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Lampisiphonia iberica gen. et sp. nov. (Ceramiales, Rhodophyta) based on morphology and molecular evidence

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Lampisiphonia iberica gen. et sp. nov. is described on the basis of specimens collected from subtidal rocky bottom habitats on the Atlantic coast of the Iberian Peninsula. The new genus was distinguished by an erect habit, pseudodichotomous branching, and 9–11 pericentral cells. Plants were bright red to brown-red in colour, 3–6 cm high, firm at the base but ultimate divisions soft and flaccid. Cortication was thick but restricted to the base of large thalli. Plants were attached to the substratum by discoid holdfasts with the tips of decumbent branches reattaching by secondary discoid holdfasts or rhizoids cut off from pericentral cells. Trichoblasts and scar cells were absent and the branching was exogenous. Tetrasporangia were arranged in straight series of up to 15 segments. Sexual structures were not observed. *Lampisiphonia* was separated from other groups and genera of *Polysiphonia sensu lato* and the tribe Polysiphonieae by a combination of features that include the absence of trichoblasts and presence of a compact basal cortication. Furthermore, *Lampisiphonia* had tetrasporangia in a straight series, a feature to date considered unique to *Polysiphonia sensu stricto*. The phylogenetic relationship of *Lampisiphonia* among the three resolved lineages of the *Polysiphonia sensu lato* was equivocal in our analyses and it was not clearly distinct from the *Neosiphonia* group and the multipericental group in morphological and molecular characters. Nevertheless, monophyly of *Lampisiphonia* and the *Polysiphonia* group was statistically rejected in small-subunit ribosomal DNA, *rbc*L and combined data with the Shimodaira–Hasegawa test. We therefore proposed *Lampisiphonia* as a new genus.

Key Words: Iberian Peninsula, *Lampisiphonia iberica*, *Neosiphonia*, *Polysiphonia*, Rhodomelaceae, Rhodophyta, Taxonomy

INTRODUCTION

Polysiphonia sensu lato is a large and cosmopolitan genus with more than 200 species (Stegenga *et al*. 1997; Guiry & Guiry 2012). *Neosiphonia* Kim & Lee (1999) was separated out and *Polysiphonia* Greville (1824) was redefined (Kim & Lee 1999). Subsequently Choi *et al*. (2001) split *Polysiphonia sensu lato* into three strongly supported clades: the '*Polysiphonia*' group, the '*Neosiphonia*' group and the 'multipericentral' group. Both vegetative and reproductive morphology are important in the taxonomy of *Polysiphonia sensu lato*, including characters such as the number of pericentral cells, the presence/absence and degree of cortication, the habit, the origin of branches (exogenous vs endogenous), the position of lateral branch development with respect to trichoblasts, the origin of the rhizoids, whether exogenous or endogenous, the rhizoid morphology, the arrangement of tetrasporangia (straight vs spiral series), the origin of spermatangial axes, as well as the size and the shape of cystocarps (Maggs & Hommersand 1993; Kim & Lee 1999; Choi *et al*. 2001; Womersley 2003; Stuercke & Freshwater 2008).

Numerous plants of an unidentified species of *Polysiphonia sensu lato* – confined to deep subtidal habitats – were found during recent subtidal collections along the Atlantic Iberian Peninsula. The morphology of this species was consistent throughout the Atlantic Iberian Peninsula sites and different collection seasons. Sexual plants were not found and most collections were sterile; however, tetrasporophytic specimens were collected at one Galician locality. Molecular analyses were conducted for comparison with other species belonging to *Polysiphonia* or *Neosiphonia*. In this work, we described a new genus and species from the Iberian Peninsula.

MATERIAL AND METHODS

Specimens were collected by scuba at 14 localities along the Atlantic Iberian Peninsula (Basque Country, Galicia and Algarve) from 2002 to 2012. In addition, herbarium

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specimens from similar habitats in the Basque Country collected in 1983 were examined. Morphological observations were made on fresh material and plants preserved in 4% formalin-seawater. Sections were made by hand with a stainless steel razor blade under a stereomicroscope. Voucher specimens were deposited at the herbaria of the Santiago de Compostela University (SANT), Basque Country University and Algarve University. Herbarium abbreviations follow Holmgren *et al*. (1990).

A collection from the type locality (November 2007) was cultured in sterile seawater (salinity = 31–32) at 16°C in 4:20 light:dark photoperiod for 8 months in an attempt to generate reproductive structures to complete the description of the species. Additional cultures were started with July 2009 collections (type locality), and grown for 4 months at 2, 10, 15, and 16°C and using 4:20, 6:18, 12:12, and 16:8 light:dark photoperiod.

Collection information and GenBank/European Molecular Biology Laboratory accession numbers for specimens used in molecular investigations are provided in Table S1. Genomic DNA was extracted from samples using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). The nuclear-encoded small-subunit (SSU) ribosomal (r)DNA and plastid-encoded *rbc*L were amplified from total genomic DNA using the polymerase chain reaction (PCR) and primer combinations of Saunders & Kraft (1994, 1996; G01/G14 and G04/G07) for SSU and Freshwater & Rueness (1994; F-rbcL/R-1150 and F-753/R-1381) and a new primer set of F993+/RrbcSP (5′-GGT ATC GTT GTA GGT AAR YTW GAA GG-3′/5′-GTC CCT TGT GTT AGT CTC AC-3′) for *rbc*L. PCR products were directly purified with LaboPass PCR Purification (COSMO Genetech, Seoul, Korea) or purified using agarose gel with QIAquick® Gel Extraction (Qiagen, Hilden, Germany) kits. Purified PCR products were sequenced using the BigDye™ terminator cycle sequencing ready reaction kit (ABI, PE Applied Biosytems, Foster City, California); sequence data were collected using an ABI PRISM 3730 DNA Analyzer, and edited using the SeqEd DNA sequence editor (ABI) software package. Edited sequences were aligned with the SeqPup multiple alignment program (Gilbert 1995) or MacClade 4 program (v.4, Maddison & Maddison 2003).

Final alignments for the SSU, *rbc*L and combined SSU and *rbc*L data were constructed for 44, 62 and 40 taxa, respectively. All alignments were edited to remove the 5′ and 3′ PCR primer regions (Freshwater & Rueness 1994; Saunders & Kraft 1994), as well as ambiguously aligned regions, to yield 1740 (SSU), 1326 (*rbc*L) and 3066 (combined) base pairs (bp) for phylogenetic inference.

Distance, maximum likelihood (ML) and parsimony analyses were completed in PAUP* 4.0*b*10 (Swofford 2002). Modeltest 3.7 (Posada & Crandall 1998; Posada & Buckley 2004) was used to determine that a general time reversible (GTR) model with a gamma correction for among-site rate variation (Γ) and invariant sites (I) is an appropriate model for our data, and this model was applied for distance and ML analyses. Distance analyses were completed with neighbor-joining (Saitou & Nei 1987) and subjected to 2000 rounds of bootstrap resampling (Felsenstein 1985). Unweighted parsimony and ML analyses (gaps treated as missing data) were completed under a heuristic search (starting trees generated from 100 and 10 random sequence additions, respectively) with tree bisection–reconnection branch swapping in effect. The robustness of internal nodes in the parsimony and ML trees was estimated with 1000 and 500 replicates of bootstrap resampling (10 and five random addition replicates per bootstrap replicate), respectively.

SSU, *rbc*L and combined data trees were also generated using MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). The GTR + Γ + I model was used and 5,000,000 generations of four chains were run, sampling every 100 generations (burn-in subsequently identified at 29,000, 42,000 and 29,000 generations for SSU, *rbc*L and combined data, respectively).

Unrooted trees were calculated in all analyses and subsequently rooted on *Bostrychia moritziana* (Phillips *et al*. 2000).

The Shimodaira–Hasegawa (1999) test (SH test), as implemented in PAUP*, was used to assess statistically a series of alternative phylogenetic hypotheses among the three resolved lineages of the *Polysiphonia sensu lato*. The following backbone constraint topologies were reconstructed:

1. Monophyly of *Lampisiphonia* and the *Neosiphonia* group [outgroups, *Polysiphonia* group, (*Neosiphonia* group, *Lampisiphonia*), multipericentral group].
2. Monophyly of *Lampisiphonia* and the multipericentral group [outgroups, *Polysiphonia* group, *Neosiphonia* group, (*Lampisiphonia*, multipericentral group)].
3. Trifurcating ancestor of *Lampisiphonia*, the *Neosiphonia* group and the multipericentral group [outgroups, *Polysiphonia* group, (*Neosiphonia* group, *Lampisiphonia*, multipericentral group)].
4. Monophyly of *Lampisiphonia* and the *Polysiphonia*-group [outgroups, (*Polysiphonia* group, *Lampisiphonia*), *Neosiphonia* group, multipericentral group].

The above constrained tree topologies were reconstructed by ignoring the uncertainty of subtree topologies. That is, tree topologies within major groups were fixed with those of ML trees and only the position of *Lampisiphonia* was changed in four constrained trees. Fixing subtree topologies is often adopted when the research focus is placed on major groups and many sequences are analyzed (e.g., Nikaido *et al*. 2003). For each SSU, *rbc*L and combined data set including sequence data from common 40 taxa only, the differences of log-likelihood scores among trees (Fig. 34A–D) and their statistical significance were measured via 1000 replicates of the resampling estimated log-likelihood procedures.

RESULTS

Lampisiphonia H.-G. Choi, Díaz Tapia & Bárbara gen. nov.

DESCRIPTION: Plants erect to decumbent pseudodichotomously branched. Thalli attached mainly by discoid holdfasts, but decumbent axes producing secondary discoid holdfast or rhizoids at the tips of branches. Rhizoids cut off from the pericentral cells. Pericentral cells 9–11. Axis ecorticated to heavily corticated near the base of large plants. Trichoblasts absent. Branching exogenous; occasionally, decumbent axes produce endogenous branches. Tetrasporangia spherical in straight series. Sexual structures unknown.

Figs 1–4. *Lampisiphonia iberica* sp. nov. Habitat and habit.
Fig. 1. Ría de A Coruña, El Grelle (type locality), November 2007, 25-m depth, with *Halopteris filicina*. Scale bar = 4 cm.
Fig. 2. Plant from El Grelle (Galicia), April 2008, pseudodichotomously branching up to seven orders. Scale bar = 2 cm.
Fig. 3. Plants from Askibille (Vizcaya, Basque country), September 2006. Scale bar = 2 cm.
Fig. 4. Plants from the Ría de Vigo (Galicia), August 2008, with cortication at the base. Scale bar = 2 cm.

ETYMOLOGY: '*Lampisiphonia*' is derived from the Spanish term 'lampiño' and refers to absence of trichoblasts.

Lampisiphonia iberica Bárbara, Secilla, Díaz Tapia & H.-G. Choi, sp. nov.

Figs 1–30

DESCRIPTION: Plants erect to decumbent, bright red to brown-red in colour, 3–6 (9) cm high, pseudodichotomously branched to 5–7 (10) orders. Thallus firm at the base and soft and flaccid toward the apex. Thalli attached mainly by discoid holdfasts, but decumbent axes producing secondary discoid holdfast or rhizoids (1000–2500 × 240 μm) at the tips of branches. Rhizoids, one to three per segment and cut off from the pericentral cells. Axes 350–900 (1300) μm in diameter at the base, to 60–200 (250) μm at the apex. Segments 0.5–2× as long as broad. Pericentral cells (7) 9–11 (12). Axis ecorticated when young, becoming heavily corticated just near base of large plants (more than 3 cm long). Cortication composed of two to five layers of cells surrounding pericentral cells, increasing toward the base. Pit connections between axial and pericentral cells conspicuous. In transverse section, external cell wall of periaxial cells striate. Trichoblasts and scar cells absent. Branching exogenous; decumbent axes occasionally producing endogenous branches. Plastids discoid (2–5 μm) to elliptical-bacillar (5–10 × 2–5 μm) densely aggregated to chained. Refractive rhomboidal inclusions (2–4 × 5–8 μm) one to three per cell. Tetrasporangia spherical (25–40 μm diameter) in straight series, up to 15 segments long. Sexual structures unknown.

HOLOTYPE: SANT-Algae 19509 (see Fig. 5, arrow), Ría de A Coruña, El Grelle, 43° 22′ 56″N, 008° 23′ 09″W, 08 November 2007, subtidal (22–26 m) over rock.

ETYMOLOGY: '*iberica*' refers to its geographical distribution.

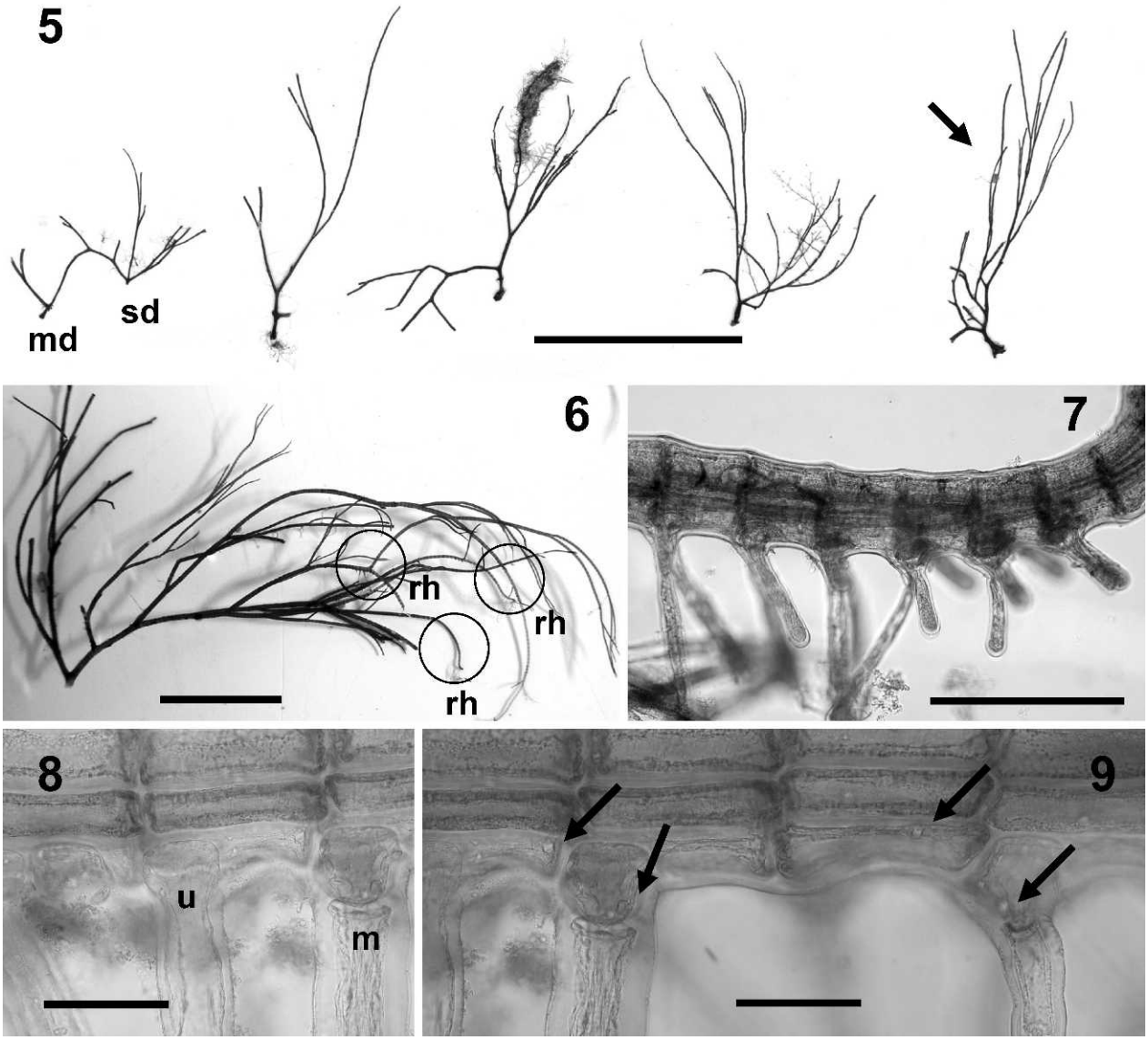
ADDITIONAL COLLECTIONS: See supplemental material.

DISTRIBUTION: Atlantic and Cantabric coast of the Iberian Peninsula.

HABITAT: Plants confined to sciophilic subtidal rocky bottoms (mainly 11–33 m depth), epilithic and epizoic in exposed and semiexposed sites, tolerating sedimentation.

MOLECULAR DATA: SSU rDNA sequences of CH1414 and CH1789 were identical (GenBank JX828168, JX828169); *rbc*L sequences of CH1414, CH1530, CH1533, CH1789, COR002 were identical (see Table S1).

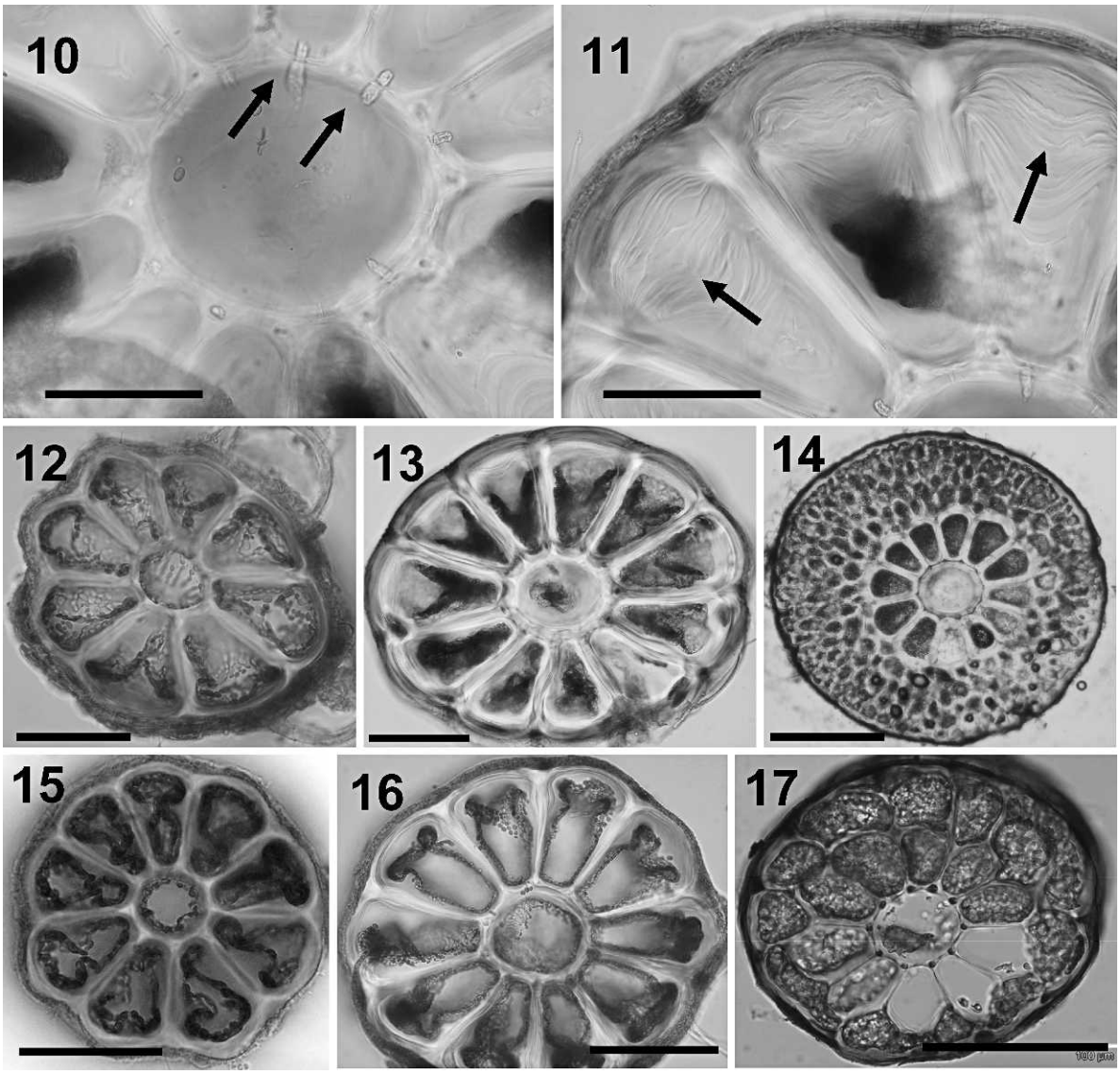
Thalli were 3–6 (9) cm high, bright red to brownish-red in colour; thalli were firm at the base, but ultimate divisions were soft and flaccid (Figs 1–6). Thalli were erect,



Figs 5–9. *Lampisiphonia iberica* sp. nov. Vegetative morphology.
Fig. 5. Images of type material, Ría de A Coruña, El Grelle, November 2007, SANT-Algae 19509, image of holotype (arrow). Main discoid holdfast (md) and secondary discoid holdfast (sc) at the tip of a decumbent axis. Scale bar = 2 cm.
Fig. 6. Specimen with decumbent axes producing rhizoids (rh). Scale bar = 1 cm.
Fig. 7. Rhizoids (one to three per segment) cut off from the proximal ends of pericentral cells of decumbent branches. Scale bar = 200 μm.
Fig. 8. Unicellular (u) and multicellular (m) rhizoids, cut off from the pericentral cells. Scale bar = 100 μm.
Fig. 9. Rhizoids cut off from the pericentral cells and refractive romboidal inclusions (arrows). Scale bar = 100 μm.

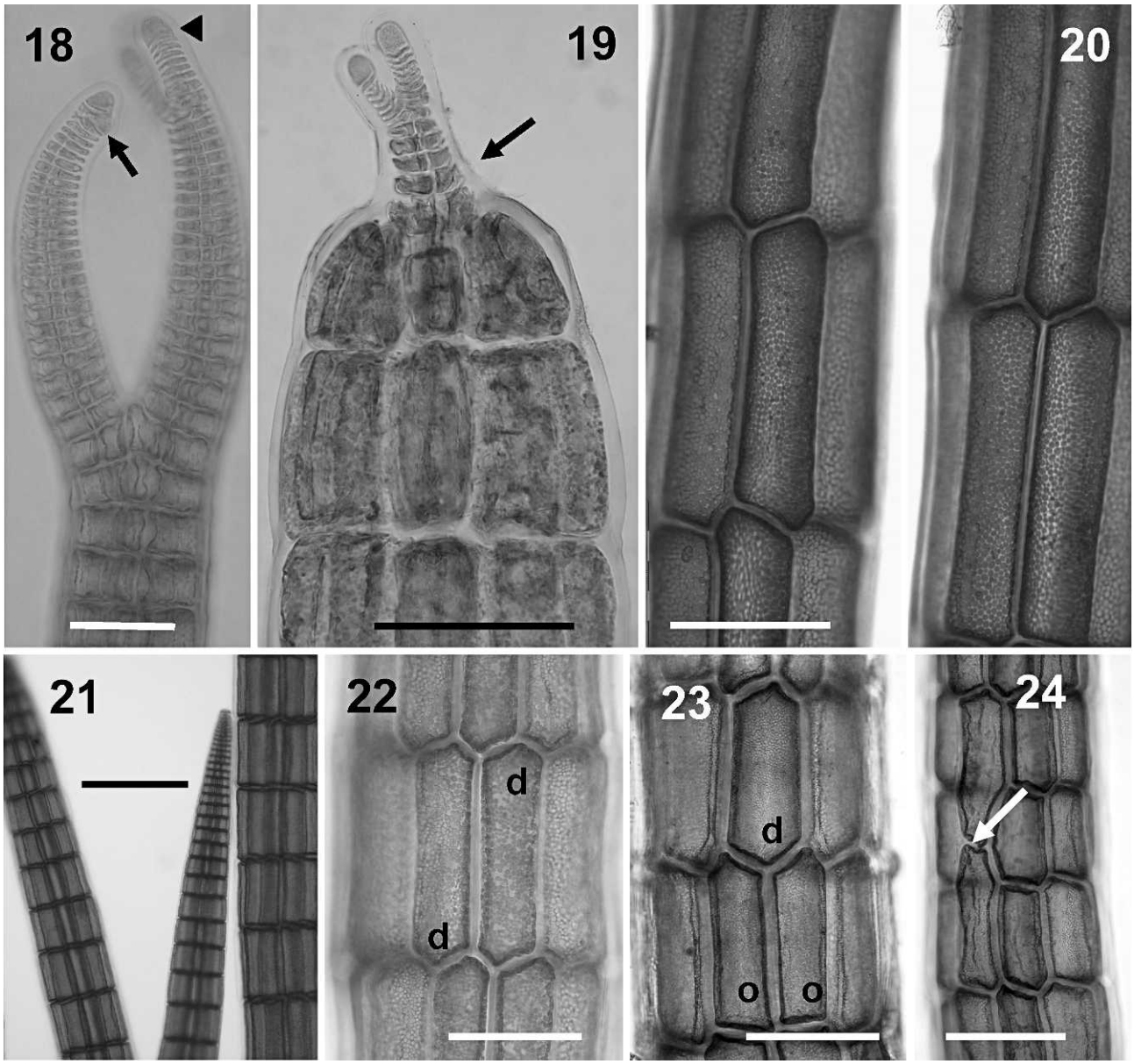
pseudodichotomously branched to 5–7 (10) orders (Figs 2–6); branches were 15–30 segments apart. The angle of branching was generally wide at the base (60–110°), and it became narrow in the middle and apical parts of the axes (10–30°). Plants were erect; initially, they were solitary with a solid main discoid (*c*. 2 mm in diameter) holdfast, but later they had decumbent axes that produced secondary discoid holdfasts at the tips (Fig. 5) and groups of colourless rhizoids (1000–2500 × 20–40 μm) (Figs 6–9). Rhizoids arose one to three per segment (Fig. 7), and they were cut off from the proximal ends of the pericentral cells. Rhizoids were usually unicellular, but occasionally they became multicellular (Fig. 8) by the presence of an isodiametric cell (60–80 μm in diameter) at their origin on pericentral cells (Fig. 9). Cortication was restricted to plants larger than 3 cm high and cortications were present only on the basal-most parts of the plants and extended to no more than the first or second orders of branching (Figs 4, 5); they were composed of two to five cortical cell layers that surrounded the pericentral cells (Figs 14, 17). In transverse section, some plants showed an asymmetrical organization of cortication that surrounded the pericentral cells (Fig. 14, 17); asymmetry was also observed in the middle axes of some plants (Figs 13, 16).

The pericentral cells numbered (7) 9–11 (12), and they occurred around a large axial cell (80–200 μm in diameter) in mid- and lower parts of axes (Figs 12–17). Axes were 350–



Figs 10–17. *Lampisiphonia iberica* sp. nov. Pericentral cells.
Fig. 10. Middle part of an axis with 12 pericentral cells and conspicuous pit connections (arrows) between the central cell and pericentral cells. Scale bar = 100 μm.
Fig. 11. External striated cell walls of pericentral cells (arrows). Scale bar = 100 μm.
Fig. 12. Section from near the apex with eight pericentral cells. Scale bar = 50 μm.
Fig. 13. Section from near the apex with nine pericentral cells. Scale bar = 50 μm.
Fig. 14. Mid-axis section with 11 pericentral cells. Scale bar = 100 μm.
Fig. 15. Mid-axis section with 10 pericentral cells. Scale bar = 100 μm.
Fig. 16. Heavily corticated basal axis section with 12 pericentral cells. Scale bar = 500 μm.
Fig. 17. Lightly corticated basal axis section with 10 pericentral cells. Scale bar = 200 μm.

900 (1300) μm in diameter at the base and branches were 240–400 (500) μm in diameter at the middle. Segments were 0.5–2× as long as broad; they were 0.3–0.5× in apical parts (Fig. 18), 0.8–2× in the middle (Figs 20–22) and 0.5–1× at the base (Figs 23, 24). The apex had a prominent dome-shaped apical cell (10–15 μm) that divided in a transverse plane and yielded up to 15 platelike segments (Fig. 18); the apex was tapered, 60–200 (250) μm in diameter. Branches were exogenous, and they were formed by oblique septa of apical cells before the division of pericentral cells; they were not associated with trichoblasts (Fig. 18). Decumbent axes occasionally produced adventitious endogenous branches. Apex regeneration from damage was common, and regeneration developed one to two orders of new polysiphonous axes (Fig. 19). Pericentral cell arrangement (opposite vs displaced) was variable along axes (Figs 20–23). Unusual pericentral cell divisions were also observed (Fig. 24). Pit connections formed between the axial and pericentral cells; cell-wall striations also were formed between these cells and these were apparent in transverse sections of plants more than 3 cm high (Figs 10, 11). Plastids were discoid (2–5 μm) to elliptical-bacilloid (5–10 × 2–5 μm), and they were densely

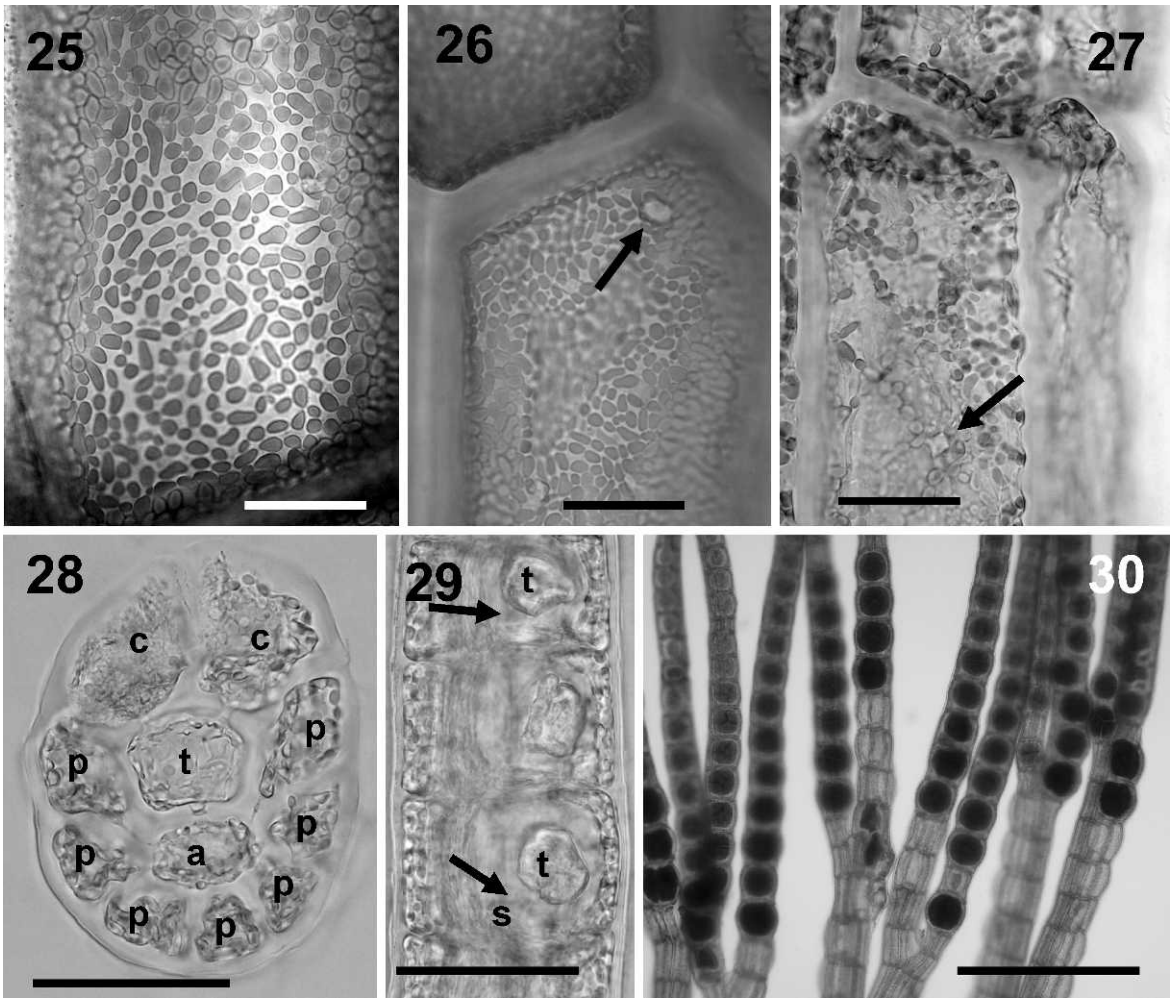


Figs 18–24. *Lampisiphonia iberica* sp. nov. Vegetative morphology.
Fig. 18. Apical tips showing prominent, transversely dividing apical cells (arrowhead). Initial cell (arrow) of a new exogenous branch. Scale bar = 50 μm.
Fig. 19. Damaged axis with a regenerated apex (arrow). Scale bar = 100 μm.
Fig. 20. Segments and pericentral cells in the mid-part of branches. Scale bar = 400 μm.
Fig. 21. Segments and pericentral cells at the apex and mid-part of branches. Scale bar = 200 μm.
Fig. 22. Middle part of branches with pericentral cells of adjacent segments displaced (d). Scale bar = 100 μm.
Fig. 23. Lower part of axis with pericentral cells directly opposed (o) or displaced (d). Scale bar = 200 μm.
Fig. 24. Lower part of axis with unusually divided pericentral cells. Scale bar = 200 μm.

aggregated or aligned in chains (Figs 25–27). Refractive romboidal inclusions (2–4 × 5–8 μm) were also present in rhizoidal cells (Fig. 9), and there were one to three per pericentral cell (Figs 26, 27). Gametophytic structures were not observed. Plants cultured in the laboratory did not produce reproductive structures.

Tetrasporangia (25–40 μm in diameter) occurred in a straight series up to 15 segments long, and they formed on the ultimate branches (Fig. 30). Each tetrasporangium had two cover cells (Fig. 28) and one basal stalk cell (Fig. 29).

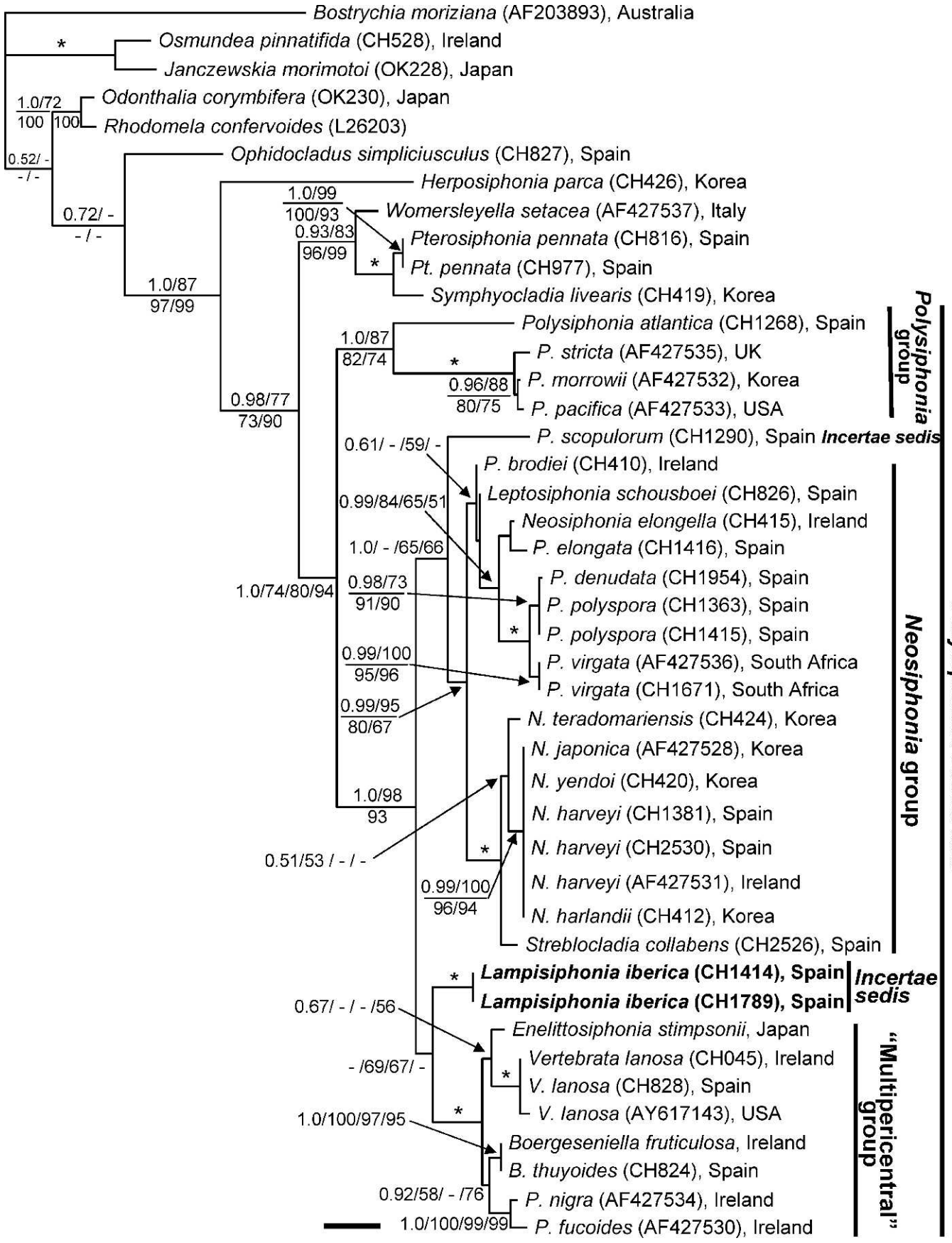
Lampisiphonia iberica was a deepwater species confined to sciophilous subtidal rocky bottoms of the Atlantic Iberian Peninsula, predominantly at 15–30-m depths in wave-exposed to semiexposed sites (Fig. 1). It occurred on hard substrata such as rock, *Mesophyllum lichenoides* (J. Ellis) M. Lemoine and *Balanus* spp., and it tolerated a layer of silt over the substratum. The species was also collected at sites



Figs 25–30. *Lampisiphonia iberica* sp. nov. Plastids, inclusions and tetrasporangia.
Fig. 25. Plastids discoid to elliptical. Scale bar = 30 μm.
Fig. 26. Plastids and refractive romboidal inclusion (arrow). Scale bar = 30 μm.
Fig. 27. Plastids in chains and refractive romboidal inclusion (arrow). Scale bar = 30 μm.
Fig. 28. Tetrasporangial branch in transverse section, axial cell (a), initial tetrasporangial cell (t), cover cells (c) and periaxial cells (p). Scale bar = 50 μm.
Fig. 29. Tetrasporangial branch in optical longitudinal section, basal stalk cells (s) joint to the initial tetrasporangial cells (t) by pit connections (arrow). Scale bar = 50 μm.
Fig. 30. Tetrasporangia in straight series. Scale bar = 300 μm.

characterized by high sedimentation at shallower depths (7–11 m). It formed isolated patches up to 40 cm in diameter that partially covered the substratum. It also occurred among gorgonians together with other typical deepwater macroalgae such as *Neurocaulon foliosum* (Meneghini) Zanardini, *Carpomitra costata* (Stackhouse) Batters, *Dictyopteris lucida* Ribera Siguan, Gómez Garreta, Pérez Ruzafa, Barceló Martí & Rull Lluch and *Halopteris filicina* (Grateloup) Kützing. Other common red algae in these mixed macrophyte subtidal communities were *Pterosiphonia parasitica* (Hudson) Falkenberg, *P. complanata* (Clemente) Falkenberg, *Chondria capillaris* (Hudson) M.J. Wynne, *Erythroglossum laciniatum* (Lightfoot) Maggs & Hommersand, *Cryptopleura ramosa* (Hudson) Kylin ex Newton, *Rhodophyllis divaricata* (Stackhouse) Papenfuss and *Zanardinia typus* (Nardo) Nardo. Generally, *L. iberica* was generally not epiphytised, but in semiexposed sites (mainly in summer) some species of hydroids as well as red algae including *Antithamnionella ternifolia* (J.D. Hooker & Harvey) Lyle, *Asparagopsis armata* Harvey as tetrasporophytic stage, *Pterothamnion pluma* (J. Ellis) Nägeli, *Antithamnion amphigenum* A.J.K. Millar, *Acrosorium ciliolatum* (Harvey) Kylin and *Microcladia glandulosa* (Solander ex Turner) Greville were found growing on plants.

Lampisiphonia iberica was abundant in collections at all localities and dates (15–50 individuals per sampling) and its populations were widely distributed along subtidal sites covering large areas 400-1600 m^2. Tetrasporangia were only observed at the Ría de Ferrol, where five tetrasporophytic plants were collected in March 2007 and only two tetrasporangial branches were found in a single individual in August 2008.


Bostrychia moriziana (AF203893), Australia
Osmundea pinnatifida (CH528), Ireland
Janczewskia morimotoi (OK228), Japan
Odonthalia corymbifera (OK230), Japan
Rhodomela confervoides (L26203)
Ophidocladus simpliciusculus (CH827), Spain
Herposiphonia parca (CH426), Korea
Womersleyella setacea (AF427537), Italy
Pterosiphonia pennata (CH816), Spain
Pt. pennata (CH977), Spain
Symphyocladia livearis (CH419), Korea
Polysiphonia atlantica (CH1268), Spain
P. stricta (AF427535), UK
P. morrowii (AF427532), Korea
P. pacifica (AF427533), USA
P. scopulorum (CH1290), Spain
P. brodiei (CH410), Ireland
Leptosiphonia schousboei (CH826), Spain
Neosiphonia elongella (CH415), Ireland
P. elongata (CH1416), Spain
P. denudata (CH1954), Spain
P. polyspora (CH1363), Spain
P. polyspora (CH1415), Spain
P. virgata (AF427536), South Africa
P. virgata (CH1671), South Africa
N. teradomariensis (CH424), Korea
N. japonica (AF427528), Korea
N. yendoi (CH420), Korea
N. harveyi (CH1381), Spain
N. harveyi (CH2530), Spain
N. harveyi (AF427531), Ireland
N. harlandii (CH412), Korea
Streblocladia collabens (CH2526), Spain
Lampisiphonia iberica (CH1414), Spain
Lampisiphonia iberica (CH1789), Spain
Enelittosiphonia stimpsonii, Japan
Vertebrata lanosa (CH045), Ireland
V. lanosa (CH828), Spain
V. lanosa (AY617143), USA
Boergeseniella fruticulosa, Ireland
B. thuyoides (CH824), Spain
P. nigra (AF427534), Ireland
P. fucoides (AF427530), Ireland
1.0/72
100
100
0.52/ -
- / -
0.72/ -
- / -
1.0/87
97/99
1.0/99
100/93
0.93/83
96/99
0.98/77
73/90
1.0/87
82/74
0.96/88
80/75
0.61/ - /59/ -
0.99/84/65/51
1.0/ - /65/66
1.0/74/80/94
0.98/73
91/90
0.99/100
95/96
0.99/95
80/67
1.0/98
93
0.51/53 / - / -
0.99/100
96/94
0.67/ - / - /56
- /69/67/ -
1.0/100/97/95
0.92/58/ - /76
1.0/100/99/99
Polysiphonia group
Incertae sedis
Neosiphonia group
Incertae sedis
"Multipericentral" group
Polysiphonia sensu lato

The basic morphology of *Lampisiphonia iberica*, basal axes growing from a discoid holdfast and the production of new decumbent and erect branches, exhibits few seasonal changes. However, larger, more profusely branched thalli (5–9 cm) were found in summer and autumn when long decumbent branches producing new discoid holdfasts and rhizoids were abundant. *L. iberica* seemed to be a hemiphanerophyceae species, with a dense cortication at the base.

Phylogeny

The SSU rRNA gene trees generated with different analysis methods were topologically similar; however, support for some branches varied greatly. A tree generated by Bayesian inference for the SSU data with 35 taxa (43 sequences) and 1740 bp [including 232 sites (13.3%) parsimony informative] is presented with posterior probabilities and bootstrap results from the distance, maximum parsimony (MP) and ML analyses appended (Fig. 31). *Polysiphonia sensu lato* was resolved as a monophyletic group with strong Bayesian posterior probability support ($B = 1.0$); however, there was only moderate distance ($D = 74\%$), MP (MP = 80%) and ML bootstrap support (ML = 94%). Two strongly supported groups were resolved within this complex: (1) a *Polysiphonia* group including *P. atlantica*, *P. morrowii, P. pacifica*, and the type species of the genus, *P. stricta*, and (2) the remainder of *Polysiphonia sensu lato*, containing species currently assigned to the genera *Polysiphonia, Neosiphonia, Streblocladia, Leptosiphonia, Boergeseniella, Enelittosiphonia*, and *Vertebrata*. A multipericentral cell group (species with 8+ pericentral cells) was resolved in all analyses with full support. *Lampisiphonia iberica* was allied with the multipericentral cell group, but with no support in Bayesian inference and ML analysis, and with only weak distance ($D = 0.69$) and MP bootstrap support (MP = 67).

A tree generated by Bayesian inference for the *rbc*L data including 45 taxa (62 sequences) and 1326 bp [including 461 sites (34.8%) parsimony informative] is presented with posterior probabilities and bootstrap results from the distance, MP and ML analyses appended (Fig. 32). Monophyly of *Polysiphonia sensu lato* was not supported in this tree as *Odonthalia corymbifera* and *Rhodomela confervoides* were resolved within the clade of *Polysiphonia sensu lato* groups, but their position within *Polysiphonia sensu lato* was weakly supported ($B = 0.76$; $D < 50$; MP < 50; ML < 50). Three strongly supported groups were resolved within this complex: (1) *Polysiphonia* group 1, consisting of *P. stricta*, the type species of the genus, *P. morrowii* and *P. pacifica* (full support all analyses); (2) *Polysiphonia* group 2, containing *P. scopulorum* and *P. atlantica* ($B = 1.0$; $D = 98$; MP = 98; ML = 100); and (3) the remainder of *Polysiphonia sensu lato*, containing species currently assigned to the genera *Polysiphonia, Neosiphonia, Streblocladia, Leptosiphonia, Enelittosiphonia, Boergeseniella*, and *Vertebrata* ($B = 1.0$; $D = 99$; MP = 99; ML = 100). Similar to the SSU analyses, *Lampisiphonia iberica* sp. nov. was grouped with the multipericentral cell clade, with only weak to moderate support ($B = 0.6$; $D = 82$; MP = 65; ML = 55).

A tree generated by Bayesian inference for the combined SSU and *rbc*L data consisting of taxa (40 sequences) and 3066 bp [including 662 parsimony informative sites (21.6%)] is presented with posterior probabilities and bootstrap results from the distance, MP and ML analyses appended (Fig. 33). *Polysiphonia sensu lato* was monophyletic with weak to strong support in analyses of the combined SSU and *rbc*L data ($B = 1.0$; $D < 50$; MP = 89; ML = 96). Three generally strongly supported groups were resolved within this complex: (1) *Polysiphonia* group 1, consisting of *P. stricta*, the type species of the genus, *P. morrowii* and *P. pacifica* (full support all analyses); (2) *Polysiphonia* group 2, containing *P. scopulorum* and *P. atlantica* ($B = 1.0$; $D < 50$; MP = 78; ML = 76), and (3) the remainder of *Polysiphonia sensu lato*, containing *L. iberica* sp. nov., *Neosiphonia* group and multipericentral cell group (full support all analyses). *Lampisiphonia iberica* sp. nov. was allied to the *Neosiphonia* group with poor support ($B = 0.73$; $D < 50$; MP < 50; ML = 58) in contrast to trees for SSU and *rbc*L data.

ML (–Ln likelihood = 6398.04381, 11,155.56820 and 18,109.04778 for SSU, *rbc*L and combined data including common 40 taxa, respectively; Table 1) produced topologies very similar to that resolved under Bayesian inference for combined data (Fig. 33). A series of phylogenetic hypotheses (Fig. 34; Table 1) was tested under the likelihood framework with the SH test. Our results indicate that the best tree that supports monophyly of *Lampisiphonia* and the *Polysiphonia* group (Fig. 34D) was statistically different from the best ML result (Fig. 34A) on the basis of SSU, *rbc*L and combined SSU and *rbc*L data, respectively. Otherwise, multiple hypotheses regarding monophyly of *Lampisiphonia* and the multipericentral group (Fig. 34B), monophyly of *Lampisiphonia* and the *Neosiphonia* group, and trifurcating ancestor of *Lampisiphonia*, the *Neosiphonia* group and the multipericentral group (Fig. 34C) were not rejected, which were not statistically worse than the best tree (Table 1).

DISCUSSION

The geographical distribution of *L. iberica* is probably wider than the current study indicates, as deep subtidal rocky habitats of the Atlantic Iberian Peninsula have not been comprehensively surveyed. The habit of *L. iberica* (pseudodichotomously branched) and its habitat (deep subtidal rocks) are characteristics that permit field recognition of the species. *Lampisiphonia iberica* exhibits the first type of prostrate development described by Kapraun (1977), wherein plants were initially erect from a discoidal base, but form secondary attachments from decumbent branches. The habit

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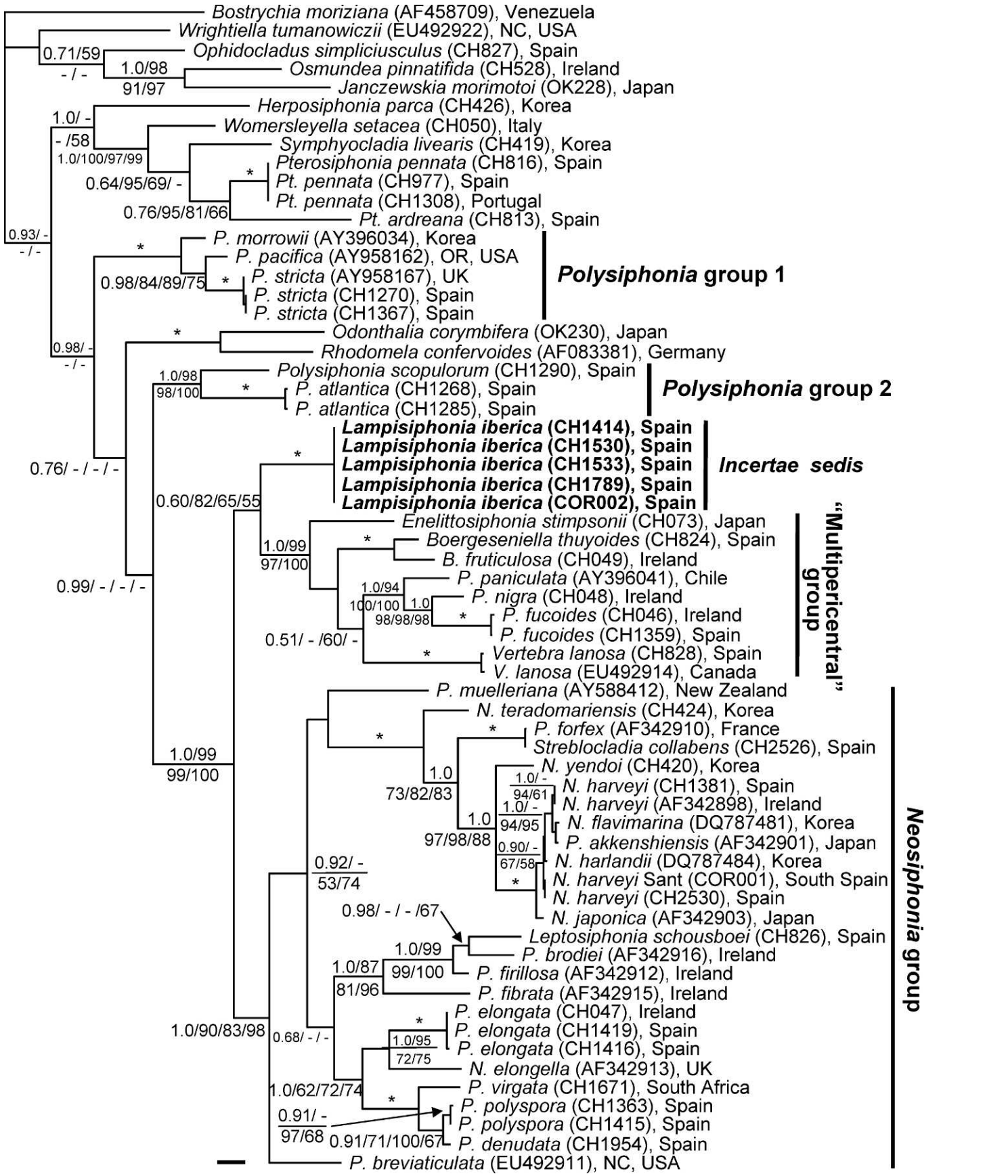
Fig. 31. Tree constructed with Bayesian inference for the SSU alignment. Values at branches represent Bayesian posterior probabilities (top left value), and 2000, 1000 and 500 bootstrap replicates each for distance, parsimony and maximum likelihood (top right and lower left and right values, respectively). Branches marked with an asterisk received 100% support in all three analyses, whereas those lacking values received less than 50% support. Scale bar = 0.01 substitutions/site.


Bostrychia moriziana (AF458709), Venezuela
Wrightiella tumanowiczii (EU492922), NC, USA
Ophidocladus simpliciusculus (CH827), Spain
Osmundea pinnatifida (CH528), Ireland
Janczewskia morimotoi (OK228), Japan
Herposiphonia parca (CH426), Korea
Womersleyella setacea (CH050), Italy
Symphyocladia livearis (CH419), Korea
Pterosiphonia pennata (CH816), Spain
Pt. pennata (CH977), Spain
Pt. pennata (CH1308), Portugal
Pt. ardreana (CH813), Spain
P. morrowii (AY396034), Korea
P. pacifica (AY958162), OR, USA
P. stricta (AY958167), UK
P. stricta (CH1270), Spain
P. stricta (CH1367), Spain
Polysiphonia group 1
Odonthalia corymbifera (OK230), Japan
Rhodomela confervoides (AF083381), Germany
Polysiphonia scopulorum (CH1290), Spain
P. atlantica (CH1268), Spain
P. atlantica (CH1285), Spain
Polysiphonia group 2
Lampisiphonia iberica (CH1414), Spain
Lampisiphonia iberica (CH1530), Spain
Lampisiphonia iberica (CH1533), Spain
Lampisiphonia iberica (CH1789), Spain
Lampisiphonia iberica (COR002), Spain
Incertae sedis
Enelittosiphonia stimpsonii (CH073), Japan
Boergeseniella thuyoides (CH824), Spain
B. fruticulosa (CH049), Ireland
P. paniculata (AY396041), Chile
P. nigra (CH048), Ireland
P. fucoides (CH046), Ireland
P. fucoides (CH1359), Spain
Vertebra lanosa (CH828), Spain
V. lanosa (EU492914), Canada
"Multipericentral" group
P. muelleriana (AY588412), New Zealand
N. teradomariensis (CH424), Korea
P. forfex (AF342910), France
Streblocladia collabens (CH2526), Spain
N. yendoi (CH420), Korea
N. harveyi (CH1381), Spain
N. harveyi (AF342898), Ireland
N. flavimarina (DQ787481), Korea
P. akkenshiensis (AF342901), Japan
N. harlandii (DQ787484), Korea
N. harveyi Sant (COR001), South Spain
N. harveyi (CH2530), Spain
N. japonica (AF342903), Japan
Leptosiphonia schousboei (CH826), Spain
P. brodiei (AF342916), Ireland
P. firillosa (AF342912), Ireland
P. fibrata (AF342915), Ireland
P. elongata (CH047), Ireland
P. elongata (CH1419), Spain
P. elongata (CH1416), Spain
N. elongella (AF342913), UK
P. virgata (CH1671), South Africa
P. polyspora (CH1363), Spain
P. polyspora (CH1415), Spain
P. denudata (CH1954), Spain
P. breviaticulata (EU492911), NC, USA
Neosiphonia group
0.71/59
- / -
1.0/98
91/97
1.0/ -
- /58
1.0/100/97/99
0.64/95/69/ -
0.76/95/81/66
0.93/ -
- / -
0.98/84/89/75
0.98/ -
- / -
1.0/98
98/100
0.76/ - / - / -
0.60/82/65/55
1.0/99
97/100
1.0/94
100/100
1.0
98/98/98
0.51/ - /60/ -
0.99/ - / - / -
1.0/99
99/100
1.0
73/82/83
1.0/ -
94/61
1.0/ -
94/95
1.0
97/98/88
0.90/ -
67/58
0.92/ -
53/74
0.98/ - / - /67
1.0/99
1.0/87
99/100
81/96
1.0/90/83/98
0.68/ - / -
1.0/95
72/75
1.0/62/72/74
0.91/ -
97/68
0.91/71/100/67

and anchorage of *L. iberica* seem to be related to its growth on hard substrata covered by silt. Rhizoids are produced at the tips of decumbent axes, which can also develop new discoid holdfasts, depending on the amount of wave motion and sedimentation rate. Thalli with secondary discoid holdfasts and few rhizoids were frequent in exposed sites (Ría de A Coruña), whereas thalli producing decumbent branches with several groups of rhizoids were prevalent in semiexposed ones. The latter was especially evident in habitats with high sedimentation (Ría de Ferrol and Ría de Vigo) where large numbers of decumbent branches with rhizoids but few secondary discoid holfasts were observed. Moreover, considerable vegetative growth in the form of apex regeneration from damaged branches was found at these sites. Dominant vegetative growth and a high capacity for thallus regeneration have been considered advantageous attributes in environments with sedimentation (Airoldi 2003). This growth strategy allows *L. iberica* to colonize broad areas of rocky subtidal habitats, where it forms large populations of ramets through vegetative growth. The ability to vegetatively propagate may help explain the persistence of populations with few tetrasporophytic and no gametophytic plants.

Lampisiphonia iberica seems to be a hemiphanerophyceae species. Along the northwest Iberian Peninsula, *Polysiphonia polyspora* (C. Agardh) Montagne, a perennial plant anchoring by discoid holdfasts, exhibits a similar phenology to *L. iberica,* with smaller individuals collected in winter, larger plants present from summer to autumn and dense cortication at the base. *Polysiphonia elongata* (Hudson) Sprengel is another species of the eastern Atlantic (Batten 1923) in which new vegetative growth originates from surviving perennial axes (Maggs & Hommersand 1993). Although these three species share similar seasonal growth cycles and habits, they differ in the number of pericentral cells, with *P. polyspora* and *P. elongata* having five to six and four pericentral cells respectively (Lancelot 1966; Maggs & Hommersand 1993).

Fertile *Lampisiphonia iberica* gametophytes were never observed despite a large number of collections in varying seasons being made from 2002 to 2010. The prevalence of fertile plants is variable among *Polysiphonia* species and there are species in which they have never been reported (e.g. Hollenberg 1944, 1968a). In the absence of sexual structures, the main features that differentiate *L. iberica* from the other species of *Polysiphonia sensu lato* are: (1) 9–11 pericentral cells; (2) plants ecorticate to heaviliy corticated, but only at the base of large plants; (3) an attachment system consisting of erect plants initially developing from a discoid base with secondary attachment of decumbent branches by rhizoids or discoid holdfasts; (4) rhizoids cut off from pericentral cells; (5) branches exogenous; (6) absence of trichoblasts and scar cells, and (7) tetrasporangia in straight series.

There are 18 *Polysiphonia sensu lato* species with a similar number of pericentral cells to that observed in *L. iberica* (Tables 2, 3). *Polysiphonia nigra* (Hudson) Batters, *P. ceramiaeformis* P. Crouan & H. Crouan, *P. simulans* Harvey, *P. subulifera* (C. Agardh) Harvey and *P. paniculata* Montagne are European species with nine or more pericentral cells. *Polysiphonia nigra* differs from the new species in being ecorticate, having an attachment system of prostrate branches with rhizoids, possessing trichoblasts, lateral branches developing in the axil of trichoblasts and having tetrasporangia arranged in spiral series (Coppejans 1981; Kapraun & Rueness 1983; Maggs & Hommersand 1993). *Lampisiphonia iberica* can be differentiated from *P. ceramiaeformis*, as it possesses forcipate apices, ecorticate axes, an attachment system of tangled prostrate axes, trichoblasts, and tetrasporangia in spiral series. *Polysiphonia simulans* can be differentiated from *L. iberica* by its short laterals, ecorticate axes, an attachment system of tangled prostrate axes, trichoblasts, and tetrasporangia in spiral series (Maggs & Hommersand 1993). *Polysiphonia subulifera* resembles *P. simulans*, and it is different from *L. iberica* in possessing abundant trichoblasts, lateral branches developing in the axils of trichoblasts, an attachment system of prostrate axes, and axes bearing short spinelike branches (Maggs & Hommersand 1993). *Polysiponia paniculata* (= *P. californica* Harvey) from the Atlantic, Pacific and Mediterranean has 9–10 pericentral cells and is abundant in subtidal habitats like *L. iberica*; however, it has an attachment system of extensive tangled prostrate axes, branches that develop in the axils of trichoblasts and tetrasporangia in spiral series (Hollenberg 1944, 1961). Some specimens of *L. iberica*, especially the upper branches (Fig. 12), resemble *P. furcellata* (C. Agardh) Harvey in colour, dichotomous branching, rhizoids cut off from pericentral cells, scarce or absent trichoblasts, and their subtidal habitat. However, *L. iberica* is more rigid with basally corticated axes more than 240 μm in diameter and 9–11 pericentral cells vs 7–8 in *P. furcellata*.

There are multiple species from the Pacific and Indian oceans (Table 3) having similar numbers of pericentral cells as *L. iberica*, but all can be distinguished by other morphological characters. The Pacific species *Polysiphonia hendryi* Gardner attaches by prostrate axes, possesses abundant trichoblasts, has branches in the axils of trichoblasts and tetrasporangia in spiral series (Hollenberg 1944; Smith 1969). *Polysiphonia confusa* Hollenberg has prostrate axes, abundant trichoblasts, branches in the axils of trichoblasts and tetrasporangia in long spiral series (Hollenberg 1944, 1961). The Japanese species *P. crassa* Okamura is epiphytic, has hyphalike filaments at the base of fronds and dense cortication throughout the thallus, even in apical parts (Segi 1951). *Polysiphonia aterrima* J.D. Hooker & Harvey (from Australia and New Zealand) has rhizoids in open connection with pericentral cells and tetrasporangia in spiral series (Adams 1994). Furthermore, *P. aterrima* can also be

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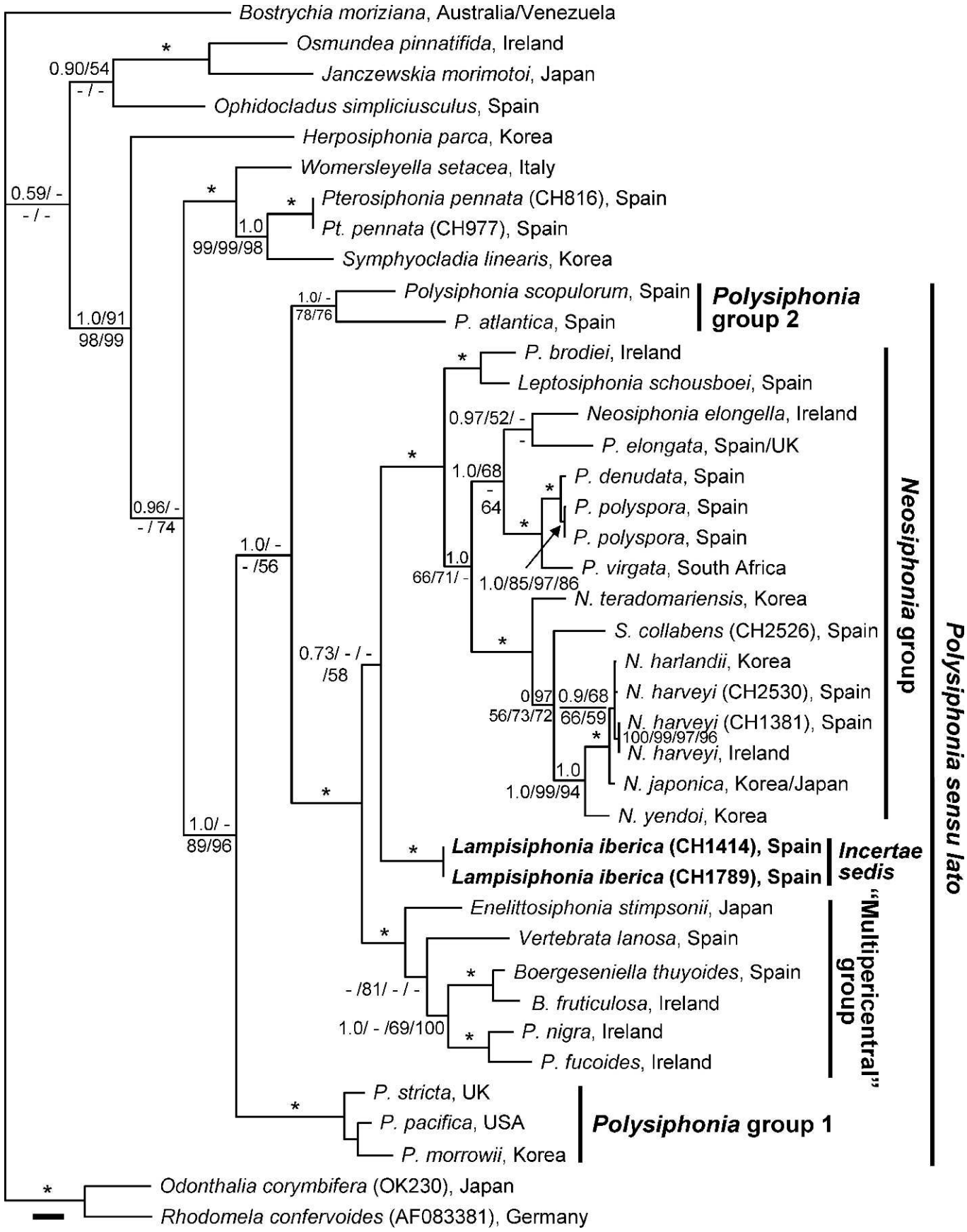
Fig. 32. Tree constructed with Bayesian inference for the *rbc*L alignment. Values at branches represent Bayesian posterior probabilities (top left value), and 2000, 1000 and 500 bootstrap replicates each for distance, parsimony and maximum likelihood (top right and lower left and right values, respectively). Branches marked with an asterisk received 100% support in all three analyses, whereas those lacking values received less than 50% support. Scale bar = 0.01 substitutions/site.


Bostrychia moriziana, Australia/Venezuela
Osmundea pinnatifida, Ireland
Janczewskia morimotoi, Japan
Ophidocladus simpliciusculus, Spain
Herposiphonia parca, Korea
Womersleyella setacea, Italy
Pterosiphonia pennata (CH816), Spain
Pt. pennata (CH977), Spain
Symphyocladia linearis, Korea
Polysiphonia scopulorum, Spain
P. atlantica, Spain
Polysiphonia group 2
P. brodiei, Ireland
Leptosiphonia schousboei, Spain
Neosiphonia elongella, Ireland
P. elongata, Spain/UK
P. denudata, Spain
P. polyspora, Spain
P. polyspora, Spain
P. virgata, South Africa
N. teradomariensis, Korea
S. collabens (CH2526), Spain
N. harlandii, Korea
N. harveyi (CH2530), Spain
N. harveyi (CH1381), Spain
N. harveyi, Ireland
N. japonica, Korea/Japan
N. yendoi, Korea
Neosiphonia group
Lampisiphonia iberica (CH1414), Spain
Lampisiphonia iberica (CH1789), Spain
Incertae sedis
Enelittosiphonia stimpsonii, Japan
Vertebrata lanosa, Spain
Boergeseniella thuyoides, Spain
B. fruticulosa, Ireland
P. nigra, Ireland
P. fucoides, Ireland
"Multipericentral" group
P. stricta, UK
P. pacifica, USA
P. morrowii, Korea
Polysiphonia group 1
Polysiphonia sensu lato
Odonthalia corymbifera (OK230), Japan
Rhodomela confervoides (AF083381), Germany

distinguished by the regular, hexagonal surface pattern of the pericentral cells (Adams 1994). Other Australian and New Zealand species such as *P. adamsiae* Womersley, *P. atricapilla* J. Agardh, *P. isogona* Harvey and *P. muelleriana* J. Agardh can be differentiated from *Lampisiphonia iberica* by the presence of trichoblasts, the development of branches in the axils of trichoblasts, the spiral arrangement of tetrasporangia and the extensive prostrate or holdfast system (Adams 1991, 1994; Womersley 2003). The Asian subtidal species, *P. tapinocarpa* Suringar, has abundant trichoblasts and development of extensive prostrate or holdfast system (Segi 1951).

Lampisiphonia iberica was not to moderately ($B = 0.60$ and <50; $D = 69$ and 82; MP = 67 and 65; ML < 50 and 55) allied to the multipericentral group including the genera *Boergeseniella*, *Enelittosiphonia* and *Vertebrata*, as well as the multipericentral *Polysiphonia* spp., *P. fucoides* and *P. nigra* in SSU and *rbc*L data (Figs 31, 32). However, it was not to moderately ($B = 0.73$; $D < 50$; MP < 50; ML < 50) allied to the *Neosiphonia* group in the combined SSU and *rbc*L data (Fig. 33). In our analyses of SSU, *rbc*L and combined SSU and *rbc*L data, a moderately to strongly supported *Neosiphonia* group included *Leptosiphonia schousboei*, *Polysiphonia denudata*, *P. elongata*, *P. polyspora* and *Streblocladia collabens* from the North Atlantic and *P. virgata* from South Africa. These genera share some key diagnostic features with the multipericentral (more than five pericentral cells except for *P. elongata*) or *Polysiphonia* groups (tetrasporangia in straight series in *P. virgata*), but on the basis of our molecular analyses, unequivocally joined with the *Neosiphonia* group. The phylogenetic relationship of *Lampisiphonia* among the three resolved lineages of the *Polysiphonia sensu lato* was equivocal in our analyses and it was not clearly distinct from *Neosiphonia* group and multipericental group in morphological and molecular characters. Nevertheless, monophyly of *Lampisiphonia* and *Polysiphonia* group was statistically rejected (Table 1) in SSU, *rbc*L and combined data including common 40 taxa with the SH test (Shimodaira & Hasegawa 1999). We therefore propose *Lampisiphonia* as a new genus.

The genus *Lampisiphonia* is characterized by a combination of morphological features that include the erect habit of the species, rhizoids cut off from pericentral cells, 9–11 pericentral cells, axes from ecorticate to densely corticated at the base of oldest plants, exogenous branches, absence of trichoblasts, and tetrasporangia in straight series. Among the features delineating *Lampisiphonia*, the absence of trichoblasts and the dense cortication are the two features that separate this genus from others in *Polyisphonia sensu lato* and the tribe Poysiphonieae. The absence of trichoblasts has only previously been reported in the genera *Vertebrata* and

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Fig. 33. Tree constructed with Bayesian inference for the combined SSU and *rbc*L alignment. Values at branches represent Bayesian posterior probabilities (top left value), and 2000, 1000 and 500 bootstrap replicates each for distance, parsimony and maximum likelihood (top right and lower left and right values, respectively). Branches marked with an asterisk received 100% support in all three analyses, whereas those lacking values received less than 50% support. Scale bar = 0.01 substitutions/site.

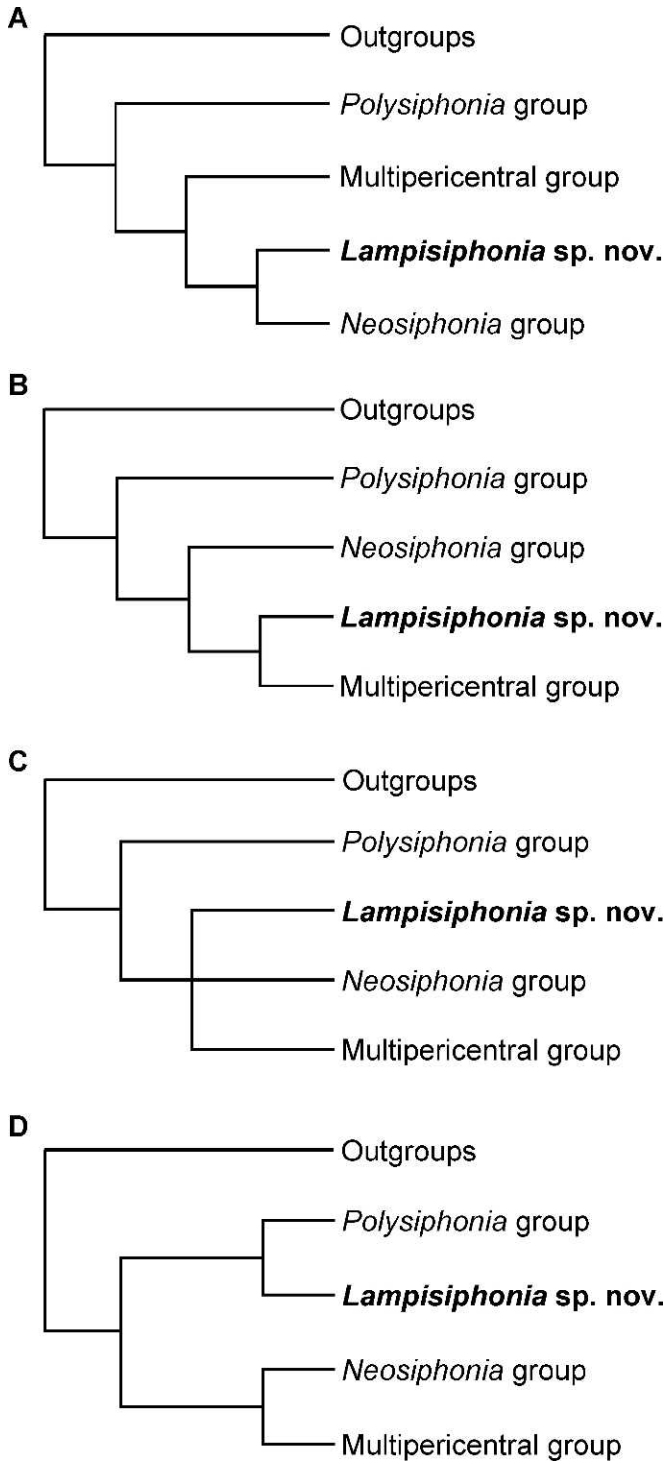

Fig. 34. A series of alternative phylogenetic hypotheses among the three resolved lineages of the *Polysiphonia sensu lato*. A. Monophyly of *Lampisiphonia* and the *Neosiphonia*-group. B. Monophyly of *Lampisiphonia* and the multipericentral-group. C. Trifurcating ancestor of *Lampisiphonia*, the *Neosiphonia*-group and the multipericentral-group. D. Monophyly of *Lampisiphonia* and the *Polysiphonia*-group.

Table 1. Results of the Shimodaira–Hasegawa (1999) tests used to evaluate alternative hypotheses among the three resolved lineages of the *Polysiphonia sensu lato* for our SSU rRNA, *rbc*L and combined SSU rRNA and *rbc*L alignments including common 40 taxa only.

Phylogenetic constraint tested[1]	−ln *L*	SSU rRNA *P*-value	−ln *L*	*rbc*L *P*-value	−ln *L*	combined *P*-value
None = best tree from ML analysis (monophyly of *Lampisiphonia* and the *Neosiphonia* group)	6398.04381	-	11,155.56820	-	18,109.04778	-
Monophyly of *Lampisiphonia* and the multipericentral group	6398.06693	0.814	11,159.24866	0.609	18,111.22206	0.692
Trifurcating ancestor of *Lampisiphonia*, the *Neosiphonia* group and the multipericentral group	6398.07773	0.794	11,163.83932	0.392	18,114.97346	0.517
Monophyly of *Lampisiphonia* and the *Polysiphonia* group	6438.56273	0.004[2]	11,263.35526	0.000[2]	18,248.44154	0.000[2]

[1] Phylogenetic constraints used are outlined in detail in the Material and Methods section.

[2] Significant difference.

Table 2. Comparison of vegetative and reproductive characters for selected species of *Polysiphonia* from the Atlantic coast, containing similar number of pericentral cells to *Lampisiphonia iberica*.

	L. iberica	*P. ceramiaeformis*	*P. exilis*	*P. howei*
Number of pericentral cells	(7) 9–11 (12)	10–12 (13)	8–11	(9) 10–14 (16)
Size thallus (cm)	3–6 (9)	2–5 (7)	2.5	0.5–1 (5.5)
Colour	bright red to brown-red	bright red, brownish red to brown, bleaching to straw-yellow	dull purplish-brown	dark brown, rosy red
Cortication	absent, except at the base	absent	absent	absent
Habit	erect; axes pseudodichotomously branched 5–7 (10) order ultimate divisions soft	dense irregularly rounded tufts. lacking distinct main axes forming corymbose apices	chiefly prostrate; densely caespitose but firm	creeping, densely matted, basal filaments entangled
Holdfast	discoid, decumbent axis produces secondary discoid	prostrate axes, becoming erect at the tips	extensive creeping system	prostrate indeterminate axes
Rhizoid anatomy	cut off from pericentral cells	cut off from pericentral cells	open conection with pericentral cells	cut off from pericentral cells
Axes and branches in diameter (μm)	apex 60–200 (250), base 350–900 (1300)	300–500	160–170	100–170
Segments (long/wide ratio)	0.5–2	1.3–2	0.5–0,75	0.5–1
Trichoblasts	not observed	frequent to scarce	few or inconspicuous to well developed	abundant
Branch origin	not associated with trichoblasts	replacing trichoblasts	replacing trichoblasts	replacing trichoblasts
Plastids	discoid to elliptical-bacillar, densely aggregated to chained	irregularly elongate to convoluted	sometimes arranged pectinately	
Spermatangial branches (long × diam. μm)	unknown	(200–300 × 30–65); lacking sterile terminal cell	50 × 150, sometimes subtended by a trichoblast	100–170 × 22–25
Cystocarps (diam. μm)	unknown	240–380, ovoid to slightly pyriform	375 × 400	*c.* 600 sessile, urceolate
Tetrasporangia (diam. μm)	25–40, straight series of 15	75–100, spiral series of 3–9	50–65, spiral sequence	40–55 (75), spiral series
Habitat	subtidal (11–30 m depth) epilithic in exposed and semiexposed sites	silty bedrock and *Ulva* sp. in pools at low water of neap tides at exposed to sheltered sites	rocks and gorgonians to low water	intertidal, over rocks, algae and corals
Geographic distribution	north of Iberian Peninsula	British Isles to northwestern France, Mediterranean and Canary Islands	western tropical and subtropical Atlantic and Pacific	Atlantic, Indian and Pacific oceans
References	this study	Lauret (1970), Maggs & Hommersand (1993), Rojas-González *et al.* (1994)	Taylor (1979), Hollenberg (1968a), Abbott (1999), Schneider & Searles (1997), Kapraun & Norris (1982)	Taylor (1945, 1979), Schneider & Searles (1991), Guimarães *et al.* (2004)

Tayloriella, and the species *P. virgata* (C.Agardh) Sprengel (Kylin 1956, as *Caradoria virgata* (C.Agardh) Kylin), wheras all other species have trichoblasts at some stage. *Vertebrata* and *Tayloriella* differ, however, in their ecorticate axes (Kylin 1956). Trichoblasts are usually more abundant in reproductive plants, and some species develop trichoblasts only in association with reproductive structures (Hollenberg 1968b). Although sexual structures in *Lampisiphonia* were not observed, trichoblasts were also absent in tetrasporophytic plants.

The cortication that characterizes *Lampisiphonia* is dense and compact, differing from that found in other species of *Polysiphonia sensu lato* where, even when well developed, the cortication is not as compact. One species whose morphological features closely match those found in *Lampisiphonia* with the exception of the compact cortications is *P. virgata* (Stegenga *et al.* 1997, ig. 235). Molecular results, however, show that *P. virgata* belongs to the *Neosiphonia* group and is not related to *Lampisiphonia*. Within the tribe Polysiphonieae similar compact cortication has been found in some genera such as *Alsidium*, *Lophurella* or *Alleynea* (Norris 1994; Womersley 2003), but these genera can be separated from *Lamphisiphonia* by having trichoblasts, amongst other features.

Another remarkable feature found in *Lampisiphonia* is the production of tetrasporangia in straight series, as in the *Polysiphonia* group, but the latter differs from *Lampisiphonia* in having rhizoids in open connection to pericentral cells and axes with four pericentral cells (Choi *et al.* 2001).

Table 2. Extended

P. nigra	*P. paniculata*	*P. simulans*	*P. subulifera*
(8) 9–13 (14)	(8) 9–12 (14)	10–13	12–13
3–20 (30)	(5) 10–20 (30)	2–8	5–20
brownish-red, reddish purple, bleaching to pale brown	brownish-red to dark reddish brown	bright red	bright red
absent	absent	absent	absent
dense cylindrical to irregularly rounded tufts, gregarious	densely tufted, soft	dense irregularly rounded tufts with a crisp, fairly rigid texture; main axes indistinct; ultimate branchlets usually short and spinelike	dense irregularly rounded tufts; main axes indistinct; ultimate branchlets usually short and spinelike
extensive prostrate axes	extensive prostrate axes	prostrate axes	prostrate axes
cut off from pericentral cells	cut off from pericentral cells	cut off from pericentral cells	cut off from pericentral cells
200–330 axes, 165–210 branches	150–240 (430)	200–300	300–500
0.7–2	1.5–2.5 (4)	0.8–1.3	
sparse or frequent	scarce to abundant	sparse	abundant
in the axils of trichoblasts	in the axils of trichoblasts	replacing trichoblasts	in the axils of trichoblasts
irregularly-shaped, discoid to convoluted		irregularly-shaped, discoid, beaded or convoluted	irregularly-shaped, beaded, elongate or convoluted
(200–300 × 50–75), replacing to trichoblast	130–250 × 50–70, without sterile apex, arising as primary branch of trichoblast	unknown	unknown
400–550, globular	350–430, globular-ovoid, urceolate	unknown	unknown
60–125, spiral series	80–110, spiral series	50–70, spiral series of 1–4	unknown
bedrock, pebbles, shells and maërl, from intertidal to subtidal (20 m)	sand-swept lower intertidal rocks and subtidal (20 m), epiphytic	epiphytic on various algae, from intertidal pools to lower intertidal, in sheltered sites	pebbles, maërl and crustose coralline algae, from lower intertidal to subtidal (20 m depth)
eastern Atlantic	Atlantic, Mediterranean, Pacific oceans	south of British Isles to northwestern France	British Isles to northwestern France, Mediterranean, Southwest Asia
Harvey (1846–51), Veldkamp (1950), Taylor (1957), Coppejans (1981), Kapraun & Rueness (1983), Maggs & Hommersand (1993)	Dawson (1944 as *P. californica*), Lauret (1970), Hollenberg (1944, 1961), Smith (1969), Abbott & Hollenberg (1976)	Harvey (1846–51), Veldkamp (1950), Lancelot (1966), Maggs & Hommersand (1993)	Harvey (1846–51), Maggs & Hommersand (1993)

Table 3. Comparison of vegetative and reproductive characters for selected species of *Polysiphonia* from the Pacific and Indian coasts, containing similar number of pericentral cells to *Lampisiphonia iberica*.

	L. iberica	*P. adamsiae*	*P. aterrima*	*P. atricapilla*	*P. collinsii*	*P. confusa*
Number of pericentral cells	(7) 9–11 (12)	(8) 10–11 (12)	10–12, hexagonal surface pattern	10–12	12–14	8–10
Size thallus (cm)	3–6 (9)	0.5–3	*c.* 12	4–12	2–4	0.8–1.3 (3)
Colour	bright red to brown-red	dull reddish brown, dark brown-red	dark brown drying black	dark brown-red	reddish-brown to nearly black	
Cortication	absent, except at the base	absent	absent	absent	absent	absent
Habit	erect; axes pseudodichotomously branched 5–7 (10) order. ultimate divisions soft	dense mats, close tufts	erect, robust, bushy, with thick, cartilaginous axes irregularly dichotomously branched	densely tufted, with a single erect, basal axis	branching frequently unilateral	
Holdfast	discoid, decumben axis produces secondary discoid	extensive system of prostrate axes	tangled clump of rhizoids	discoid, rhizoidal; basal axis suberect to very shortly prostrate;	prostrate creeping axes	prostrate axes (100–175 μm)
Rhizoid anatomy	cut off from pericentral cells	open connection with pericentral cells	open connection with pericentral cells	cut off from pericentral cells	cut off from pericentral cells	cut off from pericentral cells
Axes and branches in diameter (μm)	apex 60–200 (250), base 350–900 (1300)	apex 60–100, base 300	*c.* 2000	apex 200–300, base 700-1000	150–180	60–140
Segments (long/wide ratio)	0.5–2	(0.2) 0.5–1 (1.5)		(0.2) 0.3–0.5 (1)	2–4	1–2.5
Trichoblasts	not observed	scarce, slender	robust, often pigmented, incurved	profuse and brown	abundant	abundant
Branch origin	not associated with trichoblasts	replacing trichoblasts	-	in the axils of trichoblasts	in the axils of trichoblasts	in the axils of trichoblasts
Plastids	discoid to elliptical-bacillar, densely aggregated to chained	discoid, scattered		ribbon-shaped		
Spermatangial branches (long × diam. μm)	unknown	spermatangial branches replacing the whole trichoblast without sterile apical cells	forming one branch of the trichoblast, often paired	developing as one basal branch of a trichoblast, without a sterile apical cell when mature		unknown
Cystocarps (diam. μm)	unknown	short-stalked, ovoid-urceolate; 280–450	ovoid to globose; very large	subsessile, globular to slightly ovoid; 400–500	200–280, subsessile, ovoid	unknown
Tetrasporangia (diam μm)	25–40, straight series of 15	30–50 (60), spiral series in upper branches	short spiral series	70–100, spiral series	40–70	60–80, long spiral series
Habitat	subtidal (11–30 m depth) epilithic in exposed and semiexposed sites	rocks, low intertidal at exposed sites; ocasionally epiphytic	epiphytic, in deep intertidal pools to subtidal, at exposed sites	commonly epiphytic	saxicola	mid-intertidal rocks with sand
Geographic distribution	north of Iberian Peninsula	Australia and New Zealand	Australia and New Zealand	Australia and New Zealand	British Columbia to Southern California	California and Peru
References	this study	Adams (1991, 1994), Womersley (1979, 2003)	Adams (1991, 1994)	Womersley (1979, 2003)	Hollenberg (1944), Smith (1969)	Hollenberg (1944 as *P. inconspicua*, 1961), Abbott & Hollenberg (1976)

Table 3. Extended

P. crassa	*P. hendryi*	*P. isogona*	*P. muelleriana*	*P. nathanielii*	*P. tapinocarpa*
10–11	10–12 (14)	(7) 9–10 (12)	(8) 9–12	(5) 7–10	8–10
3.5–4.2	0.5–1 (4)	(1) 3–15 (20)	*c*. 40	10–15	6.5–10
dark redish brown	dark brown, reddish-black	dark red-brown	dark reddish brown	nearly black in dried	brownish black
present throughout thallus	absent	absent	present	absent	absent
solitary or a few tufted	branching appearing as dichotomous	slender, tufted (with numerous axes) soft and flaccid	erect, robust, cartilaginous. Ultimate divisions soft and flaccid	coarse, erect, main axes sparsely branched and very short lateral branchlets	flaccid, mucous, fine, slender filaments
-	brief prostrate axes (120–140 μm)	short system of prostrate axes	truncate holdfast or short system of prostrate axes	basal mass of tangled branches	tangled prostrate axes and decumbent filaments
-	cut off from pericentral cells	cut off from pericentral cells	-	open connection with pericentral cells	cut off from pericentral cells
c. 675	90–150 (350)	apex 30–80, erect axes 125–250 (300), prostrate axes (100) 140–250	2000–3000		225 base
0.3–0.5	0.5–2	(0.5) 0.7–3 (4)		0.5–0.7	1.4–6
	abundant	slender and short	not observed	apparently absent	abundant
-	in the axils of trichoblasts	in the axils of trichoblasts discoid, densely aggregated, occasionally in chains	-	not associated with trichoblasts	not associated with trichoblasts
	conical to fusiform (90–160 × 25–36)	spermatangial branches developing as basal branch of trichoblast (150–220)	unilateral, acropetal series, replacing the trichoblast	with very short one-celled sterile tips	
345–450, sessile, globose	350–400, subsessile, ovoid	stalked, subspherical or slightly conical to ovoid; 250–400 (600)	short-stalked, ovoid to globose	unknown	440–675, ovoid, stalked
60, in few seriate	55–65, spiral series	55–90, spiral series in upper branches	spiral series in upper branches	straight series	60, straight series
epiphytic on other algae	epiphytic	epiphytic, rock, low intertidal at sandy semiexposed sites	rocks in tidal pools to subtidal, on open coasts	rocks, low intertidal	rocks in upper subtidal
Japan	Alaska, Baja California, Mexico	Australia and New Zealand; South America	Australia and New Zealand	Pacific coast of Mexico, Southern California	Asia
Segi (1951), Yoshida (1998)	Hollenberg (1944, 1961), Smith (1969)	Adams (1991, 1994), Womersley (1979, 2003)	Adams (1991, 1994)	Hollenberg (1944, 1958, 1961), Abbott & Hollenberg (1976), Sentíes (1995)	Segi (1951)

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