia Setchell & N.L. Gardner, Dromonema, Yamadaella, Nemalion, and Liagoropsis Yamada), four species of Scinaea in the Scinaeaceae, and four genera (Galaxaura, Dicotomotaria, Trileocarpa, and Actinotrichia) in the Galaxauraceae, including 50 taxa for psaA and 18 taxa for rbcL (see Table S1). The genera Acrophaeidium, Thorea, and Batrachospermum were selected as outgroups, and their sequences were obtained from GenBank based on the studies of Yang and Boo (unpublished data), Müller et al. (2002), and Yoon et al. (2002), as well as 36 rbcL sequences of the Liagoraceae previously published by Lin et al. (2011a,b, 2014). The analyzed data matrix included 1,583 base pairs (bp) for rbcL with 517 parsimony-informative sites and 1,952 bp for psaA with 796 parsimony-informative sites. No insertion or deletion mutations were found in the two data sets of rbcL and psaA.

The topology of the MP, ML, and BA trees was largely congruent, and only the ML tree was shown for the rbcL and psaA combined data set (Fig. 1). Both ML topologies of the rbcL phylogeny and psaA phylogeny alone were similar to the rbcL+psaA combined data but with weaker statistical supports. Genera from the Liagoraceae sensu lato were clustered into four assemblages (I-IV, Fig. 1), but their phylogenetic relationships were not resolved. Clade I contained the majority of the genera analyzed, including the generitypes of Liagora, Helminthocladia, and Dromonema, the type genus for the families Liagoraceae, Helminthocladiaceae, and Dermomenataceae, respectively. The genera Yamadaella (Clade II), Nemalion (Clade III), and Liagoropsis (Clade IV) formed separate lineages and did not cluster with Liagora (Clade I). Although Yamadaella and Nemalion were clustered together with the family Scinaeaceae and A. savium, their relationships were not supported (less than 50% BP or 0.5 PP). The rbcL sequence divergence values among the genera analyzed from the Liagoraceae sensu lato ranged from 8.9% (Neoizziella vs. Macarocarpus) to 19.5% (Nemalion vs. Yamadaella), whereas the psaA gene sequence divergence values among the same genera ranged from 13.3% (Neoizziella vs. Macarocarpus) to 21.6% (Nemalion vs. Liagora).

Morphological observations. Nemalion elminthoides (Velley) Batters (1902: 59) (Fig. 2)

Basionym: Fucus elminthoides Velley (in Withering 1792: 255)

Fig. 1. *PsaA+rbcL* phylogeny: Global ML tree (*ln L* = −48817.0031) of Liagoraceae, using selection of genera from related families Scinaiaceae and Galaxauraceae within Nemaliales. Numbers above branches are Bayesian posterior probabilities (PP), ML, and MP bootstrap values (BP), respectively. Dash refers to BP values less than 50% or PP values below 0.5. Note: the names of families, genera, and the species in bold type were traditionally classified in the Liagoraceae sensu lato.
a prior lectotypification to that of Womersley (see Guiry and Guiry 2014), but this requires further investigation.

Nomenclatural note 2: This species has long passed under the epithet “helminthoides,” even though Velley (in Withering 1792: 255) did not use “h,” and Batters (1902: 59) retained this spelling when he made the transfer to Nemalion. The epithet is derived from the Greek ἐκλθιμός or ἐκλθίμος (meaning, relating to worms). Generally, when new names were formed, the “spiritus asper” (“rough breathing” or what is called an “[h]aitch” in English; see Stearn 2004:255) would have been utilized to make the prefix “helmint-” rather than “elminth-” as in, for example, the genus Helminthochorton Zanardini or Batrachospermum helminthosum Bory de Saint-Vincent. However, Art. 60.3 of the ICN (McNeill et al. 2012: 128) is quite firm on the matter: “The liberty of correcting a name is to be used with reserve, especially if the change affects the first syllable and, above all, the first letter of the name [our emphasis];” this original spelling has long been employed by Paul Silva in his Index Nominum Algarum.

Nomenclatural note 3: The generic type of Nemalion, Nemalion lubricum Duby (1830), for many years considered to be a heterotypic synonym of N. elminthoides, has been restored as a separate species by Le Gall and Saunders (2010) based on molecular evidence.

Examined specimens: Atlantic France, Saint Michel de Plouguerneau, attached on intertidal rocks, 15 July 2010, leg. C. Rodríguez-Prieto (HGI-A 9169 & HGI-A 9170, only rbcL successfully sequenced).

Habit and carposporophyte development: Thalli simple or branched, with terete axes, arising from a discoid holdfast on intertidal rocks. Carpogonial branches straight, 3- to 4-celled, borne on shoulder of supporting cell (Fig. 2A). After fertilization, gonimoblast initial cut off transversely from fertilized carpogonium; several sterile filaments cut off from supporting cell or nearby cortical cells (Fig. 2B); gonimoblast initial enlarges and divides longitudinally (Fig. 2C, arrowhead) producing on one side first gonimolobe initial and then divides longitudinally again on another side (Fig. 2D, arrowheads);
two gonimolobe initials divide obliquely or transversely producing gonimoblast filaments and cells of carpogonial branch begin to fuse (Fig. 2E); gonimolobes cut off radially new filaments by both transverse and oblique cell divisions (Fig. 2F); when mature, terminal gonimoblast cells differentiate into carposporangia; pit connections between carpogonial branch cells break down to form fusion cell (Fig. 2G).

**Dermonema virens** (J. Agardh) Pedroche and Avila Ortiz (1996: 77) (Fig. 3)

Basionym: Nemalion virens J. Agardh (1847:8).

Type locality: San Agustín, Oaxaca, Mexico.

Nomenclatural note: The generitype (monotype) of *Dermonema*, *Dermonema dichotomum* Harvey ex Heydrich (1894:289), was described by Heydrich on the basis of material collected at Kelung on the north coast of Formosa (now Keelung, Taiwan). However, Heydrich (1894:289) opted to adopt an unpublished name of Harvey’s in an exsiccatum, “Algae from Ceylon, Friendly Islands and Australia. Collected during the years 1852, 1853, 1854 and 1855.” This name is illegitimate as Heydrich placed it in synonymy under Gymnophlaea gracilis Martens, thus rendering it superfluous. This is discussed in great detail by Silva in Silva et al. (1996, pp. 915–6). Nevertheless, the type of the genus is *D. dichotomum* Harvey ex Heydrich, regardless of its legitimacy and is typified by material collected by Ferguson from Ceylon and distributed by Harvey. The question of the conspecificity of Heydrich’s material from Taiwan (upon which the concept of the genus is essentially based), Harvey’s material from Sri Lanka, and Agardh’s material of *N. virens* from Mexico remains

**Fig. 3.** *Dermonema virens*. Carposporophyte development (A, E: NTOU28032006Dv; B–D, G: NTOU09032008; F: NTOU432006); scale bars: A = 1 cm; B–C, E–G = 25 μm; D = 10 μm. (A) Thallus habit. (B) Prefertilization four-celled carpogonial branch (cb) with carpogonium (cp). (C) Early postfertilization showing carpogonial branch (cb) and presumably fertilized carpogonium (cp) cutting off a gonimoblast initial (gi) that divide obliquely (arrowhead) to produce a young gonimoblast cell. Note cortical cells (white arrows) adjacent to carpogonial branch slightly elongated. (D) Early postfertilization stage showing carpogonial branch (cb), fertilized carpogonium (cp), gonimoblast initial (gi), and young gonimoblast cells (pg) growing acropetally. (E) Later early postfertilization stage showing lateral and outward spread of gonimoblast filaments (arrows) and unmodified carpogonial branch (cb). (F) Young carposporophyte showing unilaterally developing gonimoblast filaments (black arrows) and elongating cortical filaments (white arrows). Note enlarged and darkly stained cells of carpogonial branch (cb). (G) Mature carposporophyte bearing terminal carposporangia (arrows), remaining cell walls (arrowheads) of released carposporangia and flask-shaped, fused carpogonial branch (cb).
unresolved at this time. The identity of *G. gracilis* also remains to be resolved.

Examined specimens: Taiwan: Chao Jing, Keelung City, attached on intertidal rocks, March 28, 2006 (NTOU19032006, sequenced for both *rbcL* and *psaA*), leg. S. M. Lin; Lungkeng, Kenting National Park, Pingtung Co., attached on intertidal rocks, March 9, 2008 (NTOU09032008, sequenced for both *rbcL* and *psaA*), leg. S. M. Lin; San Xin Tai, Taitung Co., attached on intertidal rocks, March 4, 2006 (NTOU432006), leg. S. M. Lin.

Habit and carposporophyte development: Thalli with terete, dichotomous axes arising from discoid holdfast (Fig. 3A) attached to rocks or coral reefs in intertidal zone. Carpogonial branches curved, three- or four-celled, medial on assimilatory filaments (Fig. 3B). After presumed fertilization, gonimoblast initial cut off transversely from fertilized carposporangium; inner cortical cells near supporting cell elongated (Fig. 3C); gonimoblast initial divided transversely several times resulting in a uniseriate series of four to six cells (Fig. 3D); these cut off gonimoblast filaments laterally (Fig. 3E) and then branched radially and carpogonial branch cells enlarged (Fig. 3F); when carposporophyte matures, terminal cells of gonimoblast filaments differentiated into carposporangia; pit connections between carpogonial branch cells broadened, forming elongated fusion cell (Fig. 3G).

*Helminthocladia australis* Harvey (1863:39)

Type locality: Fremantle, Western Australia. Lectotype: TCD 0011731.

Examined specimens: Taiwan: Taitung Co.: Three Fairy, attached on intertidal rocks, March 20, 2010 (NTOU20032010), leg. S. M. Lin; Yalulan, attached on intertidal rocks, June 15, 2007 (NTOU15062007), leg. S. M. Lin; Shanyuan, attached on intertidal rocks, June 9, 2005 (NTOU09062005, only *rbcL* successfully sequenced), leg. S. M. Lin; Jefouli, attached on intertidal rocks, March 1, 2009 (NTOU01032009, sequenced for both *rbcL* and *psaA*), leg. S. M. Lin.

Habit and carposporophyte development: Thalli 2–3 (-4) cm tall, strongly calcified, branched subdichotomously to 4–6 orders, and occur in decumbent clumps or mats in patches (Fig. 5A) or on tops of coral reefs intertidally; only monoecious gametophytes found in field. Carpogonial branches threecelled, straight, lateral from basal or proximal portion of assimilatory filaments, carpogonia with relatively short, terminal trichogynes (Fig. 5B); postfertilization stages rarely seen, restricted to lightly calcified tips of branches; after presumed fertilization, zygote cut off a gonimoblast initial laterally (Fig. 5C) followed by another lateral gonimoblast initial (Fig. 5D) from opposite side; at early stage of gonimoblast development, gonimoblast initials elongated and branched adaxially producing gonimoblast filament initials (Fig. 5E); as carposporophytes matured, gonimoblast filaments branched radially and loosely (Fig. 5F); pit connections between cells of carpogonial branch broadened to form slender fusion cell (Fig. 5G); at later stage of carposporophyte development, gonimoblast filaments further elongated and branched once or twice (Fig. 5G); up to this stage, assimilatory filaments remained unmodified and no involucral filaments cut off from cortical cells in vicinity of carposporophyte (Fig. 5G); when mature, terminal cells of gonimoblast filaments differentiated into carposporangia and two gonimoblast initial cells remained distinct (Fig. 5H, arrows); carposporangia undivided in Taiwan specimens, decussately or
Liagoropsis maxima Yamada 1944: 19 (Fig. 6)

Type locality: Babukutu, Taitung Co., Taiwan.

Holotype: SAP026365.

Examined specimens: Taiwan: Taitung Co., Shen-glan (= southern part of Babukutu), growing in subtidal zone at 1–2 m depths, March 11, 2008 (NTOU11032008), leg. S. M. Lin; Shanyuan, growing in subtidal zone at 1–2 m depths, August 3, 2003 (NTOU03082003, sequenced for both rbcL and psaA), leg. S. M. Lin, June 9, 2005 (NTOU09062005, sequenced for both rbcL and psaA), leg. S. Y. Yang.

Habit and carposporophyte development: the topotype specimens agreed with the description and illustrations of Yamada (1944) and Doty and Abbott (1964). Thalli slightly calcified (Fig. 6A), 15–50 cm tall, with one to several, cylindrical to flattened axes arising from short, terete stalk with discoid holdfast attached on coral reefs at 1–2 m depth or in tidal pools. Carposporangial branches 7- to 8-celled, single or...
Fig. 5. *Yamadaella caenomyce*. Carposporophyte development (A: NTOU20032010, B, C: NTOU15062007, D, E: NTOU09062005, F, H: NTOU01032009); scale bars: A = 2 cm; B–F = 20 μm; G, H = 50 μm. (A) Thallus habit, wet preserved. (B) Cortical filaments (co) bearing a carpogonial branch (cb). (C) Early postfertilization stage showing fertilized carpogonium (cp) cutting off a first gonimoblast initial (gi) laterally. (D) Early postfertilization stage showing fertilized carpogonium (cp) cutting off two gonimoblast initials (gi) bilaterally, and unmodified cortical cells (co). Note elongation of one of two gonimoblast initials (arrow). (E) Later stage of early postfertilization development showing fertilized carpogonium (cp) and elongating gonimoblast initials (gi), one laterally branched (arrow). (F) Young carposporophyte showing branched gonimoblast filaments (arrows) and fusing carpogonial branch (cb). Note gonimoblast initials (gi) remain distinct. (G) Immature carposporophyte showing loosely developed gonimoblasts, fused carpogonial branch (fc), and unmodified cortical cells (co). Note gonimoblast initials (arrows) remain distinct and no involucral filaments produced. (H) Nearly mature carposporophyte showing diffuse gonimoblast filaments, terminal carposporangia (arrowheads), and fused carpogonial branch (fc). Note gonimoblast initials (arrows) remain distinct.

in clusters on basal portion of assimilatory filaments (Fig. 6B); after presumed fertilization, zygote divided several times longitudinally (Fig. 6C, arrowhead) to produce gonimoblast initials, at least three gonimoblast initials cut off directly from zygote; these gonimoblast initials elongated upwardly and then divided several times transversely to produce young gonimoblast filaments (Fig. 6D); gonimoblast
filaments at first branched unilaterally (Fig. 6E) and then radially (Fig. 6F); no involucral filaments produced during carposporophyte development; carposporophytes fastigiate when fully developed (Fig. 6G); carposporangia differentiated successively from terminal cells of gonimoblast filaments (Fig. 6, E–G); no remaining cell walls from discharged carposporangia found; basal cells of assimilatory filaments adjacent to carpogonial branch have elongated (white arrows).

Note: Doty and Abbott (1964) placed L. maxima in synonymy with Liagoropsis schrammii (P.L. Crouan & H.M. Crouan) Doty & I.A. Abbott (basionym: Helminthocladia schrammii P.L. Crouan & H.M. Crouan; type locality, Guadeloupe, Lesser Antilles, Caribbean), recognizing only a single widespread species in the genus. Given the considerable geographic separation and the lack of any sequence data for Caribbean populations, we prefer to retain Yamada’s name until further collections become available.

DISCUSSION

Nemaliales, type order of subclass Nemaliophycidae T.A. Christensen, has been regarded as a natural assemblage characterized by carposporophytes that are produced directly from the carpogonium after fertilization without a generative auxiliary cell. Most families previously included in the order by Fritsch (1945) and Kylin (1956) (as “Nemalionales”)
have been raised to a higher taxonomic rank (see Huisman 2006; 2, Le Gall and Saunders 2010 for reviews), except for the Galaxauraceae and the Ligatoraceae (Chaeatangiaceae and Helminthocladiaceae, respectively, in Fritsch and Kylin). Chaeatangiaceae was elevated to ordinal status by Desikachary (1964), but this has largely been ignored. Monophyly of the order has been demonstrated by Huisman et al. (2004a,b) based on large-subunit ribosomal DNA sequences, and Huisman et al. (2004a) also added a third family, the Scinaiaceae. However, relationships among genera in the family Ligatoraceae sensu lato have remained mostly unexplored (Le Gall and Saunders 2010, Lin et al. 2011a,b).

In the present study, phylogenetic analyses of combined rbcL and psaA sequences have shown that the Ligatoraceae sensu lato is polyphyletic as the genera examined were grouped in four independent clades (see Fig. 1). Clade I contained the generitype of Ligator and the majority of genera with more-or-less well-developed involucral filaments and in which gonimoblast initials are cut off transversely or obliquely (i.e., Alakalphyce, Demonema, Iziella, Titanophycus, Helminthocladia, Trichogloeopsis).

The other three clades (II-IV) were monogenic: Yamadaella (Clade II), Nemalion (Clade III), and Ligator (Clade IV). Genera in Clade I (the Ligatoraceae sensu stricto as defined herein) have the widest geographic distribution, occurring from temperate northern Atlantic and southern Pacific Oceans through warm-water regions of the Indo-Pacific and Caribbean Sea (Abbott and Hollenberg 1976, Huisman 2006, Lin et al. 2011a, 2013, 2014). By contrast, Yamadaella (Clade II) and Ligator (Clade IV) are restricted to warm-water regions of the Indo-Pacific and western Atlantic Oceans (Yamada 1944, Doty and Abbott 1964, Abbott 1970), whereas Nemalion (Clade III) is found only in temperate regions but is widespread in both hemispheres (Womersley 1994, p. 81, Guiry and Guiry 2014, see Le Gall and Saunders 2010).

Our molecular analyses do not support the proposed subfamilial/familial relationships among genera placed in the Ligatoraceae by Doty and Abbott (1964: 451) (table 2), in which Ligator, Nemalion, Trichogloea Kützing, and Trichogloeopsis were placed in Nemaliaceae (as “Nemalionaceae”) based on their gelatinous thalli as well as their simply constructed, compact gonimoblasts. Based on our analyses, the phylogenetic relationships among the families recognized herein are incompletely resolved. However, the resurrected family Nemaliaceae includes the single genus Nemalion and is not in the clade of Ligatoraceae sensu stricto. Doty and Abbott (1964) included Demonema and Cumagloia in the subfamily Demonemae, which they did not formally place in a family although they suggested that “it would seem perhaps to be more closely related to the Helminthocladiace than to the Nemalionaceae”. The genera Demonema, Dotyophycus, and Yamadaella, which possess diffuse gonimoblasts, were later placed in the newly proposed family Dermomnemataceae by Abbott (1976), but these genera did not prove to be closely related based on our psaA+rbcL sequence analyses. Thus, strictly morphological characters such as thallus and gonimoblast morphology are clearly inadequate for grouping genera into families. However, the orientation of cell divisions of the carposporangia after presumed fertilization is clearly phylogenetically significant. For the genera clustered in Ligatoraceae sensu stricto (Clade I), the first cell division of the zygote is always transverse (i.e., Ligator spp., see Lin et al. 2011a, figs 27, 30; H. australis, Fig. 4C in this study) or slightly oblique (i.e., D. virens, Fig. 3 C and D, this study), and only a single gonimoblast initial is produced. Although the gonimoblast initial is also cut off transversely in Nemalion, the primary gonimoblast cell is produced longitudinally, rather than transversely or obliquely as is seen in the Clade I members. By contrast, two gonimoblast initials are cut off laterally from the zygote in Yamadaella (Clade II, Fig. 5 D–F), and at least three are produced by longitudinal division of the zygote in Ligator (Clade IV, Fig. 6 C and D).

Although not the focus of the present study, our analyses (Fig. 1) placed A. savianum of the order Acrochaetales as sister to the Nemaliaceae and clearly embedded within the Nemaliaceae. In their investigation of the Acrochaetales, Harper and Saunders (2002: 469) highlighted the close relationship between that order and the Nemaliaceae, suggesting that a single order could be argued, but preferring to adopt a multiorder classification based on the considerable morphological differences between the taxa. We prefer not to speculate given our very limited sample size, but this result should be investigated further.

Molecular phylogenetic analyses based on combined rbcL and psaA sequences of the currently circumscribed Ligatoraceae demonstrate that the family is polyphyletic (Fig. 1). Yamadaella (Clade II), Nemalion (Clade III), and Ligator (Clade IV) are shown to be three independent natural assemblages. Although the genera Demonema, Helminthocladia, and Cumagloia formed a strong clade sister to the other genera of Ligatoraceae sensu stricto, the post-fertilization stages examined in this study did not show significant differences from the majority of Ligatoraceae sensu stricto, in which only a single gonimoblast initial is cut off transversely from the fertilized carposporangium and their involucral filaments are produced from cortical cells adjacent to carposporophytes. Accordingly, we propose to emend the family Ligatoraceae; to reinstate the family Nemaliaceae for Nemalion; and to establish the two new families Yamadaellaceae Showe M. Lin, Rodriguez-Prieto & Huisman based on Yamadaella and Ligatoropsidaceae Showe M. Lin, Rodriguez-Prieto & Huisman based on Ligatoropsis.
irregularly filamentous tetrasporophytes that produce monosporangia and/or tetrasporangia with cruciately or decussately arranged tetraspores (Guiry 1990).

Type genus: Liagora J.V. Lamouroux (1812: 185).

Included genera: Akalaphycus, Cumagloia, Dermo-


Although the genera Cylindraxis Kraf, Gloiottus Huisman & Kraf, Sinoclada C.K. Tseng & W. Li have not been sequenced, their fertilized carpospogonia all divide transversely to produce gonimo-

blast initials, and these genera should be included in the emended Liagoraceae.

Nemaliaceae (Farlow) De Toni and Levi (1886: 212) (as “Nemaliaceae”)

Description: Thalli gelatinous and flaccid, uncalci-
fied, composed of cylindrical, simple or dichotomous axes arising from discoid holdfasts. Carpospogonia branches straight and three-celled borne on upper parts of assimilatory filaments, sometimes in place of assimilatory filaments. Gonimoblast initial single, cut off transversely from fertilized carpogonium and producing gonimoblast initials longitudinally first then obliquely and radially. Carposporophytes of compactly arranged gonimoblast initials; few sterile or involucral filaments produced from assimilatory filaments surrounding carpogonia. Cells of mature carposporophytes mostly differentiate into carposporangia; pit connections between carpospogonia branch cells break down to form fusion cells. Life history heteromorphic with large, dioecious gameto-

phyles and diminutive filamentous tetrasporophytes.

Type and only known genus: Nemalion Duby (1830: 959)

Note: Silva (1980: 85) concluded that the name Nemaliaceae was superfluous, since the family to which it was applied “implicitly included” Liagora, the type of the preexisting, valid family name Liago-

raceae Kützing. Under provisions of the Interna-
tional Code of Botanical Nomenclature in force at the time (Stafleu et al. 1978), the name Nemaliaceae was thus illegitimate and could not be used without conservation. However, a subsequent revi-
sion of the Code removed this requirement (see Nicolson and Norris 1983: 290) and the name Ne-

maliaceae is available for our concept of the family (i.e., excluding Liagora).

Yamadaellaceae Showe M. Lin, Rodríguez-Prieto & Huisman fam. nov.

Description: Thalli highly calcified, forming decumbent clumps of subdichotomous axes attached by multiple holdfasts. Carpospogonia branches three-celled, borne laterally on lower portions of assimilatory filaments. Cell sizes of carpospogonia branches relatively large compared to members of Liagoraceae. Two gonimoblast initials produced lat-
erally from carpogonia after fertilization, then elongating and branching outwardly, producing young gonimoblast filaments. Involutural filaments lacking. Pit connections between cells of carpogonial branches breaking down to form a slender fusion cell at early postfertilization stages. Fully developed carposporophytes composed of lax, laterally spreading, coarse gonimoblast filaments bearing terminal univ- ided or cruciately divided carposporangia. Spermatan- gial parental cells cut off directly from terminal cells of assimilatory filaments; each spermatangial parental cell producing 2–5 spermatangia. Life his- tory probably heteromorphic; isomorphic tetrarosporo- phytes unknown.

Type and currently only known genus: *Yamadaella* I.A. Abbott (1970: 117).

**Liagoropsis** Showe M. Lin, Rodriguez-Prieto & Huisman fam. nov.

Description: Thalli strongly lubricous, with intern- al reticula of light calcification; axes erect, with or without a short stalk. Carposporophy- nal reticula of light calcification; axes erect, with or without a short stalk. Carposporophy- nal reticula of light calcification; axes erect, with or without a short stalk. Carposporo- phytes composed of lax, laterally spreading, coarse gonimoblast filaments bearing terminal univ- ided or cruciately divided carposporangia. Sperma- tangular parental cells cut off directly from terminal cells of assimilatory filaments; each spermatan- gial parental cell producing 2–5 spermatangia. Life his- tory probably heteromorphic; isomorphic tetrarosporo- phytes unknown.

Type genus and currently only known genus: *Liagoropsis* Yamada (1944:19).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web site:

Table S1. List of species sequenced for rbcL and LSU analysis in this study and their accession numbers in GenBank.

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SHOWE-MEI LIN ET AL.


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#9: On page 12, Key to the families of the Nemaliales: The format is out of the order, at the end of each line in the key is not aligned properly.