

## Three new cryptogenic species in the tribes Polysiphonieae and Streblocladieae (Rhodomelaceae, Rhodophyta)

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**ABSTRACT:** During our sampling surveys of the tribes Polysiphonieae and Streblocladieae in Spain and Australia, three previously unrecorded species were collected. Based on molecular and morphological evidence they are proposed as new species. *Polysiphonia delicata* sp. nov. and *Polysiphonia radiata* sp. nov. belong to the Polysiphonieae and share the synapomorphy in this group, rhizoids in open connection to pericentral cells. They differ from other members of this group either by *rbcL* sequence divergences greater than 4.5% and/or by morphological characters. The third species is placed in *Melanothamnus* (tribe Streblocladieae), as *Melanothamnus pseudoforcipatus* sp. nov. In agreement with the morphological delineation of the genus, it has plastids lying only on the radial walls of pericentral cells. It can be separated from most other members of the genus by having naked segments between trichoblasts or branches and/or *rbcL* sequence divergences higher than 4%. In Galicia, Spain, both *Polysiphonia* species were mainly collected in marinas, while *M. pseudoforcipatus* was found at a site close to oyster aquaculture facilities. *Polysiphonia delicata* was also found in Victoria, Australia, and the potentially non-native status of these three species in relation to their known distribution is discussed.

**KEY WORDS:** *Cox1*, Introduction vectors, *Melanothamnus*, *Melanothamnus pseudoforcipatus* sp. nov., Non-native, *Polysiphonia*, *Polysiphonia delicata* sp. nov., *Polysiphonia radiata* sp. nov., *rbcL*, New species

### INTRODUCTION

Species introduction is one of the major environmental concerns in relation to human activities and global change (Occhipinti-Ambrogi 2007). The detection of new introductions in the marine realm is often difficult due to the complex systematics of many taxa, and consequently cryptic introductions are common (Carlton & Geller 1993). Aquaculture activities and hull fouling are considered to be the two main introduction vectors (Mineur *et al.* 2007a, b; Williams & Smith 2007; Thomsen *et al.* 2016). The species associated with different vectors can be determined by experimental work on transport of seaweeds by potential pathways such as the aquaculture trade (e.g. oysters as vectors; Mineur *et al.* 2007b) and recreational boating (e.g. yacht hulls and/or floating pontoons as vectors; Hay 1990; Mineur *et al.* 2007a, 2008). This evidence can provide a good indication of the types of organisms that can be introduced with particular vectors, so that, by comparison with native ranges, inferences can be made as to likely pathways of introduction (Mineur *et al.* 2014). Another approach is to compile species lists from habitats associated with introduction pathways. The presence of new aliens in yacht marinas is linked to fouling on recreational vessels, so rapid assessment protocols in marinas are valuable (Arenas *et al.* 2006). Monitoring areas with aquaculture installations has provided lists of new records presumed to be associated with shellfish farming

(Mineur *et al.* 2010, 2012a). A recent comprehensive meta-analysis of aquaculture activities by Grosholz *et al.* (2015) evaluated successful and unsuccessful introductions of marine aliens into California. However, the actual status of many potentially alien species is unclear – they may have been spread anthropogenically but they could instead be native, although previously unrecognized within the geographical area in question (Ruiz *et al.* 2000; McIvor *et al.* 2001). Species with an unknown or speculative origin are termed cryptogenic (Carlton 1996, 2009). They are frequently cryptic and/or small, belong to little-studied taxonomic groups and are often described taxonomically by different names in each new area (Mineur *et al.* 2012b). It has been estimated that 346 seaweed species have been introduced or are cryptogenic in one or more world regions (Thomsen *et al.* 2016).

The family Rhodomelaceae and particularly the tribes Polysiphonieae and Streblocladieae have large numbers of introduced species recorded worldwide: 41 and 18 recognized non-native or cryptogenic species, respectively (Williams & Smith 2007; Bustamante *et al.* 2015a; Thomsen *et al.* 2016). These high numbers are probably not surprising because the Polysiphonieae and the recently segregated Streblocladieae includes the largest genus of the red algae, *Polysiphonia* (200 species), as well as another 19 smaller genera (Guiry & Guiry 2016; Díaz-Tapia *et al.* 2017a,b). The Polysiphonieae and Streblocladieae are also cosmopolitan groups, distributed worldwide in a wide variety of benthic habitats. However, numbers of introduced species in the Polysiphonieae and Streblocladieae could be significantly underestimated because members of these groups are small and morphologi-

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cally similar, making them good candidates for cryptic introductions (McIvor *et al.* 2001; Geoffroy *et al.* 2012). Although small Rhodomelaceae are often individually inconspicuous, they can be invasive and become dominant in some habitats, e.g. *Womersleyella setacea* (Hollenberg) R.E.Norris in the Mediterranean Sea or *Polysiphonia morrowii* Harvey in French Brittany (Battelli & Rindi 2008; Geoffroy *et al.* 2012).

During surveys of the tribes Polysiphonieae and Streblocladieae along the Atlantic coasts of Spain, detection of new introductions was a priority, so particular efforts were made to sample near sites with known aquaculture and/or boating activities. Identification of taxa within this group is difficult due to shared characters, morphological variation and the need to compare species from distant coasts. However, DNA-assisted identification greatly facilitates this task, and molecular data have clarified the identity of specimens from different regions, resulting in the description of new species (Stuercke & Freshwater 2010) or the establishment of synonyms (Díaz-Tapia *et al.* 2013). Also some introduced species have been identified using molecular data (Mineur *et al.* 2010, 2012a; Geoffroy *et al.* 2012; Díaz-Tapia *et al.* 2013; Bustamante *et al.* 2015a). Within regions, high cryptic diversity has been discovered, e.g. four new species of the Polysiphonieae from Korea (Bustamante *et al.* 2014a, b, 2015b; Kim & Kim 2015). However, an important limitation of molecular identification tools is that to date DNA sequences are available for only approximately 25% of the currently accepted Polysiphonieae and Streblocladieae.

Here, we report on three unknown species of the tribes Polysiphonieae and Streblocladieae from north-western Spain, one of which was also found in southern Australia. These algae were collected in marinas, on piers or close to oyster farms. The aim of this paper is to clarify their taxonomic identity based on morphological characters and molecular data and to determine whether they represent new introductions into Europe and/or Australia.

## MATERIAL AND METHODS

Material of the three species described here was collected during general sampling surveys of the family Rhodomelaceae in Galicia (north-western Spain) and southern Australia (Table S1; Fig. 1). In addition to natural habitats, anthropogenically impacted sites including a range of marinas and aquaculture facilities were sampled. In total, eight marinas were explored in Galicia (Ribadeo, A Graña, Sada, Oza, Dique de Abrigo, O Portiño, Bouzas, Baiona), two piers and two marinas in Victoria (Australia) and two in Western Australia. In Spain, the vicinity of three aquaculture installations was sampled. Comparative sampling was carried out in marinas and aquaculture installations in England (Plymouth), Wales (Westfield Pill), Ireland (Lough Swilly), France (Marseille, Thau Lagoon) and Italy (Alassio).

Materials for DNA extraction were preserved in silica gel desiccant. Plants for morphological study were preserved in 4% formalin seawater at 4°C and stored in the dark. Some specimens were mounted in 20% Karo® Syrup (ACH Foods, Memphis, Tennessee USA) and 80% distilled water. Sections

for microscopic observations were made by hand using a razor blade. Voucher specimens were deposited in Herbario SANT, Universidade de Santiago de Compostela (SANT). Herbarium abbreviations follow Thiers (2016).

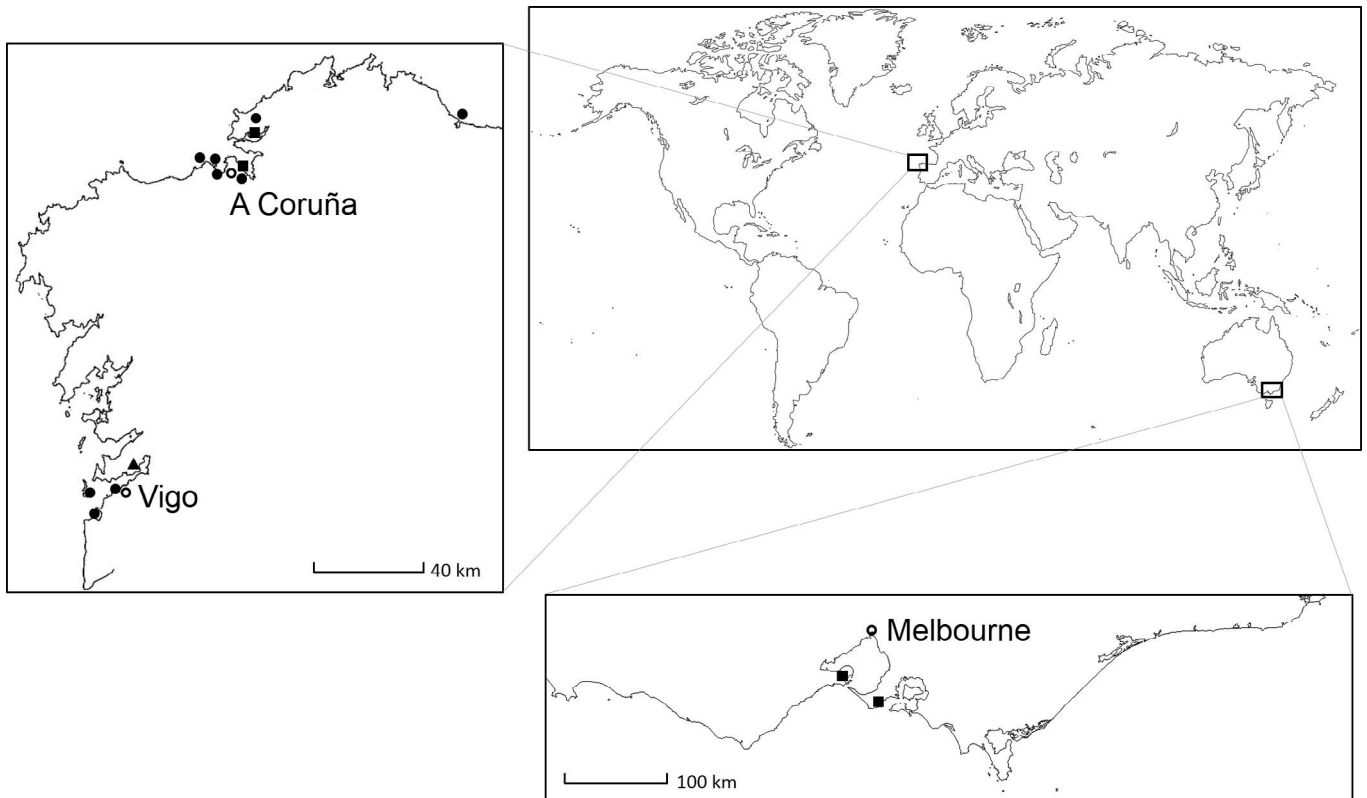
DNA was extracted from silica gel-dried material following Saunders & McDevit (2012), using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) or the Promega Wizard Magnetic 96 DNA Plant System kit (Promega, Madison, Wisconsin USA), following the manufacturer's instructions. Polymerase chain reaction (PCR) amplification was carried out for *rbcL* using the primers F7/RbcStart, F7/R893 or F57/rbcLrevNEW (Freshwater & Ruess 1994; Mamoozadeh & Freshwater 2011; Saunders & Moore 2013) and for *cox1* using the primers GwsFn/Cox1R1 (Saunders 2008; Le Gall & Saunders 2010). Reactions were performed in a total volume of 25 µl, consisting of 5 µl 5× MyTaq™ reaction buffer, 0.7 µl 10 µM of forward and reverse primers, 0.125 µl 1U/µl My Taq DNA Polymerase (Bioline, London, UK), 17.475 µl MilliQ® water 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 at Queen's University of Belfast on an AB3730xl DNA Analyzer (Applied Biosystems, Foster City, California USA) or commercially by Macrogen (Seoul, South Korea) or the sequencing service of the University of A Coruña.

In total, 32 and 19 new sequences were generated in this study for the *rbcL* and *cox1* genes, respectively. In addition, 85 sequences were downloaded from GenBank for the *rbcL* phylogenetic analysis. The sequences and their corresponding GenBank accession numbers are listed in Table S2. Sequences were aligned using Muscle in Geneious 6.1.8 (Kearse *et al.* 2012). Identical sequences and those that diverged by less than 1.1% were removed from the *rbcL* analysis. The sequences included in the final alignment were selected after considering their quality in terms of both length and the presence of ambiguous bases. Phylogenetic trees for *rbcL* were estimated with maximum likelihood (ML) using RAxML 8.1.X (Stamatakis 2014). General time reversible gamma was used as the nucleotide model; branch support was estimated with 1000 bootstrap replicates. Three species of *Symphocladia* were selected as the outgroup based on our phylogenomic analyses of the major lineages of the Rhodomelaceae, which resolve a clade formed by the Herposiphonieae and Pterosiphonieae as sister to the Polysiphonieae and Streblocladieae (Díaz-Tapia *et al.*, 2017a).

## RESULTS

### Species surveys

All samples collected on the coasts of Galicia corresponded to species identifiable as those previously reported in this area (Bárbara *et al.* 2005; Díaz-Tapia & Bárbara 2013) except the three unknown members of the Polysiphonieae and Streblocladieae reported in detail here. Two of these species (here named *Polysiphonia delicata* and *Polysiphonia radiata*) were collected in eight marinas, and one of them (*P. radiata*) was



**Fig. 1.** Distribution of *Polysiphonia radiata* (circles), *Polysiphonia delicata* (squares) and *Melanothamnus pseudoforcipatus* (triangle). Galician locations from the North to the South: Ribadeo (43°32.24'N; 7°02.12'W), A Graña (43°24.46'N; 8°15.33'W), Sada (43°21.34'N; 8°14.52'W), Oza (43°20.55'N; 8°23.00'W), Abrigo (43°22.04'N; 8°23.13'W), Portiño (43°22.22'N; 8°26.50'W), Rande (42°27.26'N; 8°39.83'W), Bouzas (42°13.43'N; 8°45.01'W), Baiona (42°07.07'N; 8°50.39'W), Cies (42°12.08'N; 8°53.40'W). Australian locations, from the West to the East: Queenscliff marina (38°15.52'S; 144°40.16'E), Flinders pier (38°28.32'S; 145°01.35'E).

also found in a maërl bed. Moreover, one of them (*P. delicata*) was found in a marina and at a pier in Victoria, Australia. The third species (*Melanothamnus pseudoforcipatus*) was collected close to Galician oyster aquaculture facilities (Fig. 1). They were not found in comparative surveys carried out in marinas or aquaculture installations in England, Wales, Ireland, France or Italy.

### Phylogeny

The RAxML phylogenetic analysis of *rbcL* sequences (Fig. 2) placed two of the unidentified species in the strongly supported clade *Polysiphonia sensu stricto* 1 of the tribe Polysiphonieae, which also includes the type of the genus, *Polysiphonia stricta*. There was 4.1–4.2% sequence divergence between the two species, which formed a weakly supported clade. There was  $\geq 4.5\%$  sequence divergence between them and all other species for which molecular data are available (Table S3). Each species has distinctive morphological characteristics within the *Polysiphonia* clade. We here propose *Polysiphonia radiata* sp. nov. from Galicia, for which 25 *rbcL* and 18 *cox1* identical sequences were generated. The second *Polysiphonia* species is here described as *Polysiphonia delicata* sp. nov. from Spain (two samples) and Australia (two samples) with only 1 bp divergence among *rbcL* sequences from the Australian and Spanish samples. We were unable to obtain *cox1* PCR products for the latter species.

The third unidentified species, which we describe here as *Melanothamnus pseudoforcipatus*, was positioned in the strongly supported *Melanothamnus* clade of the tribe Streblocladiae in the *rbcL* phylogeny (Fig. 2). The most similar species was *Melanothamnus sphaerocarpus*, which differed by 4% sequence divergence (Table S4) but the grouping was not well-supported by bootstrapping in our *rbcL* phylogeny.

### Species descriptions

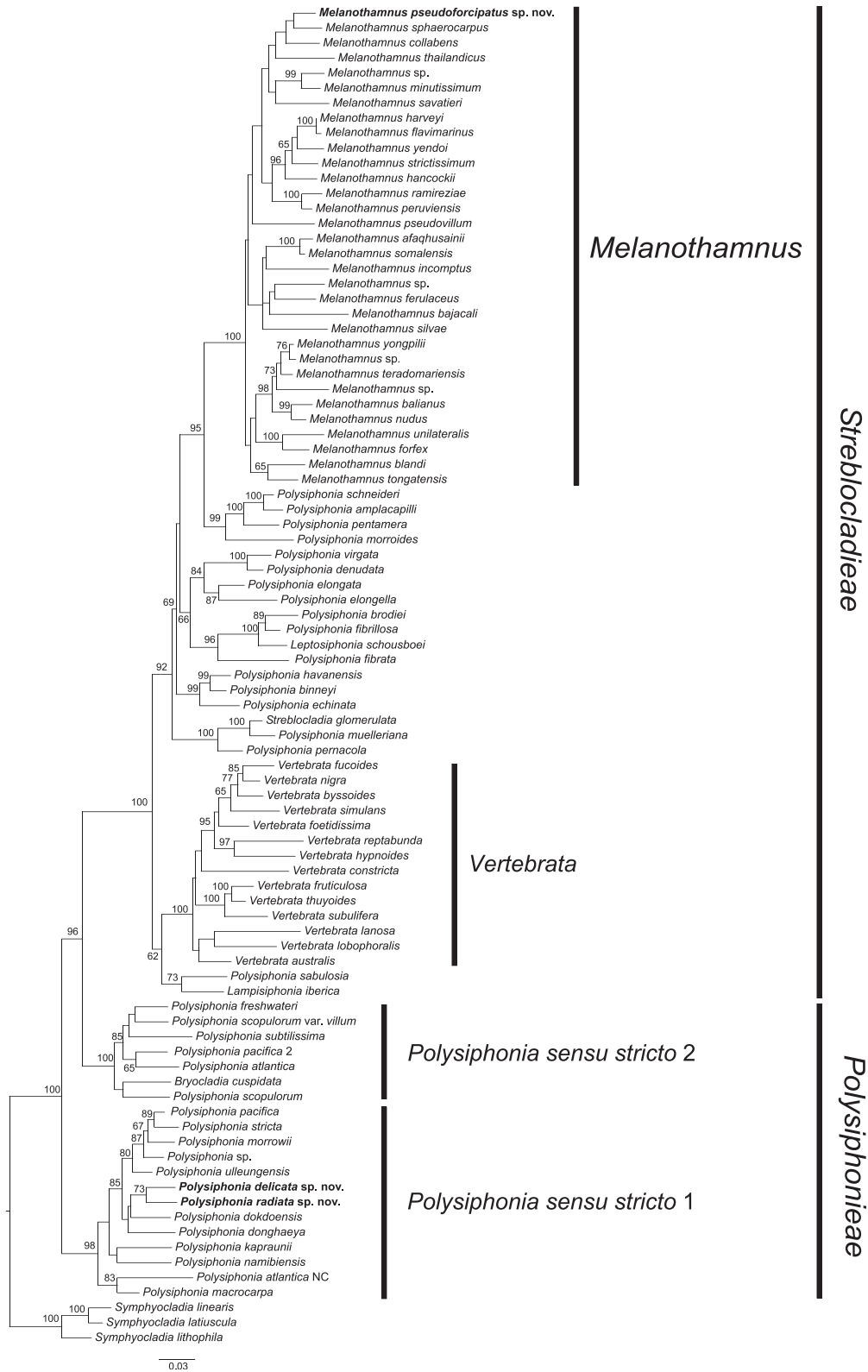
#### *Polysiphonia radiata* Díaz-Tapia sp. nov.

Figs 3–26

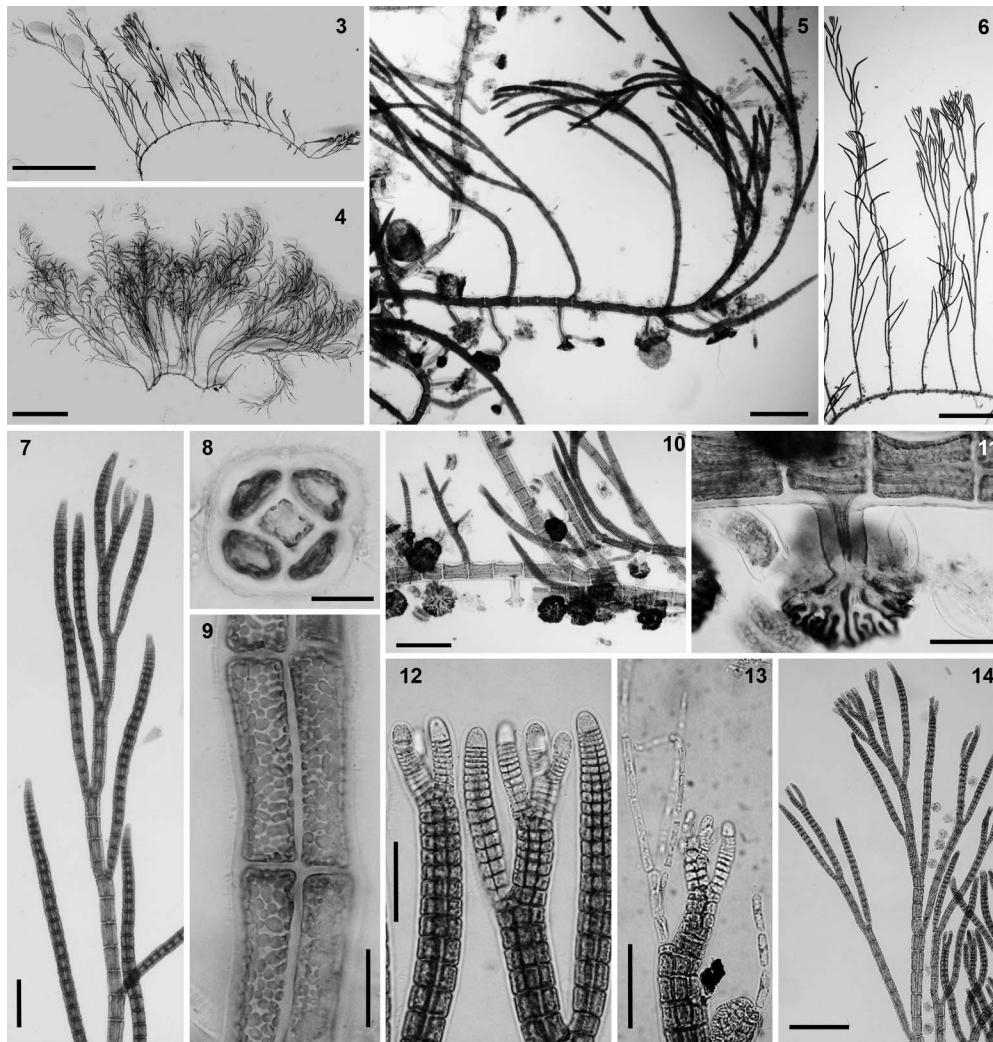
**DIAGNOSIS:** Thalli decumbent, attached by unicellular rhizoids in open connection with pericentral cells. Axes ecorticate, up to 120  $\mu\text{m}$  in diameter, with four pericentral cells. Erect axes radially and regularly branched every four to seven segments, up to two to three orders. Branches exogenous, independent of trichoblasts. Trichoblasts absent in sterile, male and tetrasporangial specimens; scarce and irregularly arranged in females. Spermatangial branches replacing trichoblasts, with or without sterile apical cells. Procarys with a four-celled carpogonial branch, cystocarps ovoid to slightly urceolate. Tetrasporangia forming straight series, with two cover cells.

**HOLOTYPE:** SANT-Algae 31120.

**TYPE LOCALITY:** Oza, A Coruña, Galicia, Spain; 43°20.55'N; 8°23.00'W.



**Fig. 2.** Phylogenetic tree estimated with ML analysis of *rbcl* sequences. Values at nodes indicate bootstrap support (BP) (only shown if > 60). Species names printed in bold correspond to the new species.



**Figs 3–14.** *Polysiphonia radiata* sp. nov., vegetative morphology.

**Figs 3–4.** Habit of a sterile (3) and tetrasporangial (4) specimen. Scale bars = 4 mm.

**Fig. 5.** Prostrate axes bearing erect branches and rhizoids. Scale bar = 400  $\mu$ m.

**Fig. 6.** Erect axes. Scale bar = 1.2 mm.

**Fig. 7.** Apical part of an erect axis with branches arranged spirally every four to five segments. Scale bar = 100  $\mu$ m.

**Fig. 8.** Cross section with an axial cell and four pericentral cells. Scale bar = 25  $\mu$ m.

**Fig. 9.** Surface view of pericentral cells with discoid plastids. Scale bar = 25  $\mu$ m.

**Fig. 10.** Prostrate axis bearing numerous rhizoids terminating in discoid pads. Scale bar = 70  $\mu$ m.

**Fig. 11.** Detail of a rhizoid, unicellular and in open connection with the pericentral cell. Scale bar = 70  $\mu$ m.

**Fig. 12.** Apex of erect axes with rounded apical cells and forming exogenous branches. Scale bar = 50  $\mu$ m.

**Fig. 13.** Apex of erect axes of a female gametophyte with a vegetative trichoblast. Scale bar = 70  $\mu$ m.

**Fig. 14.** Apex of erect axes of a sterile specimen lacking trichoblasts. Scale bar = 200  $\mu$ m.

**ETYMOLOGY:** From the Latin word ‘radiatus’ for ‘radial’ because branches are spirally arranged.

**MOLECULAR VOUCHERS:** KY620065 *rbcL*; KY620044 *cox1*.

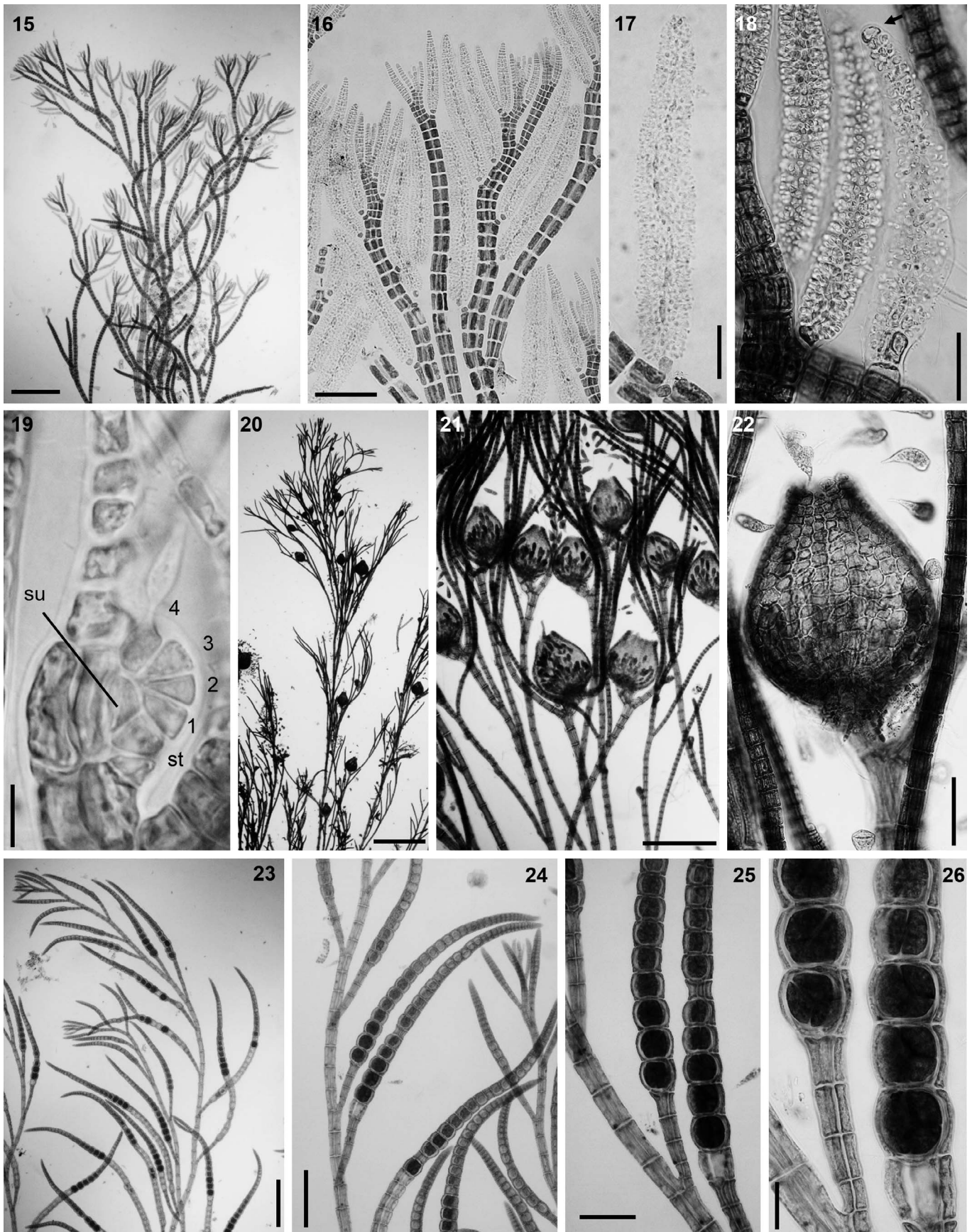
**OTHER SPECIMENS EXAMINED:** See Table S1.

### Vegetative morphology

Thalli forming small turfs up to 17 mm high. Thallus initially organized radially, growing from indeterminate erect axes, becoming decumbent and dorsiventral when developing

rhizoids in basal parts, forming extensive prostrate systems (Figs 3–5). Erect axes percurrent, producing short lateral branches every four to seven segments, in a  $\frac{1}{4}$  spiral pattern, up to two to three orders (Figs 6, 7). Tufts pink in colour, with a very flaccid texture.

Axes ecorticate, consisting of an axial cell surrounded by four pericentral cells (Fig. 8) with plastids discoid and lying on all the cell walls (Fig. 9). Prostrate axes (50–) 60–100 (–120)  $\mu$ m in diameter (Fig. 10), composed of segments Length/Diameter (L/D) 0.5–1.8. Rhizoids one per segment or scattered throughout the prostrate axes, formed on the



Figs 15–26. *Polysiphonia radiata* sp. nov., reproductive morphology.

ventral side of the prostrate axes, arising in the median or distal parts of the pericentral cells and remaining in open connection to them, unicellular, 30–70 µm in diameter and up to 900 µm long, sometimes branched, usually terminating in digitate haptera (Figs 10, 11).

Erect axes growing from rounded apical cells 12–15 µm in diameter (Fig. 12), dividing transversally or obliquely when producing naked segments or exogenous lateral branches, respectively. Axes 40–70 (–80) µm in diameter in mid and basal parts, composed of segments L/D 0.8–2.4. Trichoblasts generally absent, only observed in female thalli, scarce and irregularly arranged, up to 450 µm in length and one to two times branched (Figs 13, 14). Branches arising exogenously at the apices of the erect axes, independently from trichoblasts. Adventitious branches only occasionally observed in prostrate axes.

### Reproductive morphology

Gametophytes dioecious. Spermatangial axes densely clustered at the apices of erect axes, borne every two to four segments in a ¼ spiral (Figs 15, 16). They arise on suprabasal cells of modified trichoblasts and replace them. Spermatangial axes cylindrical, with or without one to three sterile apical cells when fully mature, (170–) 200–300 µm long and 32–40 (–48) µm in diameter (Figs 17, 18).

Procarys are formed in the apices of erect axes, on suprabasal cells of modified trichoblasts, consisting of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell and a lateral group of two sterile cells (Fig. 19). Cystocarps ovoid or slightly urceolate when mature, (190–) 210–350 µm high and (160–) 200–310 µm in diameter, with a narrow ostiole 40–80 µm wide (Figs 20–22). Carposporangia clavate, 20–30 × 60–105 µm.

Tetrasporangia in slightly thickened and often reflexed lateral branches, forming straight series of up to eight mature tetrasporangia (Figs 23, 24). They are ovate, 35–60 µm in diameter, tetrahedrally divided (Figs 25, 26), with two cover cells similar to the pericentral cells.

### Habitat and distribution

The known distribution is restricted to Galicia, north-western Spain (Fig. 1). *Polysiphonia radiata* was collected in all the eight explored marinas in this area, where it formed small tufts mainly on mussels attached to the pontoons but also on ropes or directly on the pontoons from 0 to 5 m depth. It was abundant at one site (Oza Marina) and rare in the other marinas. It was also collected in a subtidal (10 m depth) maërl bed in the Cíes Archipelago in the Galician Atlantic Islands Maritime-Terrestrial National Park where it was very scarce, forming small tufts on rhodoliths.

### *Polysiphonia delicata* Díaz-Tapia sp. nov.

Figs 27–46

**DIAGNOSIS:** Thalli decumbent, attached by unicellular rhizoids in open connection with pericentral cells. Axes with four pericentral cells, ecorticate; up to 140 µm in diameter. Erect axes spirally and regularly branched every three–six segments, up to four orders. Branches exogenous, independent of trichoblasts. Trichoblasts scarce in sterile, male and female specimens, abundant in tetrasporophytes, every two to three segments. Spermatangial branches replacing trichoblasts, terminating in a filament of four to six sterile apical cells. Procarys with a four-celled carpogonial branch. Tetrasporangia forming straight series, with two cover cells.

**HOLOTYPE:** SANT-Algae 31087.

**TYPE LOCALITY:** A Graña, Ferrol, Galicia, Spain; 43°24.46'N; 8°15.33'W.

**ETYMOLOGY:** The Latin word 'delicatus' meaning 'delicate' refers to the fine and fragile thalli.

**MOLECULAR VOUCHERS:** KY620062 *rbcL*.

**OTHER SPECIMENS EXAMINED:** see Table S1.

### Vegetative morphology

Thalli forming small turfs up to 27 mm high. Thallus initially organized radially, growing from indeterminate erect axes, becoming decumbent and dorsiventral when developing rhizoids in basal parts, forming extensive prostrate systems (Figs 27–29). Erect axes percurrent, producing short lateral branches every three to six segments, in a ¼ spiral pattern, up to four orders (Figs 30, 31). Tufts pink in colour, with a very flaccid texture.

Axes ecorticate, consisting of an axial cell surrounded by four pericentral cells (Fig. 32), with discoid plastids lying on all the cell walls (Fig. 33). Prostrate axes 60–120 (–140) µm in diameter (Fig. 34), composed of segments L/D 0.7–1.8 (–2.3). Rhizoids one per segment or scattered throughout the prostrate axes, formed ventrally on prostrate axes, arising in the mid or distal parts of the pericentral cells and remaining in open connection to them, unicellular, (20–) 30–80 (–110) µm in diameter and up to 500 µm long, usually terminating in digitate haptera (Figs 34, 35).

Erect axes growing from rounded apical cells 10–15 µm in diameter, dividing transversally, producing naked segments or obliquely, producing lateral exogenous branches (Fig. 36). Axes 60–120 (–140) µm in diameter in middle and basal parts, composed of segments L/D (0.8–) 1.3–2.2 (–2.7). Trichoblasts scarce and irregularly arranged in vegetative parts of specimens, as well as in female and

**Figs 15–16.** Upper parts of erect axes with densely clustered spermatangial branches. Scale bars: Fig. 15 = 400 µm; Fig. 16 = 100 µm.

**Figs 17–18.** Spermatangial branches, replacing trichoblasts and with (arrow) or without a sterile apical cell. Scale bars = 40 µm.

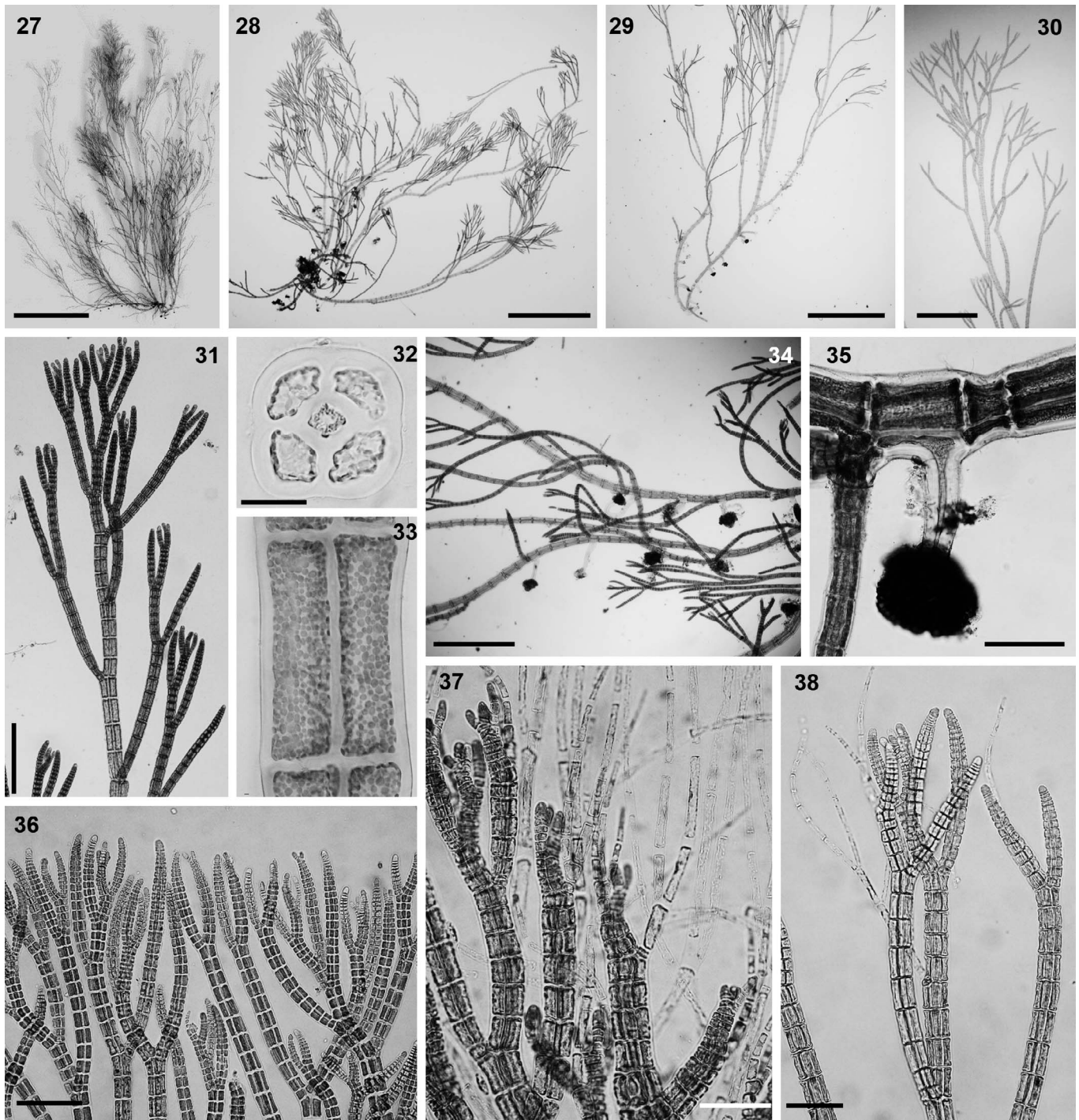
**Fig. 19.** Procary showing the supporting cell (su), bearing the four-celled carpogonial branch (1–4) and a basal sterile cell (st). Scale bar = 10 µm.

**Figs 20–21.** Erect axes with urceolate cystocarps. Scale bars: Fig. 20 = 100 µm; Fig. 21 = 400 µm.

**Fig. 22.** Detail of a cystocarp and cells of the pericarp. Scale bar = 100 µm.

**Figs 23–24.** Upper branches of erect axes, reflexed and bearing tetrasporangia in long straight series. Scale bars: Fig. 23 = 400 µm; Fig. 24 = 200 µm.

**Figs 25–26.** Detail of tetrasporangia. Scale bars: Fig. 25 = 100 µm; Fig. 26 = 40 µm.



**Figs 27–38.** *Polysiphonia delicata* sp. nov., vegetative morphology.

Figs 27–29. Habit. Scale bars: Fig. 27 = 6 mm; Figs 28–29 = 2 mm.

Fig. 30. Erect axis. Scale bar = 600  $\mu$ m.

Fig. 31. Upper part of an erect axis with branches every four to five segments. Scale bar = 200  $\mu$ m.

Fig. 32. Cross section with a small axial cell and four pericentral cells. Scale bar = 50  $\mu$ m.

Fig. 33. Surface view of pericentral cells with discoid plastids. Scale bar = 30  $\mu$ m.

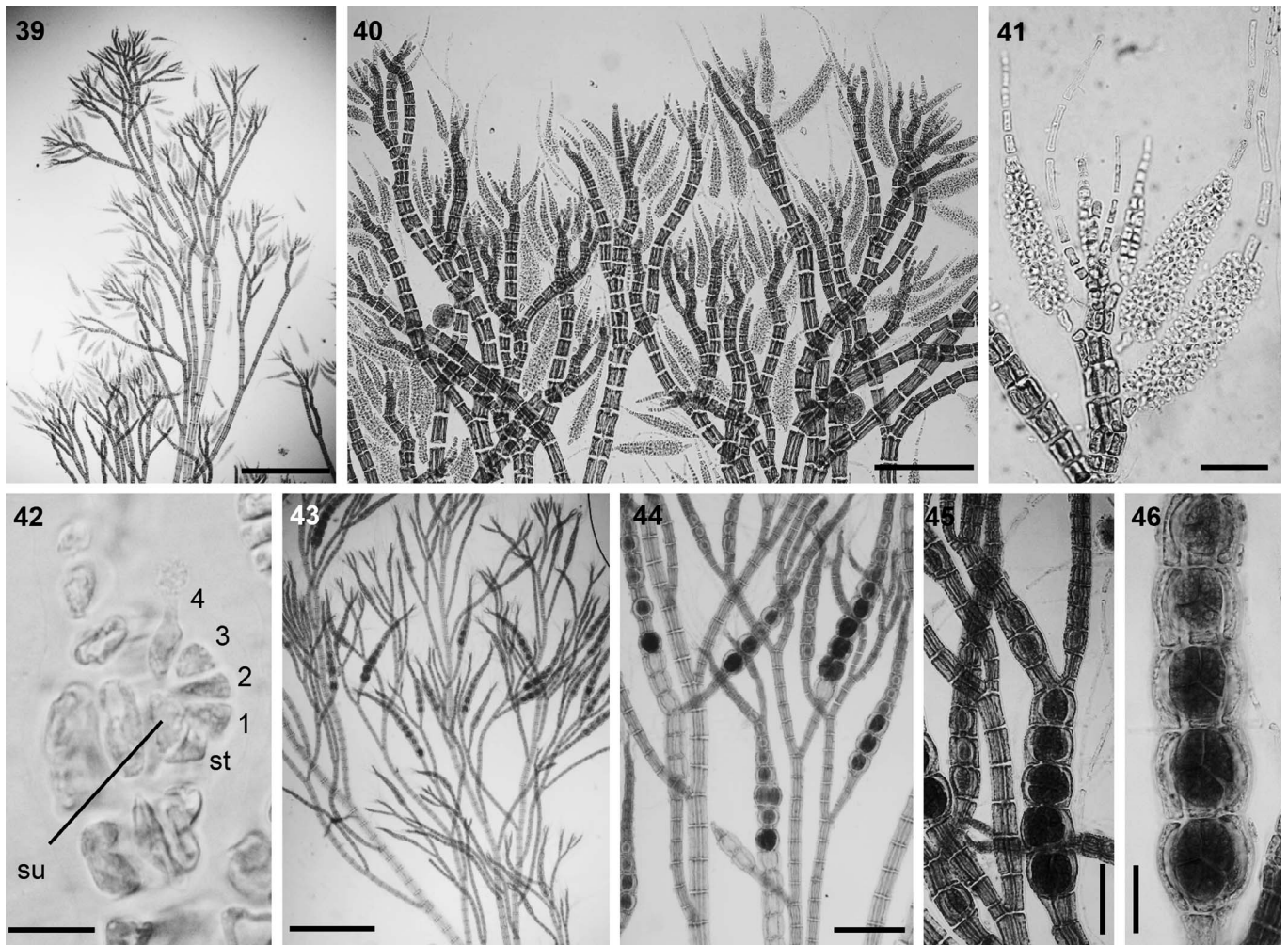
Fig. 34. Prostrate axis bearing numerous rhizoids terminating in discoid haptera. Scale bar = 600  $\mu$ m.

Fig. 35. Detail of a rhizoid, in open connection with the pericentral cell. Scale bar = 100  $\mu$ m.

Fig. 36. Apices of erect axes with rounded apical cells and forming exogenous branches. Scale bar = 100  $\mu$ m.

Fig. 37. Apex of erect axes of a tetrasporangial specimen with abundant trichoblasts. Scale bar = 50  $\mu$ m.

Fig. 38. Apex of erect axes of a sterile specimen with scarce trichoblasts. Scale bar = 30  $\mu$ m.



Figs 39–46. *Polysiphonia delicata* sp. nov., reproductive morphology.

Figs 39–40. Upper parts of erect axes with densely clustered spermatangial branches. Scale bars: Fig. 39 = 600  $\mu$ m; Fig. 40 = 200  $\mu$ m.

Fig. 41. Spermatangial branches, replacing trichoblasts and with sterile apical filament. Scale bar = 50  $\mu$ m.

Fig. 42. Procarp showing the supporting cell (su), bearing the four-celled carpogonial branch (1–4) and a basal sterile cell (st). Scale bar = 20  $\mu$ m.

Figs 43–44. Upper parts of the erect axes, with branches bearing tetrasporangia in long straight series and often pseudodichotomously branched. Scale bars: Fig. 43 = 600  $\mu$ m; Fig. 44 = 200  $\mu$ m.

Figs 45–46. Detail of tetrasporangia. Scale bars: Fig. 45 = 100  $\mu$ m; Fig. 46 = 50  $\mu$ m.

male gametophytes; abundant in tetrasporangial thalli, spirally arranged at the apices, formed every two to three segments, up to 450  $\mu$ m in length and one to two times branched (Figs 37, 38). Branches arising exogenously at the apices of the erect axes, independently from trichoblasts. Adventitious branches only occasionally observed in prostrate axes.

### Reproductive morphology

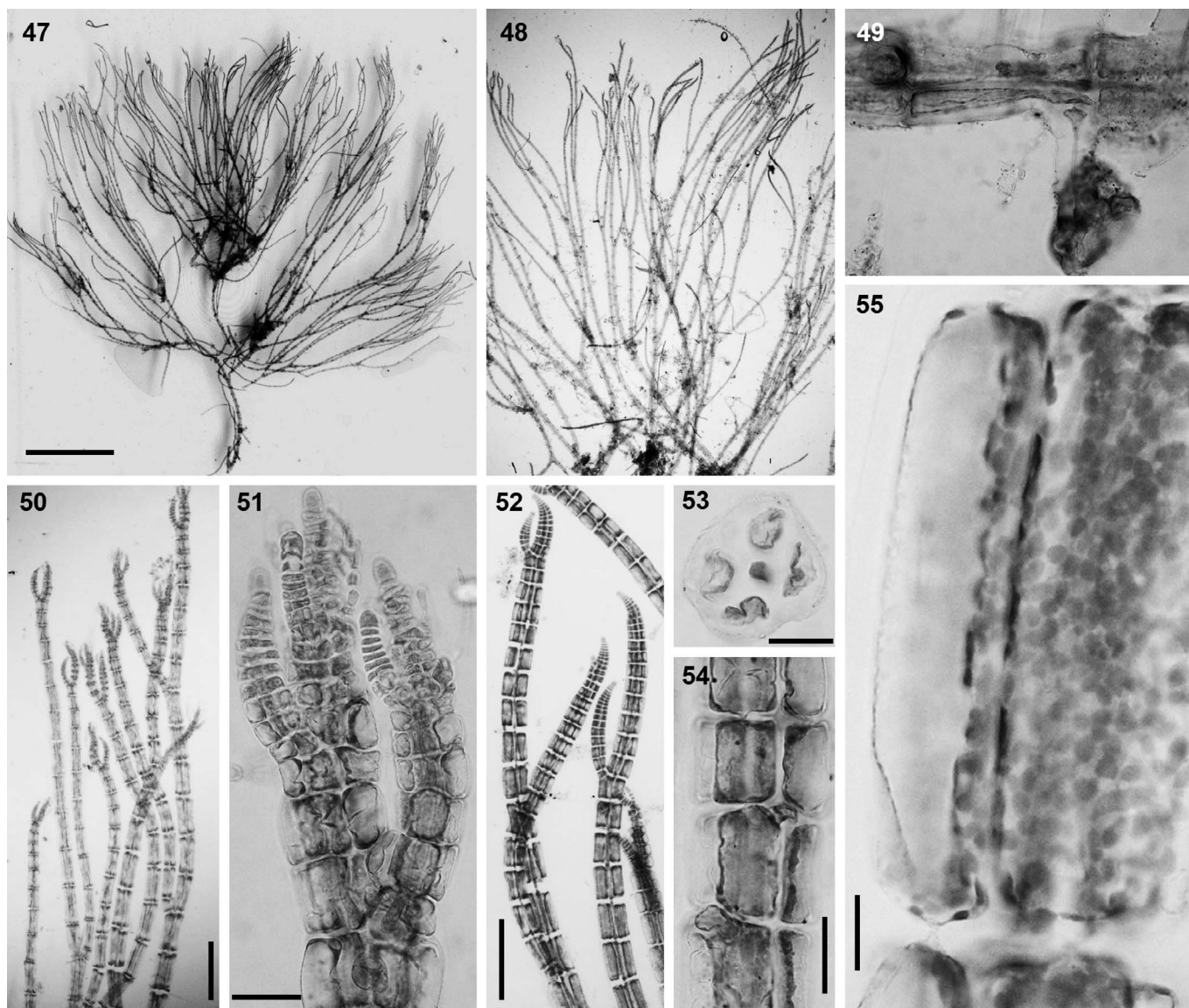
Gametophytes dioecious. Spermatangial axes densely clustered at the apices of erect axes (Figs 39, 40), borne every one to three segments in a  $\frac{1}{4}$  spiral, arising on suprabasal cells of modified trichoblasts and replacing them (Fig. 41). Spermatangial axes cylindrical with a sterile apical filament of four to six cells, 185–285 (–325)  $\mu$ m long and 35–43  $\mu$ m in diameter (Fig. 41).

Procarps formed at the apices of erect axes, on suprabasal cells of modified trichoblasts, consisting of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell and a lateral group of two sterile cells (Fig. 42). Mature cystocarps not observed.

Tetrasporangia in lateral branches that were often pseudodichotomously branched, slightly thickened, formed straight series with up to six mature tetrasporangia (Figs 43, 44). They were ovate, 35–65  $\mu$ m in diameter, tetrahedrally divided, with two cover cells similar to the pericentral cells (Figs 45, 46).

### Habitat and distribution

*Polysiphonia delicata* was found forming small tufts on pontoons and piers, either growing directly on the artificial substrata or on mussels attached to these structures. It was collected in two of the eight explored marinas in Galicia, both



**Figs 47–55. *Melanothamnus pseudoforcipatus* sp. nov.**

**Figs 47–48.** Habit. Scale bars: Fig. 47 = 4 mm; Fig. 48 = 1.4 mm.

**Fig. 49.** Rhizoid cut off from pericentral cell. Scale bar = 60 µm.

**Fig. 50.** Upper part of thalli, with scarcely branched axes. Scale bar = 300 µm.

**Fig. 51.** Detail of an apex with rounded apical cells and exogenous young branches and short trichoblasts. Scale bar = 40 µm.

**Fig. 52.** Apex with young pseudoforcipate branches. Scale bar = 200 µm.

**Fig. 53.** Cross section with a small axial cell and four pericentral cells. Scale bar = 40 µm.

**Fig. 54.** Scar cells of trichoblasts on successive segments. Scale bar = 40 µm.

**Fig. 55.** Surface view of pericentral cells with plastids lying only on radial walls. Scale bar = 15 µm.

located in the same area (Golfo Ártabro). In Australia, it was found in both artificial marina and pier sites investigated in Port Phillip Bay and Western Port (Victoria), respectively but not in the other 40 natural habitats explored in Victoria nor in marinas in Western Australia.

***Melanothamnus pseudoforcipatus* Díaz-Tapia sp. nov.**

Figs 47–55

**DIAGNOSIS:** Thalli predominantly erect, attached by rhizoids cut off from pericentral cells. Axes with four pericentral cells, eocorticate; up

to 140 µm in diameter. Plastids lying exclusively on radial walls of pericentral cells. Erect axes pseudodichotomously and irregularly branched. Branches exogenous, independent from trichoblasts. Trichoblasts absent or scarce, at irregular intervals.

**HOLOTYPE:** SANT-Algae 28188.

**TYPE LOCALITY:** Rande, Galicia, Spain; 42°27.26'N; 8°39.83'W.

**ETYMOLOGY:** Named from the Greek 'pseudo-' for 'resembling but not equalling' and the Latin 'forcipatus' for 'shaped like pincers or tongs', referring to the appearance of the apices.

MOLECULAR VOUCHERS: KY620089 *rbcL*; KY620040 *cox1*.

### Vegetative morphology

Thalli forming small dense tufts of densely entangled axes. Axes are erect, forming rhizoids in the basal parts, lacking distinct main axes, scarcely and pseudodichotomously branched (Figs 47, 48). Thallus pink in colour, texture flaccid.

Erect axes developing small unicellular rhizoids, cut off from the pericentral cells, sometimes terminating in digitate discoid pads (Fig. 49). Axes consisting of a small axial cell surrounded by four pericentral cells, ecorticate (Fig. 53), cells with plastids lying exclusively on radial walls of pericentral cells, with outer walls appearing transparent (Fig. 55). Axes growing from a rounded apical cell 15–20 µm in diameter, increasing from 70–90 µm in diameter apically to 170–190 (–220) µm basally. Segments longer than wide. Axes scarcely and pseudodichotomously branched at irregular intervals usually greater than five segments (Fig. 50). Branches formed exogenously at the apices, independent of trichoblasts (Fig. 51). Young branches often giving a pseudoforcipate aspect to the apices (Figs 50, 52). Adventitious branches not observed. Trichoblasts absent or scarce and short (Fig. 51), formed at irregular intervals, leaving a scar cell when shed (Fig. 54).

Reproduction unknown.

### Habitat and distribution

*Melanothamnus pseudoforcipatus* was collected forming tufts in the low intertidal of a single site in South Galicia (Spain) characterized by a strong tidal current. This site was near oyster aquaculture facilities.

## DISCUSSION

### Taxonomic position of the new species

*Polysiphonia radiata* and *Polysiphonia delicata* are placed in the *Polysiphonia sensu stricto* clade 1 of the tribe Polysiphonieae, with *Polysiphonia stricta*, the type of the genus (Fig. 2). In agreement with the recently redefined morphological delineation of the Polysiphonieae, both species share the synapomorphic character of having rhizoids in open connection with pericentral cells (Díaz-Tapia et al. 2017a). Furthermore, like most other members of this group they have four pericentral cells without cortication, branches independent of trichoblasts, spermatangial branches replacing trichoblasts and four-celled carpogonial branches (Kim & Lee 1999; Díaz-Tapia et al. 2017b). They are also similar in size, with decumbent thalli, and the branches are predominantly exogenous and radially arranged at regular intervals of three to seven segments. They are therefore very similar in morphology, sharing most of the main key characters that distinguish species in the Polysiphonieae and Streblocladiae (Stuercke & Freshwater 2008). However, a detailed examination of the available material reveals significant differences between the two species. Trichoblasts are generally absent in *P. radiata*, except in female plants where they are rare but they

are abundant in the tetrasporophytes of *P. delicata*. Spermatangial branches terminate in a sterile filament of four to six cells in *P. delicata*, whereas, mature spermatangial branches in *P. radiata* lack a sterile filament or have a single apical sterile cell. Branches bearing tetrasporangia are simple in *P. radiata*, while they are often pseudodichotomously branched in *P. delicata*. Consequently, in the absence of tetrasporangia or spermatangial branches, molecular data are currently needed to confidently separate this pair of species.

*Polysiphonia radiata* and *Polysiphonia delicata* are morphologically very similar to other members of the Polysiphonieae. According to our review of the literature and considering both morphological and molecular data, among approximately 200 species of the Polysiphonieae/Streblocladiae for which detailed descriptions are available, 28 with four pericentral cells belong to the Polysiphonieae (Table 1). All them share the synapomorphy of having rhizoids in open connection with pericentral cells. The two new species differ from 15 species of this group in GenBank by *rbcL* sequence divergences > 4.5%. Moreover, GenBank sequences reveal a higher diversity than currently recognized, as they include two different entities identified as *Polysiphonia pacifica*, two identified as *Polysiphonia atlantica* and a *Polysiphonia* sp. from Japan. In addition to the evidence provided by molecular data, the two new species can be morphologically distinguished from 22 species of the Polysiphonieae with four pericentral cells by at least one of the following characters: branch origin, anatomy of spermatangial branches, trichoblast abundance, tetrasporangial arrangement, thallus size, branching pattern, the presence of acuminate apical cells and hooked tendrils (Table 1). Although trichoblast abundance and arrangement can vary within a single species, they are usually consistent in species with one trichoblast per segment and, consequently, we only considered this character as diagnostic when it met this requisite and was complementary to other traits. In summary, the two new species can be distinguished from previously described species of the Polysiphonieae by molecular and/or morphological evidence (Table 1). The Korean species *Polysiphonia donghaeya* and *Polysiphonia dokdoensis* are the most morphologically similar species to *P. radiata* and *P. delicata* (Table 1), and these were placed together in a poorly supported clade (Fig. 2). *Polysiphonia delicata* can be distinguished from *P. donghaeya* and *P. dokdoensis* by the same characters noted above that separate it from *P. radiata* (sterile apical filament on spermatangial branches and pseudodichotomously branched tetrasporangial branches). Conversely, there are no key features separating *P. radiata* from the two Korean species, and identification requires molecular data.

The third new species was placed in the highly supported genus *Melanothamnus* (tribe Streblocladiae). This placement is in agreement with the morphological traits observed because, among other features, it has the synapomorphic character of plastids lying exclusively on the radial walls of pericentral cells (Díaz-Tapia et al. 2017b). As in the Polysiphonieae, numerous members of *Melanothamnus* share most of the relevant diagnostic characters for species delineation in the Polysiphonieae and Streblocladiae (Stuercke & Freshwater 2008). At present, 48 members of the Streblocladiae are included in this genus, and molecular data are available for 32 species, which differ from the new species by *rbcL* sequence divergences greater than 4%.

Table 1. Comparison of selected morphological characters for the species of the Polysiphoniae with four pericentral cells.

<i>Polysiphonia</i>	Type locality	Molecular data	Branch origin	Spermatangial branches	Trichoblasts	Tetrasporangia	Other characters	References
<i>P. delicata</i> Diaz-Tapia <i>sp. nov.</i>	A Graña, Ferrol, Atlantic Spain	+	Exogenous	Replacing trichoblasts	Rare to abundant in tetrasporophytes	Straight	Thalli < 40 mm; axes 140 µm in diam; apical cell rounded; spirally arranged branches	This work
<i>P. radiata</i> Diaz-Tapia <i>sp. nov.</i>	Oza, A Coruña, Atlantic Spain	+	Exogenous	Replacing trichoblasts	Absent (rare in female gametophytes)	Straight	Thalli < 20 mm high; axes 120 µm in diam; apical cell rounded; spirally arranged branches	This work
<i>P. atlantica</i> Kapraun & J.N.Norris	Antrim and Clare, Ireland	+	Endogenous	Replacing trichoblasts	Absent	Straight		Maggs & Hommersand (1993)
<i>P. boergeresii</i> Baardseth	Tristan da Cunha	—	Exogenous	Replacing trichoblasts	Abundant	Unknown	Spine-like lateral branches	Baardseth (1941)
<i>P. caespitosa</i> (Pocock) Hollenberg	False Bay, South Africa	—	Endogenous	Replacing trichoblasts	Absent to abundant	Straight		Pocock (1953)
<i>P. caretilla</i> Hollenberg	California, USA	—	Endogenous	Unknown	Short, irregularly arranged	Spiral		Hollenberg (1971)
<i>P. decussata</i> Hollenberg	California, USA	—	Exogenous	On a branch of trichoblasts	Well developed, not on every segment	Slightly spiral		Hollenberg (1942)
<i>P. devoniensis</i> Maggs & Hommersand	Devon, UK	+	Exogenous	On a branch of trichoblasts	Scarce to abundant, irregularly arranged	Spiral		Maggs & Hommersand (1993); Diaz-Tapia & Bárbara (2013)
<i>P. dokdoensis</i> D.E.Bustamante, B.Y.Won & T.O.Cho <sup>1</sup>	Korea	+	Exogenous	Replacing trichoblasts	Absent to rare	Straight		Bustamante <i>et al.</i> (2014a); Kim & Kim (2015), as <i>Polysiphonia muninsula</i>
<i>P. donghaeaya</i> B.Kim & M.S.Kim	Korea	+	Exogenous	Unknown	Absent to rate	Straight		Kim & Kim (2015); Bustamante <i>et al.</i> (2015b), as <i>Polysiphonia koreana</i> )
<i>P. freshwateri</i> D.E.Bustamante, B.Y.Won & T.O.Cho	Korea	+	Exogenous	Unknown	One per segment	Straight to spiral		Bustamante <i>et al.</i> (2015b)
<i>P. funebris</i> De Notaris ex J.Agardh	Genoa, Italy	—	Exogenous	Unknown	One per segment	Spiral	Alternately arranged branches	Pizzuto <i>et al.</i> (1996)
<i>P. hollenbergii</i> J.N.Norris	Baja California, Mexico	—	Exogenous	Replacing trichoblasts	Absent or little developed	Unknown	Large axes (up to 320 µm in diameter), irregular branching every 8–10 segments	Norris (2014)
<i>P. kampsaxii</i> Borgesen	Iran	—	Exogenous	On a branch of trichoblasts	Well developed	Spiral		Zahid <i>et al.</i> (1981)
<i>P. kapraunii</i> B.Stuercke & D.W.Freshwater	North Carolina, USA	+	Exogenous	On a branch of trichoblasts	Moderately abundant, not on every segment	Straight		Stuercke & Freshwater (2010)
<i>P. macrocarpa</i> (C.Agardh) Sprengel	Haiti	+	Exogenous	Unknown	Well developed but not on every segment	Straight		Mamoozadeh & Freshwater (2012)
<i>P. morrowii</i> Harvey	Japan	+	Exogenous	Replacing trichoblasts	Absent	Straight	Apical cell acuminate	Segi (1951)
<i>P. namibiensis</i> Stegenga & Engdow	Namibia	+	Exogenous	Replacing trichoblasts	Absent	Straight		Stegenga <i>et al.</i> (1997); Rull-Lluch (2002)
<i>P. pacifica</i> Hollenberg	California, USA	+	Exogenous	Replacing trichoblasts	Absent to rare	Straight	Large thalli (10–20 cm in length, 100–300 µm in diameter)	Hollenberg (1942)

Table 1. Continued

<i>Polysiphonia</i>	Type locality	Molecular data	Branch origin	Spermatangial branches	Trichoblasts	Tetrasporangia	Other characters	References
<i>P. perforans</i> Cormaci, G. Fumari, Pizzuto & Serio	Catania, Italy	—	Exogenous	Replacing trichoblasts	Absent	Straight	Erect axes simple or with a pseudodichotomy at the base	Cormaci <i>et al.</i> (1998); Alongi & Catra (2012)
<i>P. pernacola</i> N.M. Adams	New Zealand	—	Endogenous	Replacing trichoblasts	Absent	Straight		Adams (1991)
<i>P. rudis</i> J.D. Hooker & Harvey	New Zealand	—	Exogenous	Replacing trichoblasts	Well developed, one per segment	Straight	Branched up to one order	Adams (1991)
<i>P. scopulorum</i> Harvey	Rottneest Island, Western Australia	+	Endogenous	Replacing trichoblasts	Absent to well developed	Slightly spiral		Womersley (1979)
<i>P. scopulorum</i> var. <i>villum</i> (J. Agardh) Hollenberg	Mexico	+	Endogenous	Replacing trichoblasts	Absent to rare	Straight		Hollenberg (1968)
<i>P. senticulosa</i> Harvey	Washington, USA	—	Exogenous	Replacing trichoblasts in Australia	Absent	Straight	Acuminate apical cell	Hollenberg (1942, as <i>Polysiphonia pungens</i> )
<i>P. shepherdi</i> Womersley	Australia	—	Exogenous	Unknown	One per segment	Straight	Hooked tendrils	Womersley (1979, 2003)
<i>P. sotorensis</i> Hollenberg	Baja California, Mexico	—	Endogenous	Unknown	Well developed, at irregular intervals	Unknown		Hollenberg (1942)
<i>P. stricta</i> (Dillwyn) Greville	Wales, UK	+	Exogenous	Replacing trichoblasts	Absent to well developed	Straight	2–2.5 cm high and 50–300 µm in diameter	Maggs & Hommersand (1993); Diaz-Tapia & Bárbara (2013)
<i>P. subtilissima</i> Montagne	French Guiana	+	Exogenous	Replacing trichoblasts	Absent to well developed	Straight	Dark red-brown in colour	Womersley (2003)
<i>P. ulleungensis</i> D.E. Bustamante, B.Y. Won & T.O. Cho	Korea	+	Exogenous	Replacing trichoblasts	Absent to scarce in female gametophytes	Unknown		Bustamante <i>et al.</i> (2014b)

<sup>1</sup> The *rbcL* sequence divergence is only 0.1–0.2% (1–2 bp) between *Polysiphonia koreana* (KJ957811) and *Polysiphonia donghaeya* (KM053380–3); intraspecific sequence divergence: 0–0.1%, 0–1 bp. Likewise, sequence divergence between *Polysiphonia muninsula* (KM053370–9; all sequences identical) and *Polysiphonia dokdoensis* (KJ407267–8; intraspecific sequence divergence: 0.3%, 4 bp) is 0.1–0.4%, 1–5 bp. So *P. koreana* and *P. muninsula* appear to be taxonomic synonyms. Only two of the four species were included in this table and the phylogenetic analysis.

Table 2. Comparison of selected morphological characters and availability of molecular data for species currently assigned to *Melanothamnus* that have four pericentral cells.

<i>Melanothamnus</i>	Country of the type locality	Molecular data	Cortication	Habit	Trichoblasts	References
<i>M. pseudoforcipatus</i> Díaz-Tapia <i>sp. nov.</i>	Rande, Atlantic Spain	+	—	Erect	Absent or scarce and at irregular intervals	This work
<i>M. afaqhussainii</i> Shameel	Pakistan	+	+	Erect	Absent (old apices), several segments apart (young apices)	Afaq-Husain & Shameel (2000)
<i>M. apiculatus</i> (Hollenberg) Díaz-Tapia & Maggs	Hawaii, USA	—	—	Erect	On every segment	Hollenberg (1968); Kim & Abbott (2006)
<i>M. bajacali</i> (Hollenberg) Díaz-Tapia & Maggs	Baja California, Mexico	+	—	Erect	On every segment	Hollenberg (1961)
<i>M. blandii</i> (Harvey) Díaz-Tapia & Maggs	Australia	+	—	Decumbent	On every segment	Womersley (1979)
<i>M. cheloniae</i> (Hollenberg & J.N.Norris) Díaz-Tapia & Maggs	Gulf of California, Mexico	—	—	Erect	On every segment	Hollenberg & Norris (1977); Norris (2014)
<i>M. decumbens</i> (T.Segi) Díaz-Tapia & Maggs	Japan	—	+	Erect	On every segment	Segi (1951); Kim (2003)
<i>M. eastwoodiae</i> (Setchell & N.L.Gardner) Díaz-Tapia & Maggs	Baja California, Mexico	—	—	Erect	On every segment	Norris (2014)
<i>M. ecorticatus</i> (R.E.Norris) Díaz-Tapia & Maggs	Hawaii, USA	—	—	Erect	On every segment	Norris (1994)
<i>M. ferulaceus</i> (Suhr ex J.Agardh) Díaz-Tapia & Maggs	East coast of Mexico; North America; Guadeloupe; Australia; Marquesas Islands; Hawaii, USA	+	—	Erect	On every segment	Mamoozadeh & Freshwater (2012)
<i>M. flavimarinus</i> (M.-S.Kim & I.K.Lee) Díaz-Tapia & Maggs	Korea	+	+	Erect	On every segment	Kim & Lee (1999)
<i>M. gorgoniae</i> (Harvey) Díaz-Tapia & Maggs	Florida, USA	—	—	Erect	On every segment	Kapraun (1979)
<i>M. hancockii</i> (E.Y.Dawson) Díaz-Tapia & Maggs	Baja California, Mexico	+	+	Erect	On every segment	Dawson (1944)
<i>M. harlandii</i> (Harvey) Díaz-Tapia & Maggs	Hong Kong	+	+	Erect	On every segment	Kim (2003)
<i>M. harveyi</i> (Bailey) Díaz-Tapia & Maggs	Connecticut, USA	+	+	Erect	On every segment	Maggs & Hommersand (1993)
<i>M. hawaiiensis</i> (Hollenberg) Díaz-Tapia & Maggs	Hawaii, USA	—	—	Erect	On every segment	Hollenberg (1968); Kim & Abbott (2006)
<i>M. incomptus</i> (Harvey) Díaz-Tapia & Maggs	South Africa	+	—	Decumbent	On every segment	Stegenga <i>et al.</i> (1997)
<i>M. japonicus</i> (Harvey) Díaz-Tapia & Maggs	Japan	+	+	Erect	On every segment	Segi (1951)
<i>M. masonii</i> (Setchell & N.L.Gardner) Díaz-Tapia & Maggs	Baja California, Mexico	—	—	Erect	On every segment	Hollenberg & Norris (1977)
<i>M. minutissimus</i> (Hollenberg) Díaz-Tapia & Maggs	Baja California, Mexico	+	—	Decumbent	On every segment	Hollenberg (1942)
<i>M. nanus</i> (A.J.K.Millar) Díaz-Tapia & Maggs	New South Wales, Australia	—	+	Erect	—	Millar (1990)
<i>M. nudus</i> (N.R.Mamoozadeh & D.W.Freshwater) D.W.Freshwater	Panama	+	—	Not specified	Absent	Mamoozadeh & Freshwater (2012)
<i>M. platycarpus</i> (Børgesen) Díaz-Tapia & Maggs	India	—	—	Decumbent	On every segment	Børgesen (1934)

Table 2. Continued

<i>Melanothamnus</i>	Country of the type locality	Molecular data	Cortication	Habit	Trichoblasts	References
<i>M. pseudovillum</i> (Hollenberg) Díaz-Tapia & Maggs	Johnston Islands	+	—	Decumbent	Mostly on every segment	Hollenberg (1968)
<i>M. ramireziae</i> (D.E.Bustamante, B.Y.Won & T.O.Cho) Díaz-Tapia & Maggs	Peru	+	—	Erect	On every segment	Bustamante <i>et al.</i> (2012)
<i>M. savatieri</i> (Hariot) Díaz-Tapia & Maggs	Japan	+	+	Erect	On every segment	Segi (1951)
<i>M. silvae</i> (D.E.Bustamante, B.Y.Won & T.O.Cho) Díaz-Tapia & Maggs	Bali	+	—	Decumbent	On every segment	Bustamante <i>et al.</i> (2013)
<i>M. simplex</i> (Hollenberg) Díaz-Tapia & Maggs	California, USA	+	—	Decumbent	On every segment	Hollenberg (1942)
<i>M. somalensis</i> Bornet & Falkenberg	Somalia	+	+	Erect	Several segments apart	Wynne & Banaimoon (1990)
<i>M. sparsus</i> (Setchell) Díaz-Tapia & Maggs	Tahiti	—	—	Prostrate	On every segment	Hollenberg (1968)
<i>M. sphaerocarpus</i> (Børgesen) Díaz-Tapia & Maggs	Virgin Islands	+	—	Erect	On every segment	Hollenberg (1968)
<i>M. strictissimus</i> (J.D.Hooker & Harvey) Díaz-Tapia & Maggs	New Zealand	+	+	Erect	On every segment	Adams (1991)
<i>M. thailandicus</i> (N.Muangmai & C.Kaewsuralikhit) Díaz-Tapia & Maggs	Thailand	+	—	Erect	Scarce	Muangmai <i>et al.</i> (2014)
<i>M. tongatensis</i> (Harvey ex Kützing) Díaz-Tapia & Maggs	Tonga	+	—	Erect	On every segment	Mamoozadeh & Freshwater (2012)
<i>M. unilateralis</i> (Levring) Díaz-Tapia & Maggs	Juan Fernandez Island	+	+	Erect	On every segment	Levring (1941); pers. obs.
<i>M. upolensis</i> (Grunow) Díaz-Tapia & Maggs	Samoa	—	—	Decumbent	Every 2–3 segments	Hollenberg (1968)
<i>M. yendoi</i> (T.Segi) Díaz-Tapia & Maggs	Japan	+	—	Decumbent	On every segment	Segi (1951)
<i>M. yongpili</i> (Kim & Kim) Díaz-Tapia & Maggs	Korea	+	—	Erect	On every segment	Kim & Kim (2016)

Morphologically, the new species can be distinguished from all other members of *Melanothamnus* by at least one of the following characters: number of pericentral cells, cortication, habit and trichoblast arrangement (Table 2). *Melanothamnus pseudoforcipatus* differs from most species of *Melanothamnus*, which have trichoblasts on every segment (Table 2). In fact, this was one of the characters proposed for delineating *Neosiphonia*, a genus recently subsumed into *Melanothamnus* (Díaz-Tapia *et al.* 2017b).

In this paper, we propose three new species based on comparisons of molecular and morphological data with previously described species of *Polysiphonia* and *Melanothamnus* for which a minimum of information is available. A common problem in algal taxonomy is the existence of a long list of previously described taxonomic entities with uncertain status (De Clerck *et al.* 2013). In *Polysiphonia*, although usually neglected when describing new taxa, this is an intractable problem. In addition to the species for which molecular data and/or detailed morphological descriptions are available, there are another 100 *Polysiphonia* species currently accepted

taxonomically (Guiry & Guiry 2016) with largely unknown morphology. Most of them were described more than a century ago, and further information has never been published. In addition, there are another 180 provisional entries in Algaebase (Guiry & Guiry 2016) with uncertain taxonomy and identity. Finally, some species currently placed in *Polysiphonia* may belong to *Melanothamnus* but currently available information is insufficient to determine the genus of the Streblocladiae to which they should be assigned. Therefore, we recognize that the new species proposed here may have been previously described from elsewhere with different names. Despite this, we consider the approach of providing a valid name for these species more suitable than the alternative of the accumulating well-defined but unnamed entities.

#### Are the new species non-native in their known distribution areas?

The three new species described here are considered cryptogenic, as it is uncertain whether they are native or introduced

(Carlton 1996, 2009). They meet several of the criteria (Chapman & Carlton 1991; Ribera & Boudouresque 1995) for consideration as non-native in Europe, and also in Australia in the case of *Polysiphonia delicata*. First, there is evidence they have not been reported previously in their known distributional range. The seaweed diversity of Galicia, the Spanish region where they were collected, has been intensively explored over the last 30 years, with particular emphasis on the Polysiphonieae and Streblocladiae for the last 15 years (e.g. Bárbara *et al.* 2005; Díaz-Tapia & Bárbara 2013). Likewise, the Southern Australian Polysiphonieae and Streblocladiae were studied in detail by Womersley (1979, 2003). However, considering the small size of the three new species, we cannot rule out the possibility that they were present in these regions but were previously overlooked.

Second, *Polysiphonia radiata* and *Polysiphonia delicata* were predominantly collected on pontoons of marinas or piers, and, therefore, boat traffic (hull fouling) might be the most probable introduction vector. Association with artificial habitats may be a general indicator of non-native status (Chapman & Carlton 1991). *Polysiphonia radiata* has a wide distribution in Galicia, as it was collected in all the explored marinas and was very abundant in one of them (Oza). Furthermore, we detected a small population of *P. radiata* in Galicia, in a subtidal maërl bed in the Cies archipelago of the Galician Atlantic Islands Maritime-Terrestrial National Park where, paradoxically, non-native seaweeds are common, probably associated with the high volume of nearby aquaculture activities, fishing and boating (Peña & Bárbara 2006). *Polysiphonia delicata* was found in two distant geographical areas (Spain and Australia), and different *rbcL* haplotypes were detected in the respective regions (0.1% sequence divergence, 1 bp), which is consistent with potential separate introduction events. Once introduced, the habitat where they were found suggests that the same vector might contribute to the expansion of their local range. To our knowledge, *P. delicata* has a limited distribution around Melbourne (Victoria, Australia) and in the Golfo Ártabro (Galicia, Spain). It was always scarce, and we found no evidence that this species occurs in natural habitats. Differences in the distribution range of these two species in Galicia suggest either that *P. radiata* might have been introduced earlier or that it might be spreading faster. Both species are well established in Galicia, as they were detected for the first time in 2011 and 2013, and further collections were made in 2016.

*Melanothamnus pseudoforcipatus* was only found once, at a Galician site close to oyster aquaculture facilities. Additional evidence that this species may be introduced is that the genus *Melanothamnus* is predominantly Pacific and the other two species of this genus reported in Europe, *Melanothamnus harveyi* and *Melanothamnus collabens*, are most probably old introductions from the Pacific Ocean (McIvor *et al.* 2001; Díaz-Tapia & Bárbara 2013; Díaz-Tapia *et al.* 2017b). We explored the areas close to the site where it was detected without finding other material, suggesting that it was recently introduced, and dispersal has not yet occurred.

Galicia and Australia have favourable conditions for seaweed introductions, for which aquaculture and hull fouling are widely recognized as the major vectors (Mineur *et al.* 2007a, b, 2008; Thomsen *et al.* 2016). Galicia is the Spanish region with the largest aquaculture production, c. 270,000

tonnes per year of mussels and other molluscs (oysters, clams, cockles and scallops) and c. 7500 tonnes of turbot and other fish (Xunta de Galicia 2015). Furthermore, along its 1720 km of shoreline there are five ports and 122 fishing harbours and marinas (Ente Público Portos de Galicia 2013). In fact, practically all the non-native species recorded in Atlantic Europe are present in this region (Bárbara *et al.* 2005, 2008). In Australia, aquaculture is not a major activity; although c. 1500 tonnes of abalone and mussels are produced per year in Victoria, mainly in Port Phillip Bay and Western Port Bay (Savage 2015). Also, there are several big harbours in the main coastal cities, and many introduced seaweeds have been recorded in Australia (Williams & Smith 2007).

These three new species of the tribes Polysiphonieae and Streblocladiae contribute to the discovery of the high cryptic diversity hidden in these tribes of the Rhodomelaceae. Considering their habitat and distribution, they have most been probably introduced into Galicia (north-western Spain) and one of them also into Victoria (Australia) through hull fouling or aquaculture activities. As a result, the list of known introduced or cryptogenic Polysiphonieae and Streblocladiae (Thomsen *et al.* 2016; Bustamante *et al.* 2015a) is expanded to 21 species. These three species are cryptogenic: their native area is uncertain, which is not uncommon in seaweeds and other introduced marine organisms (Carlton & Geller 1993; Mineur *et al.* 2012a). In Europe, a high proportion of introduced species originated from south-east Asia, particularly Japan, arriving as hitch-hikers via the oyster trade (Mineur *et al.* 2014). We do not know the origins of our three new cryptogenic species but explorations of cryptic diversity within Polysiphonieae in Asia have recently revealed four new species tribes Polysiphonieae and Streblocladiae in Korea (Bustamante *et al.* 2014a, b, 2015b; Kim & Kim 2015). We therefore suggest the likelihood that our new species will also be detected in Asia, where their genetic diversity is predicted to be higher.

The three new species are not invasive in their known distribution at present, and they were not found in the marinas explored in England, Wales, Ireland, Alassio (Italy), Marseille (France) and Western Australia. However, *Polysiphonia radiata* has already spread widely in Galicia, and *Polysiphonia delicata* is found in two distant countries, so their range may expand further under favourable circumstances.

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## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at <http://dx.doi.org/10.2216/17-17.1.s1>.

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