Morphological evolution and classification of the red algal order Ceramiales inferred using plastid phylogenomics

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ABSTRACT

The order Ceramiales contains about one third of red algal diversity and it was classically classified into four families according to morphology. The first phylogenies based on one or two molecular markers were poorly supported and failed to resolve these families as monophyletic. Nine families are currently recognized, but relationships within and among them are poorly understood. We produced a well-resolved phylogeny for the Ceramiales using plastid genomes for 80 (28 newly sequenced) representative species of the major lineages. Three of the previously recognized families were resolved as independent monophyletic lineages: Ceramiaceae, Wrangeliaceae and Rhodomelaceae. By contrast, our results indicated that the other six families require reclassification. We propose the new order Inkyuleeales, a new circumscription of the Callithamniaceae to include the Spyridiaceae, and a new concept of the Delesseriaceae that includes the Sarcomeniaceae and the Dasyaceae. We also investigated the evolution of the thallus structure, which has been important in the classical delineation of families. The ancestor of the Ceramiales was a monosiphonous filament that evolved into more complex morphologies several times independently during the evolutionary history of this hyperdiverse lineage.

Keywords: Evolution; Morphology; New order; New subfamily; Phylogenomics; Rhodophyta

1. Introduction

The order Ceramiales, with 2,689 species currently recognized, accounts for more than a third of red algal species diversity (Guiry and Guiry, 2019). The Ceramiales is morphologically defined by details of its anatomy (the uniaxial thallus structure, the presence of periaxial cells and the post-fertilization formation of the auxiliary cell directly from the supporting cell) (Maggs and Hommersand, 1993; Womersley, 1998). Nägeli (1847) first named the order Ceramiales, and the earliest family level classification into Ceramiaceae, Delesseriaceae and Rhodomelaceae was proposed by Oltmanns (1904). Subsequently, the family Dasyaceae was segregated from the Rhodomelaceae (Rosenberg, 1933). These families were defined based on a combination of vegetative and reproductive characters (Kylin, 1956; Womersley, 1998; 2003; Hommersand, 1963; Maggs and Hommersand, 1993). The Ceramiaceae form monosiphonous filaments (Fig. 1A, see the Glossary in supplementary data 1) and, when present in vegetative structures, the periaxial cells only cover parts of the axial cells. The Rhodomelaceae has a polysiphonous structure (the periaxial cells cover the full length of the axial cells) with axes developing monopodially (Fig. 1B-C, see Fig. S1A and the Glossary in supplementary data 1). The Dasyaceae also has a polysiphonous structure, but axes develop sympodially (Fig. 1H-I, see Fig. S1B and the Glossary in supplementary data 1) and bear unusual pigmented monosiphonous branches. The Delesseriaceae includes blade-like species that consist of polysiphonous axes in which the lateral periaxial cells divide to form the blade (Fig. 1L-M). These basic structures define the respective families, but in some representatives have evolved into networks of filaments (Fig. 1F), corticate terete (Fig. 1H) or blades (Fig. 1J-K), and parasites (Fig. 1G). In general, members of these families are easily recognized based on the thallus structure. However, some species or groups of species exhibit characters interpreted as “intermediate” between several families. For example, *Halydictyon* has been considered to be related to the Delesseriaceae, Dasyaceae and the Rhodomelaceae (Womersley, 2003, as *incertae sedis*), *Sonderella* has been included in the Rhodomelaceae and the Delesseriaceae (Womersley, 1965) and the “*Sarcomenia* group” has morphological similarities with the Rhodomelaceae and Delesseriaceae and was finally segregated into the new family Sarcomeniaceae (Womersley and Shepley, 1959; Womersley, 2003).

The classification of the Ceramiales into five families based on morphology was maintained until the first molecular phylogenies including members of several families were constructed based on 18S rRNA gene sequences (Choi et al., 2002, 2008). These phylogenies resolved some lineages with high support, but they did not correspond with the previously established families. The Rhodomelaceae was the only taxon unequivocally resolved as monophyletic in these phylogenies. By contrast, other families were split into several clades and relationships among them remained unresolved. Based on these poorly supported phylogenies for the Ceramiales, four families were segregated for the Ceramiaceae (Callithamniaceae, Wrangeliaceae, Spyridiaceae and Inkyuleeaceae) (Choi et al., 2008). The Dasyaceae was divided into two subfamilies: the Dasyoideae and the Heterosiphonioideae (Choi et al., 2002). Likewise, in the Delesseriaceae, based on LSU and *rbc*L phylogenies, three subfamilies were recognized: Phycodryoideae, Nitophylloideae and Delesserioideae (Lin et al., 2001). Of these, only the Phycodryoideae was fully supported, while the other two subfamilies were not resolved as monophyletic in all analyses. These studies clearly indicate that better resolved phylogenies are needed to improve our understanding of phylogenetic relationships among the major lineages of Ceramiales and arrive at a stable classification.

The Ceramiales includes a high diversity of morphological structures that makes it a good candidate model for evolutionary studies. The structure of the female apparatus and post-fertilization development have been classically used in the Ceramiales and other red algae as key characters to infer relationships and establish a classification (Falkenberg, 1901; Rosenberg, 1933; Hommersand, 1963; Kylin, 1956; Díaz-Tapia and Bárbara, 2011). Their uniformity within major groups and the variability among them has been confirmed in phylogenetic analyses (Saunders and Hommersand, 2004; Choi et al., 2008; Yang et al., 2016). By contrast, the evolutionary patterns of vegetative structures in the Ceramiales have barely been discussed. Hommersand (1963) proposed that the ancestor of the order was a monosiphonous filamentous species that gave rise to two major monosiphonous lineages differing in branching pattern and the female structures. According to Hommersand (1963), eight lineages evolved independently from one of these two lineages: their morphologies maintained this basic structure or evolved into more complex types of thalli.

High throughput sequencing (HTS) techniques allow us to sequence complete organellar genomes and their potential usefulness in resolving challenging phylogenies has been demonstrated in several groups of animals and plants (e.g. Kocot et al., 2018; Sun et al., 2018). These tools have also successfully been applied in the resolution of difficult phylogenies in the red algae (Costa et al., 2016; Díaz-Tapia et al., 2017; Iha et al. 2018; Saunders et al., 2018; Pasella et al. 2019). Therefore, phylogenomic approaches could contribute to the construction of a well-supported phylogeny for the Ceramiales, providing a basis for reassessing its classification. The objective of this paper is to produce a molecular phylogeny for the order Ceramiales based on plastid genome data for representative taxa of the main clades of the order and suitable outgroups. We aim to reassess the classification of the Ceramiales based on joint interpretation of this phylogeny and morphological characters relevant to the delineation of families. Furthermore, we aim to understand the evolution of thallus structure, on which the current classification of the Ceramiales has been established.

2. Material and methods

2.1. Taxon sampling

To identify the main lineages of the order Ceramiales, we considered previously published phylogenies, as well as the most recent proposed classifications based on molecular data (Lin et al., 2001; Choi et al., 2002, 2008; Díaz-Tapia et al., 2017; Table S1). An additional classification was published recently (Athanasiadis, 2016), but it does not include molecular phylogenetic analyses and several proposals are questionable because they are in stark contrast with what we know from molecular work. We selected one to four species of each major lineage for phylogenomic analyses. Plastid genomes are available in GenBank for 64 species of the Ceramiales, mostly in the family Rhodomelaceae (Díaz-Tapia et al., 2017; Pasella et al., 2019; Salomaki and Lane, 2019). In order to balance the number of taxa for the Ceramiales lineages, we excluded from our analyses 12 of the previously published genomes (ten Rhodomelaceae and two *Membranoptera* species) so that major lineages were represented by one to four species. Furthermore, we selected 28 additional species of the order Ceramiales to be newly analysed using high-throughput sequencing. Our taxon selection includes representative species of the type genera for all the families or subfamilies, with the exception of the subfamilies Phycodryoideae and Heterosiphonieae and the family Sarcomeniaceae. However, representative species included in our analyses for the Phycodryoideae and Heterosiphonieae were unequivocally placed in the same lineages as their respective type genera (Lin et al., 2001; Choi et al., 2002, 2008). For the Sarcomeniaceae we included two genera in our taxon selection whose relationship with *Sarcomenia* has not been studied using molecular tools. However, we constructed an *rbc*L tree (not shown) including *Sarcomenia*, *Platysiphonia* and other related species according to our results and they formed a highly supported clade (98%). Our outgroup selection included 12 species representative of the red algal orders most closely related to the Ceramiales (Verbruggen et al. 2010; Yang et al. 2016). Plastid genomes of three of them were newly determined here.

2.2. Data collection

Total DNA was isolated with an adapted cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987) as described in Cremen et al. (2016). Barcoded sequencing libraries (350 nt) of the 28 DNA extracts were prepared with the TruSeq DNA HT Sample preparation Kit (Illumina, USA). Because the Verbruggen lab carries out organellar genome projects for 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; Díaz-Tapia et al., 2017). Libraries were sequenced on Illumina Hiseq X platform, generating 150 nt paired-end reads. 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 S2.

2.3. Sequence alignment and phylogenetic analyses

We assembled a dataset consisting of 92 plastid genomes: the 28 newly sequenced for the order Ceramiales, 52 previously published for the order Ceramiales, and 12 genomes for other Rhodymeniophycidae to be used as outgroups (Table S2).

In total, 208 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. 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 WAG+Γ models for the nucleotide and amino-acid alignments, respectively, using 100 traditional bootstrap replicates (Felsenstein, 1985). Further analyses were carried out to assess the sensitivity of these analyses to model choice (LG, CPREV).

2.4. Analysis of trait evolution

Species were classified into seven morphological groups that represent the thallus structure in the order Ceramiales. These groups are 1) monosiphonous filaments (filaments formed by a single cell row, Fig. 1A), 2) polysiphonous filaments (filaments formed by an axial cell row surrounded by periaxial cells that cover partially or completely the axial cells, with periaxial cells producing a row of flanking cells in some species, Fig. 1B-E), 3) corticate terete (corticate polysiphonous filaments, in which the cortication covers more than 70% of the thallus and medullary and cortical layers are distinguishable in cross sections, Fig. 1H-I), 4) membranous blades (blades formed by a layer of cells, Fig. 1L-M), 5) blades formed by a network of filaments (blade-like species composed of axial filaments with monosiphonous branches that fuse to form a network, Fig. 1F), 6) thick leathery (corticate blades, Fig. 1J-K), and 7) parasites (Fig. 1G).

Trait evolution analyses were carried out in R 3.3.2 (R Core Team, 2013), using the geiger and phytools packages (Harmon et al., 2008; Revell, 2011). Equal rates (ER), symmetrical, all rates different and a user-defined model were fitted and compared with the AICc criterion. The user-defined model is a variant of the equal rates model in which some biologically implausible transitions are disallowed, i.e. transitions from parasite to non-parasite, from blade and leathery to monosiphonous, and from monosiphonous directly to blade and leathery. Ancestral state estimation was carried out with the make.simmap function in phytools using 100 simulations, and the ER and user-defined model.

3. Results

3.1. Phylogenetic relationships and classification

Among the newly assembled genomes, seven were complete, eight could not be confirmed as circular but we recovered all or nearly all the genes for them, and 13 were partial (see Table S2). Moreover, we determined three complete genomes of other Rhodymeniophycidae to be used as outgroups (Table S2). Our analyses also included 52 plastid genomes of the Ceramiales and nine of other Rhodymeniophycidae that were downloaded from GenBank (Table S2). A concatenated alignment of 92 species and 208 genes, amounting to 160,464 nucleotides or 53,488 amino acid positions, was analysed.

Plastid phylogenomics resolved the relationships among the major lineages of the Ceramiales with full support for the vast majority of branches (Fig. 2). The topology was robust to analysing the data as nucleotides or amino acids (Fig. 2 *vs.* S3). Only five branches received bootstrap support lower than 85.

The core of the Ceramiales was resolved in a monophyletic group that received full support. *Inkyuleea* is the only taxon currently assigned to the Ceramiales that was not placed within this clade. Conversely, it was resolved with high support as sister to *Schimmelmannia* of the order Acrosymphytales. *Inkyuleea* includes three species and is the only genus of the family Inkyuleeaceae.

The core of the Ceramiales contains five major clades. The families Ceramiaceae, Wrangeliaceae and Rhodomelaceae were monophyletic and received full support. The Callithamniaceae, including *Spyridia* (at present in the Spyridiaceae), formed a clade with very high statistical support (97). The families Dasyaceae and Delesseriaceae as currently delineated were not resolved as monophyletic. The clade indicated as Delesseriaceae in our phylogeny was fully supported and was composed of species previously assigned to the families Sarcomeniaceae, Dasyaceae and Delesseriaceae. Within this clade, six lineages are distinguished, of which five correspond to the subfamilies of the Delesseriaceae (Delesserioideae, Phycodryoideae and Nitophylloideae) and Dasyaceae (Dasyoideae and Heterosiphonoideae). The sixth lineage is proposed as the new subfamily Sarcomenioideae and includes *Malaconema* and *Platysiphonia* (former Sarcomeniaceae), as well as *Apoglossum*, *Caloglossa*, *Taenioma* (formerly in the Delesserioideae) and *Halydictyon* (formerly *incertae sedis*).

3.2. Evolution of the thallus structure

The trait evolution analyses indicated that the less parameter-rich models equal rates (ER) and custom models outperformed the more parameter-rich symmetric (SYM) and all rates different (ARD) models based on the uncorrected and corrected Akaike Information Criteria (AIC and AICc, Table 1). The ancestral character estimation indicated that the ancestor of the Ceramiales was a monosiphonous filamentous species (Figs 3 and S4) and this condition is present in the earliest diverged lineages of the order. Polysiphonous filaments, corticated terete, blade-like and leathery thalli, and networks of filaments evolved in independent lineages several times in the evolution of the Ceramiales. Only the plastid genome of one parasitic species was available at the time of writing, but parasites are known in multiple lineages of the family and this condition also evolved multiple times independently in the Ceramiales. The ancestral state estimation using the custom rate matrix (Fig. 3) and equal rates (Fig. S4) models produced similar results. The main difference between them is that the common ancestor of the Rhodomelaceae and Delesseriaceae was resolved, respectively, as polysiphonous or with high likelihoods of being blade-like.

4. Discussion

Our analyses of plastid genomes provide the first well-resolved phylogeny for the order Ceramiales. This is an important step forward in understanding the evolution and classification of this order that accounts for one third of red algal diversity (Guiry and Guiry, 2019). It contrasts with previous phylogenies based on one or a few molecular markers that failed to resolve relationships among most lineages (Lin et al., 2001; Choi et al., 2002, 2008). Our phylogeny is used to reassess family level classification and to analyse the evolution of thallus structure in this order.

4.1. Phylogenetic relationships and classification

Inkyuleea was resolved in our phylogeny as sister to *Schimmelmannia* (order Acrosymphytales). Accordingly, we propose the erection of the new order Inkyuleeales.

Its segregation from the Ceramiales has been suggested before but was not formally proposed due to uncertainties about its phylogenetic relationships with other red algae (Choi et al., 2008). The segregation of this new order is also supported by its morphological characters. The thallus structure of *Inkyuleea* resembles the Ceramiales, but the post-fertilization development of gonimoblasts includes the formation of an auxiliary branch on the supporting cell (Wollaston, 1974). This character resembles the auxiliary branches formed in the Acrosymphytales (Saunders et al., 2016) and differs from the single auxiliary cell produced in the Ceramiales (Wollaston, 1974). Other vegetative and reproductive characters that differ between the Inkyuleeales and Acrosymphytales include isomorphic *vs.* heteromorphic life histories, with crustose tetrasporophytes in the latter (Withall and Saunders, 2007; Saunders et al., 2016).

Earlier molecular phylogenies of the Ceramiaceae led to its division into four families (plus the Inkyuleeaceae, discussed above) (Choi et al., 2002, 2008). However, our phylogeny resolved them in three independent clades corresponding to the Ceramiaceae, Wrangeliaceae and Spyridiaceae/Callithamniaceae. The last two families were unequivocally resolved as sister, leading to our proposal for their merger into a single family, the Callithamniaceae having priority. The Spyridiaceae contains a single free-living genus with 15 species, characterized by distinctive vegetative and reproductive structures, and its placement in the Ceramiales has even been questioned (Choi et al., 2008). However, Hommersand (1963) confirmed its relationship with the tribes Crouanieae and Callithamnieae (both in the Callithamniaceae) based on several morphological features, including a synapomorphy: the complete absence of sterile cells in the procarps of the female apparatus. The Ceramiaceae and Wrangeliaceae were supported in our phylogeny, which resolved all the available representatives of these families in two independent clades. These two families were delineated based on a combination of vegetative and reproductive characters by Choi et al. (2008) with a special emphasis on the position and structure of the procarps. Relationships among genera, and implicitly among tribes, within each family differed considerably with respect to previous phylogenies (Choi et al., 2008). For example, *Wrangelia* (tribe Wrangelieae) was resolved in our tree as sister to *Plumaria* (tribe Ptiloteae), while it was placed as sister to the Griffithsieae in previous phylogenies (Choi et al., 2008). Our phylogeny shows that the tribe Griffithsieae needs reassessment. *Bornetia* is currently included in that tribe (Athanasiadis, 1996), but it forms a clade with members of the Spermothamnieae (*Spermothamnion, Ptilothamnion*) and Sphondylothamnieae (*Sphondylothamnion*); the Griffithsieae (represented by *Anotrichium* in our tree) is sister to the clade formed by the Compsothamnieae (*Compsothamnion*) and the Spongoclonieae (*Pleonosporium*). The Wrangeliaceae, Ceramiaceae and Callithamniaceae include 926 species in 141 genera and 29 tribes. Their classification is mainly based on morphological characters, while phylogenetic relationships have been scarcely studied.

Our phylogenomic analyses show that the Dasyaceae and Delesseriaceae as previously circumscribed are paraphyletic and require re-classification, confirming previous indications based on single marker phylogenies (Choi et al., 2002). Members of these two families together with the Sarcomeniaceae are placed in our phylogeny in a fully supported clade. The classification adopted after the publication of the first molecular phylogenetic analyses subdivided the Delesseriaceae and Dasyaceae into five subfamilies (Lin et al., 2001; Choi et al., 2002). Four of these subfamilies were supported in our phylogeny and they were morphologically characterized in previous works (Lin et al., 2001; Choi et al., 2002; Wynne, 2013). However, some genera currently included in the Delesserioideae (*Caloglossa*, *Taenioma*, *Apoglossum*) were resolved in a lineage not closely related to the type genus of the subfamily in a clade with species of the Sarcomeniaceae and *Halydictyon* (*incertae sedis*, Womersley, 2003). This result is unsurprising considering that the alliance between these genera and the Delesserioideae was previously unsupported (Lin et al., 2001; Choi et al., 2002). Therefore, our results demonstrate the necessity of taxonomic revision and we propose to merge the three families, the Delesseriaceae having priority. The current subfamilial classification is maintained and the Sarcomenioideae is recognized as a subfamily that also includes the genera *Caloglossa*, *Taenioma*, *Apoglossum* and *Halydictyon*. We also propose the new tribe Halydictyeae for the genus *Halydictyon*, which differs from other Ceramiales by unusual morphological characters (Womersley, 2003).

The Delesseriaceae and Rhodomelaceae were resolved as sister families in our phylogeny, an alliance that had been previously suggested based on their morphological similarities (Rosenberg, 1933; Hommersand, 1963). They share a polysiphonous structure that in some lineages evolved into a foliose thallus, have a uniform procarp structure, and the gonimoblast is surrounded by a pericarp. In general, the majority of representatives from both families are easily distinguishable by their thallus structure. However, both are highly diverse groups (ca. 500 and 1000 species, respectively) and several of the characters that are typical for one of the families are not present in all the members or are also present in a few members of the other family. For example, trichoblasts (unpigmented monosiphonous apical branches) are exclusive to the Rhodomelaceae, but some species lack them. The periaxial cells are formed by circular or alternate divisions in the Rhodomelaceae and Delesseriaceae, respectively, but alternate divisions are also present in some Delesseriaceae (some Sarcomenioideae). The high morphological diversity within each family makes it difficult to establish a universal delineation. Still, there are synapomorphies in the Delesseriaceae as here redefined and the Rhodomelaceae related to the development of the carposporophyte: 1) the auxiliary cell produces a single gonimoblast initial in the Rhodomelaceae, while it divides transversely forming several gonimoblast initials in the Delesseriaceae; and 2) the gonimoblast is monopodially branched in the Delesseriaceae, while in the Rhodomelaceae it branches sympodially (Rosenberg, 1933; Hommersand, 1963).

4.2. Selection of taxonomic ranks

Alternatives to our proposed classification of the Ceramiales and the Delesseriaceae clade are possible. One of them would consist of raising the five subfamilies of the former Delesseriaceae and Dasyaceae to the family level, maintaining the Sarcomeniaceae. A second alternative would be raising the five families we are recognizing to ordinal level, reorganizing the lower taxonomic levels (subfamilies and tribes). Most current classifications combine classical phenotypic observations with phylogenies to establish taxonomic levels (Avise and Johns, 1999). Accordingly, we delineated the family Delesseriaceae based on its forming a neatly defined clade that can be characterized by synapomorphic morphological characters. Taxonomic ranks are arbitrary and, when taxa are considered individually, the attributed rank does not alter the interpretation of the information they provide about characters and systematic relationships (Avise and Johns, 1999; Giribet et al., 2016; Kraichak et al., 2017). However, this can be a source of bias when establishing comparisons among distant taxa or when using taxonomic surrogacy, i.e. using counts of families or genera to measure biodiversity (Kraichak et al., 2017; Sigwart et al., 2017).

Our proposed classification for the Ceramiales includes families that are in turn subdivided into tribes, and moreover the Delesseriaceae has an intermediate taxonomic rank, subfamilies (Table S1), which might be actually equivalent to the tribes in other families. In the Delesseriaceae, both taxonomic ranks are needed, as they represent well-defined morphological groups and, when studied, they were supported in phylogenies (Lin et al., 2001; Choi et al., 2002; Wynne, 2013). The necessity for standardization of taxonomic ranks used in biological classification among organisms has been hotly debated (e.g. Avise and Johns, 1999; Giribet et al., 2016; Kraichak et al. 2017). Temporal banding, i.e. the use of a standardized time of divergence for the assignment of taxonomic ranks, has been proposed as a universal system (Hennig, 1966; Avise and Johns, 1999). However, this method requires time-calibrated phylogenies, which are rare in the red algae probably because the fossil record is scarce and phylogenies are commonly difficult to resolve using few molecular markers. Further work is needed to evaluate whether the taxonomic ranking for the Ceramiales includes unequal ranks considering the divergence time of taxa.

4.3. Thallus structure evolution

The ancestral state estimation suggests that the ancestor of the Ceramiales was a monosiphonous filamentous species, the simplest morphological architecture of the order. This agrees with previous interpretations based on morphology that proposed that the order derives from a primitive Ceramiaceae (Hommersand, 1963). The monosiphonous condition persists in the earliest diverged lineages of the Ceramiales (Ceramiaceae, Callithamniaceae and Wrangeliaceae). Other more complex thallus structures that characterize the extant Ceramiales displayed a complex evolutionary pattern. Polysiphonous filaments are thought to have evolved through a reduction of whorled branches to single cells (Hommersand, 1963). According to our inferred ancestral states, this happened independently in three clades of the Ceramiales (some Ceramiaceae, some Callithamniaceae, and Rhodomelaceae/Delesseriaceae).

Blade-like thalli evolved independently in the Rhodomelaceae and the Delesseriaceae. Developmentally, these thalli are thought to have evolved from polysiphonous filaments by the division of the lateral periaxial cells, leading to the formation of wings, or by the fusion of lateral branches (Womersley, 2003; Wynne, 2013). The estimation of the ancestor of the Rhodomelaceae and Delesseriaceae differed between the analyses using custom rate matrix and equal rates models (polysiphonous or with high likelihoods of being blade-like respectively). Considering that a direct transition from monosiphonous to blade-like seems developmentally unlikely in the Ceramiales, we are of the opinion that the ancestral states inferred from the custom model are more realistic.

Blade-like thalli were inferred to have been ancestral to two lineages of Delesseriaceae that form networks of filaments by cell adhesion between branches. Corticate terete and leathery thalli are thought to have evolved by the development of a cortex covering the cylindrical or blade-like thalli in several lineages of the Wrangeliaceae, Delesseriaceae and Rhodomelaceae (Womersley, 1998, 2003; Wynne, 2002, 2013), a hypothesis that is supported by our inferred ancestral states. Furthermore, a secondary reduction of the wings of blades occurred twice in the Delesseriaceae. It led to the formation of polysiphonous filaments in which a flanking row of cells still persists in all or part of the thallus (*Taenioma* and *Platysiphonia/Malaconema*) (Womersley, 2003, Wynne, 2013). Parasites have extremely reduced thalli and have evolved independently in multiple lineages of the Ceramiales (Preuss et al., 2017; Preuss and Zuccarello, 2019; Salomaki and Lane, 2019).

The evolution of thallus morphology from simple filaments to more complex structures implies a decrease in the thallus surface to volume ratio. High surface to volume ratios are correlated in extant seaweeds with rapid nutrient uptake and growth rates, which are higher in filamentous and blade-like species than in corticated terete and leathery species (Littler and Littler, 1980; Hurd et al., 2014). Therefore, the morphological diversification of thallus structures may have allowed the Ceramiales to also diversify into different ecological niches. Evolutionary drivers of morphological change in seaweeds are poorly understood, but it has been hypothesized that grazing pressure and CO_2 availability could have been involved in the morphological diversification during the Ediacaran (541-635 My) (Xiao and Dong, 2006). Similar drivers might be the underlying cause of the evolution of the Ceramiales, but a better understanding of environmental controls over development as well as more detailed insights into the timing of Ceramiales diversification will be needed to evaluate this hypothesis in more detail.

5. Conclusions

For the first time, the relationships of the Ceramiales are reconstructed in a well-supported phylogeny. This adds to the growing body of evidence that phylogenomics and transcriptomics can resolve challenging phylogenies in the red algae (Costa et al., 2016; Díaz-Tapia et al., 2017; Oliveira et al., 2018; Saunders et al., 2018; Pasella et al., 2019). Of the nine families currently recognized, three were supported as previously circumscribed. However, six families required reclassification. The families proposed in this work are distinguished by synapomorphic characters of the female structures or carposporophyte development. The thallus structure, however, which was traditionally used to distinguish the families, displayed complex evolutionary dynamics. Some thallus types evolved multiple times in independent lineages of the family, leading to misinterpretations of the affinities among taxa. The thallus structure in the Ceramiales increased in complexity during its evolution from a monosiphonous filamentous ancestor, and this process is likely to be linked to niche differentiation.

In this work, we reassessed the family level classification of the Ceramiales based on a selection of 80 species representative of the previously recognized major lineages. However, a large number of genera and species of this order have never been investigated using molecular tools. Thus, further studies will be required to acquire a more comprehensive understanding of the Ceramiales and reassess their classification at lower taxonomic levels. Large knowledge gaps remain, particularly in the families Wrangeliaceae, Callithamniaceae and Ceramiaceae, which are probably the most understudied groups of the red algae although they contain almost 1,000 currently recognized species. This is in part due to a large proportion of species in these groups being small in size (< 5 mm) so their morphological and molecular study requires additional effort compared with larger red algae.

6. Formal taxonomy

Table S1 provides a summary of the family level classification of genera and tribes in the Ceramiales as a result of our work, as well as a comparison with the previous classification according to studies that included phylogenetic analyses. Below we provide descriptions for taxa that require emendation, as well as the new taxa.

6.1. Inkyuleeales Díaz-Tapia & Maggs, ord. nov.

Diagnosis: Thalli erect, uniaxial, consisting of uniseriate filaments with rhizoidal cortication in lower axes. Each axial cell bearing three whorl branchlets, one major opposite two minor. Life history triphasic, with isomorphic gametophytes and tetrasporophytes. In female gametophytes, carpogonial branches formed adaxially on basal cells of major branchlets. After fertilization, the supporting cell producing a 3-4-celled auxiliary branch and the subapical cell fusing with the carpogonium.

Type family: Inkyuleeaceae H.-G.Choi, Kraft & G.W.Saunders (2008, pp 1040-1041)

6.2. Delesseriaceae Bory (1828, p. 181), fam. emend. Díaz-Tapia & Maggs

Thalli foliose, terete or compressed, attached by rhizoids with multicellular haptera or a discoid fibrous rhizoidal holdfast. Structure uniaxial, growth through divisions of an apical cell that produces an axial filament whose cells divide longitudinally forming 4-11 periaxial cells. In the Delesserioideae, Nitophylloideae, Phycodryoideae and Sarcomenioideae, lateral periaxial cells undergo oblique divisions, forming one or several orders of cell rows to produce a membrane or a row of flanking cells. In the Heterosiphonioideae and Dasyoideae axial filament clothed by pigmented monosiphonous branches. In *Halydictyon* (Sarcomenioideae), thallus consisting of a network of monosiphonous filaments. Spermatangia formed on the thallus surface or on monosiphonous branches. Procarps consisting of a supporting cell, a lateral sterile group of 1-7 cells, a four-celled carpogonial branch and a basal sterile group of 1-4 cells. The fertilized carpogonium cutting off two connecting cells, one of which fuses with the auxiliary cell. The auxiliary cell produces several gonimoblast initials. Gonimoblasts monopodially branched and bearing carposporangia, cystocarps ostiolate. Tetrasporangia formed in sori or stichidia, tetrahedrally divided.

Type genus: *Delesseria* J.V.Lamouroux (1813, p. 122)

6.3. Callithamniaceae Kützing (1843, p. 370), fam. emend. Díaz-Tapia & Maggs

Thalli filamentous, uniaxial, with each axial cell bearing determinate or indeterminate branches arranged distichously, radially or in whorls. In female gametophytes, procarps four-celled and sterile cells completely absent. Gonimolobes with or without sterile involucral cells.

Type genus: *Callithamnion* Lyngbye (1819, p. 123)

6.4. Sarcomenioideae Díaz-Tapia & Maggs, subfam. nov.

Thalli foliose, terete with flanking cells throughout the entire or part of the thallus or net-like in *Halydictyon*. Structure uniaxial, growth through divisions of an apical cell that produces an axial filament whose cells form a network (*Halydictyon*) or divide longitudinally forming four periaxial cells; the lateral periaxial cells undergo oblique divisions forming one to several orders of cell rows that produce a membrane or a row of flanking cells. Intercalary divisions in primary cell rows absent. In female gametophytes, procarps restricted to primary cell rows, consisting of a four-celled carpogonial branch with two groups of sterile cells.

Type genus: *Sarcomenia* Sonder (1845, p. 56)

6.5. Halydictyeae Díaz-Tapia & Maggs, trib. nov.

Thalli consisting of a network of monosiphonous filaments formed by the union of lateral cells. Fertile axes in female gametophytes producing four pericentral cells that bear a carpogonial branch. Spermatangial axes ovate and compressed, with sterile marginal cells. Two tetrasporangia per segment formed on compressed stichidia that consist of an axial cell and four pericentral cells.

Type genus: *Halydictyon* Zanardini (1843, pp 52-53)

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Figure legends

Fig. 1. Types of thallus structure in the Ceramiales. (A) Monosiphonous filament, *Antithamnionella ternifolia*. (B, C) Polysiphonous filament, in cross section (C) with an axial cell (a) surrounded by periaxial cells (p), *Polysiphonia scopulorum*. (D, E) Polysiphonous filament, in cross section (E) with an axial cell (a) surrounded by periaxial cells (p), and flanking cells (f) cut off from lateral periaxial cells, *Taenioma* sp. (F) Network of filaments, *Halydictyon mirabile*. (G) Parasite, *Choreocolax polysiphonieae* (arrow) on *Vertebrata lanosa*. (H, I) Corticate terete axes, in cross section (I) with an axial cell (a) surrounded by periaxial cells (p), a layer of medullary cells (m) and cortical cells (c), *Dasyphonia japonica*. (J, K) Leathery thalli, in cross section (K) with an axial cell (a) surrounded by periaxial cells (p), a layer of medullary cells (m) and cortical cells (c), *Alsidium seaforthii*. (L, M) Blade-like thalli, in cross section (L) with an axial cell (a) surrounded by pericentral cells (p) and a blade (b) formed by division of the lateral periaxial cells, *Cryptopleura ramosa*. Scale bars: 30 μm in Fig. A; 450 in Fig. B; 60 μm in Fig. C; 200 μm in Figs D, F and I; 75 μm in Fig. E; 350 μm in Fig. G; 8 mm in Fig. H; 2 cm in Fig. J; 300 μm in Fig. K; 250 μm in Fig. L; 1.5 cm in Fig. M.

Fig. 2. Phylogeny of the order Ceramiales indicating families and the order Inkyuleeales with light or dark shaded areas; the unshaded area corresponds to the outgroup. New taxa are indicated with bold font. RAxML tree based on nucleotide alignment of the 208 concatenated genes from the plastid genome. All branches have full bootstrap support, except where bootstrap values are indicated on branches. In the Delesseriaceae bars show the subfamilial level classification and the former familial classification of each

species is indicated (DA = Dasyaceae, DE = Delesseriaceae, SA = Sarcomeniaceae, IS = *incertae sedis*).

Fig. 3. Ancestral character estimation for the thallus structure in the Ceramiales. Pie charts indicate proportion of reconstructed character histories under a used-defined model in which some transitions were disallowed.

Supplemental data 1. Glossary and Figs S1-2.

Supplemental Fig. S1. (A) *Vertebrata byssoides*, thallus with monopodial growth. (B) *Dasya gunniana*, thallus with sympodial growth. Images taken from Harvey (1847, 1850).

Supplemental Fig. S2. *Rhodomela virgata*. Procarp and gonimoblast development. (A) Procarp consisting on a supporting cell (su), a sterile cell (st), and a carpogonial branch (1-3) bearing the carpogonium (cp). (B) After the fertilization, the supporting cell (su) produces an auxiliary cell (aux). (C) Cystocarp consisting on the gonimoblast (g) resulting from the divisions of the auxiliary cell that is surrounded by the pericarp (p). Images taken from Kylin 1956.

Supplemental Fig. S3. Phylogeny of the order Ceramiales indicating families and the order Inkyuleeales with light or dark shaded areas; the unshaded area corresponds to the outgroup. New taxa are indicated with bold font. RAxML tree based on amino acid alignment of the 208 concatenated genes from the plastid genome. All branches have full bootstrap support, except where bootstrap values are indicated on branches. In the Delesseriaceae bars show the subfamilial level classification and the former familial classification of each species is indicated (DA = Dasyaceae, DE = Delesseriaceae, SA = Sarcomeniaceae, IS = *incertae sedis*).

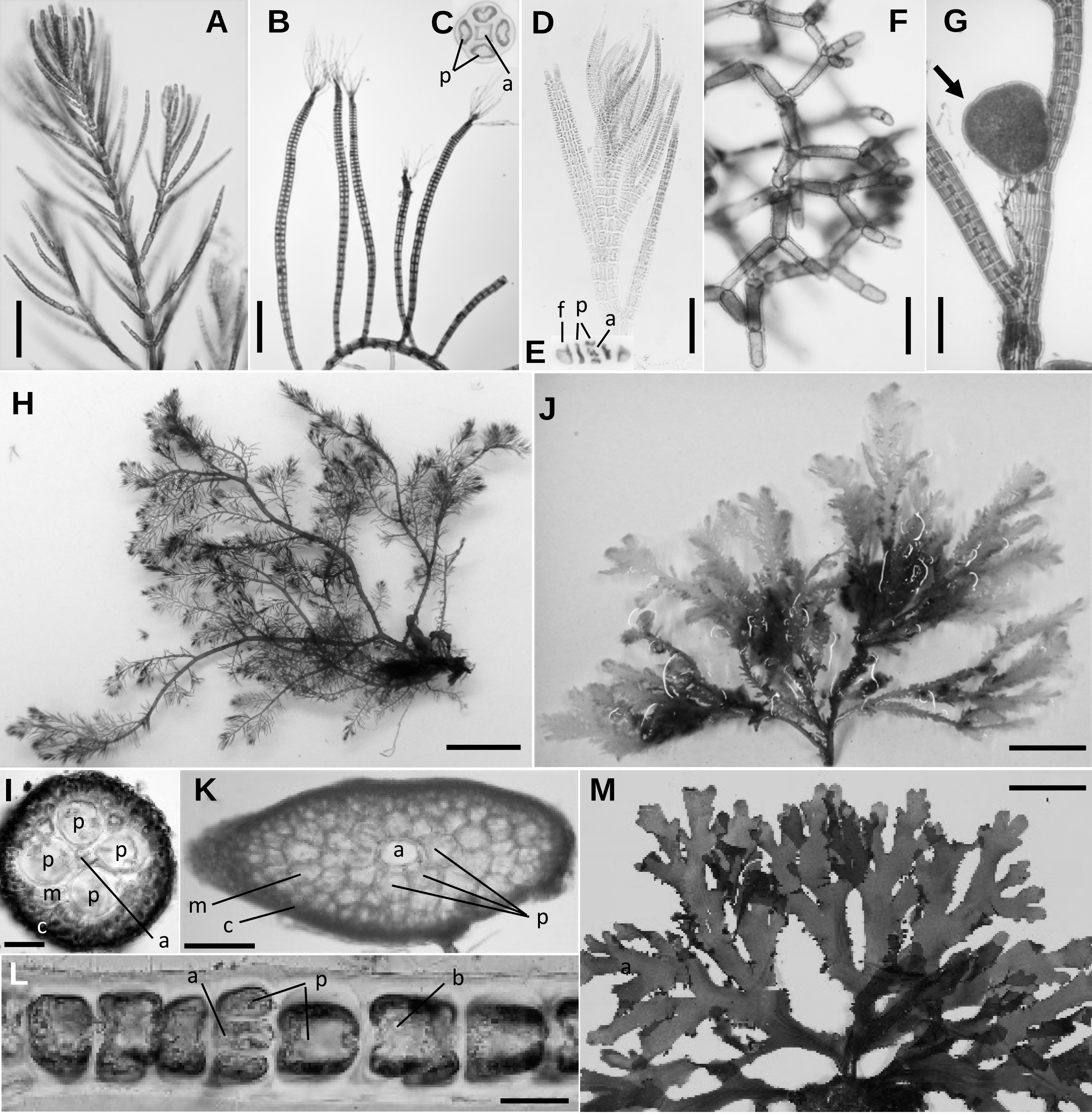
Supplemental Fig. S4. Ancestral character estimation for the thallus structure in the Ceramiales. Pie charts indicate proportion of reconstructed character histories under an equal rates model.

Supplemental Table S1. Classification of genera and tribes of the Ceramiales in works including molecular data (excluding the Rhodomelaceae, see Díaz-Tapia et al. 2017).

Supplemental Table S2. GenBank accession numbers of the plastid genomes included in the phylogenetic analysis.

Table 1. Comparison of models of trait evolution showing that the parameter-rich symmetrical (SYM) and all rates different (ARD) models are outperformed (higher AIC and AICc) by the simpler equal rates (ER) and custom models. The log-likelihood is given under lnL, number of model parameters as k along with the uncorrected and sample-size corrected Akaike Information Criterion (AIC and AICc).

	lnL	k	AIC	AICc
ER	-97.28011	1	196.5602	196.6109
SYM	-85.51230	21	213.0246	228.6856
ARD	-79.35069	42	242.7014	337.7540
custom	-99.70025	1	201.4005	201.4511


A
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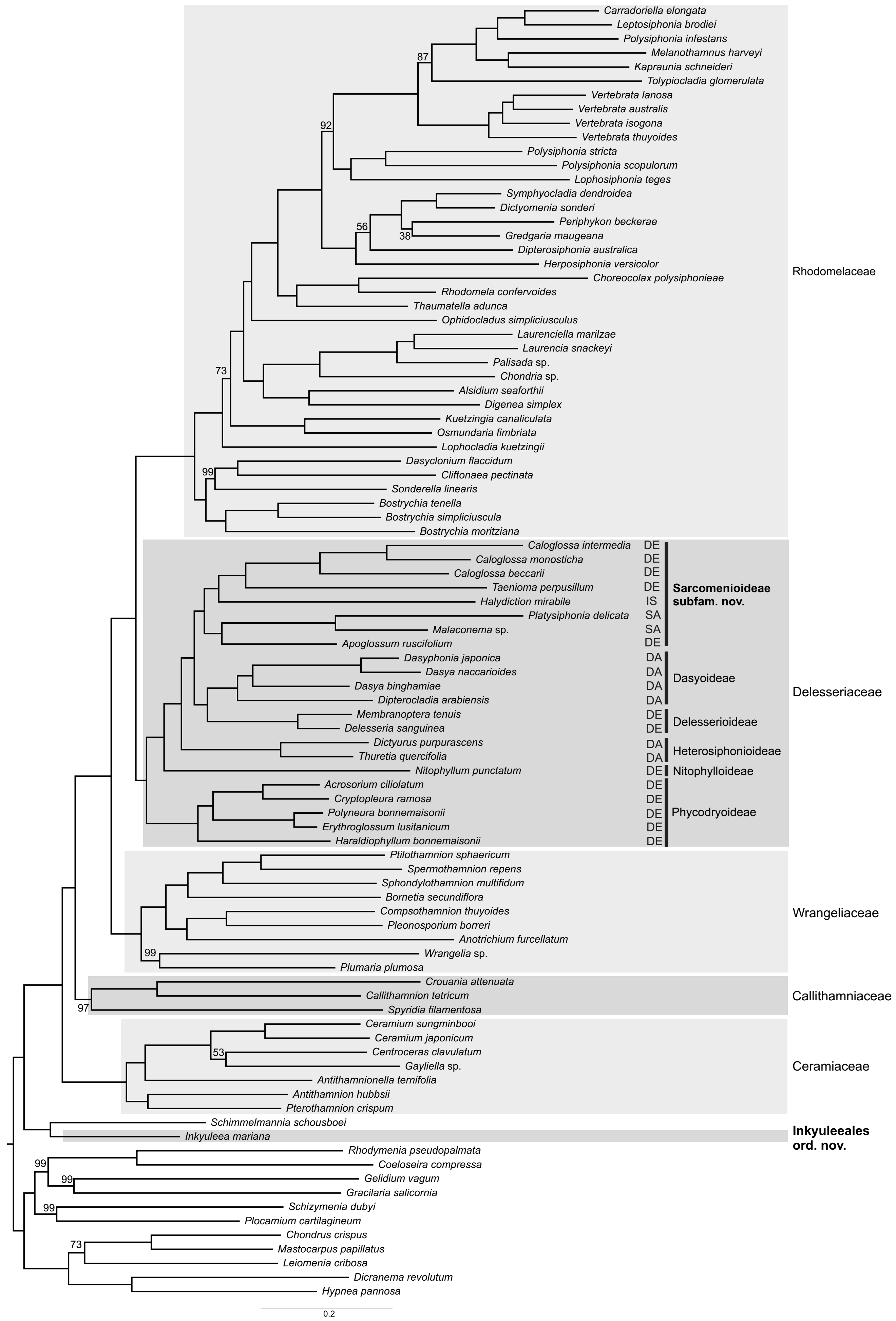
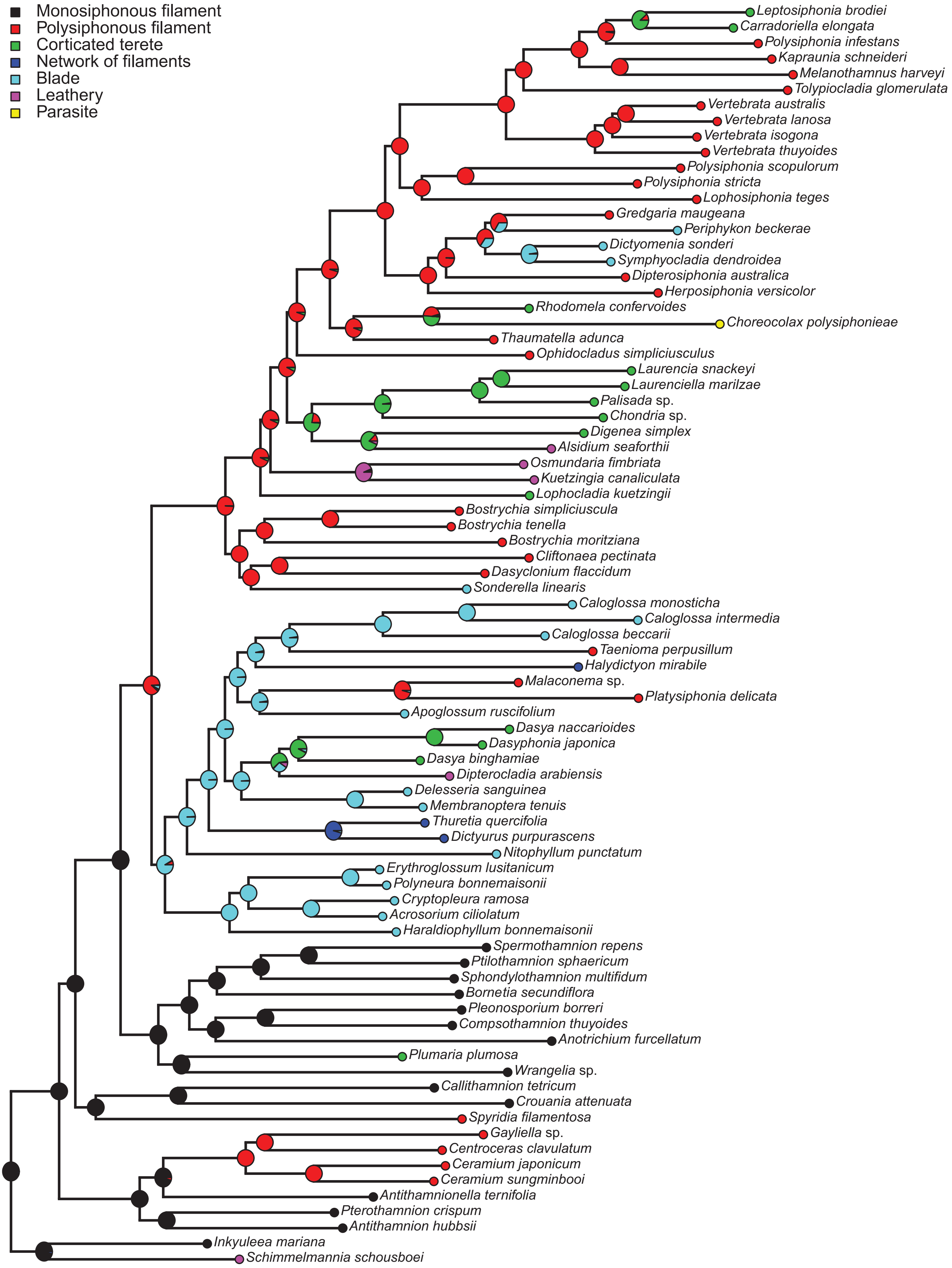

Carradoriella elongata
Leptosiphonia brodiei
Polysiphonia infestans
Melanothamnus harveyi
Kapraunia schneideri
Tolypiocladia glomerulata
87
Vertebrata lanosa
Vertebrata australis
Vertebrata isogona
Vertebrata thuyoides
92
Polysiphonia stricta
Polysiphonia scopulorum
Lophosiphonia teges
Symphyocladia dendroidea
Dictyomenia sonderi
Periphykon beckerae
56
38
Gredgaria maugeana
Dipterosiphonia australica
Herposiphonia versicolor
Choreocolax polysiphonieae
Rhodomela confervoides
Thaumatella adunca
Ophidocladus simpliciusculus
Laurenciella marilzae
Laurencia snackeyi
Palisada sp.
73
Chondria sp.
Alsidium seaforthii
Digenea simplex
Kuetzingia canaliculata
Osmundaria fimbriata
Lophocladia kuetzingii
Dasyclonium flaccidum
99
Cliftonaea pectinata
Sonderella linearis
Bostrychia tenella
Bostrychia simpliciuscula
Bostrychia moritziana
Rhodomelaceae
Caloglossa intermedia DE
Caloglossa monosticha DE
Caloglossa beccarii DE
Taenioma perpusillum DE
Halydiction mirabile IS
Platysiphonia delicata SA
Malaconema sp. SA
Apoglossum ruscifolium DE
Sarcomenioideae subfam. nov.
Dasyphonia japonica DA
Dasya naccarioides DA
Dasya binghamiae DA
Dipterocladia arabiensis DA
Dasyoideae
Membranoptera tenuis DE
Delesseria sanguinea DE
Delesserioideae
Dictyurus purpurascens DA
Thuretia quercifolia DA
Heterosiphonioideae
Nitophyllum punctatum DE
Nitophylloideae
Acrosorium ciliolatum DE
Cryptopleura ramosa DE
Polyneura bonnemaisonii DE
Erythroglossum lusitanicum DE
Haraldiophyllum bonnemaisonii DE
Phycodryoideae
Delesseriaceae
Ptilothamnion sphaericum
Spermothamnion repens
Sphondylothamnion multifidum
Bornetia secundiflora
Compsothamnion thuyoides
Pleonosporium borreri
Anotrichium furcellatum
99
Wrangelia sp.
Plumaria plumosa
Wrangeliaceae
Crouania attenuata
Callithamnion tetricum
97
Spyridia filamentosa
Callithamniaceae
Ceramium sungminbooi
Ceramium japonicum
53
Centroceras clavulatum
Gayliella sp.
Antithamnionella ternifolia
Antithamnion hubbsii
Pterothamnion crispum
Ceramiaceae
Schimmelmannia schousboei
Inkyuleea mariana
Inkyuleeales ord. nov.
99
Rhodymenia pseudopalmata
Coeloseira compressa
99
Gelidium vagum
Gracilaria salicornia
99
Schizymenia dubyi
Plocamium cartilagineum
Chondrus crispus
73
Mastocarpus papillatus
Leiomenia cribosa
Dicranema revolutum
Hypnea pannosa
0.2


Monosiphonous filament
Polysiphonous filament
Corticated terete
Network of filaments
Blade
Leathery
Parasite
Leptosiphonia brodiei
Carradoriella elongata
Polysiphonia infestans
Kapraunia schneideri
Melanothamnus harveyi
Tolypiocladia glomerulata
Vertebrata australis
Vertebrata lanosa
Vertebrata isogona
Vertebrata thuyoides
Polysiphonia scopulorum
Polysiphonia stricta
Lophosiphonia teges
Gredgaria maugeana
Periphykon beckerae
Dictyomenia sonderi
Symphyocladia dendroidea
Dipterosiphonia australica
Herposiphonia versicolor
Rhodomela confervoides
Choreocolax polysiphonieae
Thaumatella adunca
Ophidocladus simpliciusculus
Laurencia snackeyi
Laurenciella marilzae
Palisada sp.
Chondria sp.
Digenea simplex
Alsidium seaforthii
Osmundaria fimbriata
Kuetzingia canaliculata
Lophocladia kuetzingii
Bostrychia simpliciuscula
Bostrychia tenella
Bostrychia moritziana
Cliftonaea pectinata
Dasyclonium flaccidum
Sonderella linearis
Caloglossa monosticha
Caloglossa intermedia
Caloglossa beccarii
Taenioma perpusillum
Halydictyon mirabile
Malaconema sp.
Platysiphonia delicata
Apoglossum ruscifolium
Dasya naccarioides
Dasyphonia japonica
Dasya binghamiae
Dipterocladia arabiensis
Delesseria sanguinea
Membranoptera tenuis
Thuretia quercifolia
Dictyurus purpurascens
Nitophyllum punctatum
Erythroglossum lusitanicum
Polyneura bonnemaisonii
Cryptopleura ramosa
Acrosorium ciliolatum
Haraldiophyllum bonnemaisonii
Spermothamnion repens
Ptilothamnion sphaericum
Sphondylothamnion multifidum
Bornetia secundiflora
Pleonosporium borreri
Compsothamnion thuyoides
Anotrichium furcellatum
Plumaria plumosa
Wrangelia sp.
Callithamnion tetricum
Crouania attenuata
Spyridia filamentosa
Gayliella sp.
Centroceras clavulatum
Ceramium japonicum
Ceramium sungminbooi
Antithamnionella ternifolia
Pterothamnion crispum
Antithamnion hubbsii
Inkyuleea mariana
Schimmelmannia schousboei

Supplemental data 1. Glossary. Extracted from Maggs & Hommersand, 1993, with permission.

Auxiliary cell Cell that receives the diploid nucleus from the carpogonium and gives rise to the gonimoblast (Fig. S2B).

Carpogonial branch Filament bearing the female gametangium, the carpogonium (Fig. S2A).

Carpogonium Female gametangium (Fig. S2A).

Corticated With a secondary cortex formed by development of filaments within, and sometimes outside, the outer walls of primary filaments (Figs 1H-K).

Cystocarp The gonimoblast tissue and surrounding gametophytic pericarp tissue; structure from which carpospores are released (Fig. S2C).

Gonimoblast Tissue which develops on the female plant after fertilization and which ultimately produces carposporangia (Fig. S2C).

Gonimoblast initial The first cell arising from a fertilized carpogonium or diploidized auxiliary cell, which develops into the gonimoblast.

Monopodial Development in which the primary axis is maintained as the main line of growth and other axes are produced from it (Fig. S1A in supplementary material).

Monosiphonous Consisting of a single row of cells, with or without cortication but without periaxial cells (cf. polysiphonous) (Fig. 1A).

Periaxial (= pericentral) cell One of a number, often a ring, of cells cut off from and surrounding an axial cell.

Pericarp That part of the cystocarp produced by the female gametangial thallus and forming a covering to the developing gonimoblast (Fig. S2C).

Polysiphonous With each axial cell surrounded, usually over its entire length, with a particular number of periaxial cells (Figs 1B-E).

Note: Polysiphonous often refers only to a thallus structure with the axial cell surrounded over its entire length by periaxial cells.

Supporting cell Cell bearing one or more carpogonial branches (Fig. S2A).

Sympodial Development in which the primary axis is continually being replaced by lateral axes which become temporarily dominant, but soon are replaced by their own laterals (Fig. S1B).

Uniaxial Containing only a single axial filament.

References:

Maggs, C.A., Hommersand, M.H., 1993. Seaweeds of the British Isles. Volume 1. Rhodophyta. Part 3A. Ceramiales. HMSO, London.

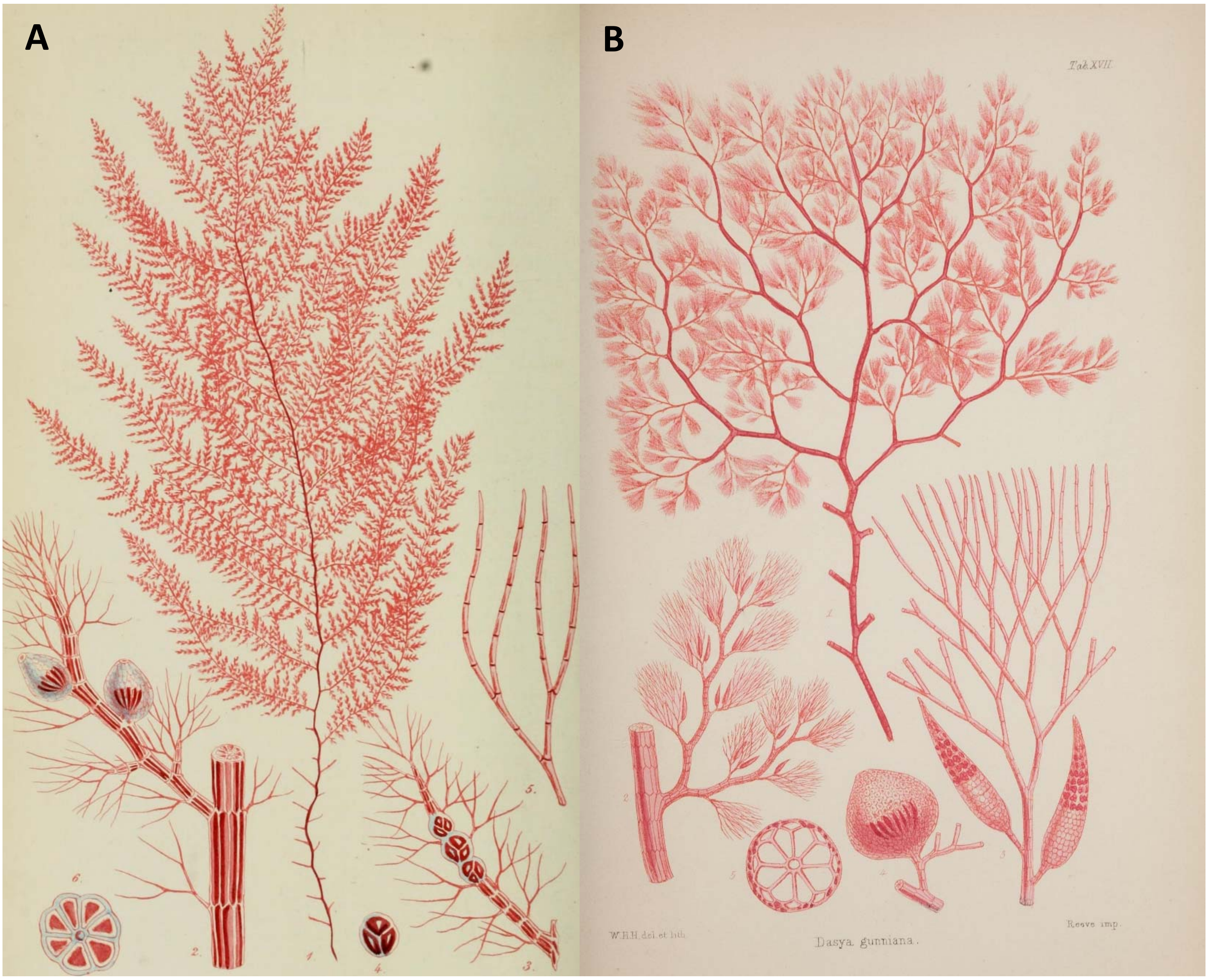


Fig. S1. **A**, *Vertebrata byssoides*, thallus with monopodial growth. **B**, *Dasya gunniana*, thallus with sympodial growth. Images taken from Harvey (1847, 1850).

References

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Harvey, W.H. (1850). Phycologia britannica, or, a history of British sea-weeds: containing coloured figures, generic and specific characters, synonymes, and descriptions of all the species of algae inhabiting the shores of the British Islands. Reeve & Benham, London. 271-318 pp.

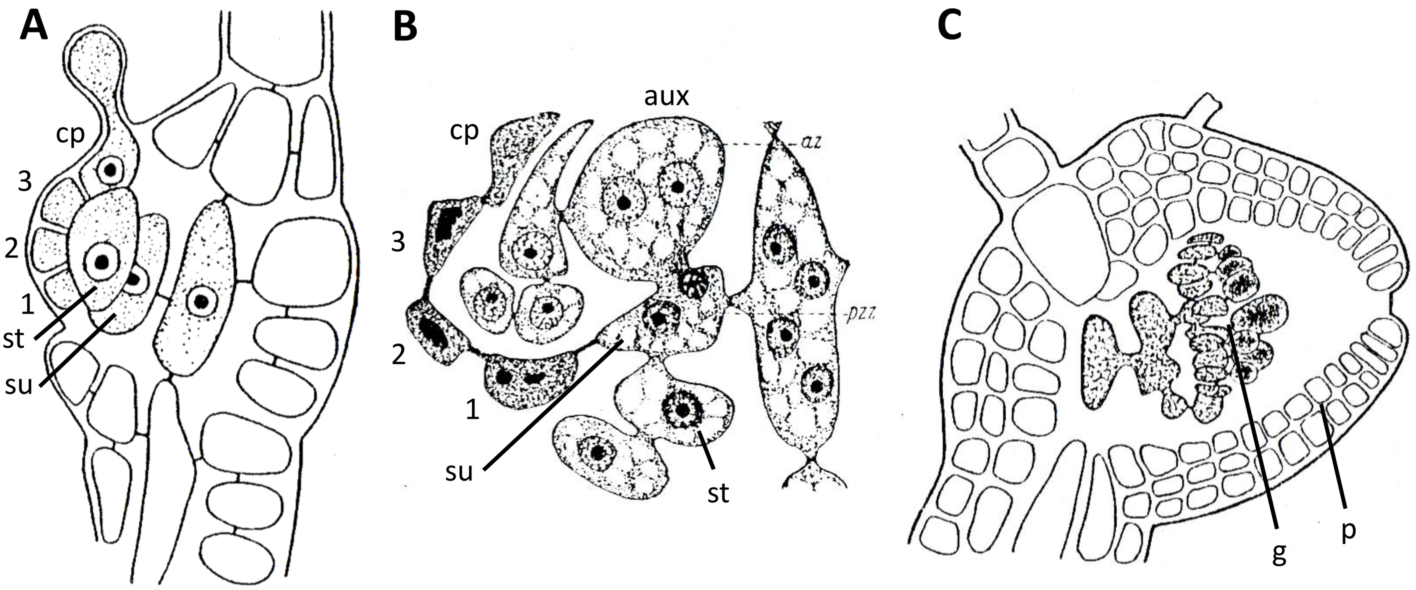
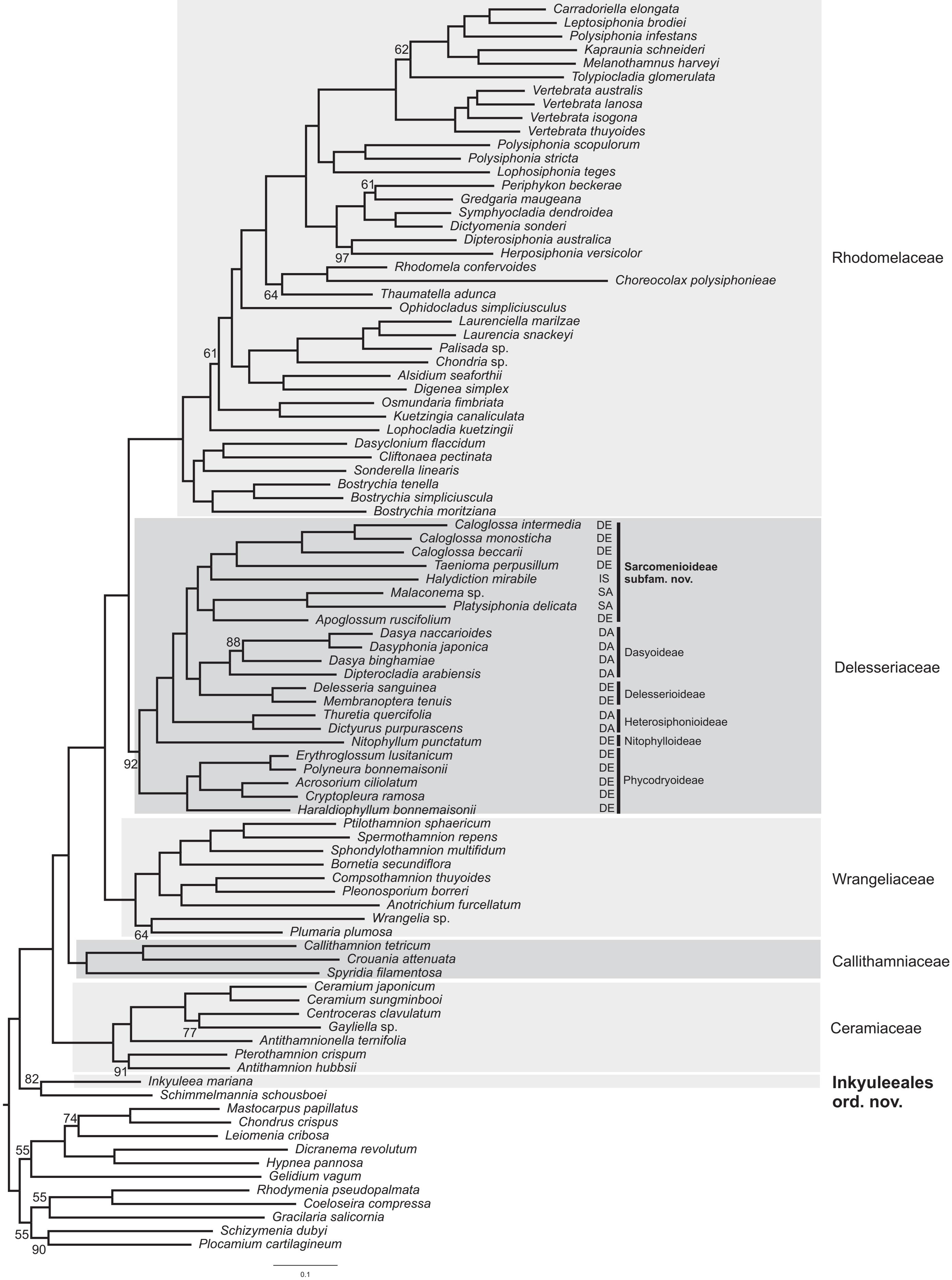


Fig. S2. *Rhodomela virgata*. Procarp and gonimoblast development. A, Procarp consisting on a supporting cell (su), a sterile cell (st), and a carpogonial branch (1-3) bearing the carpogonium (cp). B, After the fertilization, the supporting cell (su) produces an auxiliary cell (aux). C, Cystocarp consisting on the gonimoblast (g) resulting from the divisions of the auxiliary cell that is surrounded by the pericarp (p). Images taken from Kylin 1956.

References

Kylin, H. (1956). Die Gattungen der Rhodophyceen. C.W.K. Gleerups, Lund. 673 pp.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Polysiphonia infestans
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Melanothamnus harveyi
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62
Vertebrata australis
Vertebrata lanosa
Vertebrata isogona
Vertebrata thuyoides
Polysiphonia scopulorum
Polysiphonia stricta
Lophosiphonia teges
61
Periphykon beckerae
Gredgaria maugeana
Symphyocladia dendroidea
Dictyomenia sonderi
Dipterosiphonia australica
Herposiphonia versicolor
97
Rhodomela confervoides
Choreocolax polysiphonieae
64
Thaumatella adunca
Ophidocladus simpliciusculus
Laurenciella marilzae
Laurencia snackeyi
Palisada sp.
61
Chondria sp.
Alsidium seaforthii
Digenea simplex
Osmundaria fimbriata
Kuetzingia canaliculata
Lophocladia kuetzingii
Dasyclonium flaccidum
Cliftonaea pectinata
Sonderella linearis
Bostrychia tenella
Bostrychia simpliciuscula
Bostrychia moritziana
Rhodomelaceae
Caloglossa intermedia DE
Caloglossa monosticha DE
Caloglossa beccarii DE
Taenioma perpusillum DE
Halydiction mirabile IS
Malaconema sp. SA
Platysiphonia delicata SA
Apoglossum ruscifolium DE
Sarcomenioideae subfam. nov.
88
Dasya naccarioides DA
Dasyphonia japonica DA
Dasya binghamiae DA
Dipterocladia arabiensis DA
Dasyoideae
Delesseria sanguinea DE
Membranoptera tenuis DE
Delesserioideae
Thuretia quercifolia DA
Dictyurus purpurascens DA
Heterosiphonioideae
Nitophyllum punctatum DE
Nitophylloideae
92
Erythroglossum lusitanicum DE
Polyneura bonnemaisonii DE
Acrosorium ciliolatum DE
Cryptopleura ramosa DE
Haraldiophyllum bonnemaisonii DE
Phycodryoideae
Delesseriaceae
Ptilothamnion sphaericum
Spermothamnion repens
Sphondylothamnion multifidum
Bornetia secundiflora
Compsothamnion thuyoides
Pleonosporium borreri
Anotrichium furcellatum
Wrangelia sp.
64
Plumaria plumosa
Wrangeliaceae
Callithamnion tetricum
Crouania attenuata
Spyridia filamentosa
Callithamniaceae
Ceramium japonicum
Ceramium sungminbooi
Centroceras clavulatum
Gayliella sp.
77
Antithamnionella ternifolia
Pterothamnion crispum
91
Antithamnion hubbsii
Ceramiaceae
82
Inkyuleea mariana
Schimmelmannia schousboei
Inkyuleeales ord. nov.
74
Mastocarpus papillatus
Chondrus crispus
Leiomenia cribosa
55
Dicranema revolutum
Hypnea pannosa
Gelidium vagum
Rhodymenia pseudopalmata
55
Coeloseira compressa
Gracilaria salicornia
55
Schizymenia dubyi
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Plocamium cartilagineum
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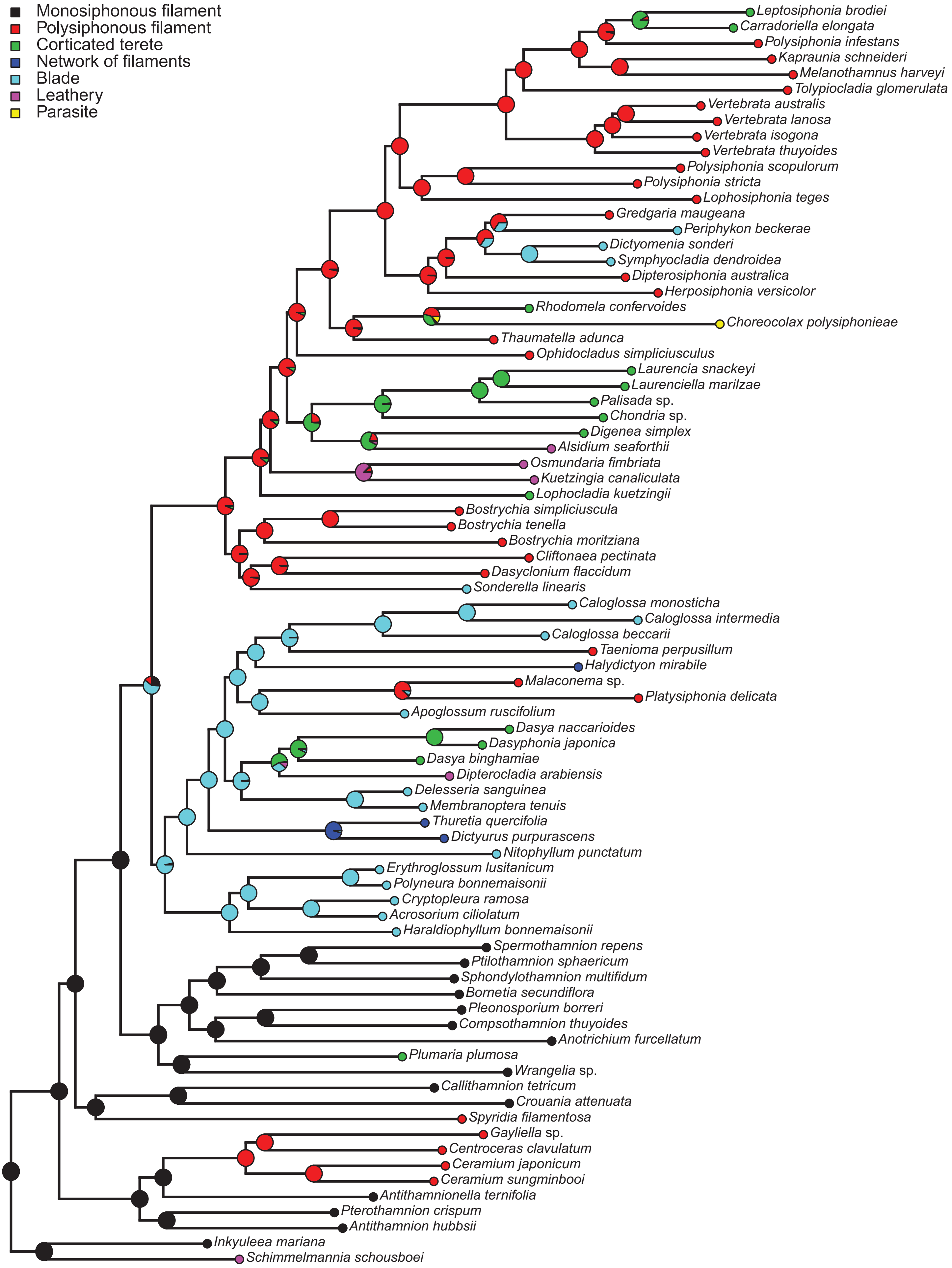
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Laurenciella marilzae
Palisada sp.
Chondria sp.
Digenea simplex
Alsidium seaforthii
Osmundaria fimbriata
Kuetzingia canaliculata
Lophocladia kuetzingii
Bostrychia simpliciuscula
Bostrychia tenella
Bostrychia moritziana
Cliftonaea pectinata
Dasyclonium flaccidum
Sonderella linearis
Caloglossa monosticha
Caloglossa intermedia
Caloglossa beccarii
Taenioma perpusillum
Halydictyon mirabile
Malaconema sp.
Platysiphonia delicata
Apoglossum ruscifolium
Dasya naccarioides
Dasyphonia japonica
Dasya binghamiae
Dipterocladia arabiensis
Delesseria sanguinea
Membranoptera tenuis
Thuretia quercifolia
Dictyurus purpurascens
Nitophyllum punctatum
Erythroglossum lusitanicum
Polyneura bonnemaisonii
Cryptopleura ramosa
Acrosorium ciliolatum
Haraldiophyllum bonnemaisonii
Spermothamnion repens
Ptilothamnion sphaericum
Sphondylothamnion multifidum
Bornetia secundiflora
Pleonosporium borreri
Compsothamnion thuyoides
Anotrichium furcellatum
Plumaria plumosa
Wrangelia sp.
Callithamnion tetricum
Crouania attenuata
Spyridia filamentosa
Gayliella sp.
Centroceras clavulatum
Ceramium japonicum
Ceramium sungminbooi
Antithamnionella ternifolia
Pterothamnion crispum
Antithamnion hubbsii
Inkyuleea mariana
Schimmelmannia schousboei

Table S1. Classification of genera and tribes of the Ceramiales mainly based in works including molecular data (excluding the Rhodomelaceae, see Díaz-Tapia et al. 2017).

Choi et al. (2001)	Lin et al. (2001)	Choi et al. (2002)	Genera and tribes proposed after Lin et al. 2001, Choi et al. 2001, 2002	This work
CERAMIALES				**INKYULEEALES**
Inkyuleeaceae				**Inkyuleeaceae**
Inkyuleea				*Inkyuleea*
				CERAMIALES
Ceramiaceae			**Ceramiaceae**	**Ceramiaceae**
Antithamnieae				Antithamnieae
Antithamnion				*Antithamnion*
Ceramieae				Ceramieae
			[1]*Campylaephora*	
Carpoblepharis				
Centroceras				*Centroceras*
Ceramium				*Ceramium*
			[2]*Corallophila*	
			[2]*Gayliella*	*Gayliella*
			[2]*Herpochondria*	
			[2]*Microcladia*	
			[2]*Reinboldiella*	
Dohrnielleae				Dohrnielleae
Antithamnionella				*Antithamnionella*
Heterothamnieae				
Heterothamnion				
Pterothamnieae				Pterothamnieae
Pterothamnion				*Pterothamnion*
Incertae sedis				
Tetrathamnion				
Callithamniaceae			**Callithamniaceae**	**Callithamniaceae**
Callithamnieae				Callithamnieae
Aglaothamnion				
			[3]*Aristoptilon*	
Callithamnion				*Callithamnion*
			[3]*Carpothamnion*	
Diapse				
			[3]*Falklandiella*	
			[3]*Georgiella*	
Crouanieae				Crouanieae
Crouania				*Crouania*
			[4]*Gulsonia*	
			[3]*Heteroptilon*	
			[3]*Ptilocladia*	
Euptiloteae				
Euptilota				
Seirospora				
			[3]*Sciurothamnion*	
Rhodocallideae				

Rhodocallis				

Choi et al. (2001)	Lin et al. (2001)	Choi et al. (2002)	Genera and tribes proposed after Lin et al. 2001, Choi et al. 2001, 2002	This work
Spyridiaceae				
				Spyridieae
Spyridia				*Spyridia*
Wrangeliaceae			**Wrangeliaceae**	**Wrangeliaceae**
Compsothamnieae				Compsothamnieae
Compsothamnion				*Compsothamnion*
Dasythamnionella				
Dasyphileae				
Dasyphila				
Muellerena				
Griffithsieae				Griffithsieae
Anotrichium				*Anotrichium*
				Bornetia
Griffithsia				
Halurus				
Monosporeae				
Monosporus				
Ptiloteae				Ptiloteae
Neoptilota				
Plumaria				*Plumaria*
Ptilota				
Spermothamnieae				Spermothamnieae
Lejolisia				
				[5]*Ptilothamnion*
Spermothamnion				*Spermothamnion*
Sphondylothamnieae				Sphondylothamnieae
Involucrana				
Shepleya				
				Sphondylothamnion
Spongoclonieae				Spongoclonieae
Pleonosporium				*Pleonosporium*
Spongoclonium				
Wrangelieae				Wrangelieae
Wrangelia				*Wrangelia*
Incertae sedis				
Warrenia				

Choi et al. (2001)	Lin et al. (2001)	Choi et al. (2002)	Genera and tribes proposed after Lin et al. 2001, Choi et al. 2001, 2002	This work
	Delesseriaceae		**Delesseriaceae**	**Delesseriaceae**
	Delesserioideae		**Delesserioideae**	**Sarcomenioideae subfam. nov.**
	Sarcomenieae			Sarcomenieae
	Sarcomenia			
				Malaconema
				Platysiphonia
	Caloglosseae			Caloglosseae
	Caloglossa			*Caloglossa*
				Taenioma
	Apoglosseae			Apoglosseae
	Apoglossum			*Apoglossum*
	Paraglossum			
			[6]*Phrix*	
	Claudea group		[7]Claudeeae	
	Vanvoorstia			
				Halydictyeae trib. nov.
				Halydictyon
				Delesserioideae
	Hemineureae		[8]Botryocarpeae	
	Botryocarpa			
	Hemineura			
	Laingia			
	Marionella			
	Patulophycus			
	Pseudophycodrys			
	Hypoglosseae			
	Hypoglossum			
	Zellera			
	Bartoniella			
	Branchioglossum			
	Delesserieae			Delesserieae
	Delesseria			*Delesseria*
			[8]Membranoptereae	Membranoptereae
	Membranoptera			*Membranoptera*
	Grinnellieae			
	Grinnellia			
			[9]Wynneophycuseae	
			[9]*Wynneophycus*	

Choi et al. (2001)	Lin et al. (2001)	Choi et al. (2002)	Genera and tribes proposed after Lin et al. 2001, Choi et al. 2001, 2002	This work
	Phycodryoideae		**Phycodryoideae**	**Phycodryoideae**
	Phycodryeae			Phycodryeae
	Cladodonta			
				Erythroglossum
	Heterodoxia			
			[10]*Hymenenopsis*	
	Nienburgia			
	Phycodrys			
	Polyneura			*Polyneura*
	Womersleya			
	Myriogrammeae			Myriogrammeae
	Myriogramme			
			[11]*Neoharaldiophyllum*	
	Haraldiophyllum			*Haraldiophyllum*
	Schizoserideae			
	Abroteia			
	Drachiella			
	Neuroglossum			
	Schizoseris			
	Cryptopleureae			Cryptopleureae
	Acrosorium			*Acrosorium*
	Cryptopleura			*Cryptopleura*
	Hymenena			
	Botryoglossum			
	Nitophylloideae		**Nitophylloideae**	**Nitophylloideae**
	Nitophylleae			Nitophylleae
			[12]*Augophyllum*	
	Nitophyllum			*Nitophyllum*
			[8]Valeriemayeae	
	Calonitophyllum			
	Polyneuropsis			
	Valeriemaya			
	Martensieae			
	Martensia			
	Opephyllum			
		Dasyaceae		
		Heterosiphonoideae		**Heterosiphonoideae**
		Dictyurus		*Dictyurus*
		Heterosiphonia		
		Thuretia		*Thuretia*
		Dasyoideae		**Dasyoideae**
		Dasya		*Dasya*
		Dasyphonia		*Dasyphonia*
				Dipterocladia
		Eupogodon		
		Rhodoptilum		

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Lin, S.M., Fredericq, S., Hommersand, M. H., 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and rbcL sequences, including the Phycodryoideae, subfam. nov. J. Phycol., 37, 881-899.

Table S2. GenBank accession numbers of the plastid genomes included in the phylogenetic analysis.

Species	Collection site; date; habitat; collectors / Publication	ID	Genbank accesion number	Length when incomplete/number of CDSs
Acrosorium ciliolatum	Díaz-Tapia et al. 2017	-	MF101411	
Alsidium seaforthii	Díaz-Tapia et al. 2017, as *Bryothamnion*	PD644	MF101430	
Anotrichium furcellatum	Perbes, A Coruña, Spain; 15.i.2017; intertidal; PD	PD2933	MK814609	170.239 / 192
Antithamnion hubsii	Baiona, Pontevedra, Spain; 3.v.2016; pontoon in a marina; PD	PD2206	MK814610	152.684 / 175
Antithamnionella ternifolia	Dique Abrigo, A Coruña, Spain; 12.iii.2017; pontoon in a marina; PD	PD2956	MK814608	
Apoglossum ruscifolium	Perbes, A Coruña, Spain; 15.i.2017; intertidal; PD	PD2929	MK814611	30.774 / 31
			MK814612	30.076 / 35
			MK814613	21.752 / 25
			MK814614	84,340 / 97
Bornetia secundiflora	Perbes, A Coruña, Spain; 15.i.2017; intertidal; PD	PD2926	MK814615	
Bostrychia moritziana	Díaz-Tapia et al. 2017	JW3660	MF101419	
Bostrychia simpliciuscula	Díaz-Tapia et al. 2017	JW3897	MF101421	
Bostrychia tenella	Díaz-Tapia et al. 2017	JW3079	MF101417	
Callithamnion tetricum	Perbes, A Coruña, Spain; 15.i.2017; intertidal; PD	PD2927	MK814616	166.571 / 188
Caloglossa beccarii	Díaz-Tapia et al. 2017	JW4523	MF101422	
Caloglossa intermedia	Díaz-Tapia et al. 2017	JW3535	MF101418	
Caloglossa monosticha	Díaz-Tapia et al. 2017	JW3046	MF101416	
Carradoriella elongata	Díaz-Tapia et al. 2017, as *Polysiphonia*	PD547	MF101427	
Centroceras clavulatum	Swan Bay, Victoria, Australia; 23.xi.2017; intertidal; HV	HV06547	MK814617	36.045 / 38
			MK814618	53.556 / 63
			MK814619	76.977 / 89
Ceramium japonicum	Lee et al. 2016a	-	KX284719	
Ceramium sungminbooi	Hughey & Boo 2016	UC2050592	NC031211	
Chondria sp.	Díaz-Tapia et al. 2017	PD1582	MF101451	
Choreocolax polysiphonieae	Salomaki et al. 2015	-	KP308096	

Cliftonaea pectinata	Díaz-Tapia et al. 2017	PD1561	MF101450	
Compsothamnion thuyoides	Margaritas, A Coruña, Spain; 16.i.2017; intertidal; PD	PD2939	MK814620	10.141 / 4
			MK814621	2.624 / 3
			MK814622	3.765 / 5
			MK814623	8.533 / 6
			MK814624	6.559 / 9
			MK814625	1.501 / 1
			MK814626	6.831 / 8
			MK814627	9.408 / 4
			MK814628	3.795 / 5
			MK814629	5.858 / 6
			MK814630	26.665 / 46
			MK814631	4.317 / 6
Crouania attenuata	San Pedro, A Coruña, Spain; 17.i.2017; intertidal; PD	PD2952	MK814632	
Cryptopleura ramosa	Perbes, A Coruña, Spain; 15.i.2017; intertidal; PD	PD2928	MK814633	10.593 / 4
			MK814634	8.392 / 11
			MK814635	33.660 / 38
			MK814636	4.623 / 5
			MK814637	11.939 / 14
			MK814638	79.318 / 91
			MK814639	14.233 / 20
Dasya binghamiae	Tamayo & Hughey 2016	UC2050572	KX247284	
Dasya naccarioides	Díaz-Tapia et al. 2017	PD888	MF101436	
Dasyclonium flaccidum	Díaz-Tapia et al. 2017	PD1087	MF101443	
Dasyphonia japonica	Margaritas, A Coruña, Spain; 16.i.2017; boat rope; PD	PD2948	MK814640	15.673 / 13
			MK814641	50.129 / 60
			MK814642	44.819 / 44
			MK814643	32.671 / 54
			MK814644	7.348 / 10
			MK814645	8.458 / 11
			MK814646	5.683 / 7

Delesseria sanguinea	Ringstead Bay, Dorset, UK; 13.iv.2017; drift; CAM	PD3001	MK814647	21.358 / 24
			MK814648	15.105 / 20
			MK814649	77.308 / 91
			MK814650	15.713 / 19
Dictyomenia sonderi	Díaz-Tapia et al. 2017	PD1725	MF101455	
Dictyurus purpurascens	Matemwe, Zanzibar, Tanzania; 9.ii.2008; reef crest	TZ0704	MK814652	
Digenea simplex	Díaz-Tapia et al. 2017	PD1820	MF101465	
Dipterocladia arabiensis	Díaz-Tapia et al. 2017	DHO101	MF101408	
Dipterosiphonia australica	Díaz-Tapia et al. 2017	PD1107	MF101444	
Erythroglossum lusitanicum	Margaritas, A Coruña, Spain; 16.i.2017; intertidal; PD	PD2950	MK814653	38.724 / 43
			MK814654	22.504 / 21
			MK814655	20.537 / 17
			MK814656	16.556 / 19
			MK814657	20.199 / 24
			MK814658	37.047 / 51
Gayliella sp.	Coral Bay, Western Australia; v.2013; epiphyte on *Laurencia snackeyi*; JFC	JFC0032b	MK814659	147.381 / 175
Gredgaria maugeana	Díaz-Tapia et al. 2017	PD1230	MF101446	
Halydictyon mirabile	Sausset les Pins, Marseilles, France; 11.v12011; IB & MV	SANT-25966	MK814660	15.821 / 18
			MK814661	5.197 / 7
			MK814662	8.365 / 15
			MK814663	10.717 / 9
			MK814664	17.718 / 14
			MK814665	14.680 / 17
			MK814666	10.748 / 14
			MK814667	17.639 / 32
			MK814668	12.589 / 12
			MK814669	7.882/ 11

Haraldiophyllum bonnemaisonii	Morás, Lugo, Spain; 15.xi.2004; intertidal; IB	S15261	MK814670	10.293 / 4
			MK814671	5.281 / 7
			MK814672	3.667 / 5
			MK814673	15.595 / 12
			MK814674	4.494 / 6
			MK814675	17.690 / 13
			MK814676	20.257 / 23
			MK814677	24.021 / 37
			MK814678	6.993 / 8
			MK814679	4.177 / 6
Herposiphonia versicolor	Díaz-Tapia et al. 2017	PD852	MF101434	
Kapraunia schneideri	Díaz-Tapia et al. 2017, as *Polysiphonia*	PD1720	MF101454	
Kuetzingia canaliculata	Díaz-Tapia et al. 2017	PD1540	MF101449	
Laurencia snackeyi	Verbruggen & Costa 2015	JFC0032	LN833431	
Laurenciella marilzae	Díaz-Tapia et al. 2017	H.1501	MF101410	
Leptosiphonia brodiei	Díaz-Tapia et al. 2017, as *Polysiphonia*	PD516	MF101425	
Lophocladia kuetzingii	Díaz-Tapia et al. 2017	PD1509	MF101448	
Lophosiphonia teges	Díaz-Tapia et al. 2017	PD1823	MF101457-64	
			MF101466	
Malaconema sp.	Queenscliff, Victoria, Australia; 03.xii.2016; drift; PD	PD2901	MK814682	6.754 / 1
			MK814683	1.327 / 2
			MK814684	1.249 / 1
			MK814685	1.872 / 2
			MK814686	5.017 / 2
			MK814687	4.120 / 5
			MK814688	2.438 / 4
			MK814689	9.145 / 3
			MK814690	3.639 / 5
			MK814691	5.899 / 5
			MK814692	1.657 / 3
			MK814693	3.468 / 6

			MK814694	5.886 / 10
			MK814695	2.016 / 3
			MK814696	4.039 / 4
			MK814697	3.016 / 5
			MK814698	2.524 / 2
Melanothamnus harveyi	Díaz-Tapia et al. 2017	PD890	MF101437	
Membranoptera tenuis	Hughey et al. 2017	UC266439	KP675983	
Nitophyllum punctatum	Perbes, A Coruña, Spain; 15.i.2017; intertidal; PD	PD2930	MK814699	
Ophidocladus simpliciusculus	Díaz-Tapia et al. 2017	PD949	MF101440	
Osmundaria fimbriata	Díaz-Tapia et al. 2017	JW2841	MF101415	
Palisada sp.	Díaz-Tapia et al. 2017	PD1686	MF101453	
Periphykon beckerae	Díaz-Tapia et al. 2017 (as *P. beckeri*)	JH1427	MF101413	
Platysiphonia delicata	Díaz-Tapia et al. 2017	H.1445	MF101409	
Pleonosporium borreri	Margaritas, A Coruña, Spain; 16.i.2017; intertidal; PD	PD2941	MK814700	36.942 / 38
			MK814701	54.388 / 62
			MK814702	77.716 / 90
Plumaria plumosa	As Amorosas, A Coruña, Spain; 19.i.2017; intertidal; PD	PD2953	MK814703	7.535 / 2
			MK814704	5.898 / 6
			MK814705	2.520 / 3
			MK814706	21.584 / 22
			MK814707	79.630 / 91
			MK814708	4.305 / 6
			MK814709	10.675 / 10
Polysiphonia infestans	Díaz-Tapia et al. 2017	PD763	MF101432	
Polysiphonia scopulorum	Díaz-Tapia et al. 2017	PD899	MF101438	
Polysiphonia stricta	Díaz-Tapia et al. 2017	PD550	MF101428	
Polyneura bonnemaisonii	Mudeford, Dorset, UK; 16.iv.2017; drift; CAM	PD2997	MK814710	10.135 / 4
			MK814711	4.926 / 7
			MK814712	6.353 / 10
			MK814713	16.133 / 15
			MK814714	18.051 / 14

			MK814715	6.714 / 6
			MK814716	10.112 / 10
			MK814717	17.019 / 21
			MK814718	2.973 / 5
Pterothamnion crispum	San Ciprián, Xove, Lugo, Spain; 21.vii.2015; subtidal (5 m); IB	SANT-29588	MK814719	7.603 / 11
			MK814720	20.685 / 21
			MK814721	4.837 / 7
			MK814722	27.993 / 23
			MK814723	30.660 / 45
			MK814724	15.472 / 19
			MK814725	2.583 / 2
			MK814726	3.075 / 1
Ptilothamnion sphaericum	Margaritas, A Coruña, Spain; 16.i.2017; intertidal; PD	PD2949	MK814727	5.109 / 8
			MK814728	11.026 / 14
			MK814729	30.626 / 36
			MK814730	20.726 / 17
			MK814731	14.322 / 15
			MK814732	9.144 / 9
			MK814733	30.672 / 44
			MK814734	3.156 / 5
Rhodomela confervoides	Díaz-Tapia et al. 2017	PD508	MF101424	
Sonderella linearis	Díaz-Tapia et al. 2017	PD1151	MF101445	
Spermothamnion repens	Margaritas, A Coruña, Spain; 16.i.2017; intertidal; PD	PD2951	MK814735	
Sphondylothamnion multifidum	Punta Insua, Carnota, A Coruña, Spain; 21.iv.2017; subtidal (2 m); IB	PD2995	MK81473	
Spyridia filamentosa	Díaz-Tapia et al. 2017	PD1020	MF101441	
Symphyocladia dendroidea	Díaz-Tapia et al. 2017	JW3780	MF101420	
Taenioma perpusillum	Díaz-Tapia et al. 2017	PD1676	MF101452	
Thaumatella adunca	Díaz-Tapia et al. 2017	PD1388	MF101447	
Thuretia quercifolia	Díaz-Tapia et al. 2017	PD1024	MF101442	

Tolypiocladia glomerulata	Díaz-Tapia et al. 2017	PD1825	MF101467	
Vertebrata australis	Díaz-Tapia et al. 2017	PD931	MF101439	
Vertebrata isogona	Díaz-Tapia et al. 2017	PD831	MF101433	
Vertebrata lanosa	Salomaki et al. 2015	-	KP308097	
Vertebrata thuyoides	Díaz-Tapia et al. 2017	PD546	MF101426	
Wrangelia sp.	Pebbly beach, Victoria, Australia; 9.xi.2016; intertidal; PD, HV & KD	PD2766	MK814737	10.289 / 5
			MK814738	8.500 / 12
			MK814739	19.311 / 18
			MK814740	10.967 / 13
			MK814741	35.573 / 33
			MK814742	40.772 / 56
OUTGROUP				
Chondrus crispus	Collén et al. 2013	PCG	NC020795	
Coeloseira compresa	Kilpatrick & Hughey 2015	UC2050599	KU053957	
Dicranema revolutum	Australia, Victoria, Pt Lonsdale; 8.xi.2014; epiphyte of *Amphibolis antarctica*; VRM	VRM0320	MK814651	
Gelidium vagum	Lee et al. 2016b	-	NC029859	
Gracilaria salicornia	Campbell et al. 2014	ARS08332	NC023785	
Hypnea pannosa	Australia, Queensland, Heron Island; 14.v.2015; reef flat	HV5551	MK814680	
Inkyuleea mariana	Merry Island, Victoria, Australia; 29.xii.2014; drift; PD	PD1141	MK814743	
Leiomenia cribosa	Australia, Victoria, Queenscliff, Popes Eye; 8.xii.2014; subtidal reef	HV05337	MK814681	
Mastocarpus papillatus	Sissini et al. 2016	UC2050562	KX525588	
Plocamium cartilagineum	Lee et al. 2016a	-	KX284727	
Rhodymenia pseudopalmata	Lee et al. 2016a	-	KX284709	
Schimmelmannia schousboei	Lee et al. 2016a	-	KX284711	
Schizymenia dubyi	Lee et al. 2016a	-	KX284712	

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