J. Phycol. **53,** 920–937 (2017)

DOI: 10.1111/jpy.12553

ANALYSIS OF CHLOROPLAST GENOMES AND A SUPERMATRIX INFORM RECLASSIFICATION OF THE RHODOMELACEAE (RHODOPHYTA)[1]

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With over a thousand species, the Rhodomelaceae is the most species-rich family of red algae. While its genera have been assigned to 14 tribes, the high-level classification of the family has never been evaluated with a molecular phylogeny. Here, we reassess its classification by integrating genome-scale phylogenetic analysis with observations of the morphological characters of clades. In order to resolve relationships among the main lineages of the family we constructed a phylogeny with 55 chloroplast genomes (52 newly determined). The majority of branches were resolved with full bootstrap support. We then added 266 *rbc*L, 125 18S rRNA gene and 143 *cox*1 sequences to construct a comprehensive phylogeny containing nearly half of all known species in the family (407 species in 89 genera). These analyses suggest the same subdivision into higher-level lineages, but included many branches with moderate or poor support. The circumscription for nine of the 13 previously described tribes was supported, but the Lophothalieae, Polysiphonieae, Pterosiphonieae and Herposiphonieae required revision, and five new tribes and one resurrected tribe were segregated from them. Rhizoid anatomy is highlighted as a key diagnostic character for the morphological delineation of several lineages. This work provides the most extensive phylogenetic analysis of the Rhodomelaceae to date and successfully resolves the relationships among major clades of the family. Our data show that organellar genomes obtained through high-throughput sequencing produce well-resolved phylogenies of difficult groups, and their more general application in algal systematics will likely permit deciphering questions about classification at many taxonomic levels.

Key index words: **chloroplast genome; classification; phylogenomics; red algae; Rhodomelaceae; Rhodophyta; tribes**

***Abbreviations*: 18S, small subunit ribosomal RNA gene; *cox*1, cytochrome oxidase subunit 1; *rbc*L, ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit**

The Rhodomelaceae is the largest family of the red algae, with 1,054 species and 149 genera recognized (Guiry and Guiry 2017). The number of species is probably underestimated as new taxa are often described when detailed studies using molecular data are performed (e.g., Sherwood et al. 2010, Machín-Sánchez et al. 2016, Savoie and Saunders 2016, Díaz-Tapia et al. 2017a). Moreover, there is a large number of synonyms and taxonomic entities of uncertain status, particularly in the most diverse genera such as *Polysiphonia* and *Laurencia* (Guiry and Guiry 2017). Most of these unknown entities correspond to species described in the 18th and 19th centuries and a proper reassessment may lead to the resurrection of some of these taxa. The enormous species count in the family is mirrored in high morphological diversity, particularly of vegetative organization. Thalli range from a wide variety of simple, filiform architectures to more complex pseudoparenchymatous structures, as well as diminutive parasites. The family is distinguished from other Ceramiales by a combination of vegetative and reproductive characters (Maggs and Hommersand 1993, Womersley 2003). The most significant trait is the polysiphonous structure (axial cell surrounded by several pericentral cells) with monopodially developed axes.

The Rhodomelaceae nom. cons. was established by Areschoug (1847) as a grouping of 10 genera of which only four are currently retained in the

[1]Received 25 March 2017. Accepted 16 May 2017. First Published Online 31 May 2017. Published Online 11 July 2017, Wiley Online Library (wileyonlinelibrary.com).

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[Correction added on October 4, 2017, after first online publication: Minor algal nomenclature corrections on p. 934]

Editorial Responsibility: K. Müller (Associate Editor)

family. The first classification of the Rhodomelaceae into tribes was provided by Schmitz (1889) and later updated in Engler (1892) and in Schmitz and Falkenberg (1897). Subsequently, Falkenberg (1901) published a monumental monograph with a more extensive and detailed integrative study of the family. The 73 genera recognized by Falkenberg were classified into 12 "Familien" (equivalent to tribes) and two unnamed groups, while five remained unplaced (Tables S1 and S2 in the Supporting Information). A major modification in Falkenberg's classification was the resurrection of the family Dasyaceae Kützing (later supported by Rosenberg 1933) for a group that he considered a tribe (as "Familie") of the Rhodomelaceae. Kylin (1956), in his classification of the red algal genera, essentially followed Falkenberg's treatise, but also proposed five new "Gruppen" (equivalent to tribes: *Levringiella*, *Picconiella*, *Placophora*, *Streblocladia* and *Pleurostichidium*).

The most recent comprehensive classification of the family (Hommersand 1963) recognized 13 tribes and maintained three genera in an uncertain position (Tables S1 and S2). Comparing Hommersand's (1963) treatise with Falkenberg's (1901) monograph, the circumscription of the tribes Amansieae, Rhodomeleae, Heterocladieae, Chondrieae, Laurencieae, Polyzonieae and Bostrychiae is the same. Although the Lophothalieae was recognized in both classifications, Hommersand (1963) included in it seven genera that Falkenberg had placed in other tribes or in the unnamed groups, as well as seven genera described after 1901. Likewise, the Pterosiphonieae was recognized by both the authors, but two of its genera (*Aphanocladia* and *Pollexfenia*) were placed in the Polysiphonieae by Hommersand. A major difference between these monographs is that Hommersand merged the tribes Polysiphonieae and Herposiphonieae. Hommersand also maintained the separation of the tribes Pleurostichidieae and Streblocladieae proposed by Kylin (1956). In addition to the tribal classification, Hommersand (1963) proposed three subfamilies (Bostrychioideae, Rhodomeloideae, and Polysiphonioideae), of which only the first two were maintained in a subsequent publication (Maggs and Hommersand 1993).

Later work on the Rhodomelaceae focused on particular taxa within the family and resulted in the recognition of 58 new or resurrected genera that were placed in previously established tribes or remain unplaced (Tables S1 and S2). Furthermore, the Brongniartelleae was segregated from the Lophothalieae (Parsons 1975), the tribe Neotenophyceae was described for the parasitic genus *Neotenophycus* (Kraft and Abbott 2002), and the Sonderelleae was established for two genera previously assigned to the Delesseriaceae (Phillips 2001).

Since the introduction of molecular tools for macroalgal systematics, some taxa of the Rhodomelaceae have been studied in attempts to clarify relationships among genera within the Polysiphonieae (Choi et al. 2001, Bárbara et al. 2013, Díaz-Tapia et al. 2017b), Pterosiphonieae (Savoie and Saunders 2016), Bostrychieae (Zuccarello and West 2006), Laurencieae (Nam et al. 1994, Martin-Lescanne et al. 2010, Cassano et al. 2012, Metti et al. 2015, Machín-Sánchez et al. 2016, Rousseau et al. 2017), Amansieae (Phillips 2002a,b, 2006, Phillips and De Clerck 2005), Heterocladieae (Phillips et al. 2000) and Pleurostichidieae (Phillips 2000). Collectively, these studies have demonstrated that the traditionally employed molecular markers (18S rRNA and *rbc*L genes) are unable to fully resolve phylogenies, especially at the taxonomic levels of genera and tribes. This problem is particularly obvious in the Polysiphonieae (Díaz-Tapia et al. 2017b) and Bostrychieae (Zuccarello and West 2006). Other tribes (Chondrieae, Polyzonieae, Herposiphonieae, Lophothalieae) have been almost completely ignored in phylogenetic studies, and a molecular phylogeny of the whole family has never been attempted. Therefore, the current tribal classification of the family is still based almost entirely on morphological characters and the correlation between morphological and phylogenetic groups has not yet been tested.

Organellar phylogenomics is a valuable approach to resolving difficult phylogenies or deep level relationships in numerous groups of organisms (i.e., Ma et al. 2014, Lu et al. 2015, Leliaert et al. 2016). In the red algae, the chloroplast genome is very large (~180 kb), with a highly conserved structure that includes the most diverse set of genes (~200) known in the Archaeplastida (Janouškovec et al. 2013). However, red algae are still underrepresented in genome datasets, despite promising results whenever they have been applied to phylogenetic studies (Costa et al. 2016, Lee et al. 2016).

The objective of this work is to produce the first comprehensive molecular phylogeny of the Rhodomelaceae and use it to evaluate and update the high-level classification of the family. Our approach relied on resolving phylogenetic relationships among the major lineages of the Rhodomelaceae using phylogenomics based on 45 (42 newly sequenced) chloroplast genomes for selected representative taxa of the main clades of the family, as well as 11 chloroplast genomes of other Ceramiales (10 newly sequenced) to be used as outgroups. In order to get a better phylogenetic view on the rich species diversity of the family, we assembled a second dataset of 407 species in 89 genera based on more comprehensive sampling of the *rbc*L, 18S rRNA and *cox*1 genes, and constructed a phylogeny constrained using the genome-scale tree as a backbone. In order to re-evaluate the tribal classification of the Rhodomelaceae we interpreted both phylogenies along with morphological characters relevant to the delineation of tribes.

MATERIALS AND METHODS

Taxon sampling. To identify the main lineages of the family Rhodomelaceae we constructed an *rbc*L phylogenetic tree including the ca. 500 sequences available in GenBank, as well as ca. 1,000 new sequences generated in our study according to methods described in Saunders and McDevit (2012). In generating new sequences, we sampled extensively in Australia, where the diversity of the Rhodomelaceae is particularly high, with nearly all tribes represented, but from where very little molecular data were available. Using a preliminary tree from this densely sampled dataset, we selected one to four species of each major lineage for high throughput sequencing. For the highly diverse (300 spp.) yet very poorly resolved tribe Polysiphonieae, 14 species were sequenced. This resulted in a total of 52 selected species (42 Rhodomelaceae and 10 other Ceramiales as outgroup). Three previously recognized tribes (Pleurostichidieae, Heterocladieae and the parasitic Neotenophyceae) were excluded as we could not collect new material for them. These are small tribes, containing one, three and one species, respectively.

Data collection. Total DNA was isolated with an adapted cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). In summary, samples were incubated for 1 h in CTAB buffer with proteinase K and extracted with 24:1 chloroform:isoamyl alcohol. DNA was precipitated using 80% isopropanol at 4°C for 1 h and eluted in 0.1 TE buffer.

Barcoded sequencing libraries (350 nt) of the 51 DNA extracts were prepared with the TruSeq Nano LT kit (Illumina, San Diego, California, USA). Because the Verbruggen lab carries out organellar genome projects of both red and green algae, we pooled DNA extracts of red and green algae prior to library preparation, resulting in reduced costs, and the assembled genomes were separated using bioinformatics (e.g., Costa et al. 2016). Libraries were sequenced either on Illumina HiSeq 2000 at the Genome Center of the Cold Spring Harbor Marine Laboratory or Illumina NextSeq at Georgia Genomics Facility. Assembly and annotation of the genomes were performed as previously described (Verbruggen and Costa 2015, Marcelino et al. 2016). GenBank accession numbers for annotated genomes are provided in Table S3 in the Supporting Information.

Sequence alignment and phylogenetic analyses. We assembled a dataset consisting of the 51 newly sequenced chloroplast genomes, an incomplete genome (*Polysiphonia teges*) for which we recovered 79 genes and the four genomes previously published for the order Ceramiales (Salomaki et al. 2015, Verbruggen and Costa 2015, Hughey and Boo 2016). All protein-coding genes were aligned at the amino acid level using MAFFTv7.245 (Katoh and Standley 2013) using default settings and checked visually in Geneious 6.1.7 (Biomatters, Auckland, New Zealand). Nucleotide alignments were constructed based on the inferred amino acid alignments using TranslatorX (Abascal et al. 2010). Alignments were then concatenated and phylogenetic trees inferred with maximum likelihood (ML) in RAxML v8.0.26 (Stamatakis 2014) with GTR+Γ and CPREV+Γ+F models for the nucleotide and amino acid alignments, respectively, and using 100 traditional bootstrap replicates (Felsenstein 1985). Further analyses were carried out to assess the sensitivity of these analyses to model choice (LG, WAG) and partitioning of the data into codon positions.

While the chloroplast genome dataset serves to infer a solid backbone for the initial diversification of the family, it represents less than 5% of the species in the family. To obtain a tree with higher species diversity, we assembled a dataset containing 266 *rbc*L, 125 18S rRNA gene and 143 *cox*1 sequences for additional species, as well as 56 *rbc*L, 54 18S rRNA gene and 51 *cox*1 sequences for species included in the genome-scale phylogeny. Genbank accession numbers for these sequences are provided in Table S4 in the Supporting Information. The total number of species in this tree was 418: 407 members of the Rhodomelaceae and 11 representatives of related families as an outgroup. The three genes were available for 89 species, but there was a substantial amount of missing data in this matrix (45%). A binary constrained phylogeny was constructed using the RAxML chloroplast genome phylogeny based on the nucleotide alignment (constructed as explained above) as the backbone and adding the concatenated alignment with the *rbc*L, 18S rRNA gene and *cox*1 sequences. Data were analyzed using rapid bootstrapping in RAxML and a GTR + CAT model (Stamatakis 2014). Data were partitioned to allow the more densely sampled genes (*rbc*L, *cox*1, and 18S rRNA gene) to have different model parameters than the remaining genes from the chloroplast genome data. Furthermore, *cox*1 and *rbc*L genes were each divided into two partitions based on codon positions (1st + 2nd, 3rd).

RESULTS AND DISCUSSION

We determined 41 complete chloroplast genomes for the Rhodomelaceae, a partial genome for *Polysiphonia teges* (79 genes) and 10 complete genomes for other Ceramiales to be used as outgroups. The genomes were identical in structure to those previously reported for the group (Salomaki et al. 2015, Verbruggen and Costa 2015), and a detailed description of the new genomes will be provided elsewhere. For this study, we required only the gene data to build alignments, and from our 52 new genomes plus 4 downloaded from GenBank, a concatenated alignment of 56 taxa and 194 genes (146,187 nucleotides) was obtained.

Chloroplast phylogenomics resolved the relationships among the major lineages of Rhodomelaceae with full support for the vast majority of branches (Fig. 1). The topology was robust to analyzing the data as nucleotides or amino acids (Fig. 1 vs. Fig. S1 in the Supporting Information), different models of sequence evolution (WAG, LG; data not shown) and partitioning strategies (genes, codon positions, both combined; data not shown). The position of *Thaumatella adunca* is the only exception, as it was resolved with high support as sister to the Rhodomeleae in the nucleotide tree while its relationships within the family were unresolved in the amino acid tree (Fig. S1). These phylogenies include representative taxa for ten of the eleven tribes recognized in Falkenberg's (1901) classification, as well as for the Sonderelleae established by Phillips (2001). While a number of these tribes form well-supported clades in the genome-scale phylogenies, some split into different, unrelated lineages. For example, the genera *Digenea* and *Bryothamnion* are not closely related to other members of the Polysiphonieae where they are currently placed but form a separate, early-branching and well-supported lineage. Similarly, the genus *Thaumatella* is not grouped with the Lophothalieae but forms an early-branching lineage. We propose a new

tribe and the resurrection of an existing tribe for both of these early-branching lineages. The Polysiphonieae as traditionally defined forms a monophyletic clade with 92% bootstrap support in our tree, but it consists of two divergent lineages and we propose their recognition as tribes (Streblocladieae and Polysiphonieae). *Ophidocladus*, previously thought to be related to genera belonging to the Polysiphonieae, is resolved as an isolated taxon that should also be placed in its own tribe. *Herposiphonia* and *Dipterosiphonia*, two lineages currently in the Herposiphonieae, are grouped together in the trees but with poor support in the nucleotide phylogeny (55%; Fig. 1), and we propose to place them in separate tribes. The delineation of these four new tribes and the Alsidieae is further discussed below. The proposals to divide the family into three subfamilies (Bostrychioideae, for the tribe Bostrychieae; Rhodomeloideae, for the tribes Rhodomeleae, Lophothalieae, Heterocladieae and Polyzonieae; and Polysiphonioideae, for the tribes Amansieae, Chondrieae, Laurencieae, Lophosiphonieae nom. nud., Pleurostichidieae, Polysiphonieae, Pterosiphonieae and Streblocladieae nom. nud.; Hommersand 1963) or two subfamilies (Bostrychioideae for the tribe Bostrychieae, and Rhodomeloideae for the other tribes; Maggs and Hommersand 1993) are not supported in the genome-scale phylogeny.

With the aim of getting a more comprehensive phylogenetic view of this species-rich family, we constructed a constrained tree using the nucleotide genome-scale tree as backbone and adding 266 *rbc*L, 125 18S rRNA gene and 143 *cox*1 sequences corresponding to 407 species and 89 genera of the Rhodomelaceae (Fig. S2 in the Supporting Information). A schematic representation of the tree (Fig. 2) shows that while it is congruent with the genome-scale tree, many branches were resolved with only moderate or low bootstrap support. In this tree we recognized the same tribes from the genome-scale tree except for Bostrychieae, which was paraphyletic with respect to Heterocladieae. In addition, there was a range of additional early-branching lineages without close relatives. These include the formerly recognized tribes Pleurostichidieae and Heterocladieae, the genus *Ophidocladus*, for which we propose the tribe Ophidocladeae, *Thaumatella*, for which we propose the Thaumatelleae and *Cladurus*, for which we propose the Cladureae. There were also three early-branching species (*Micropeuce strobiliferum*, *Heterodasya mucronata* and *Wilsonosiphonia howei*) whose tribal assignment requires further work. The Heterocladieae was resolved among taxa of the Bostrychieae, rendering the latter paraphyletic. However, support for this placement was very low, and it most probably resulted from missing data, because only 18S rRNA gene sequences were available for the Heterocladieae, and there were only five 18S rRNA gene sequences for the Bostrychieae (*Bostrychia simpliciuscula*, *B. tenella*, of the Peripherohapteron clade in Fig. S2; and *B. moritziana*, *Bostrychiocolax* and *Dawsoniocolax* of the Cladohapteron clade in Fig. S2).

Below we discuss in more detail the classification that emerged from our phylogenies. We will present the groups in the order they appear in Figure 2, from the bottom upwards. Each tribe is morphologically defined by a combination of vegetative and reproductive characters and for detailed descriptions for previously established tribes we refer to Falkenberg (1901), Hommersand (1963), Womersley (2003) and for the Sonderelleae to Phillips (2001). The brief descriptions provided below for each tribe are intended to highlight easily recognizable characters, as well as propose new key characters needed to delineate some tribes. A summary of the key morphological characters delineating tribes is presented in Table S5 in the Supporting Information. More detailed descriptions of the new tribes are provided in the "Formal taxonomy" section at the end of the paper.

The Sonderelleae is an endemic Australasian tribe that includes two monospecific genera (*Sonderella* and *Lembergia*). Thalli consist of linear blades with a dorsiventral structure formed by three or four pericentral cells, the two laterals producing the ecorticate blade, and one or two pseudopericentrals. They lack trichoblasts; procarps and spermatangia are formed on the blade surface; and there are two tetrasporangia per segment in stichidia. Before placement in their own tribe by Phillips (2001) based on an 18S rRNA gene phylogeny, *Sonderella* and *Lembergia* had been thought to be related, to the tribe Amansieae (Harvey 1859, Lindauer 1949, Womersley 1965, 2003) and the family Delesseriaceae (Schmitz 1889, Saenger et al. 1971). Both species of the Sonderelleae were represented in the taxon-rich tree and the tribe was resolved as monophyletic with high support (Fig. 2 and Fig. S2). The genome-scale tree included *Sonderella*, and evidenced its sister relationship with the tribe Polyzonieae. Phillips (2001) had already predicted this because these are the only two tribes of the family in which three pericentral cells can be observed in certain vegetative structures of some species.

The Polyzonieae includes 17 species in five genera (*Cliftonaea*, *Dasyclonium*, *Echinosporangium*, *Leveillea* and *Polyzonia*) with an Indo-Pacific distribution, characterized by an elaborate structure. Thalli are strongly dorsiventral and consist of indeterminate ecorticate or corticate axes with 6 or 7 pericentral cells, bearing determinate laterals in a regular pattern. The determinate laterals have 3 pericentral cells and are simple, branched or foliose. Trichoblasts are persistent and pigmented (*Cliftonaea* and *Echinothamnion*), deciduous and unpigmented (*Leveillea*) or absent (*Dasyclonium* and *Polyzonia*). Spermatangial structures arise on determinate laterals with a sterile marginal flank, procarps and cystocarps are formed on branches or on the basal cell

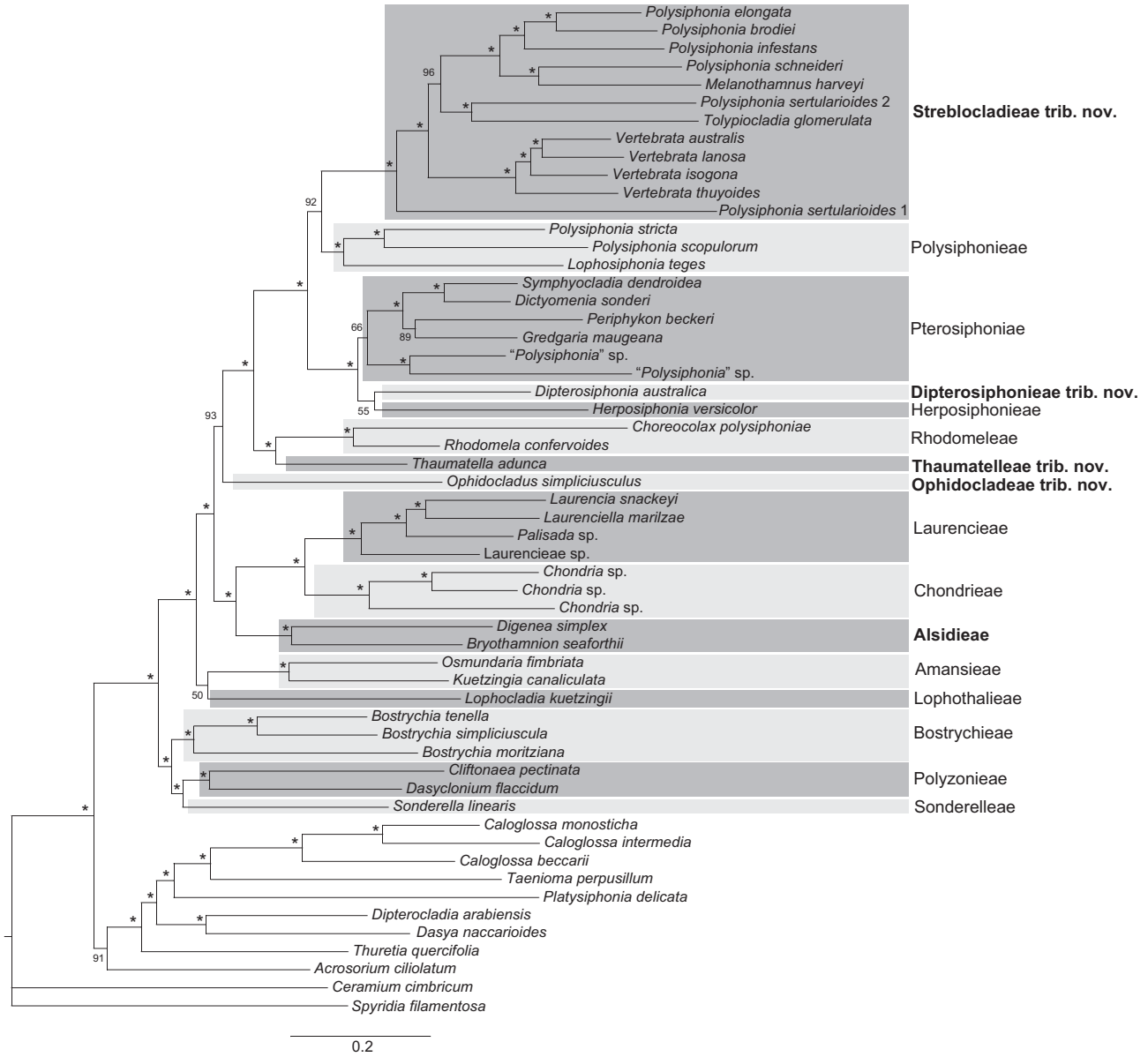

FIG. 1. Phylogeny of the family Rhodomelaceae indicating tribes with light or dark shaded areas; the unshaded area corresponds to the outgroup. Resurrected (Alsidieae) and new tribes are indicated with bold font. RAxML tree based on nucleotide alignment of the 198 concatenated genes from the chloroplast genome. All branches have full bootstrap support (*), except those where bootstrap values are indicated on branches.

of a trichoblast, and the tetrasporangia are in stichidia. The genome-scale tree resolved *Cliftonaea pectinata* and *Dasyclonium flaccidum* in a strongly supported clade (Fig. 1). Likewise, our taxon-rich tree including representatives of four genera resolved the Polyzonieae as monophyletic, although with low support (Fig. 2 and Fig. S2). Our results are in line with the general agreement regarding the generic composition of the tribe (Falkenberg 1901, Scagel 1953, Hommersand 1963). Interestingly, our data revealed significant cryptic diversity in *Dasyclonium incisum* (three species from Australia and one from South Africa – *rbc*L sequence divergence >2.7%), as well as in *Leveillea jungermannioides* (two species from Australia differing from a Korean specimen – sequence divergence >2.1%; the type locality is in the Red Sea).

The Heterocladieae is an Australian tribe with three species in the single genus *Heterocladia*, the delineation of which has been widely accepted in all previous classifications (Falkenberg 1901, Hommersand 1963, Phillips et al. 2000). It is distinguished from other Rhodomelaceae by having four pericentral cells that divide longitudinally forming 7–8 cells around the axial cell, with cortical and rhizoidal cells giving rise to a pseudoparenchymatous thallus

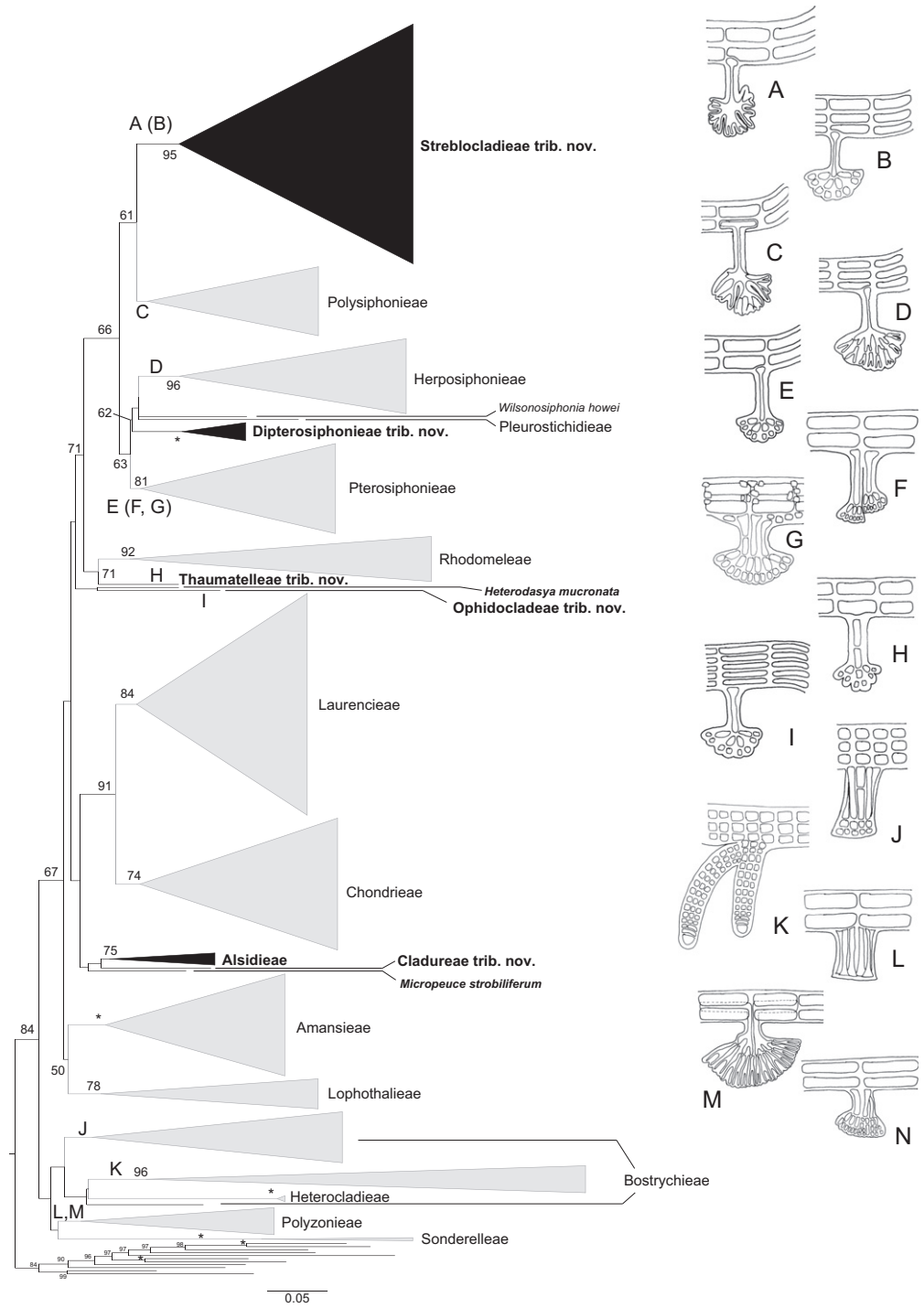

FIG. 2. Compressed phylogeny of 16 clades and eight isolated taxa. The width of each triangle is proportional to the number of species from that clade included in the analysis. The RAxML tree used the genome-scale phylogeny based on nucleotides as a constraint and incorporated 322 *rbc*L, 179 18S rRNA gene and 194 *cox*1 sequences for a total of 418 species. Bootstrap values are indicated on branches when 100 (*) or ≥50%. Black triangles and bold names represent resurrected (Alsidieae) and new tribes, while gray triangles represent previously recognized tribes. The complete phylogeny is presented in Figure S2. Schematic representations of the rhizoid anatomy and cladohapteron (panel K) are provided indicating their corresponding tribes with capital letters, when applicable (basal discs characterize tribes without diagrams). Panel N corresponds to *Veleroa subulata*, which was not included in our phylogeny and is currently placed in the Lophothalieae.

that bears pigmented trichoblasts. The procarps and spermatangial branches are formed on trichoblasts and one tetrasporangium per segment develops in stichidia. Our taxon-rich tree included 18S rRNA gene sequences for the three known species and, in agreement with Phillips et al. (2000), the tribe was

resolved as monophyletic (Fig. 2 and Fig. S2). However, it was placed together with members of the Bostrychieae in an unsupported clade, which is probably an artifact resulting from missing data. Therefore, the relationship of this tribe to other members of the family should be considered unresolved.

All earlier classifications recognized the Bostrychieae for the genus *Bostrychia*, as it is clearly distinguished morphologically from other Rhodomelaceae (Falkenberg 1901, Hommersand 1963). It is distributed worldwide, often in brackish environments, and is mainly characterized by its filiform habit, consisting of axes with pericentral cells dividing transversely to form tiers, the basal cell of which remains pit-connected with the axial cell. *Bostrychia* lacks trichoblasts, but has monosiphonous branches. The spermatangia and procarps are formed on determinate branches, with a particular development of female structures, and tetrasporangia form in whorls in stichidia. Furthermore, the two parasitic genera *Dawsoniocolax* and *Bostrychiocolax* were included in the tribe based on their phylogenetic affinities (Zuccarello et al. 2004). The three *Bostrychia* species for which we obtained the complete chloroplast genome were resolved in a strongly supported clade (Fig. 1). In the taxon-rich tree (Fig. 2 and Fig. S2) all *Bostrychia* species were placed together in an unsupported clade, which in turn contains two major clades, a species without close relatives and the Heterocladieae. The first clade, which was poorly supported, was composed of 17 species of *Bostrychia* (Peripherohapteron-clade in Fig. S2), but the second one received high support, and contained eight *Bostrychia* species and the two monospecific parasitic genera *Dawsoniocolax* and *Bostrychiocolax* (Cladohapteron-clade in Fig. S2). These clades were named from and are in agreement with the two major groups delineated in the Bostrychieae based on the anatomy of attachment organs: peripherohapteron and cladohapteron (Zuccarello and West 2006). As discussed above, and considering the clear morphological differences, the positioning of the Heterocladieae among the Bostrychiae is very likely to be an artifact explained by the lack of overlapping markers from the two tribes in our dataset.

The tribe Lophothalieae included ten genera in Falkenberg's (1901) classification. Subsequently, Hommersand (1963) added another 14, seven that had been placed in different tribes by Falkenberg and seven described since 1901. Later, five newly described genera were allocated to this tribe (Joly and de Oliveira Filho 1966, Wynne and Norris 1982, Noble and Kraft 1983, Millar 2000a, Huisman 2001). More recently, the two *Brongniartella* species were transferred to *Vertebrata* in the Polysiphonieae (here Streblocladieae), based on phylogenetic studies (Díaz-Tapia et al. 2017b). Therefore, the Lophothalieae currently encompasses 28 genera, each containing only one to seven species. The tribe is distributed worldwide and mainly characterized by thalli consisting of terete and radially branched axes that bear pigmented and persistent trichoblasts. Genera are delineated by characters such as the presence and degree of cortication, number of pericentral cells, trichoblast anatomy, number of sterile groups in procarps, tetrasporangial arrangement, and number and origin of cover cells (pre- or postsporangial; Parsons 1975, Millar 2000a, Womersley 2003). On the other hand, nine genera are parasites and Hommersand (1963) placed them in this tribe mainly because they form tetrasporangia in stichidia. Our taxon-rich tree (Fig. S2) resolved with moderate support a clade including *Lophothalia hormoclados*, as well as species of *Doxodasya*, *Lophocladia*, *Murrayella*, *Spirocladia* and *Wrightiella*. This clade is represented in our genome-scale tree by *Lophocladia kuetzingii* and its phylogenetic relationships within the family are still unclear (Fig. 1). Furthermore, *Heterodasya mucronata* and *Micropeuce strobiliferum* are two rogue taxa in the taxon-rich tree placed as sisters to the Alsidieae and the Ophidocladeae with low support (Fig. 2 and Fig. S2). More gene sequences are needed to resolve the phylogenetic relationships of these two taxa and clarify whether they are in the Lophothalieae or if they should be placed in different tribes.

Our phylogenies showed that the tribe Lophothalieae is not monophyletic as currently circumscribed. In addition to the above-mentioned clade and these two rogue taxa, *Thaumatella* (*Veleroa) adunca* was placed as sister to the Rhodomeleae with strong support in the genome-scale tree (Fig. 1). These results, together with the placement of *Brongniartella* in *Vertebrata* (as *V. byssoides* and *V. australis* in Fig. S2; Díaz-Tapia et al. 2017b), demonstrate that pigmented and persistent trichoblasts have evolved independently in several lineages of the family and further morphological traits are needed to redefine the tribe. Two schemes for subdividing the Lophothalieae have been proposed, though not generally accepted. Parsons (1975) segregated the Brongniartelleae from the Lophothalieae based on the number of sterile groups in the procarps (2/1), the absence/presence of post-sporangial tetrasporangial cover cells, and trichoblasts branched in a single plane/spirally branched/unbranched. However, Womersley and Parsons (2003) merged them again into a single tribe, suggesting that a tribal character may be the formation of tetrasporangia in stichidia without trichoblasts (*Lophocladia*, *Haplodasya*) versus tetrasporangia on normal branches. This second proposal is not supported in our phylogeny, as *Lophocladia* is closely related to *Spirocladia barodensis* which has tetrasporangial stichidia bearing trichoblasts. Interestingly, and although the Brongniartelleae is not supported in our phylogeny as a monophyletic taxon, four genera (*Brongniartella*, *Micropeuce*, *Veleroa* and *Heterodasya*, among the five

currently recognized) that Parsons attributed to this tribe, and are represented in our taxon-rich tree, were not placed in the Lophothalieae clade. Therefore, the morphological delineation proposed by Parsons (1975) for the Lophothalieae is consistent with our phylogenies. However, some of the key reproductive characters are poorly known in several species or genera, our analysis only included representatives of nine of 19 non-parasitic genera currently assigned to the tribe, and the phylogenetic relationships of *Micropeuce* and *Heterodasya* are unresolved. Therefore, it is not yet possible to provide an accurate delineation for the Lophothalieae and further morphological and molecular studies are needed to clarify the systematics of this group. The systematics of *Thaumatella (Veleroa) adunca*, a morphologically distinctive species with respect to other Lophothalieae, is discussed below.

The circumscription of the Amansieae is identical in Falkenberg (1901) and Hommersand (1963), who both assigned nine genera to this tribe. Four new genera have subsequently been included in the Amansieae (Wilson and Kraft 2000, Phillips 2002b, 2006). The tribe includes about 60 species, and is particularly diverse on Australian and South African shores. It is characterized by pseudoparenchymatous thalli, mostly complanate or leaf-like, with strong dorsiventrality involving trichoblasts arising adaxially at the apices and, in most species, the differentiation of pericentral cells into lateral, dorsal and ventral positions. The procarps and spermatangial branches are formed on modified trichoblasts and the tetrasporangia, two per segment, usually form in stichidia. Our genome-scale and taxon-rich trees (Figs. 1, 2 and Fig. S2), including representatives of two and 13 genera, respectively, resolved the Amansieae as monophyletic with strong support. Although relationships among species within the tribe in the taxon-rich tree are in general not well supported, our data suggest that a revision is needed at generic level, as *Amansia*, *Vidalia* and *Osmundaria* are apparently not monophyletic.

The monospecific genus *Cladurus*, endemic to Australia, was included in the tribe Chondrieae in earlier classifications (Falkenberg 1901, Hommersand 1963). However, Gordon-Mills and Womersley (1987) and Womersley (2003) considered that it did not belong to this tribe because spermatangial branches are cylindrical instead of plate-like, as is characteristic in the Chondrieae. Furthermore, this genus is distinguished from other Rhodomelaceae by its terete thalli with five pericentral cells, pseudoparenchymatous construction with light cortication so that the segments are conspicuous in surface view in branches, cystocarps arising on short axillary branches and tetrasporangia borne in stichidia. This species was only included in the taxon-rich tree, where it was placed as sister to the Alsidieae, but with low support. Considering the peculiar morphological characters of this genus and its ambiguous relationships with other members of the family, we propose the Cladureae trib. nov.

The small genera *Alsidium*, *Bryothamnion* and *Digenea* (8, 3 and 2 species, respectively) were previously included in the Polysiphonieae (Falkenberg 1901, Hommersand 1963). The genome-scale tree (Fig. 1) placed *Bryothamnion* and *Digenea* in a single clade with strong support, sister to the clade formed by the Chondrieae and Laurencieae. The taxon-rich phylogeny (Fig. S2) resolved *Alsidium*, *Bryothamnion* and *Digenea* in a moderately supported clade. These three genera have pseudoparenchymatous thalli with 5–12 pericentral cells, forming axes of indeterminate growth usually clothed with short determinate branches. Furthermore, they differ from the Polysiphonieae by having plate-like spermatangial branches without sterile margins (Falkenberg 1901, Børgesen 1920, Norris 1994). These spermatangial branches resemble the typical ones of the Chondrieae, however, in the Chondrieae they have marginal sterile cells and all species have 5 pericentral cells. Therefore, based on the morphology and the phylogeny we propose the resurrection of the tribe Alsidieae for these three genera (discussed in the formal taxonomic treatment below). According to the taxon-rich tree, two main clades are resolved in the tribe and *Alsidium* is not monophyletic. Considering that *A. corallinum* from the Mediterranean is the type of the genus, *A. cymatophilum* from Hawaii must be transferred to *Digenea*. Furthermore, the separation between *Bryothamnion* and *Alsidium* requires further investigation.

Generic composition of the Chondrieae was very similar in Falkenberg's (1901) and Hommersand's (1963) classifications. Falkenberg included six genera, one later transferred to the Lophothalieae by Hommersand (1963), who also added two newly described genera. Subsequently, *Waldoia* and the parasitic genera *Ululania*, *Benzaitenia* and *Jantinella* were included in this tribe (Taylor 1962, Morrill 1976, Apt and Schlech 1998, Kurihara et al. 2010). The genome-scale phylogeny (Fig. 1) includes three *Chondria* species that constitute a monophyletic clade. Similarly, the taxon-rich phylogeny (Fig. S2) includes representatives from nine of the 11 genera currently assigned to the tribe which, except for *Cladurus* (see above), are resolved in a monophyletic clade with moderate support. Our data also suggest that the tribe requires a revision at the genus level because neither *Chondria*, currently including 80 species, nor *Acanthophora* (7 species), is monophyletic.

The tribe Laurencieae was likewise very similar in generic composition in Falkenberg's (1901) and Hommersand's (1963) classifications, encompassing the large genus *Laurencia* (145 currently recognized species), *Rodriguezella* and the parasitic *Janczewskia*. Subsequently, six other genera were reinstated or segregated for groups of species previously assigned to *Laurencia* (Nam et al. 1994, Nam 2007, Martin-

Lescanne et al. 2010, Cassano et al. 2012, Metti et al. 2015, Rousseau et al. 2017). The genome-scale and taxon-rich phylogenies (Fig. 1 and Fig. S2), with representatives of seven genera, resolved all members of the tribe as a single clade that, respectively, received high or moderate support.

A close relationship between the Chondrieae and Laurencieae was previously predicted in evolutionary reconstructions of the family based on morphological characters (Falkenberg 1901, Hommersand 1963) and is strongly supported in our phylogenies (Figs. 1 and 2). Both tribes are distributed worldwide and characterized by pseudoparenchymatous thalli, such that the segments and pericentral cells are not distinguishable in surface view. They differ in the number of pericentral cells (5 in the Chondrieae and 2 or 4 in the Laurencieae) and the anatomy of the male structures (plate-like spermatangial branches with sterile marginal cells in the Chondrieae and modified trichoblasts or filaments immersed in apical depressions in the Laurencieae).

Ophidocladus simpliciusculus was included in Falkenberg's (1901) classification in the "*Lophosiphonia* group" (Lophosiphonieae nom. nud. in Hommersand 1963), a group of genera with dorsiventral prostrate and erect ecorticate terete axes and exclusive production of endogenous branches. Although this group resembles various tribes in some of its characters, it could not be assigned to any of them (Falkenberg 1901). *Ophidocladus simpliciusculus* is separated out in our genome-scale and taxon-rich phylogenies (Figs. 1 and 2) and it has numerous characters that make it unique within the family, such as a large axial cell surrounded by up to 28 pericentral cells, alternately arranged trichoblasts and spermatangial structures covering the two basal dichotomies of a trichoblast (Saenger 1971, Díaz-Tapia and Bárbara 2013). On the basis of its morphology and our molecular evidence we propose Ophidocladeae trib. nov. for this monospecific genus.

The genus *Veleroa* is currently placed in the tribe Lophothalieae (Dawson 1944, Hommersand 1963); *V. adunca* is the only one of the seven species in this genus included in our analysis (as *T. adunca*; see below). It was placed as a taxon without close relatives, sister to the Rhodomeleae, with high and moderate support in the genome-scale and taxon-rich trees (Figs. 1 and 2), respectively. The type species of *Veleroa* is *V. subulata* from California and the genus is characterized by ecorticate axes with four pericentral cells, pigmented unbranched trichoblasts and one tetrasporangium per segment on branches bearing trichoblasts (Dawson 1944). *Veleroa adunca*, in contrast, has branched trichoblasts (Womersley and Parsons 2003). Furthermore, the detailed description of *V. subulata* (Abbott and Ballantine 2012) based on topotype material reveals additional important differences between them. *V. adunca* has rhizoids cutoff from a single pericentral cell as multicellular, but uniseriate, filaments that terminate in a multicellular discoid pad (Fig. 2H); rhizoids in *V. subulata* have multiseriate rhizoidal filaments formed from two adjoining pericentral cells (Abbott and Ballantine 2012; Fig. 2N). Also, the spermatangial organs differ in these species – they are ovoid, with a single basal sterile cell in *V. adunca*, while they are cylindrical with long basal and apical sterile filaments in *V. subulata*. When Womersley and Parsons (2003) transferred *Dasya adunca* to *Veleroa* they also placed *Thaumatella disticha*, the type of the genus, in synonymy. They argued that the characters used by Kylin (1956) to separate *Thaumatella* from *Veleroa* (i.e., branching patterns) were misinterpreted. However, differences in the anatomy of rhizoids and spermatangial organs suggest that *V. adunca* and *V. subulata* most probably belong to different genera. Therefore, we propose to resurrect the genus *Thaumatella* for *V. adunca*. Furthermore, considering the position of this species in the phylogeny and its unique rhizoid anatomy (differing from other *Veleroa* – see also *V. mangeana*; Millar 2000b, Schneider et al. 2010 – and members of the Lophothalieae, when information is available), we propose the Thaumatelleae trib. nov.

The Rhodomeleae includes *Rhodomela* and *Odonthalia*, both placed in this tribe by Falkenberg (1901) and Hommersand (1963), as well as the subsequently described *Neorhodomela* (Masuda 1982) and *Beringiella* (Wynne 1980). Their distribution is restricted to cold shores of the Northern Hemisphere. They are characterized by having pseudoparenchymatous thalli, with 6–7 pericentral cells dividing transversely and the apical cell retaining the pit connection with the axial cell. The taxon-rich tree (Fig. 2), including representatives of three genera, resolves the tribe in a highly supported clade. Furthermore, three parasites (*Harveyella*, *Leachiella* and *Choreocolax*), whose placement in the Rhodomelaceae was clarified in Zuccarello et al. (2004), were also resolved in this tribe in our phylogeny. The tribe is represented in the genome-scale tree (Fig. 1) by *R. confervoides* and *C. polysiphoniae* and was placed as sister to *Thaumatella* and in turn to the Polysiphonieae.

The Pterosiphonieae was erected by Falkenberg (1901) for six genera that share a bilateral branching pattern, with the branches congenitally fused to the main axes to a varying extent, ranging from filiform to foliose thalli. They have procarps and spermatangia on modified trichoblasts and tetrasporangia on lateral branches. Hommersand (1963) pointed out that *Tayloriella*, *Rhodomelopsis* and *Carradoria* (as *Carradoriella*) of the Polysiphonieae, all erected after 1901, must be in this tribe although they lack congenital fusion of branches. Consequently, he redefined the tribe mainly by the alternate-distichous branching pattern and the absence of vegetative trichoblasts, and he transferred

Aphanocladia and *Pollexfenia* to the Polysiphonieae. Only two genera have subsequently been placed in this tribe, *Xiphosiphonia*, recently segregated from *Pterosiphonia,* and *Heterostroma* (Kraft and Wynne 1992, Savoie and Saunders 2016).

The taxon-rich tree (Fig. S2), including 10 representatives of the 12 genera assigned to the tribe at one time, resolved a moderately supported clade comprising *Pterosiphonia* and seven other genera previously assigned to the Pterosiphonieae. However, *Pterochondria* and *Carradoria* (as *P. virgata*) were placed in the Polysiphonieae (here clade Streblocladieae) despite having the characters of the tribe Pterosiphonieae. Furthermore, the clade Pterosiphonieae included five additional genera, *Lophurella*, *Echinothamnion* and *Periphykon*, currently included in the Polysiphonieae, *Gredgaria* of the Herposiphonieae and *Womersleyella* currently lacking tribal assignment. Also, an unidentified species of Pterosiphonieae was resolved in this clade with high support, and three other *Polysiphonia*-like species were placed as sister to this clade with low support (their taxonomic identity at generic and species level requires further work).

This tribe is represented in the genome-scale tree (Fig. 1) by members of four genera (*Symphyocladia*, *Dictyomenia*, *Periphykon* and *Gredgaria*) that form a strongly supported clade, which in turn is sister with moderate support to the clade formed by two *"Polysiphonia"* spp. Among the genera placed for the first time in the Pterosiphonieae, *Gredgaria* is the only one that meets Falkenberg's or Hommersand's criteria for delineating the tribe, despite being included by Womersley (2003) in the Herposiphonieae. In contrast, the other genera or species have trichoblasts, and/or branches spirally arranged and not congenitally fused with the main axes (Hollenberg 1967, Womersley 2003, P. Díaz-Tapia, pers. obs.). Therefore, the morphological criteria used for distinguishing the Pterosiphoniae from the Polysiphonieae are not supported. While all genera with an alternate branching pattern and congenitally fused branches are in the Pterosiphonieae (except *Pterochondria*), the tribe also includes several members with spirally arranged branches not congenitally fused with the main axes. Also the presence/absence of trichoblasts varies among members of the tribe. However, a character that we found uniformly in all the species placed in this tribe in our phylogeny is that rhizoids are cutoff from the distal (and proximal in *Gredgaria* and Pterosiphonieae sp.) ends of the pericentral cells, and the rhizoidal filament terminates in several cells forming a multicellular discoid pad (Fig. 2, E–G). This character is distinctive with respect to the Polysiphonieae and Streblocladieae, in which the rhizoids are unicellular and are formed from the mid-proximal ends of the pericentral cells. *Lampisiphonia* is the only known exception among the species placed in the Streblocladieae in our phylogeny, as it has multicellular rhizoids (some rhizoids of the thallus have multicellular filaments, and discoid pads are multicellular when mature). However, they are formed from the proximal ends of the pericentral cells (Fig. 2B; Bárbara et al. 2013).

The Herposiphonieae is found worldwide and is characterized by a dorsiventral and filiform habit, thalli consisting of ecorticate axes with 4–16 pericentral cells and the exclusive production of endogenous branches with defined sequences of determinate and indeterminate branches. Procarps and spermatangia are formed on modified trichoblasts and tetrasporangia on determinate branches. The tribe was erected by Falkenberg (1901) for seven genera, but Hommersand (1963) merged it with the Polysiphonieae, distinguishing the genera of this tribe as "dorsiventral Polysiphonieae." *Streblocladia* and the parasite *Microcolax* were moved to a separate "Gruppe" by Kylin (1956), which was recognized as the tribe Streblocladieae nom. nud. by Hommersand (1963), as discussed below. On the other hand, four genera described since 1963 (*Herposiphoniella*, *Ditria*, *Gredgaria* and *Tiparria*) have been attributed to the Herposiphonieae (Hollenberg 1967, Womersley 2003). In summary, nine genera are currently assigned to the tribe Herposiphonieae, of which *Herposiphonia* contains 56 species, *Dipterosiphonia* seven and the other genera only one to three species. They are distinguished by distinct branching patterns. Three of them were included in our analysis, but *Gredgaria* was transferred to the Pterosiphonieae (see above). The other two, *Herposiphonia* and *Dipterosiphonia*, were placed together in a poorly supported clade, sister to the Pterosiphonieae in the genome-scale tree (Fig. 1). The taxon-rich tree placed them, with *Wilsonosiphonia* and *Pleurostichidium*, in a poorly supported clade (Fig. 2).

Pleurostichidium is a morphologically very distinctive monospecific genus placed in its own tribe, the Pleurostichidieae, for which Phillips (2000) provided a detailed characterization. Considering that the Dipterosiphonieae and Herposiphonieae clades are strongly supported, the early divergence of these two lineages as well as the Pleurostichidieae, and the extent to which *Pleurostichidium* differs morphologically from the Herposiphonieae, we propose the segregation of the tribe Dipterosiphonieae from the Herposiphonieae. The tribal assignment of *Wilsonosiphonia* requires a better understanding of its phylogenetic relationships and further studies using more gene data are needed. The Dipterosiphonieae and Herposiphonieae differ from the Polysiphonieae and share with the Pterosiphonieae rhizoids cutoff from the distal end of pericentral cells. All have multicellular discoid pads, which have the same structure in the Dipterosiphonieae and Pterosiphonieae. In contrast, in the Herposiphonieae, discoid pads consist of a digitate structure formed by an extension of the rhizoidal filament

that divides to form small apical cells (Fig. 2D). Furthermore, the Herposiphonieae is characterized by its distinctive regular pattern of the formation of determinate and indeterminate branches, often in a 3:1 sequence. The tribe Dipterosiphonieae, in contrast, is distinguished by producing alternate pairs of determinate branches. However, as only seven species are currently known, it remains to be determined whether this branching pattern applies more generally.

The Polysiphonieae is the largest tribe of the Rhodomelaceae and has a worldwide distribution. Falkenberg (1901) included 11 genera characterized by filiform thalli, heavily corticated in a few species, with branches radially organized and trichoblasts deciduous and unpigmented. Subsequently, another 11 newly described or resurrected genera, three of them parasitic, were included in this tribe. While some genera in this tribe (*Echinothamnion*, *Lophurella*, *Digenea*, *Alsidium*, *Bryothamnion*) are here transferred to other tribes (see discussion on Alsidieae and Pterosiphonieae), the vast majority are placed in a monophyletic clade that was resolved with high and moderate support in the genome-scale and taxon-rich phylogenies, respectively (Figs. 1, 2 and Fig. S2; Strebloclad ieae and Polysiphonieae clades). Two major lineages were resolved within this clade and we propose to segregate the tribe Strebloclad ieae from the Polysiphonieae. They are distinguished by the synapomorphic trait of having rhizoids cutoff from the mid-proximal end of the pericentral cells (Strebloclad ieae; Fig. 2A) versus rhizoids in open connection with the pericentral cells (Polysiphonieae; Fig. 2C; Kim and Lee 1999, Choi et al. 2001, Díaz-Tapia et al. 2017b).

The Polysiphonieae clade contains the type of the genus *Polysiphonia* (*P. stricta*) and it was termed *Polysiphonia* sensu stricto in previous phylogenetic studies of the tribe (Choi et al. 2001, Bárbara et al. 2013, Díaz-Tapia et al. 2017b). These studies emphasized the existence of two major clades within *Polysiphonia* sensu stricto (here named *Polysiphonia* and *Bryocladia/Falkenbergiella* in Fig. S2), and they were resolved as monophyletic or paraphyletic in previous works depending on the taxon selection and the molecular marker(s) considered. *Polysiphonia* and *Bryocladia/Falkenbergiella* are represented in our genome-scale tree by *P. stricta* and *P. scopulorum* and are definitively resolved as a monophyletic clade sister to Strebloclad ieae (Fig. 1). In the taxon-rich tree (Fig. S2) *Polysiphonia* and *Bryocladia/Falkenbergiella* contain eight and 10 species and are resolved as two highly supported clades. The clade containing *P. stricta* corresponds to the genus *Polysiphonia* and all the species have four pericentral cells, are decumbent or erect and have predominantly exogenous branches. The other clade is morphologically more variable and includes species with a dorsiventral or radial structure, with predominantly exogenous or endogenous branches, and with four or more (*Bryocladia*) pericentral cells. The generic assignment of this second clade requires further studies including analysis of material of *Falkenbergiella capensis* from South Africa (currently included in *Lophosiphonia*), with morphological traits (four pericentral cells, dorsiventral, with endogenous branches) that indicate it may be included in this clade, and the scarcely known *Bryocladia cervicornis* from Java. These two species are the types of their corresponding genera, *Bryocladia* pre-dating *Falkenbergiella*.

In addition to these two previously recognized groups in *Polysiphonia* sensu stricto, *P. teges* was also resolved in this clade (Fig. 1). In the taxon-rich phylogeny (Fig. S2), *P. teges* is closely related to *Lophosiphonia simplicissima* and *L. obscura* sensu (1956, with six pericentral cells; see Rueness 1971, Silva et al. 1996, Díaz-Tapia and Bárbara 2013, for a further discussion on the taxonomic identity of this species), the type species of *Lophosiphonia*. We propose to maintain the generic attribution of this clade to *Lophosiphonia* and transfer *P. teges* to this genus. *Lophosiphonia* was erected by Falkenberg (in Schmitz and Falkenberg 1897) to group species with a secondary dorsiventral structure and predominantly endogenous branches. However, the validity of this circumscription has been discussed (Díaz-Tapia and Bárbara 2013 and references therein) and finally rejected on the basis of molecular and morphological evidence, as species meeting these criteria have very different affinities with other members of the Polysiphonieae (e.g., *L. reptabunda* is in *Vertebrata* and *L. scopulorum* in *Bryocladia/Falkenbergiella*). The main character distinguishing *Lophosiphonia* sensu stricto from the Strebloclad ieae is that rhizoids are in open connection with the pericentral cells. It differs from other Polysiphonieae by having more than four pericentral cells (*Bryocladia* is an exception). Furthermore, the characters proposed by Falkenberg to delineate this genus are shared by all members of the clade, and are also present in other genera. *Lophosiphonia prostrata* is also resolved as sister to this clade but with moderate support and it differs from other *Lophosiphonia* species because it is always epiphytic on brown algae in the Zonarieae, with the apices curled over the host, growing synchronously with it, and is completely prostrate except for the branches bearing reproductive structures (Womersley 2003). We propose *Epizonaria* gen. nov. for this species. Our phylogenies reveal that Falkenberg's "*Lophosiphonia* group" (equivalent to Hommersand's tribe Lophosiphonieae nom. nud.) is not phylogenetically supported, as the type species of the genus *Lophosiphonia* is placed with high support in the Polysiphonieae. Among the genera included in the *Lophosiphonia* group by Falkenberg, *Ctenosiphonia* has been merged with *Vertebrata* (Díaz-Tapia et al. 2017b) and *Pleurostichidium* and *Ophidocladus* represent separate tribes (Hommersand 1963, this study). Finally, in addition to the three clades discussed above, three small unidentified

Polysiphonia-like species collected on Australian coral reefs and at Rottnest Island (Western Australia) were resolved as sisters to the *Bryocladia*/*Falkenbergiella* clade with low support. They are very similar in morphology to other small Polysiphonieae, as they have four pericentral cells and unicellular rhizoids in open connection to pericentral cells. They may constitute new genera, but further studies are required.

The Streblocladieae clade includes eight genera, as well as a number of clades and taxa for which generic assignment needs further investigation. The parasite *Aiolocolax pulchellus* was placed in this clade but with low support. Previously this species was considered incertae sedis, even at family level (Pocock 1956). Here, we propose the tribe Streblocladieae for this clade. This name was used before by Kylin (1956) (as *Streblocladia* "Gruppe") and by Hommersand (1963; Streblocladieae nom. nud.) but, as discussed above, we propose a different circumscription, defined by unicellular rhizoids cutoff from the mid-proximal ends of pericentral cells. Kylin's and Hommersand's concept was of a tribe containing species similar to *Polysiphonia* but with primary dorsiventrality, which is not supported in our phylogeny. The genus *Streblocladia*, including the type species *S. glomerulata*, is placed among radially branched species in phylogenetic analyses (Díaz-Tapia et al. 2017b; Fig. S2).

CONCLUSIONS

The phylogenies presented here are based on the most comprehensive molecular dataset analyzed to date for the family Rhodomelaceae, both in terms of number of genes (198 for the genome-scale phylogeny) and number of taxa (407 for the taxon-rich phylogeny). The relationships among the major clades of the family received very strong support in the genome-scale phylogeny including 44 species from 16 tribes (11 previously established and five proposed here), demonstrating the strength of chloroplast genome data to resolve challenging phylogenies in the red algae. Conversely, the taxon-rich phylogeny resolved the majority of branches with moderate to low support, suggesting that the chloroplast genomes of many more species are required to fully understand the phylogeny of the family. An integrative analysis of the two phylogenies and the morphological characters of the identified lineages have led us to thoroughly evaluate previous classification schemes and propose the first subdivision of the family Rhodomelaceae into tribes supported by molecular data.

The genome-scale and taxon-rich molecular phylogenies of the family Rhodomelaceae supported recognition of the 12 tribes previously proposed in Falkenberg's (1901) and Hommersand's (1963) classifications, as well as the tribe Sonderelleae (Phillips 2001). By contrast, the Lophosiphonieae and the division of the family into subfamilies proposed by Hommersand (1963) and Maggs and Hommersand (1993) were not supported. Our analysis, representing 89 genera of the Rhodomelaceae, corroborates the previously established circumscriptions of the tribes Sonderelleae, Polyzonieae, Heterocladieae, Bostrychieae, Amansieae, Rhodomeleae, Chondrieae (except *Cladurus*, as predicted by Womersley 2003), Laurencieae and Pleurostichidieae. A very different scenario emerged for the tribes Lophothalieae, Pterosiphonieae, Herposiphonieae and Polysiphonieae. Not surprisingly, these four tribes include most of the members of the family with terete ecorticate or slightly corticate filiform thalli (aside from the Bostrychieae and some Polyzonieae), the simplest morphological architecture in the Rhodomelaceae.

A tribe is resurrected (Alsidieae) and three new tribes are here proposed (Dipterosiphonieae, Thaumatelleae, and Streblocladieae) to accommodate genera previously placed in the Lophothalieae, Herposiphonieae, Polysiphonieae and Pterosiphonieae. Furthermore, several genera are transferred from the Polysiphonieae (and Streblocladieae) to the Pterosiphonieae and vice versa. Therefore, the morphological delineation of these tribes requires reassessment and we propose rhizoid anatomy as a key diagnostic character. Free rhizoids are the attachment structures of most Rhodomelaceae, while basal discs have evolved in the largest species. Although rhizoids are small structures, they are morphologically very variable, as previously described (e.g., Hollenberg 1967, Womersley 2003, Zuccarello and West 2006, Bustamante et al. 2017; Fig. 2). However, their relevance in delineating tribes was not previously highlighted (see McIvor 2000). Rhizoid anatomy is particularly useful in delineating the tribes Streblocladieae, Polysiphonieae, Herposiphonieae and Pterosiphonieae/Dipterosiphonieae (Table S5; Fig. 2), as some of their species are very similar in other morphological characters. Furthermore, the Thaumatelleae, Polyzonieae, and Bostrychieae also have distinctive rhizoids (Fig. 2), although there are many other key characters for delineating them at the tribal level.

The resurrected tribe Alsidieae is recognized as independent from the Polysiphonieae and is characterized by having corticate and radially branched indeterminate axes and plate-like spermatangial branches. The delineation of the tribe Lophothalieae is more problematic, as it is not monophyletic as originally conceived (*Brongniartella* was merged with *Vertebrata* and *Thaumatella* was segregated as a separate tribe). Furthermore, the relationships of *Micropeuce* and *Haplodasya* within the family are still unclear and further investigations, including more extensive taxon and gene sampling, are needed to clarify their tribal placement and determine the actual circumscription of the tribe Lophothalieae. Finally, the genus *Ophidocladus*, previously included

in the Lophosiphonieae nom. nud., was also allocated to its own tribe. In addition to the tribal level results emerging from this work, our phylogenies showed that an integrative review at the genus level is especially needed in the tribes Chondrieae, Amansieae and Strebloclad ieae.

The family Rhodomelaceae includes 48 species of parasites in 26 genera separated from non-parasitic species. However, the few previous investigations on parasites involving molecular data and our phylogenies (including 10 species and 9 genera) have all demonstrated that they are often closely related to non-parasitic species and their separation as independent genera is not always supported (Zuccarello et al. 2004, Kurihara et al. 2010, Preuss et al. 2017). The available molecular data suggest that species of *Janczewskia*, *Benzaitenia*, *Ululania*, *Dawsoniocolax* and *Bostrychiocolax* belong to genera with non-parasitic type species. We do not make nomenclatural proposals here, pending revisions of these genera. In contrast, our data support the recognition of *Leachiella*, *Harveyella, Choreocolax* and *Aiolocolax* as separate genera, but their phylogenetic relationships within the tribes are still not well resolved. These four genera previously lacked tribal attributions and our phylogenies revealed that the first three are in the Rhodomeleae, while *Aiolocolax* is in the Strebloclad ieae. In addition to the above-mentioned taxa, five parasitic genera are unclassified at a tribal level, one was placed in an independent tribe and 11 were included in five other tribes. Assigning parasitic genera to tribes based on morphological characters is not supported. For example, nine parasitic genera were included in the Lophothalieae mainly because they have tetrasporangia in whorls (Hommersand 1963), but several parasites with sporangia in whorls do not belong in the Lophothalieae (e.g., *Aiolocolax*, *Ululania*). The morphological characters of parasites, with very reduced vegetative structures, are markedly different from the non-parasitic members of their corresponding tribes and are always exceptions to the morphological delineations established for the tribes.

Besides the tribal classification of the Rhodomelaceae, Falkenberg (1901, p. 700) and Hommersand (1963, p. 343) reconstructed the phylogenetic relationships among tribes based on morphology. While the combination of the wide variety of morphological characters is reliable for delineating tribes, reconstructing their phylogenetic relationships on this basis it is much more difficult. Interpretations provided by Falkenberg (1901) and Hommersand (1963) agreed in recognizing the Bostrychieae on the basis of the phylogeny and considering the Laurencieae and the Chondrieae as closely related tribes, which were all supported in our molecular phylogeny (Fig. 1). Otherwise, their interpretations differed greatly and also are very different from our results (Fig. 1). For example, the Polyzonieae was considered related to the Rhodomeleae by Hommersand (1963), Falkenberg (1901) allied this tribe to the Herposiphonieae and our phylogeny resolved it as sister to the Sonderelleae and the Bostrychieae (Fig. 1). Differences between morphological and molecular phylogenies may result from the fact that characters classically used for establishing tribal relationships (e.g., dorsiventrality, pigmented trichoblasts, reproductive structures on specialized branches) evolved independently several times in the history of the family.

In addition to the 407 species and 89 genera represented in our phylogeny, there are currently 647 species and 60 genera in the family Rhodomelaceae for which molecular data are not available at present. Further investigations are needed to unravel their phylogenetic relationships and reassess their classification. Among them, some taxa, such as *Pachychaeta*, *Rhodolachne*, *Stichothamnion*, *Oligocladus*, and "*Lophosiphonia*" *mexicana*, have very unusual morphological characteristics (Weber-van Bosse 1911, Dawson 1944, Hommersand 1963, Vroman 1967, Womersley and Bailey 1970, Wynne 1970). This study provides the first global phylogenetic study of the family Rhodomelaceae, but much work remains, especially at lower taxonomic levels, to fully understand the systematics of the most diverse family of the red algae.

FORMAL TAXONOMY

Taxonomic proposals at tribe level.

Cladureae Díaz-Tapia & Maggs, trib. nov.

Diagnosis: Thalli erect, attached by a holdfast, radially branched, with a protruding apical cell surrounded by deciduous trichoblasts. Axes terete, with 5 (−6) pericentral cells, 1–3 layers of cortical cells developing close to the apices, but the segments remaining obvious throughout branches in surface view. Rhizoids cut off from pericentral cells, surrounding the axial and pericentral cells in older parts of the thallus. Spermatangial branches cylindrical, arising on branches of trichoblasts; cystocarps formed on axillary branchlets; one tetrasporangium per segment, cutoff from the pericentral cells, formed in stichidia arising in axils of lateral branches.

Type and only genus: *Cladurus* Falkenberg in Schmitz and Falkenberg 1897: 435.

Dipterosiphonieae Díaz-Tapia & Maggs, trib. nov.

Diagnosis: Thalli entirely or largely prostrate, formed by axes of indeterminate growth bearing branches of determinate growth in alternate pairs. Rhizoids cutoff from the distal ends of pericentral cells of prostrate axes, terminating in multicellular haptera. Axes with 4–10 pericentral cells, ecorticate. All branches exogenous. Trichoblasts, when present, only on determinate branches, deciduous. Spermatangial branches cylindrical, formed on modified trichoblasts; one tetrasporangium per segment in determinate branches. Cystocarps ovoid.

Type and only genus: *Dipterosiphonia* F.Schmitz and Falkenberg 1897: 463.

Ophidocladeae Díaz-Tapia & Maggs, trib. nov.

Diagnosis: Thalli dorsiventral, consisting of an extensive prostrate system bearing rhizoids ventrally and erect axes dorsally. Rhizoids cutoff from the middle or proximal ends of pericentral cells, terminating in multicellular discoid pads. Axes ecorticate; erect axes composed of a large axial cell and 16–28 pericentrals. All branches endogenous. Trichoblasts deciduous, alternately arranged. Spermatangial structures formed on branched trichoblasts, each covering the two basal dichotomies, with a quadrifurcate appearance; procarps formed on trichoblasts, with two sterile groups, cystocarps ovoid; two tetrasporangia per segment in lateral branches, with two cover cells.

Type and only genus: *Ophidocladus* Falkenberg in Schmitz and Falkenberg 1897: 461.

Streblocladieae Díaz-Tapia & Maggs, trib. nov.

Hommersand's (1963) proposal of the Streblocladieae was invalid because it lacked a formal description. Considering that we are proposing a very different concept for the tribe than that established by Hommersand (1963) and formerly by Kylin (1956; as *Streblocladia* "Gruppe"), here we propose a new tribe.

Diagnosis: Thalli predominantly erect, decumbent or dorsiventral (erect and prostrate axes). Axes with 4–24 pericentral cells, ecorticate or corticate. Rhizoids cutoff from mid-proximal ends of pericentral cells, normally unicellular (multicellular in *Lampisiphonia*), occasionally absent in largest species and in the obligate epiphyte *Vertebrata lanosa*. Trichoblasts deciduous and unpigmented when mature (except *V. byssoides* and *V. australis*). Spermatangial branches cylindrical, borne on modified trichoblasts or on one or two branches of trichoblasts; procarps formed on modified trichoblasts, with two sterile groups; one tetrasporangium per segment (two in *Leptosiphonia* and *Ctenosiphonia*) on main axes or lateral branches.

Type genus: Streblocladia F.Schmitz in Schmitz and Falkenberg 1897: 457–458.

Other genera of this tribe included in our molecular analysis: Aiolocolax M.A.Pocock 1956: 22, *Lampisiphonia* H.G.Choi, Díaz-Tapia & Bárbara in Bárbara et al. 2013: 138, *Leptosiphonia* Kylin 1956: 509, *Melanothamnus* Bornet & Falkenberg in Falkenberg 1901: 684, *Pterochondria* Hollenberg 1942: 532–533, *Polyostea* Ruprecht 1850: 231, *Tolypiocladia* F.Schmitz in Schmitz and Falkenberg 1897: 441–442, *Vertebrata* S.F.Gray 1821: 338.

Thaumatelleae Díaz-Tapia & Maggs, trib. nov.

Diagnosis: Thalli predominantly prostrate, radially branched, with 4 ecorticate pericentral cells. Rhizoids cutoff from pericentral cells, with a uniseriate multicellular filament terminating in multicellular haptera. Trichoblasts pigmented and persistent, branched 1–3 times. Spermatangial branches formed on trichoblasts, often several per trichoblast, ovoid, lacking basal and sterile apical cells; cystocarps stongly urceolate; one tetrasporangium per segment on lateral branches bearing trichoblasts.

Type and only genus: Thaumatella (Falkenberg) Kylin 1956: 511.

Amended descriptions of tribes.

Alsidieae Ardissone 1883: 352.

Diagnosis: Thalli erect, attached by a holdfast or a basal crust, consisting of axes of indeterminate growth, radially branched, and clothed in some species with branches of determinate growth. Trichoblasts, if present, deciduous. Axes terete or complanate, with 5–12 pericentral cells, corticated from close to the apices with 1–2 layers of cortical cells. Spermatangial branches plate-like, lacking sterile marginal cells; one tetrasporangium per segment. Cystocarps globose.

Type: *Alsidium* C.Agardh 1827: 639.

Other genera of this tribe included in our molecular analysis: *Digenea* C.Agardh 1822: 388–389, *Bryothamnion* Kützing 1843: 433.

Nomenclatural note: although J.Agardh (1863) provided a diagnosis for the tribe Alsideae, he included this "tribus" and other tribes as sections of the Ordo Rhodomeleae so it is not valid under ICBN Art. 37.6–8 which states that names of taxa with misplaced rank are invalid. Therefore, the first valid publication of the tribe Alsideae was by Ardissone (1883).

Herposiphonieae F.Schmitz and Falkenberg 1897: 457.

Description: Thalli formed by axes of indeterminate growth, prostrate or partially erect, which bear axes of determinate growth that are simple and erect. Rhizoids cutoff from the distal ends of pericentral cells of prostrate axes, terminating in multicellular haptera that consist of the extension of the rhizoidal filament into a digitate structure that divides to form small terminal cells. Axes with 6–16 pericentral cells, ecorticate. All branches exogenous, formed on consecutive segments in a pattern that consists of one branch of indeterminate growth followed by three determinate branches. Some species have naked segments and more determinate branches separate indeterminate axes. Trichoblasts only on determinate branches, deciduous and unpigmented when mature. Spermatangial branches cylindrical, formed on modified trichoblasts; cystocarps terminal or subterminal on determinate branches; one tetrasporangium per segment on determinate branches.

Genus of this tribe included in our molecular analysis: *Herposiphonia* Nägeli 1846: 238.

Polysiphonieae F.Schmitz 1889: 447.

Description: Thalli predominantly erect, decumbent or dorsiventral (erect and prostrate axes). Axes with 4 (−7 to 11) pericentral cells, ecorticate. Rhizoids in open connection with pericentral cells, unicellular. Trichoblasts, when present, deciduous and unpigmented at maturity. Spermatangial branches cylindrical, formed on modified trichoblasts or on

one or two branches of trichoblasts; procarps formed on modified trichoblasts, with two sterile groups; one tetrasporangium per segment on main axes or lateral branches with two or three cover cells. Cystocarps globose or ovoid.

Genera of this tribe included in our molecular analysis: *Bryocladia* F.Schmitz in Schmitz and Falkenberg 1897: 442, *Epizonaria* Díaz-Tapia & Maggs gen. nov., *Lophosiphonia* Falkenberg in Schmitz and Falkenberg 1897: 459–460, *Polysiphonia* Greville 1823: 210.

Pterosiphonieae Falkenberg 1901: 261.

Description: Thalli ranging from largely prostrate to erect, bilaterally or radially branched, usually with erect axes of determinate growth bearing determinate laterals that remain completely free, or are congenitally fused with the main axes to different degrees, forming foliose thalli in genera with branches fused along the whole length with the main axes. Attachment by holdfasts in the largest species or by rhizoids cut-off from the distal ends of pericentral cells of prostrate axes (in some genera also from proximal ends in adjoining pericentral cells), terminating in multicellular haptera formed by cell divisions at the end of the rhizoidal filament. Axes with 4–14 pericentral cells, ecorticate to heavily corticate. Trichoblasts varying from rare and formed only on reproductive branches, to common in determinate branches, deciduous. Spermatangial branches cylindrical, formed on modified trichoblasts; one tetrasporangium per segment on determinate branches, with two pre-sporangial and one post-sporangial cover cells.

Genera of this tribe included in our molecular analysis: *Amplisiphonia* Hollenberg 1939: 380, *Aphanocladia* Falkenberg in Schmitz and Falkenberg 1897: 444, *Dictyomenia* Greville 1830: l, *Echinothamnion* Kylin 1956: 506, *Gredgaria* Womersley 2003: 314–315, *Lophurella* Schmitz in Schmitz and Falkenberg 1897: 440–441, *Periphykon* Weber-van Bosse 1929: 255, *Pollexfenia* Harvey 1844: 431, *Pterosiphonia* Falkenberg in Schmitz and Falkenberg 1897: 443, *Rhodomelopsis* M.A.Pocock 1953: 34, *Symphyocladia* Falkenberg in Schmitz and Falkenberg 1897: 443–444, *Womersleyella* Hollenberg 1967: 213, *Xiphosiphonia* Savoie and Saunders 2016: 933.

Taxonomic proposals at genus level.

Epizonaria Díaz-Tapia & Maggs, gen. nov.

Diagnosis: Vegetative thalli entirely prostrate, attached by unicellular rhizoids in open connection with the pericentral cells. Axes with four pericentral cells, ecorticate. Reproductive structures on short erect axes. Trichoblasts, if present, on erect branches, deciduous. Spermatangial branches on modified trichoblasts; cystocarps terminal on erect branches, ovoid to slightly urceolate; one tetrasporangium per segment.

Type species: Epizonaria prostrata (Harvey) Díaz-Tapia & Maggs, comb. nov.

Basionym: Polysiphonia prostrata Harvey 1855, p. 540: Some account of the marine botany of the colony of western Australia. *Trans. R. Ir. Acad.* 22:525–66.

Synonyms: Lophosiphonia prostrata (Harvey) Falkenberg; *Falkenbergiella prostrata* (Harvey) Kylin.

Etymology: From the Greek prefix epi (on) and the genus name *Zonaria*, as the type species of the genus is epiphytic on members of the Zonarieae.

Amended descriptions of genera.

Lophosiphonia Falkenberg in Schmitz and Falkenberg 1897: 459–460.

Description: Thalli consisting of prostrate and erect axes, endogenously branched. Axes ecorticate, with 6–7 pericentral cells. Rhizoids in open connection with pericentral cells, unicellular. Trichoblasts deciduous when present. Spermatangial branches cylindrical, formed on modified trichoblasts; cystocarps ovoid; one tetrasporangium per segment.

Type species: Lophosiphonia obscura (C.Agardh) Falkenberg in Schmitz and Falkenberg 1897: 460.

Species of this genus included in our molecular analysis: *L. simplicissima* Díaz-Tapia in Díaz-Tapia and Bárbara 2013: 356, *Lophosiphonia teges* (Womersley) Díaz-Tapia & Maggs, comb. nov.

Taxonomic proposals at species level.

Digenea cymatophila (R.E.Norris) Díaz-Tapia & Maggs, comb. nov.

Basionym: Alsidium cymatophilum R.E.Norris 1994, p. 434: Some cumophytic Rhodomelaceae (Rhodophyta) occurring in Hawaiian surf. *Phycologia* 33:434–43.

Lophosiphonia teges (Womersley) Díaz-Tapia & Maggs, comb. nov.

Basionym: Polysiphonia teges Womersley 1979: 494, Southern Australian species of *Polysiphonia* Greville (Rhodophyta). *Aust. J. Bot.* 27:459–528.

PDT acknowledges support by the postdoctoral programmes Axudas de apoio á etapa inicial de formación posdoutoral do Plan I2C (Xunta de Galicia). This work was supported by grants from the Australian Research Council (FT110100585), the Australian Biological Resources Study (RFL213-08) and also benefited from funding provided by the University of Melbourne (UoM-FAPESP SPRINT grant), the Holsworth Wildlife Research Endowment and the Bush Blitz program (Australian Biological Resources Study). This research was supported by use of the computational facilities of Melbourne Bioinformatics (project UOM0007) and the Nectar Research Cloud, a collaborative Australian research platform supported by the National Collaborative Research Infrastructure Strategy (NCRIS). We warmly thank the members of the Verbruggen lab (J. Costa, V. Marcelino, M. Brookes, C. Cremen and C. Jackson) for providing samples and assistance during field and lab work. We are grateful to J. Huisman, M. Terradas, F. Rindi, M. Toyota Fujii, V. Cassano, N. Robinson, R. Carballeira for supplying materials and Y. Metti for sharing some sequences. We thank M.D. Guiry for advice on nomenclatural issues.

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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:

Figure S1. Phylogeny of the family Rhodomelaceae indicating tribes with light or dark shaded areas, the unshaded area corresponds to the outgroup. Resurrected (Alsidieae) and new tribes are indicated with bold font. RAxML tree based on protein alignment of the 198 concatenated genes from the chloroplast genome. All branches have full bootstrap support (*), except those where bootstrap values are indicated on branches.

Figure S2. Phylogeny of the family Rhodomelaceae. The RAxML tree used the genome-scale phylogeny based on nucleotides as a constraint and incorporated 322 *rbc*L, 179 18S rRNA gene and 194 *cox*1 sequences for a total of 418 species. Bootstrap values are indicated on branches 100 (*) or ≥50%. Species names in bold correspond to type species of genera. Note: *Dasyclonium incisum, Leveillea jungermannioides, Dipterosiphonia dendritica* and *Herposiphonia tenella* are the type species of their corresponding genera, but considering the cryptic diversity found in these species, we are unable to determine at present which of them, if any, should be considered as the type.

Table S1. Alphabetical list of genera currently recognized in the Rhodomelaceae indicating their tribal placement in Falkenberg's (1901) and Hommersand's (1963) classifications and the tribal assignment of genera described after 1963, as well as the positions resulting from this work.

Table S2. Generic composition of the rhodomelacean tribes in Falkenberg's (1901) and Hommersand's (1963) classifications. The tribal placement of genera described after 1963 is also indicated, as well as the generic composition of tribes resulting from this work. N.d. = no data; d.p. = different position.

Table S3. GenBank accession numbers of the chloroplast genomes included in the phylogenetic analysis.

Table S4. GenBank accession numbers of the sequences included in the phylogenetic analysis. Numbers printed in bold correspond to newly determined sequences.

Table S5. Key morphological characters delineating the tribes of the Rhodomelaceae.