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: Coxl, 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; Diaz-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-
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 (Diaz-Tapia et al. 2013). Also some introduced species have been identified using molecular data (Mineur et al. 2010, 2012a; Geoffroy et al. 2012; Diaz-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 Portoño, 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 & McDevitt (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 & Rueness 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 *Symphyocladia* 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 (Diaz-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; Diaz-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
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 ≥ 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 Streblocladieae 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 Diaz-Tapia sp. nov.

Figs 3–26

DIAGNOSIS: Thalli decumbent, attached by unicellular rhizoids in open connection with pericentral cells. Axes ecorticate, up to 120 μm 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. Procarps 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.
ETYMOLOGY: From the Latin word 'radiatus' for 'radial' because branches are spirally arranged.

MOLECULAR VOUCHERS: KY620065 rbcL; KY620044 coxl.

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 ¼ spiral pattern, up to two to three orders (Figs 6, 7). Tufts pink in colour, with a very flaccid texture.

Axes ecoricate, 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) μm 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
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).

Procarps 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 ones.

Habitat and distribution

The known distribution is restricted to Galicia, northwestern 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 from 0 to 5 m depth. It was abundant at one site (Oza Marina) and rare in the other marinas. It was also collected from 0 to 5 m depth. It was abundant at one site (Oza Marina) and rare in the other marinas.

**Polysiphonia delicata Diaz-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. Procarps 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 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 μm.

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

Fig. 32. Cross section with a small axial cell and four pericentral cells. Scale bar = 50 μm.

Fig. 33. Surface view of pericentral cells with discoid plastids. Scale bar = 30 μm.

Fig. 34. Prostrate axis bearing numerous rhizoids terminating in discoid haptera. Scale bar = 600 μm.

Fig. 35. Detail of a rhizoid, in open connection with the pericentral cell. Scale bar = 100 μm.

Fig. 36. Apices of erect axes with rounded apical cells and forming exogenous branches. Scale bar = 100 μm.

Fig. 37. Apex of erect axes of a tetrasporangial specimen with abundant trichoblasts. Scale bar = 50 μm.

Fig. 38. Apex of erect axes of a sterile specimen with scarce trichoblasts. Scale bar = 30 μm.
male gametophytes; abundant in tetrasporangial thalli, spirally arranged at the apices, formed every two to three segments, up to 450 μm 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 ¼ 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) μm long and 35–43 μm in diameter (Fig. 41).

Figs 39–40. Upper parts of erect axes with densely clustered spermatangial branches. Scale bars: Fig. 39 = 600 μm; Fig. 40 = 200 μm.
Fig. 41. Spermatangial branches, replacing trichoblasts and with sterile apical filament. Scale bar = 50 μm.
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 μm.

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 μm; Fig. 44 = 200 μm.
Figs 45–46. Detail of tetrasporangia. Scale bars: Fig. 45 = 100 μm; Fig. 46 = 50 μm.

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 μm 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
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, ecorticate; 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.
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 pseudofurcate 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 Streblocladieae (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/Streblocladieae 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 Streblocladieae). 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 Streblocladieae (Stuercke & Freshwater 2008). At present, 48 members of the Streblocladieae 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%.
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<td><em>P. delicata</em></td>
<td>A Graña, Ferrol, Atlantic Spain</td>
<td>+ Exogenous</td>
<td>Replacing</td>
<td>Rare to abundant in</td>
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<td>Thalli &lt; 40 mm; axes 140 μm in diam; apical cell rounded; spirally arranged branches</td>
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<td><em>P. radiata</em></td>
<td>Oza, A Coruña, Atlantic Spain</td>
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<td>Absent (rare in female gametophytes)</td>
<td>Straight</td>
<td>Thalli &lt; 20 mm high; axes 120 μm in diam; apical cell rounded; spirally arranged branches</td>
<td>This work</td>
<td></td>
</tr>
<tr>
<td><em>P. atlantica</em></td>
<td>Antrim and Clare, Ireland</td>
<td>+ Exogenous</td>
<td>Replacing</td>
<td>Absent</td>
<td>Straight</td>
<td></td>
<td>Mggs &amp; Hommersand (1993)</td>
<td></td>
</tr>
<tr>
<td><em>P. boergeseni</em></td>
<td>Tristan da Cunha</td>
<td>— Exogenous</td>
<td>Replacing</td>
<td>Abundant</td>
<td>Unknown</td>
<td>Spine-like lateral branches</td>
<td>Baardseth (1941)</td>
<td></td>
</tr>
<tr>
<td><em>P. caespitosa</em></td>
<td>False Bay, South Africa</td>
<td>— Endogenous</td>
<td>Replacing</td>
<td>Absent to abundant</td>
<td>Straight</td>
<td></td>
<td>Pocock (1953)</td>
<td></td>
</tr>
<tr>
<td><em>P. carettia</em></td>
<td>California, USA</td>
<td>— Endogenous</td>
<td>Unknown</td>
<td>Short, irregularly</td>
<td>Spiral</td>
<td></td>
<td>Hollenberg (1971)</td>
<td></td>
</tr>
<tr>
<td><em>P. decussata</em></td>
<td>California, USA</td>
<td>— Exogenous</td>
<td>On a branch of trichoblasts</td>
<td>Well developed, not on every segment</td>
<td>Slightly spiral</td>
<td></td>
<td>Hollenberg (1942)</td>
<td></td>
</tr>
<tr>
<td><em>P. devoniensis</em></td>
<td>Devon, UK</td>
<td>+ Exogenous</td>
<td>On a branch of trichoblasts</td>
<td>Scarce to abundant, irregularly arranged</td>
<td>Spiral</td>
<td></td>
<td>Mggs &amp; Hommersand (1993); Diaz-Tapia &amp; Bárbara (2013)</td>
<td></td>
</tr>
<tr>
<td><em>P. dokdoensis</em></td>
<td>Korea</td>
<td>+ Exogenous</td>
<td>Replacing</td>
<td>Absent to rare</td>
<td>Straight</td>
<td></td>
<td>Bustamante et al. (2014a); Kim &amp; Kim (2015, as <em>Polysiphonia muninsula</em>)</td>
<td></td>
</tr>
<tr>
<td><em>P. donghaeya</em></td>
<td>Korea</td>
<td>+ Exogenous</td>
<td>Unknown</td>
<td>Absent to rare</td>
<td>Straight</td>
<td></td>
<td>Kim &amp; Kim (2015); Bustamante et al. (2015b, as <em>Polysiphonia koreana</em>)</td>
<td></td>
</tr>
<tr>
<td><em>P. freshwater</em></td>
<td>Korea</td>
<td>+ Exogenous</td>
<td>Unknown</td>
<td>One per segment</td>
<td>Straight to spiral</td>
<td></td>
<td>Bustamante et al. (2015b)</td>
<td></td>
</tr>
<tr>
<td><em>P. funebris</em></td>
<td>Genoa, Italy</td>
<td>— Exogenous</td>
<td>Unknown</td>
<td>One per segment</td>
<td>Spiral</td>
<td>Alternately arranged branches</td>
<td>Pizzuto et al. (1996)</td>
<td></td>
</tr>
<tr>
<td><em>P. hollenbergii</em></td>
<td>Baja California, Mexico</td>
<td>— Exogenous</td>
<td>Replacing</td>
<td>Absent or little</td>
<td>Unknown</td>
<td>Large axes (up to 320 μm in diameter), irregular branching every 8-10 segments</td>
<td>Norris (2014)</td>
<td></td>
</tr>
<tr>
<td><em>P. kampsaxii</em></td>
<td>Iran</td>
<td>— Exogenous</td>
<td>On a branch of trichoblasts</td>
<td>Well developed</td>
<td>Spiral</td>
<td></td>
<td>Zahid et al. (1981)</td>
<td></td>
</tr>
<tr>
<td><em>P. kapraunii</em></td>
<td>North Carolina, USA</td>
<td>+ Exogenous</td>
<td>On a branch of trichoblasts</td>
<td>Moderately abundant, not on every segment</td>
<td>Straight</td>
<td></td>
<td>Stuercke &amp; Freshwater (2010)</td>
<td></td>
</tr>
<tr>
<td><em>P. macrocarpa</em></td>
<td>Haiti</td>
<td>+ Exogenous</td>
<td>Unknown</td>
<td>Well developed but</td>
<td>Spiral</td>
<td></td>
<td>Mamoozadeh &amp; Freshwater (2012)</td>
<td></td>
</tr>
<tr>
<td><em>P. morrowii</em></td>
<td>Japan</td>
<td>+ Exogenous</td>
<td>Replacing</td>
<td>Absent</td>
<td>Straight</td>
<td>Apical cell acuminate</td>
<td>Sugi (1951)</td>
<td></td>
</tr>
<tr>
<td><em>P. namibensis</em></td>
<td>Namibia</td>
<td>+ Exogenous</td>
<td>Replacing</td>
<td>Absent</td>
<td>Straight</td>
<td></td>
<td>Stegenga et al. (1997); Rull Lluch (2002)</td>
<td></td>
</tr>
<tr>
<td><em>P. pacifica</em></td>
<td>California, USA</td>
<td>+ Exogenous</td>
<td>Replacing</td>
<td>Absent to rare</td>
<td>Straight</td>
<td>Large thalli (10–20 cm in length, 100–300 μm in diameter)</td>
<td>Hollenberg (1942)</td>
<td></td>
</tr>
<tr>
<td>Polysiphonia</td>
<td>Type locality</td>
<td>Molecular data</td>
<td>Branch origin</td>
<td>Spermatangial branches</td>
<td>Trichoblasts</td>
<td>Tetrasporangia</td>
<td>Other characters</td>
<td>References</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>--------------------------------</td>
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<td>------------------------</td>
<td>--------------</td>
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<td>-----------------------------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td><em>P. perforans</em> Cormaci, G.Fumari, Pizzuto &amp; Serio</td>
<td>Catania, Italy</td>
<td>—</td>
<td>Exogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent</td>
<td>Straight</td>
<td>Erect axes simple or with a pseudodichotomy at the base</td>
<td>Cormaci <em>et al.</em> (1998); Alongi &amp; Catra (2012)</td>
</tr>
<tr>
<td><em>P. pernacola</em> N.M. Adams</td>
<td>New Zealand</td>
<td>—</td>
<td>Endogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent</td>
<td>Straight</td>
<td>Straight</td>
<td>Adams (1991)</td>
</tr>
<tr>
<td><em>P. rudis</em> J.D. Hooker &amp; Harvey</td>
<td>New Zealand</td>
<td>—</td>
<td>Exogenous</td>
<td>Replacing trichoblasts</td>
<td>Well developed, one per segment</td>
<td>Straight</td>
<td>Branched up to one order</td>
<td>Adams (1991)</td>
</tr>
<tr>
<td><em>P. scopulorum</em> Harvey</td>
<td>Rottnest Island, Western Australia</td>
<td>+</td>
<td>Endogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent to well developed</td>
<td>Slightly spiral</td>
<td></td>
<td>Womersley (1979)</td>
</tr>
<tr>
<td><em>P. scopulorum</em> var. villum (J.Agardh) Hollenberg</td>
<td>Mexico</td>
<td>+</td>
<td>Endogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent to rare</td>
<td>Straight</td>
<td></td>
<td>Hollenberg (1968)</td>
</tr>
<tr>
<td><em>P. senticulosa</em> Harvey</td>
<td>Washington, USA</td>
<td>—</td>
<td>Exogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent</td>
<td>Straight</td>
<td>Acuminate apical cell</td>
<td>Hollenberg (1942, as <em>Polysiphonia pungens</em>)</td>
</tr>
<tr>
<td><em>P. shepherdii</em> Womerskey</td>
<td>Australia</td>
<td>—</td>
<td>Exogenous</td>
<td>Unknown</td>
<td>One per segment</td>
<td>Straight</td>
<td>Hooked tendrils</td>
<td>Womersley (1979, 2003); Hollenberg (1942)</td>
</tr>
<tr>
<td><em>P. sonorensis</em> Hollenberg</td>
<td>Baja California, Mexico</td>
<td>—</td>
<td>Exogenous</td>
<td>Unknown</td>
<td>Well developed, at irregular intervals</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. stricta</em> (Dihwyn) Greville</td>
<td>Wales, UK</td>
<td>+</td>
<td>Exogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent to well developed</td>
<td>Straight</td>
<td>2-25 cm high and 50-300 μm in diameter</td>
<td>Maggs &amp; Hommersand (1993); Díaz-Tapia &amp; Bárbara (2013)</td>
</tr>
<tr>
<td><em>P. subtilissima</em> Montagne</td>
<td>French Guiana</td>
<td>+</td>
<td>Exogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent to well developed</td>
<td>Straight</td>
<td>Dark red-brown in colour</td>
<td>Womersley (2003)</td>
</tr>
<tr>
<td><em>P. ulleungensis</em> D.E. Bostamante, B.Y. Won &amp; T.O. Cho</td>
<td>Korea</td>
<td>+</td>
<td>Exogenous</td>
<td>Replacing trichoblasts</td>
<td>Absent to scarce in female gametophytes</td>
<td>Unknown</td>
<td></td>
<td>Bustamante <em>et al.</em> (2014b)</td>
</tr>
</tbody>
</table>

1 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.

<table>
<thead>
<tr>
<th>Melanothamnus</th>
<th>Country of the type locality</th>
<th>Molecular data</th>
<th>Cortication</th>
<th>Habit</th>
<th>Trichoblasts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pseudoforcipatus</em> Diaz-Tapia sp. nov.</td>
<td>Rande, Atlantic Spain</td>
<td>+</td>
<td>—</td>
<td>Erect</td>
<td>Absent or scarce and at irregular intervals</td>
<td>This work</td>
</tr>
<tr>
<td><em>M. afaghussainii</em> Shameel</td>
<td>Pakistan</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>Absent (old apices), several segments apart (young apices)</td>
<td>Afiaq-Husain &amp; Shameel (2000)</td>
</tr>
<tr>
<td><em>M. bajacali</em> (Hollenberg) Diaz-Tapia &amp; Maggs</td>
<td>Baja California, Mexico</td>
<td>+</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Hollenberg (1961)</td>
</tr>
<tr>
<td><em>M. blandi</em> (Harvey) Diaz-Tapia &amp; Maggs</td>
<td>Australia</td>
<td>+</td>
<td>—</td>
<td>Decumbent</td>
<td>On every segment</td>
<td>Womersley (1979)</td>
</tr>
<tr>
<td><em>M. eastwoodiae</em> (Setchell &amp; N.L. Gardner) Diaz-Tapia &amp; Maggs</td>
<td>Baja California, Mexico</td>
<td>—</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Norris (1994)</td>
</tr>
<tr>
<td><em>M. ferulaceus</em> (Suhr ex J.Agardh) Diaz-Tapia &amp; Maggs</td>
<td>East coast of Mexico; North America; Guadeloupe; Australia; Marquesas Islands; Hawaii, USA</td>
<td>+</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Mamoozadeh &amp; Freshwater (2012)</td>
</tr>
<tr>
<td><em>M. gorgoniae</em> (Harvey) Diaz-Tapia &amp; Maggs</td>
<td>Florida, USA</td>
<td>—</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Kapraun (1979)</td>
</tr>
<tr>
<td><em>M. hancockii</em> (E.Y.Dawson) Diaz-Tapia &amp; Maggs</td>
<td>Baja California, Mexico</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>On every segment</td>
<td>Dawson (1944)</td>
</tr>
<tr>
<td><em>M. harlandii</em> (Harvey) Diaz-Tapia &amp; Maggs</td>
<td>Hong Kong</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>On every segment</td>
<td>Kim (2003)</td>
</tr>
<tr>
<td><em>M. incomptus</em> (Harvey) Diaz-Tapia &amp; Maggs</td>
<td>South Africa</td>
<td>+</td>
<td>—</td>
<td>Decumbent</td>
<td>On every segment</td>
<td>Stegenga et al. (1997)</td>
</tr>
<tr>
<td><em>M. japonicae</em> (Harvey) Diaz-Tapia &amp; Maggs</td>
<td>Japan</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>On every segment</td>
<td>Segi (1951)</td>
</tr>
<tr>
<td><em>M. masonii</em> (Setchell &amp; N.L.Gardner) Diaz-Tapia &amp; Maggs</td>
<td>Baja California, Mexico</td>
<td>—</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Hollenberg &amp; Norris (1977)</td>
</tr>
<tr>
<td><em>M. minutissimus</em> (Hollenberg) Diaz-Tapia &amp; Maggs</td>
<td>Baja California, Mexico</td>
<td>+</td>
<td>—</td>
<td>Decumbent</td>
<td>On every segment</td>
<td>Hollenberg (1942)</td>
</tr>
<tr>
<td><em>M. platycarpus</em> (Børgesen) Diaz-Tapia &amp; Maggs</td>
<td>India</td>
<td>—</td>
<td>—</td>
<td>Decumbent</td>
<td>On every segment</td>
<td>Børgesen (1934)</td>
</tr>
</tbody>
</table>
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 Streblocladieae 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.

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**Table 2. Continued**

<table>
<thead>
<tr>
<th><em>Melanothamnus</em></th>
<th>Country of the type locality</th>
<th>Molecular data</th>
<th>Cortication</th>
<th>Habit</th>
<th>Trichoblasts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pseudovillum</em> (Hollenberg) Diaz-Tapia &amp; Maggs</td>
<td>Johnston Islands</td>
<td>+</td>
<td>—</td>
<td>Decumbent</td>
<td>Mostly on every segment</td>
<td>Hollenberg (1968)</td>
</tr>
<tr>
<td><em>M. savatieri</em> (Hariot) Diaz-Tapia &amp; Maggs</td>
<td>Japan</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>On every segment</td>
<td>Segi (1951)</td>
</tr>
<tr>
<td><em>M. simples</em> (Hollenberg) Diaz-Tapia &amp; Maggs</td>
<td>California, USA</td>
<td>+</td>
<td>—</td>
<td>Decumbent</td>
<td>On every segment</td>
<td>Hollenberg (1942)</td>
</tr>
<tr>
<td><em>M. somalensis</em> Bornet &amp; Falkenberg</td>
<td>Somalia</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>Several segments apart</td>
<td>Wynne &amp; Banaimoon (1990)</td>
</tr>
<tr>
<td><em>M. sparsus</em> (Setchell) Diaz-Tapia &amp; Maggs</td>
<td>Tahiti</td>
<td>—</td>
<td>—</td>
<td>Prostrate</td>
<td>On every segment</td>
<td>Hollenberg (1968)</td>
</tr>
<tr>
<td><em>M. sphaerocarpus</em> (Børgesen) Diaz-Tapia &amp; Maggs</td>
<td>Virgin Islands</td>
<td>+</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Hollenberg (1968)</td>
</tr>
<tr>
<td><em>M. strictissimus</em> (J.D. Hooker &amp; Harvey) Diaz-Tapia &amp; Maggs</td>
<td>New Zealand</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>On every segment</td>
<td>Adams (1991)</td>
</tr>
<tr>
<td><em>M. tongatensis</em> (Harvey ex Kützing) Diaz-Tapia &amp; Maggs</td>
<td>Tonga</td>
<td>+</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Mamoozadeh &amp; Freshwater (2012)</td>
</tr>
<tr>
<td><em>M. unilateralis</em> (Levring) Diaz-Tapia &amp; Maggs</td>
<td>Juan Fernandez Island</td>
<td>+</td>
<td>+</td>
<td>Erect</td>
<td>On every segment</td>
<td>Levring (1941); pers. obs.</td>
</tr>
<tr>
<td><em>M. upolensis</em> (Grunow) Diaz-Tapia &amp; Maggs</td>
<td>Samoa</td>
<td>—</td>
<td>—</td>
<td>Decumbent</td>
<td>Every 2–3 segments</td>
<td>Hollenberg (1968)</td>
</tr>
<tr>
<td><em>M. yendoi</em> (T. Segi) Diaz-Tapia &amp; Maggs</td>
<td>Japan</td>
<td>+</td>
<td>—</td>
<td>Decumbent</td>
<td>On every segment</td>
<td>Segi (1951)</td>
</tr>
<tr>
<td><em>M. yongpilii</em> (Kim &amp; Kim) Diaz-Tapia &amp; Maggs</td>
<td>Korea</td>
<td>+</td>
<td>—</td>
<td>Erect</td>
<td>On every segment</td>
<td>Kim &amp; Kim (2016)</td>
</tr>
</tbody>
</table>
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 Streblocladieae for the last 15 years (e.g. Bárbara et al. 2005; Díaz-Tapia & Bárbara 2013). Likewise, the Southern Australian Polysiphonieae and Streblocladieae 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 maerl 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 rHeL 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 Arztabro (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 Streblocladieae contribute to the discovery of the high cryptic diversity hidden in these tribes of the Rhodomelaceae. Considering their habitat and distribution, they have most probably been 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 Streblocladieae (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 Streblocladieae 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

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