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PHYLOGENOMIC ANALYSIS OF PSEUDOCRYPTIC DIVERSITY REVEALS THE NEW GENUS *DELTALSIA* (RHODOMELACEAE, RHODOPHYTA)[1]

Pilar Díaz-Tapia [2,]

Coastal Biology Research Group, Faculty of Sciences and Centre for Advanced Scientific Research (CICA), University of A Coruña, 15071 A Coruña, Spain
Instituto Español de Oceanografía (IEO-CSIC), Centro Oceanográfico de A Coruña, Paseo Marítimo Alcalde Francisco Vázquez, 10, 15001 A Coruña, Spain

Iván Rodríguez-Buján

Coastal Biology Research Group, Faculty of Sciences and Centre for Advanced Scientific Research (CICA), University of A Coruña, 15071 A Coruña, Spain

Christine A. Maggs

Queen's University Marine Laboratory, Portaferry, Newtownards BT22 1PF, UK

and Heroen Verbruggen

School of BioSciences, University of Melbourne, Victoria 3010, Australia

Molecular analyses, in combination with morphological studies, provide invaluable tools for delineating red algal taxa. However, molecular datasets are incomplete and taxonomic revisions are often required once additional species or populations are sequenced. The small red alga *Conferva parasitica* was described from the British Isles in 1762 and then reported from other parts of Europe. *Conferva parasitica* was traditionally included in the genus *Pterosiphonia* (type species *P. cloiophylla* in Schmitz and Falkenberg 1897), based on its morphological characters, and later transferred to *Symphyocladia* and finally to *Symphyocladiella* using molecular data from an Iberian specimen. However, although morphological differences have been observed between specimens of *Symphyocladiella parasitica* from northern and southern Europe they have yet to be investigated in a phylogenetic context. In this study, we collected specimens from both regions, studied their morphology and analyzed *rbc*L and *cox*1 DNA sequences. We determined the phylogenetic position of a British specimen using a phylogenomic approach based on mitochondrial and plastid genomes. Northern and southern European populations attributed to *S. parasitica* represent different species. *Symphyocladiella arecina* sp. nov. is proposed for specimens from southern Europe, but British specimens were resolved as a distant sister lineage to the morphologically distinctive *Amplisiphonia*, so we propose the new genus *Deltalsia* for this species. Our study highlights the relevance of using materials collected close to the type localities for taxonomic reassessments, and showcases the utility of genome-based phylogenies for resolving classification issues in the red algae.

Key index words: **Ceramiales; mitochondrial genomes; morphological plasticity; new species; plastid genomes; pseudocryptic species; *Pterosiphonia*; Pterosiphonieae; *Symphyocladiella*; turf-forming species**

***Abbreviations*: CTAB, cetyltrimethylammonium bromide; HTS, high-throughput sequencing; ML, maximum likelihood**

Red algal species description and classification were primarily based on morphological features until the late 20th century (e.g., Schmitz and Falkenberg 1897, Kylin 1956). The first applications of molecular tools to reassess red algal classification, typically using one or two molecular markers, resulted in the reassignment of numerous taxa (e.g., Saunders and Kraft 1994, 1996, Choi et al. 2002, Harper and Saunders 2002). However, evolutionary relationships among lineages have often remained uncertain due to the low resolution of commonly used molecular markers (Verbruggen et al. 2010).

The development of high-throughput sequencing (HTS) techniques has made organellar genome

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[2]Author for correspondence: e-mail p.diaz@udc.es
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sequencing cost-effective (Oliveira et al. 2018). Red algal plastid genomes contain about 200 genes and have been used to construct well-resolved phylogenies, greatly outperforming single-locus phylogenies (Costa et al. 2016, Díaz-Tapia et al. 2017c, 2019, Preuss et al. 2020). Mitochondrial genomes contain far fewer genes, about 25, and have also been used in phylogenetic studies (Nan et al. 2017, Iha et al. 2018, Kim et al. 2021). Both plastid and mitochondrial phylogenies have solved difficult problems in classification, demonstrating the enormous potential of phylogenomic approaches for advancing algal systematics (Costa et al. 2016, Díaz-Tapia et al. 2017c, 2019, Lyra et al. 2021).

At the species level, the application of integrative taxonomy, combining morphological and molecular observations, has rapidly accelerated red algal species discovery (Leliaert et al. 2014). As a result, numerous overlooked, cryptic and pseudocryptic species have been described in the last 15 years (e.g., Schneider et al. 2017, Guimarães et al. 2019, Díaz-Tapia et al. 2020a, 2021, Cabrera et al. 2021, Rodríguez-Buján et al. 2021). Molecular tools are particularly useful for detecting cryptic species, as well as for distinguishing between pseudocryptic species and species that exhibit high phenotypic plasticity (Díaz-Tapia et al. 2020b, Gurgel et al. 2021). Accordingly, current practice in red algal taxonomy uses a combination of molecular and morphological information.

Despite increased sequencing of red algal DNA in recent years, many species have not yet been molecularly characterized. Consequently, taxonomic proposals are often based on incomplete molecular datasets, and taxonomic revisions may be required once additional species or specimens are sequenced. For example, the recently described *Pterosiphonia arenosa* from Korea was placed in synonymy with *Symphyocladiella spinifera* based on subsequent sequencing of topotype material of the latter from South America (Kim et al. 2012b, Bustamante et al. 2016a). Likewise, once sequences were available for previously published generitypes, some genera have been merged, such as *Neosiphonia* with *Melanothamnus* and *Coronaphycus* with *Corynecladia* (Kim and Lee 1999, Metti et al. 2015, Díaz-Tapia et al. 2017b, Cassano et al. 2019).

Members of the Pterosiphonieae include genera with problematic tribe-level placement due to reinterpretation of morphological character traits in successive classifications. Also, some genera and pseudocryptic species have recently been described based on integrative approaches (Savoie and Saunders 2016, Díaz-Tapia et al. 2017c, 2020b, Bustamante et al. 2019). Of the genera in the Pterosiphonieae, *Pterosiphonia* (type species *P. cloiophylla*) has the largest number of described species (Guiry and Guiry 2022). It included eight species with a bilateral branching pattern in Falkenberg's monograph of the Rhodomelaceae (Falkenberg 1901), but expanded to 36 species names after the transfer of taxa from other genera and the description of new species (Guiry and Guiry 2022). Of these, 19 species were transferred to *Symphyocladia*, *Xiphosiphonia* and five other rhodomelacean genera and seven species are considered heterotypic synonyms of other taxa (Savoie and Saunders 2016, Guiry and Guiry 2022). In turn, a further analysis of *Symphyocladia* led to the segregation of *Symphyocladiella* (Bustamante et al. 2019). As a result, *Pterosiphonia* at present includes ten species (Guiry and Guiry 2022).

One of the species traditionally placed in *Pterosiphonia* (as *P. parasitica)* is *Conferva parasitica*, originally described from the British Isles as an epiphyte on other seaweeds (Hudson 1762, Falkenberg 1901). It was subsequently reported along Northeast Atlantic coasts from Norway to Morocco as well as in the Mediterranean Sea (Maggs and Hommersand 1993, Gómez Garreta et al. 2001, Díaz-Tapia and Bárbara 2013). It was recently transferred to *Symphyocladia*, and subsequently to *Symphyocladiella* based on molecular data from an Iberian specimen (Savoie and Saunders 2016, Bustamante et al. 2019). There are minor but consistent morphological differences between populations in the British Isles and Iberian and Mediterranean populations (Falkenberg 1901, Maggs and Hommersand 1993, Díaz-Tapia and Bárbara 2013, García-Redondo et al. 2016, as *P. parasitica*). The aims of this study are (1) to analyze the morphological variability of *S. parasitica* in a phylogenetic context to determine whether it corresponds to plasticity or indicates the existence of pseudocryptic species, and (2) to reassess the generic assignment of *S. parasitica* using a phylogenomic approach.

MATERIALS AND METHODS

Collections and morphological observations. Nine samples tentatively identified as *Symphyocladiella parasitica* were collected from 2011 to 2021 in the UK (4 samples), Norway (2 samples) and Atlantic Spain (3 samples; Table S1 in the Supporting Information). Part of each sample was preserved in silica gel for DNA extraction, while the remaining material was preserved for morphological study in 4% formalin seawater at 4°C and stored in the dark.

For morphological observations, specimens were mounted in 20% Karo® Syrup (ACH Foods, Memphis, TN, USA) and 80% distilled water. Sections for microscopic observations were made by hand using a razor blade. Morpho-anatomical studies were performed using light microscopy. Voucher specimens were deposited in the herbarium of the University of Santiago de Compostela (SANT); herbarium acronyms follow Thiers (2022, continuously updated). Current names and taxonomic synonyms were checked in AlgaeBase (Guiry and Guiry 2022).

DNA extraction and preliminary analyses of the rbc*L gene.* DNA was extracted from silica gel-dried material using an adapted cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987, Cremen et al. 2016). PCR amplification was carried out for *rbc*L gene using the primers F7/R753, F645/rbcSstart, F57/rbcLrevNEW and F492 or F2/R1464 (Freshwater and Rueness 1994, Gavio and Fredericq 2002, Saunders

and Moore 2013, Díaz-Tapia et al. 2018). Reactions were performed in a total volume of 25 μL, consisting of 1× MyTaqTM reaction buffer, 0.28 μM of forward and reverse primers, 0.125 units My TaqTM DNA Polymerase (Bioline, London, UK) and 1 μL template DNA. The PCR profile consisted of initial denaturation (93°C for 3 min), 35 cycles of denaturation (94°C for 30 s), primer annealing (45°C for 30 s), and extension (74°C for 90 s), and final extension (74°C for 5 min). The PCR products were purified and sequenced commercially by Macrogen Inc. (Madrid, Spain). In total, nine *rbc*L sequences were newly determined in this study (Table S1).

Newly determined sequences of the *rbc*L gene were aligned with previously published sequences of species of the tribe Pterosiphonieae to achieve an initial assessment of the relationships of *Symphyocladiella parasitica* and other members of this tribe using an IQ-Tree phylogeny (Fig. S1 in the Supporting Information).

Organellar phylogenomics. Based on the preliminary *rbc*L gene tree that included *Symphyocladiella parasitica* sequences from northern and southern Europe, *S. parasitica* was placed in a highly supported clade including the genera *Amplisiphonia*, *Dictyomenia*, *Pterosiphonia*, *Rhodomelopsis*, *Symphyocladia*, *Symphyocladiella, Tayloriella* and *Xiphosiphonia*. This clade has been consistently resolved with high support in previously published phylogenies based on plastid genomes and single-/two-marker analyses (Savoie and Saunders 2016, Díaz-Tapia et al. 2017c, Bustamante et al. 2019). Along with *S. parasitica* from the British Isles, we selected one species of each genus in this clade for phylogenomic analyses using either previously published or newly determined organellar genomes using high throughput sequencing (Table S2 in the Supporting Information). We also used *"Melanothamnus" gigas*, *"Polysiphonia"* sp., *Herposiphonia versicolor* and *Dipterosiphonia australica* as outgroups based on Díaz-Tapia et al. (2017c).

DNA, extracted as described in the previous section, was used to prepare barcode sequencing libraries (350 nt) with the TruSeq Nano LT kit (Illumina, San Diego, CA, USA) or NEBNext® DNA Library Prep Kit (NEB, Ipswich, MA, USA). Libraries were sequenced either on Illumina HiSeq 2000 at the Genome Center of the Cold Spring Harbor Marine Laboratory, Illumina NextSeq at Georgia Genomics Facility or Illumina SBS at Novogene. 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.

We assembled a dataset consisting of the 15 newly determined mitochondrial genomes for the family Rhodomelaceae (Table S2). A second dataset included seven newly determined plastid genomes and eight previously published (Table S2). All protein coding genes were aligned at the amino acid level using MAFFTv7.245 (Katoh and Standley 2013) with default settings and checked visually in Geneious 7.0.6 (Biomatters, Auckland, New Zealand). Nucleotide alignments were constructed based on the inferred amino acid alignments using TranslatorX (Abascal et al. 2010), and the alignments were concatenated. Models of nucleotide evolution were selected based on the Bayesian Information Criterion using ModelFinder in IQ-Tree (Kalyaanamoorthy et al. 2017). The models TVM + F + R4 and GTR + F + R6 were used for the mitochondrial and plastid datasets, respectively (Tavaré 1986, Posada 2003). Maximum likelihood (ML) phylogenetic trees were inferred in IQ-TREE v2.1.2 (Minh et al. 2020) and branch support was determined using 1000 replicates for nonparametric bootstrap (Felsenstein 1985) and ultrafast bootstrap (Hoang et al. 2018).

To obtain a phylogenetic tree including species for which full organellar genomes are not available, we also assembled a dataset containing 20 and 27 additional *cox*1 and *rbc*L sequences, respectively, of species belonging to the studied genera that were downloaded from GenBank (Table S1). One sequence was included per species, selecting the longest one when several were available, with the exception of *Symphyocladiella dendroidea* for which one sequence per haplotype was included. We separately aligned sequences of *rbc*L and *cox*1 genes using Geneious 7.0.6, and sequence divergence among species and genera was calculated as uncorrected p-distances. We concatenated both alignments and a phylogeny was constructed from this dataset using the plastid genome phylogeny (as explained above) as a backbone constraint. This approach leverages the strong signal of the plastid genomes while adding into the phylogeny the extra sequences of 25 species based on the *cox*1 and *rbc*L data available for them. Data were analyzed as plastid and mitochondrial datasets, using the TIM2 + F + I + G4 model (Posada 2003).

RESULTS

There was very little structural variation among species in either plastid or mitochondrial genomes (Tables S3 and S4 in the Supporting Information). The aligned data set of plastid and mitochondrial genomes consisted of 192 and 23 concatenated genes amounting to 143,901 and 17,706 nucleotides, respectively, from 15 rhodomelacean species, including 11 species of the tribe Pterosiphonieae and four other species in the family. Topologies of plastid and mitochondrial phylogenies were identical and both were generally well-resolved (Fig. 1). However, while plastid phylogeny resolved all nodes with full or high support, several nodes remained unresolved in the mitochondrial tree. In particular, the relationship between *Symphyocladiella parasitica* (as *Deltalsia* in Fig. 1) from the British Isles and *Amplisiphonia pacifica* was unsupported in the mitochondrial phylogeny.

The constrained tree using the plastid nucleotide genome-scale tree as backbone and adding concatenated *cox*1 and *rbc*L gene sequences of 25 additional species (Fig. 2) was congruent with the genome-scale trees. Within the Pterosiphonieae clade, samples of *Symphyocladiella parasitica* from the British Isles (the type locality of this species), and from the Iberian Peninsula were placed in two distantly related clades. Our phylogeny strongly supports the placement of the British *S. parasitica* as sister of *Amplisiphonia* (100% bootstrap support), rejecting the possibility that it belongs to the genus *Symphyocladiella*. Accordingly, we propose the new genus *Deltalsia* to accommodate the species originally described from England as *Conferva parasitica*. Uncorrected genetic divergence between *Deltalsia* and *Amplisiphonia* was 5.5–5.9% and 10.3–11.1% in the *rbc*L and *cox*1 genes, respectively. These values are similar to or higher than the sequence divergences among other genera of the same lineage in the Pterosiphonieae (Table S5 in the Supporting Information). The Iberian *S. parasitica* was resolved as sister to *S. dendroidea* with full support, and we propose the new species *S. arecina*.

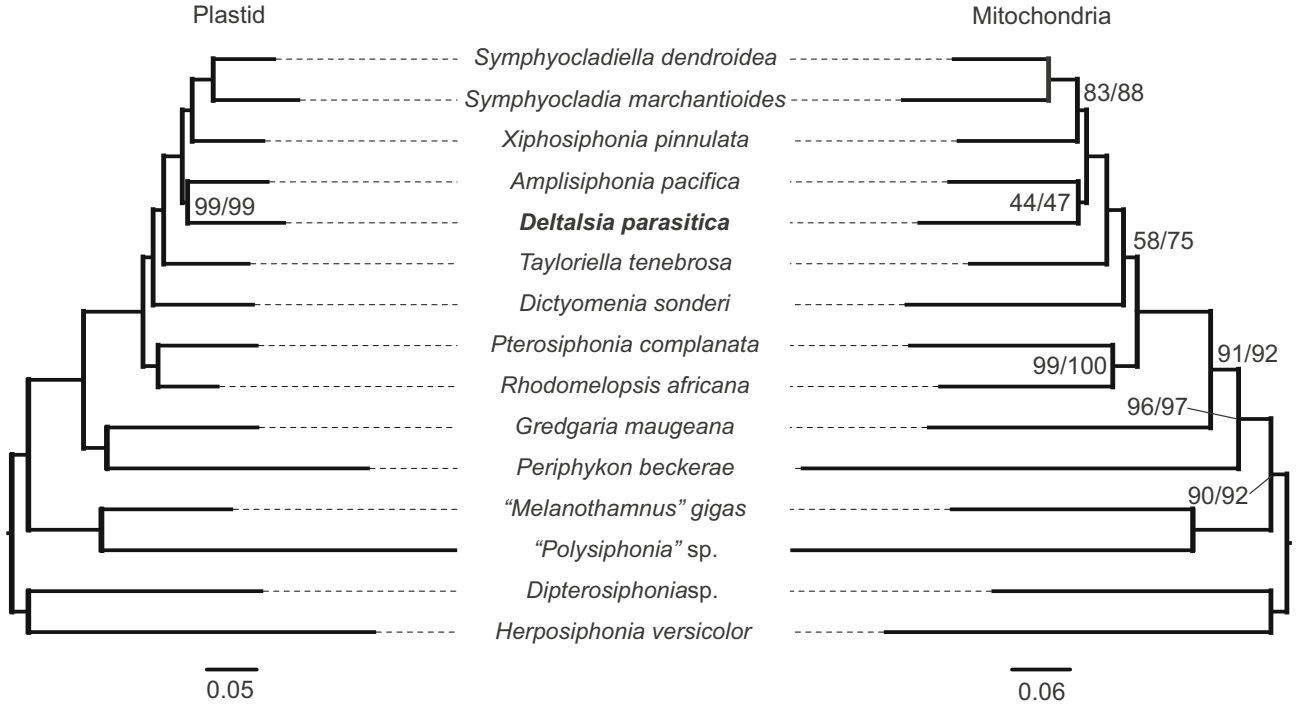

FIG. 1. ML phylogeny of the tribe Pterosiphonieae based on nucleotide alignment of the 189 and 23 concatenated genes from the plastid (left) and mitochondrial (right) genomes. All branches have full bootstrap support except those where bootstrap values are indicated on branches (as nonparametric/ultrafast bootstrap).

The uncorrected genetic distance in the *cox*1 gene between *Deltalsia parasitica* and *Symphyocladiella arecina* was 11.4% (68 base pairs, bp), and 3.2% (19 bp) between *S. arecina* and *S. dendroidea*. Unfortunately, only one *cox*1 gene sequence was available for each species, and the potential intraspecific variability for this molecular marker could not be assessed. We also compared the uncorrected genetic distance of the *rbc*L gene, for which considerably more sequences are available. Sequences of *D. parasitica* from northern Europe, including the six newly determined in our study as well as a GenBank sequence (MN184567) from Norway labeled as *Symphyocladia* sp. were identical. Likewise, the four sequences of *S. arecina* from southern Europe were identical. By contrast, the 29 sequences of *S. dendroidea* represented seven haplotypes that diverged by 0.4–1% (3–9 bp). Sequences of *D. parasitica* and *S. arecina* diverged by 5.9–6.6% (79–86 bp). The distance between *S. arecina* and the seven haplotypes of the closely related *S. dendroidea* was 1–1.7% (8–17 bp).

Taxonomic proposals. Deltalsia *Díaz-Tapia & Rodríguez-Buján gen. nov.*: Diagnosis: *Deltalsia* is distinguished from other genera in the tribe Pterosiphonieae by thalli composed of prostrate and erect axes that are triangular in shape and alternately branched every 2 segments; ecorticate axes have 6–8 pericentral cells and are cylindrical or subcylindrical; the erect axes and the first-order branches are of indeterminate growth with apical cells of branches that typically are level with or remain below the main apical cell; the branches of the first order coalesced with main axes over 0.7–1.3 segments, bearing 2–3 orders of branches of determinate growth, alternately arranged; vegetative trichoblasts are absent; and tetrasporangia are arranged in spiral series.

Etymology: From the Greek *"delta"* referring to the triangular outline of the erect axes of the type species and the Latin *"alsia"* referring to the cold-water affinity of the type species.

Type species: *Deltalsia parasitica* (Hudson) Díaz-Tapia & Rodríguez-Buján comb. nov.

Deltalsia parasitica *(Hudson) Díaz-Tapia & Rodríguez-Buján comb. nov. (Fig. 3)*: Basionym: *Conferva parasitica* Hudson 1762: 486. *Flora anglica; exhibens plantas per regnum angliae sponte crescentes, distributas secundum systema sexuale*: cum *differentiis specierum, synonymis auctorum, nominibus incolarum, solo locorum, tempore florendi, officinalibus pharmacopoeorum*. Prostant venales apud J. Nourse in the Strand, et C. Moran in Covent-Garden, London.

Neotype: BM-K, Herb. Lightfoot, BM000807112 (designated by Maggs and Hommersand 1993).

Neotype locality: Yorkshire, England, United Kingdom (Maggs and Hommersand 1993).

Homotypic synonyms: *Hutchinsia parasitica* (Hudson) C.Agardh, *Polysiphonia parasitica* (Hudson) Greville, *Pterosiphonia parasitica* (Hudson) Falkenberg,

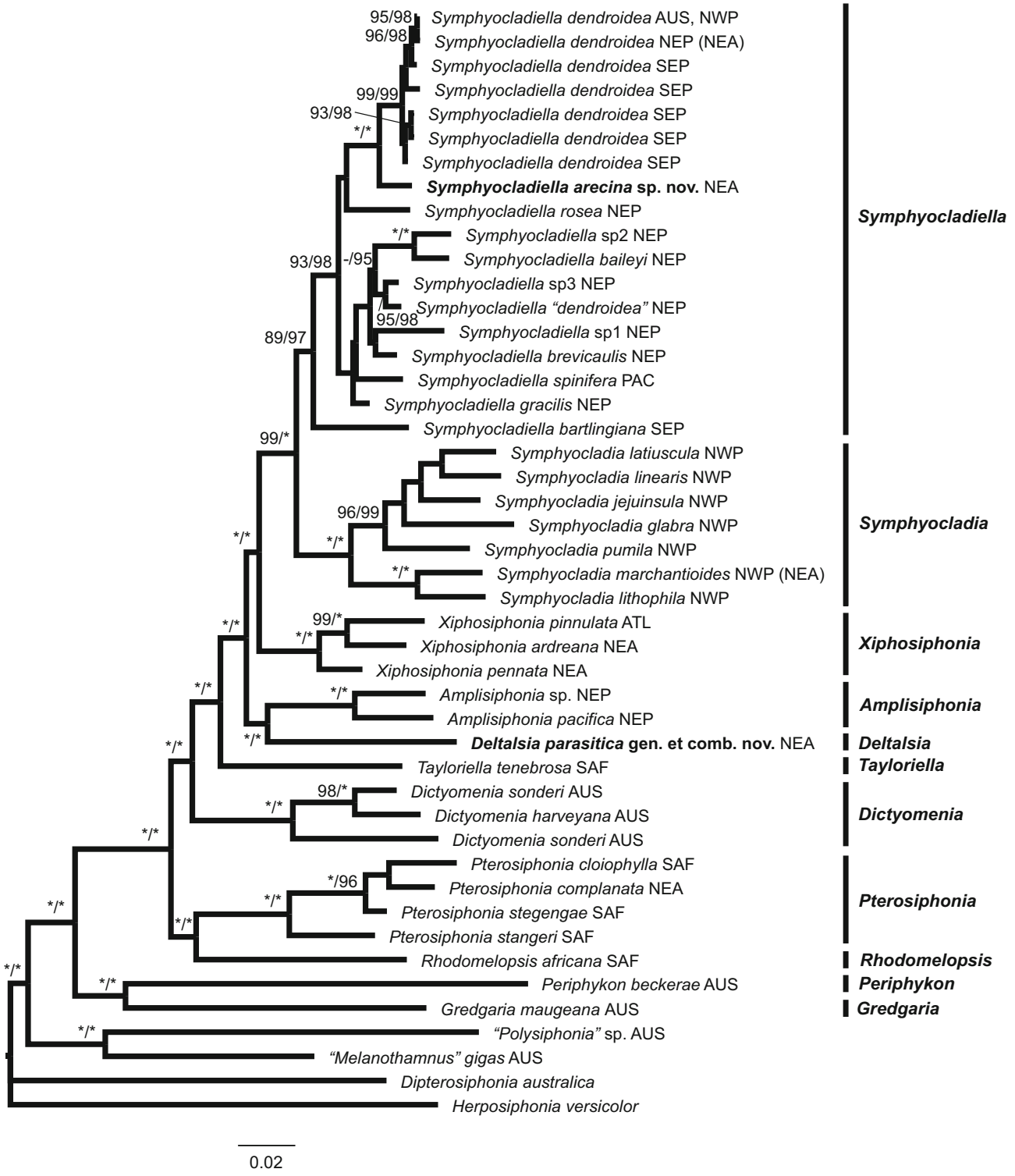

FIG. 2. ML tree of the tribe Pterosiphonieae using the genome-scale plastid phylogeny based on nucleotides as a constraint and incorporating *cox*1 and *rbc*L gene sequences for additional 31 species. Branch support values are indicated on branches as nonparametric bootstrap/ultrafast bootstrap when ≥85% and >95%, respectively; asterisks represent full support. Codes after species names indicate their distribution confirmed by molecular information, including between brackets introduced regions (ATL: Atlantic; AUS: Australia; NEA: northeastern Atlantic; NEP: northeastern Pacific; NWP: northwestern Pacific; PAC: Pacific; SAF: southern Africa; SEP: southeastern Pacific).

Symphyocladia parasitica (Hudson) Savoie & G.W.Saunders, *Symphyocladiella parasitica* (Hudson) D.Bustamante, B.Y.Won, S.C.Lindstrom & T.O.Cho, *Vertebrata parasitica* (Hudson) Kuntze.

Heterotypic synonyms: *Hutchinsia moestingii* Lyngbye, *Polysiphonia moestingii* (Lyngbye) Sprengel (Guiry and Guiry 2022).

Description: Thalli forming tufts up to 5 cm high, consisting of a short system of prostrate axes bearing rhizoids ventrally and erect axes dorsally. Thalli bright red in color with a rigid texture. Erect axes with distinct main axes that bear 3–4 orders of branches alternately arranged (Fig. 3a). The length of the lateral branches decreases upward, so that the erect axes have a triangular outline (Fig. 3b).

Axes cylindrical or subcylindrical, ecorticate, with 6–8 pericentral cells (Fig. 3, c and d). Prostrate axes bearing rhizoids at irregular intervals. Rhizoids cut-off from pericentral cells, consisting of a filament often terminating in a discoid pad composed of filaments up to four cells in length (Fig. 3e).

Erect axes growing from apical cells 10–20 μm in diameter, increasing to 230–350 μm in diameter in mid and basal parts. Erect axes and first-order branches of indeterminate growth, growing by the division of domed apical cells. Apical cells of young branches typically level with or not reaching the apical cells of main axes (Fig. 3f), occasionally overtopping them. Erect axes bearing branches every 2 segments; branches of the first order coalesced with main axis over 0.7–1.3 axial segments (Fig. 3g). Branches of second or higher orders of determinate growth, terminated by triangular apical cells once growth is complete (Fig. 3h). Vegetative trichoblasts absent.

Tetrasporangia subspherical, 100–120 × 100–130 μm, formed on last-order determinate branches in short spiral series that are often interrupted, distorting branches when mature (Fig. 3i). Tetrasporangia with two presporangial and one post sporangial cover cells (Fig. 3j). Male and female reproductive structures not observed in this work,

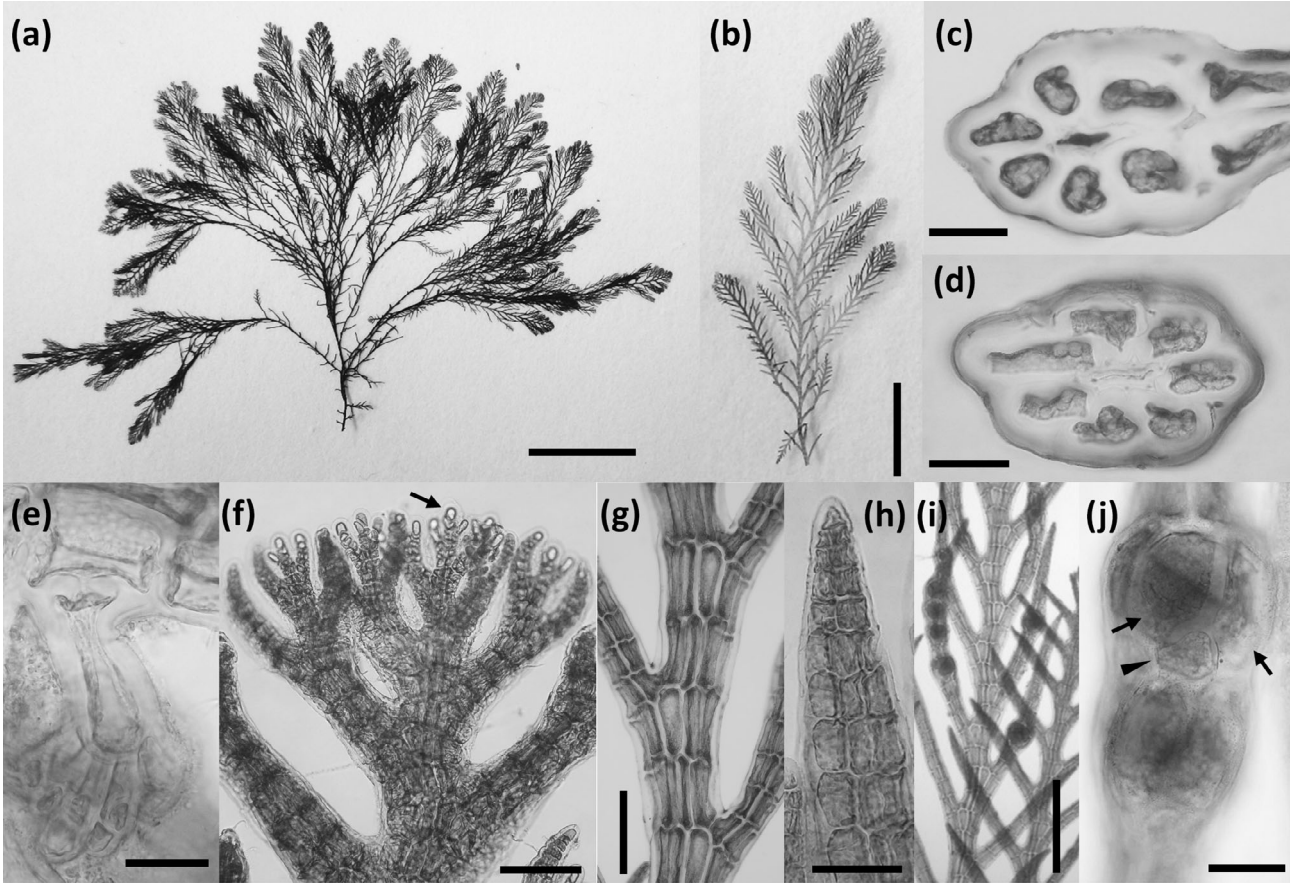

FIG. 3. *Deltalsia parasitica*. (a) Habit. (b) Erect axes with lateral branches of determinate growth decreasing in length upward. (c and d) Cross-section of erect axes with six and seven pericentral cells. (e) Rhizoid cut-off from a pericentral cell, terminating in a multicellular discoid pad. (f) Apex of an erect axis with apical cells of lateral branches at the level of the apical cell (arrow) of the main axes. (g) Main erect axes with determinate branches coalescent over 0.7 segments. (h) Branch of second-order with a triangular apical cell. (i) Tetrasporangia forming spiral series on last-order branches. (j) Tetrasporangia with two presporangial (arrows) and one postsporangial (arrowhead) cover cells. Scale bars: a, 1 cm; b, 5 mm; c, 70 μm; d, 60 μm; e, 30 μm; f, 80 μm; g, 270 μm; h, 50 μm; i, 670 μm; j, 60 μm.

but previously described for the British Isles (Maggs and Hommersand 1993).

Symphyocladiella arecina *Díaz-Tapia & Rodríguez-Buján sp. nov. (Fig. 4)*: Etymology: Referring to the resemblance of the outline morphology of erect axes to the leaves of the land plant *Areca*.

Holotype: Collected from sand-covered low intertidal rocks on 22 March 2011, leg. Pilar Díaz-Tapia and Ignacio Bárbara. SANT-Algae 25,631.

Type locality: La Arena, Basque Country (Spain).

Additional specimens examined: Table S1.

Description: Thalli forming turfs up to 3 cm high, consisting of an extensive system of prostrate axes bearing rhizoids ventrally and erect axes dorsally (Fig. 4a). Thalli pink to brownish red in color with a fairly rigid texture. Erect axes with a distinct main axis bearing 2–3 orders of alternately arranged branches (Fig. 4a). The length of the lateral branches decreases at the tips, maintaining a similar length throughout most of the main erect axes (Fig. 4a).

Axes ecorticate, with (7-) 8–9 pericentral cells (Fig. 4b). Prostrate axes cylindrical, with rhizoids cut-off from pericentral cells, consisting of a filament often terminating in a discoid pad composed of filaments of up to 3 cells in length (Fig. 4c).

Erect axes subcylindrical, with main axes of indeterminate growth, terminating in domed apical cells 15–22 μm in diameter, increasing to 250–400 μm in diameter in median and basal parts. Lateral branches of determinate growth formed every 2 segments, with domed apical cells that become triangular once growth is complete (Fig. 4, d and e). Apical cells of the main axes and young lateral branches overtopped by lateral branches (Fig. 4, d and f). Occasionally, some first-order lateral branches, formed at irregular intervals, are of indeterminate growth (Fig. 4a). Branches of the first order coalesce with main axis over 2–3 axial segments (Fig. 4, g and h). Vegetative trichoblasts absent.

Reproductive structures not reported here, but have previously been described for specimens from Atlantic Spain (Díaz-Tapia and Bárbara 2013, García-Redondo et al. 2016).

GenBank accession number of the holotype: KF648524 (*cox*1 gene), OP186026 (*rbc*L gene).

Habitat: *Symphyocladiella arecina* was found from the low intertidal to 20 m depth. In the intertidal, it grows as part of algal turfs on sand-covered rocks, usually mixed with other species such as *Rhodothamniella floridula*, *Xiphosiphonia pinnulata* and *X. ardreana*. In the subtidal, it grows in a variety of habitats including subtidal seaweed assemblages growing in *Zostera marina* meadows, subtidal rocky substrates often covered by sediments, maërl beds and pebbles.

DISCUSSION

Symphyocladiella arecina *sp. nov.: A pseudocryptic species*. Molecular analyses of populations of *"Symphyocladiella" parasitica* from northern and southern Europe showed that this taxon name actually involves two species (*Deltalsia parasitica* and *S. arecina*) that are only distantly related phylogenetically. This finding is unsurprising considering the morphological differences previously highlighted between northern and southern European populations of *"Symphyocladiella" parasitica* (Falkenberg 1901,

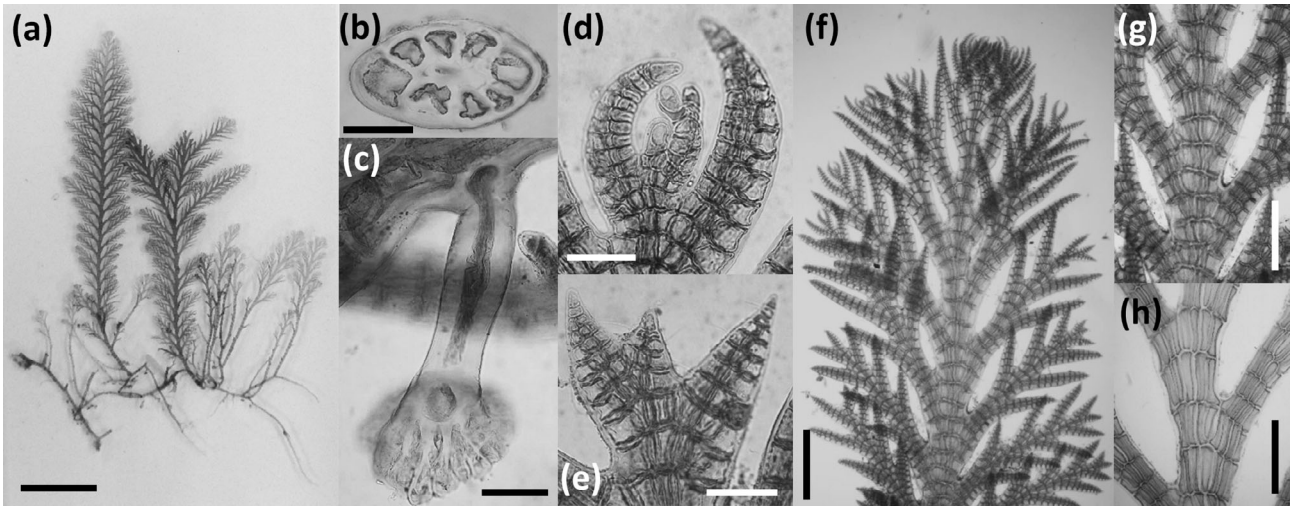

FIG. 4. *Symphyocladiella arecina*. (a) Habit. (b) Cross-section of an erect axis with eight pericentral cells. (c) Rhizoid cut-off from a pericentral cell, terminating in a multicellular discoid pad. (d) Apex of an erect axis, with a domed apical cell overtopped by young branches. (e) Apex of a mature determinate branch with a triangular apical cell. (f) Apical part of an erect axis. (g and h) Erect axis bearing branches every two segments that are coalesced with the main axis over 2–3 segments. Scale bars: a, 5 mm; b, 80 μm; c, 60 μm; d, 50 μm; e, 60 μm; f, 450 μm; g, 300 μm; h, 400 μm.

Díaz-Tapia and Bárbara 2013, García-Redondo et al. 2016; as *Pterosiphonia parasitica*). *Deltalsia parasitica* and *S. arecina* can be distinguished by the type of growth of first-order branches, the number of pericentral cells, the coalescence between first-order branches and the main erect axes, and the arrangement of tetrasporangial series (Table 1). Interestingly, the identification of Atlantic and Mediterranean forms of the species has been discussed and some of these differences were illustrated in floristic accounts from Naples and the British Isles (Harvey 1848, Falkenberg 1901). However, these morphological differences were not considered sufficient to distinguish species, and Atlantic and Mediterranean forms have been recorded under the same name throughout Europe (Falkenberg 1901). Therefore, *S. arecina* is an example of pseudocryptic species rather than a morphological form of *D. parasitica*. This is in line with the finding of numerous new red algal species for which morphological variability was traditionally interpreted as intraspecific plasticity, but are actually different species discovered by the application of molecular approaches (Freshwater and Shahnaz 2019, Díaz-Tapia et al. 2020b, Gurgel et al. 2021).

Symphyocladiella arecina was, instead, more closely related to and morphologically more similar to the Pacific species *S. dendroidea* (Table 1). The seven known haplotypes of *S. dendroidea* are more closely related to each other than any of these haplotypes is to *S. arecina*, even though the uncorrected p-distances between the haplotypes of *S. dendroidea* (0.4–1%) slightly overlap with their distance from the only known haplotype of *S. arecina* (1–1.7%). Moreover, *S. arecina* and *S. dendroidea* can be morphologically distinguished by the thallus length, the number of pericentral cells, and the occurrence of vegetative trichoblasts and cortication (Table 1). In spite of these differences, most of which can overlap, small specimens of *S. dendroidea* can be very similar to *S. arecina*, and molecular analysis is recommended for accurate identification of specimens <3 cm. This is particularly relevant in Europe, where *S. dendroidea* has been recorded as an introduced species (Boudouresque and Verlaque 2008 as *Pterosiphonia tanakae*, Díaz-Tapia et al. 2018).

Our molecular data showed that *Deltalsia parasitica* is restricted to northern Europe, while the presence of *Symphyocladiella arecina* has only been demonstrated in the northern Iberian Peninsula. However, the available morphological information for specimens from Atlantic coasts of Morocco and the Mediterranean suggests that they correspond to *S. arecina* (Falkenberg 1901, Gayral 1958). *Deltalsia parasitica* has been also recorded as *Pterosiphonia* in the Canary Islands, Azores, Angola and South Africa (Guiry and Guiry 2022), but morphological descriptions from these regions could not be found. Moreover, the variety *Pterosiphonia parasitica var. australis* was described from Brazil and later transferred to *Symphyocladia* to be finally placed in *Symphyocladiella* (Joly et al. 1967, Wynne 2017, 2022). This variety clearly differs from *D. parasitica* but is more similar to *S. dendroidea* (Table 1). In its original description, it was separated from *S. dendroidea* based on its shorter coalescence between main axes and branches (3 vs. 4 segments) and the higher number of second-order branches growing on each first-order axis (6–10 vs. > 20; Joly et al. 1967). However, recent descriptions of *S. dendroidea* show that it can have a shorter branch coalescence and more second-order branches (Uwai and Masuda 1999a, as *Pterosiphonia tanakae*; Bustamante et al. 2016a, as *P. dendroidea*), overlapping with the characters proposed for separating the variety. Accordingly, its placement as a variety of *D. parasitica* is untenable

TABLE 1. Comparison of selected morphological characters among species/varieties that are morphologically most similar to *Deltalsia parasitica* and *Symphyocladiella arecina*.

	Deltalsia parasitica	*Symphyocladiella arecina*	*Symphyocladiella dendroidea*	*Symphyocladiella parasitica* var. *australis*
Thallus length (cm)	5	1–3	1–7	4
Growth of first-order branches	Indeterminate	Determinate; occasionally indeterminate	Determinate and indeterminate	Determinate (indeterminate not described or illustrated)
Pericentral cells	6–8	(7-) 8–9	7–12	6–10
Coalescence between branches and main axes (segments)	0.7–1.3	2–3	1.5–2.5	3
Trichoblasts	Absent	Absent	Present	-
Cortication	Absent	Absent	Basal parts of largest specimens	Basal parts of largest specimens
Series of tetrasporangia	Interrupted, distorting branches	Continuous, not distorting branches	Continuous, not distorting branches	Interrupted, not distorting branches
References	3, 5, 7	2, 3, 7	1, 6	4

- = not described.

References: (1) Bustamante et al. 2016b (as *Pterosiphonia dendroidea*); (2) Díaz-Tapia and Bárbara 2013; (3) Falkenberg 1901; (4) Joly et al. 1967; (5) Maggs and Hommersand 1993; (6) Uwai and Masuda 1999a (as *P. tanakae*); (7) This study.

and molecular analysis of specimens of this variety are required to clarify its taxonomic identity and its relationship with *S. dendroidea*.

All other species of *Symphyocladiella* are restricted to Pacific coasts, with the only exception being the introduction of *S. dendroidea* to Europe (Boudouresque and Verlaque 2008 as *Pterosiphonia tanakae*; Díaz-Tapia et al. 2018, Guiry and Guiry 2022). The occurrence of *S. arecina* in southern Europe, disjunct from the natural range of its congeners, is puzzling. A plausible explanation is that *S. arecina* could be a human-mediated old introduction in southern Europe. Numerous cryptic or cryptogenic species have been reported in the Ceramiales (Williams and Smith 2007, Thomsen et al. 2016, Díaz-Tapia et al. 2017a, Sherwood et al. 2020, Piñeiro-Corbeira et al. 2020a,b), highlighting the need to improve molecular datasets in this diverse red algal order for which only a small proportion of the diversity has been molecularly characterized. However, the introduction hypothesis cannot be confirmed for *S. arecina*, as its potential distribution beyond southern Europe is at present unsupported by the available molecular information. In this regard, in addition to Brazilian specimens of *S. parasitica* var. *australis*, further molecular data for *Symphyocladiella* spp. from Namibia and Pacific South America could be particularly relevant. In Namibia, specimens very similar in morphology to *S. arecina* have been described as *Pterosiphonia* cf. *dendroidea* (Rull Lluch 2002). In Pacific South America, *S. dendroidea* has been reported from Peru to southern Chile (Ramírez and Santelices 1991), but the potential genetic variability of *S. dendroidea*, or even the possible occurrence of cryptic species, has not been investigated in detail. The only molecular data from Peru and the Chilean Juan Fernández archipelago showed a relatively high genetic diversity in the *rbc*L gene congruent with the distribution of the collected samples (Díaz-Tapia et al. 2018).

Phylogenetic position of Deltalsia parasitica *gen. et comb. nov.*. Our phylogenomic analysis resolved *Deltalsia parasitica* as sister to *Amplisiphonia*. The latter genus includes a single recognized species and an undescribed cryptic species uncovered in recent molecular diversity surveys in Pacific North America (Savoie and Saunders 2016). These two species, *Amplisiphonia pacifica* and *Amplisiphonia* sp., are characterized by a blade-like habit as the result of the complete fusion of axes, clearly differing from *Deltalsia* in which the fusion between branches and the main axes is restricted to 0.7–1.3 segments (Hollenberg 1939, Savoie and Saunders 2016). Moreover, *Amplisiphonia* has a lower number of pericentral cells than *Deltalsia* (5 vs. 6–8). Given the relevant morphological differences between these sister lineages, we propose a new genus.

Morphologically, *Deltalsia* is more similar to nonsister genera such as *Pterosiphonia, Symphyocladia*, *Symphyocladiella* and *Xiphosiphonia* than to *Amplisiphonia*. Among the similarities are that erect axes are mostly subcylindrical or complanate (thalli are foliose in some *Symphyocladia* spp.) and that thalli are composed of a main axis with branches alternately arranged at regular intervals (Maggs and Hommersand 1993, Kim et al. 2012a, Savoie and Saunders 2016, Bustamante et al. 2019). These four genera have been distinguished by a combination of morphological characters that primarily include the number of pericentral cells, the presence or absence of cortication and the extent of the congenital fusion between axes and branches (Table 2). These three characters clearly separate *Deltalsia* from *Pterosiphonia* and *Symphyocladia*, but show overlap of *Deltalsia* with *Symphyocladiella* and *Xiphosiphonia* (Table 2). The most relevant character that distinguishes *Deltalsia* from other similar Pterosiphonieae is the triangular shape of the thallus due to first-order branches having indeterminate growth. The other genera have first-order branches with only determinate growth, or branches of determinate and indeterminate growth alternating along the main axis. Moreover, the simple first-order branches of *Xiphosiphonia*, and the straight arrangement of tetrasporangia in several genera, also differ from *Deltalsia* (Table 2).

Interestingly, even though *Deltalsia* has a very similar body plan to *Xiphosiphonia* and *Symphyocladiella*, they do not constitute a monophyletic group. The clade that includes these three genera also contains *Amplisiphonia* and *Symphyocladia*, so it encompasses high morphological diversity, from filamentous species with thin axes and a short congenital fusion between branches and the main axes (*Deltalsia*, *Xiphosiphonia*, *Symphyocladiella*) to species with complete fusion resulting in foliose thalli (*Amplisiphonia* and some *Symphyocladia*). Evolutionary schemes based on morphological characters and ancestral character phylogenetic reconstructions of the order Ceramiales suggest that the ancestor of the Pterosiphonieae was filamentous (Falkenberg 1901, Díaz-Tapia et al. 2019). Accordingly, it is plausible to hypothesize that filamentous genera retained the ancestral traits, and the foliose character has evolved several times independently in the clade.

Beyond the interest of the taxonomic results here presented, our work further shows the utility of phylogenomic approaches to resolve classification issues in red algae (e.g., Costa et al. 2016, Díaz-Tapia et al. 2017c, 2019, Lyra et al. 2021). Our preliminary analyses based on the *rbc*L gene, the most widely used molecular marker in red algal phylogenetic reconstructions, placed *Deltalsia* as sister to *Amplisiphonia*, but this relationship was unsupported. A similar result was obtained from phylogenetic reconstructions based on the less gene-rich mitochondrial genome (23 genes) whereas the plastid genome (192 genes) resolved all nodes with full or high support. Therefore, the generic placement of *D. parasitica* would have remained uncertain based on *rbc*L gene or mitochondrial genome phylogenies, while

TABLE 2. Comparison of selected morphological characters among the genera *Amplisiphonia*, *Deltalsia*, *Pterosiphonia*, *Symphyocladia*, *Symphyocladiella* and *Xiphosiphonia*.

	Amplisiphonia	*Deltalsia*	*Pterosiphonia*	*Symphyocladia*	*Symphyocladiella*	*Xiphosiphonia*
Pericentral cells	5	6–8	5	6–10	7–14	8–11
Cortication	Absent	Absent	Incomplete throughout all thalli to heavy	Absent or present	Absent or present from restricted to basal parts to heavy	Absent
Coalescence between branches and main axes	Complete	0.7–1.3 segments	2–8 segments	≥ 3 segments to complete	1–3 segments	1–3 segments
Vegetative trichoblasts	Absent	Absent	Absent	Absent or present	Absent or rare	Absent or rare
First-order branches growth; branching orders	Indeterminate; N/A	Indeterminate, 2–3 times branched	Determinate and indeterminate, 2–3 times branched	Determinate and indeterminate; N/A	Determinate and simple; or determinate and indeterminate; 1–4 times branched	Determinate; simple
Tetrasporangia	Straight	Spiral	Straight or spiral	Straight	Straight	Spiral
Sterile cells in spermatangial branches	1	1–3	0–1	0–2	1–2	2–3
Cystocarps	Ovoid to globular	Ovoid to globular	Globular	Ovoid to globular	Ovoid	Ovoid to globular
Length of rhizoidal pad filaments	-	1–4 cells	1–5 cells	1–4 cells	1–5 cells	1–5 cells
References	7, 8;	14, this work	5, 14, 15, 16, P. Díaz-Tapia pers. obs. of *P. complanata*	4, 9, 10, 11, 17, P. Díaz-Tapia pers. obs. of *S. marchantioides*	1, 2, 3, 13, 15	6, 14, P. Díaz-Tapia, pers. obs.

N/A = not applicable; – = not described.

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our tree based on plastid genomes resolved its phylogenetic relationships with high support, providing the basis for confident taxonomic reassessments. By constructing a tree using the well-resolved plastid phylogeny as backbone constraint and adding *rbc*L and *cox*1 gene sequences for additional species, we obtained a taxon-rich phylogeny that includes species for which organellar genomes are unavailable. In our case, this tree received full or high support for most branches, and allowed us to combine newly determined genomes using HTS with gene data that have been incorporated in public databases during the last two decades. While use of organelle genome-based phylogenetics is now a proven method to obtain well-resolved phylogenies, and is remarkably cost-effective (Oliveira et al. 2018), it has not yet been widely applied. Expanding red algal genomic datasets could greatly advance our understanding of their systematics and evolution (Bhattacharya et al. 2015).

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DATA AVAILABILITY STATEMENT

DNA sequences were deposited in NCBI GenBank under accession numbers OP748263-OP748284 (organellar genomes) and OP186025-OP186033, OP7 64600 (*rbc*L sequences).

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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. Maximum likelyhood phylogeny the tribe Pterosiphonieae based on the *rbc*L gene. Scale bar represents substitution per site. Branch support values correspond to UFbootstrap.

Table S1. GenBank accession numbers and collection information of the *cox*1 and *rbc*L gene sequences used in phylogenetic analyses.

Table S2. GenBank accession numbers and collection information of the plastid and mitochondrial genomes used in phylogenomic analyses. Newly determined genomes are printed in bold.

Table S3. Information on complete mitochondrial genomes.

Table S4. Information on complete plastid genomes.

Table S5. Uncorrected p-distances (%) in the *cox*1 (down) and *rbc*L (above) genes among genera of the Pterosiphonieae more closely related to *Deltalsia.*