

cryptogamie

Algologie

2020 • 41 • 8

Molecular assessment of the tribes Streblocladiaceae and Polysiphonieaceae (Rhodomelaceae, Rhodophyta) in the British Isles reveals new records and species that require taxonomic revision

**Cristina PIÑEIRO-CORBEIRA, Christine A. MAGGS, Fabio RINDI,
Francis BUNKER, Lin BALDOCK & Pilar DÍAZ-TAPIA**

art. 41 (8) — Published on 5 June 2020
www.cryptogamie.com/algologie

**PUBLICATIONS
SCIENTIFIQUES**



DIRECTEUR DE LA PUBLICATION / *PUBLICATION DIRECTOR*: Bruno DAVID
Président du Muséum national d'Histoire naturelle

RÉDACTRICE EN CHEF / *EDITOR-IN-CHIEF*: Line LE GALL
Muséum national d'Histoire naturelle

ASSISTANTE DE RÉDACTION / *ASSISTANT EDITOR*: Audrina NEVEU (algo@cryptogamie.com)

MISE EN PAGE / *PAGE LAYOUT*: Audrina NEVEU

RÉDACTEURS ASSOCIÉS / *ASSOCIATE EDITORS*

Ecoevolutionary dynamics of algae in a changing world

Stacy KRUEGER-HADFIELD

Department of Biology, University of Alabama, 1300 University Blvd, Birmingham, AL 35294 (United States)

Jana KULICHOVA

Department of Botany, Charles University, Prague (Czech Republic)

Cecilia TOTTI

Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona (Italy)

Phylogenetic systematics, species delimitation & genetics of speciation

Sylvain FAUGERON

UMI3614 Evolutionary Biology and Ecology of Algae, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Av. Bernardo O'Higgins 340, Santiago (Chile)

Marie-Laure GUILLEMIN

Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia (Chile)

Diana SARNO

Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli (Italy)

Comparative evolutionary genomics of algae

Nicolas BLOUIN

Department of Molecular Biology, University of Wyoming, Dept. 3944, 1000 E University Ave, Laramie, WY 82071 (United States)

Heroen VERBRUGGEN

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

Algal physiology & photosynthesis

Janet KÜBLER

California State University Northridge, Department of Biology, California State University, Northridge, CA 91330-8303 (United States)

Prokaryotic algae

Nico SALMASO

IASMA Research and Innovation Centre, Fondazione Mach-Istituto Agrario di S. Michele all'Adige, Limnology and River Ecology, Via E. Mach, 1, 38010 San Michele all'Adige, Trento (Italy)

Vitor VASCONCELOS

Faculdade de Ciências da Universidade do Porto and CIIMAR, Rua do Campo Alegre, s/n, 4169-007 Porto (Portugal)

COUVERTURE / *COVER*:

Polysiphonia morrowii Harvey.

Cryptogamie, Algologie est indexé dans / *Cryptogamie, Algologie is indexed in*:

- Aquatic Sciences & Fisheries Abstracts Part I.
- Biological Abstracts
- Chemical Abstracts
- Current Contents
- Marine Science Contents Tables (FAO)
- Science Citation Index
- Publications bibliographiques du CNRS (Pascal).

Cryptogamie, Algologie est distribué en version électronique par / *Cryptogamie, Algologie is distributed electronically by*:

- BioOne® (<http://www.bioone.org/loi/crya>)

Cryptogamie, Algologie est une revue en flux continu publiée par les Publications scientifiques du Muséum, Paris
Cryptogamie, Algologie is a fast track journal published by the Museum Science Press, Paris

Les Publications scientifiques du Muséum publient aussi / *The Museum Science Press also publishes*:

Adansonia, Geodiversitas, Zoosystema, Anthropolzoologica, European Journal of Taxonomy, Naturae, Cryptogamie sous-sections *Bryologie, Mycologie*.

Diffusion – Publications scientifiques Muséum national d'Histoire naturelle

CP 41 – 57 rue Cuvier F-75231 Paris cedex 05 (France)

Tél. : 33 (0)1 40 79 48 05 / Fax : 33 (0)1 40 79 38 40

diff.pub@mnhn.fr / <http://sciencepress.mnhn.fr>

© Publications scientifiques du Muséum national d'Histoire naturelle, Paris, 2020

ISSN (imprimé / *print*): 0181-1568 / ISSN (électronique / *electronic*): 1776-0984

Molecular assessment of the tribes Strebloladiaceae and Polysiphoniaceae (Rhodomelaceae, Rhodophyta) in the British Isles reveals new records and species that require taxonomic revision

Cristina PIÑEIRO-CORBEIRA

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

Christine A. MAGGS

Queen's University Belfast, Marine Laboratory, Portaferry, Newtownards BT22 1PF (United Kingdom)

Fabio RINDI

Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona (Italy)

Francis BUNKER

MarineSeen, Estuary Cottage, Bentlass, Hundleton, Pembrokeshire, Wales SA71 5RN (United Kingdom)

Lin BALDOCK

Dorset Wildlife Trust, Brooklands Farm, Forston, Dorchester DT2 7AA (United Kingdom)

Pilar DÍAZ-TAPIA

Coastal Biology Research Group, Faculty of Sciences and Centre for Advanced Scientific Research (CICA), University of A Coruña, 15071, A Coruña (Spain) and Instituto Español de Oceanografía (IEO), Centro Oceanográfico de A Coruña, Aptdo. 130, 15080, A Coruña (Spain) pdiaz@udc.es (corresponding author)

Submitted on 16 December 2019 | Accepted on 23 April 2020 | Published on 5 June 2020

Piñeiro-Corbeira C., Maggs C. A., Rindi F., Bunker F., Baldock L. & Díaz-Tapia P. 2020. — Molecular assessment of the tribes Strebloladiaceae and Polysiphoniaceae (Rhodomelaceae, Rhodophyta) in the British Isles reveals new records and species that require taxonomic revision. *Cryptogamie, Algologie* 41 (8): 55-72. <https://doi.org/10.5252/cryptogamie-algologie2020v41a8>. <http://cryptogamie.com/algologie/41/8>

KEY WORDS

Vertebrata,
Polysiphonia,
British Isles,
cox1,
rbcl,
distribution,
introduced species,
pseudo-cryptic diversity,
new record,
new combination.

ABSTRACT

The use of molecular tools often shows that regional species diversity differs from what we know from assessments based on morphological identifications. The seaweed flora of the British Isles has been well established over a long period from foundational work published during the XVIII-XIX centuries to more recent revisions based on molecular tools. The application of these tools (primarily *rbcl* sequences), alongside morphological observations, to the study of the tribes Strebloladiaceae and Polysiphoniaceae in the British Isles led us to three new records of species, as well as two species that require taxonomic revision. *Polysiphonia morrowii* Harvey, *P. delicata* Díaz-Tapia and *Vertebrata tripinnata* (Harvey) Kuntze are recorded for the first time in the British Isles.

Finding *P. morrowii* and *P. delicata*, which are considered introduced or cryptogenic in Europe, is not surprising and these new records improve our knowledge of their distribution. *Vertebrata tripinnata* had previously been recorded in southern Europe and collecting it on the northern coast of Ireland considerably expands its known distribution. We also found that *rbcl* and *cox1* sequences for the morphologically divergent *V. simulans* (Harvey) Kuntze and *P. ceramiiiformis* P.Crouan & H.Crouan (which has never been transferred to *Vertebrata*) from the British Isles were identical, and we propose to reduce the latter to a synonym of the former. Finally, we found two pseudo-cryptic species represented in specimens morphologically assigned to *V. fruticulosa* (Wulfen) Kuntze, and we propose the resurrection of *V. martensiana* (Kützinger) Piñeiro-Corbeira, Maggs & Díaz-Tapia. This work further evidences the relevance of reassessing red algal species diversity using molecular tools, even in regions where floras are considered well-known.

RÉSUMÉ

L'inventaire moléculaire des tribus Strebloladidae et Polysiphonieae (Rhodomelaceae, Rhodophyta) dans les îles Britanniques révèle de nouveaux signalements et des espèces qui nécessitent une révision taxonomique. L'utilisation d'outils moléculaires montre souvent que la diversité régionale des espèces diffère de ce que nous savons des évaluations basées sur des identifications morphologiques. La flore d'algues des îles Britanniques a été bien établie sur une longue période, depuis les travaux fondamentaux publiés au cours du XVIII^e et XIX^e siècle jusqu'aux révisions plus récentes basées sur les outils moléculaires. L'application de ces outils (principalement des séquences *rbcl*), parallèlement aux observations morphologiques, à l'étude des tribus Strebloladidae et Polysiphonieae dans les îles Britanniques nous a conduit à répertorier trois espèces qui n'étaient pas encore référencées dans la flore locale ainsi que deux espèces qui nécessitent une révision taxonomique. *Polysiphonia morrowii* Harvey, *P. delicata* Díaz-Tapia et *Vertebrata tripinnata* (Harvey) Kuntze sont recensées pour la première fois dans les îles Britanniques. La découverte de *P. morrowii* et *P. delicata*, qui sont considérés comme introduits ou cryptogéniques en Europe, n'est pas surprenante et ces nouveaux signalements améliorent notre connaissance de leur répartition. *Vertebrata tripinnata* avait été précédemment enregistrée dans le sud de l'Europe et sa collecte sur la côte nord de l'Irlande élargit considérablement sa répartition connue. Nous avons également constaté que les séquences *rbcl* et *cox1* morphologiquement divergentes de *V. simulans* (Harvey) Kuntze et *P. ceramiiiformis* P.Crouan & H.Crouan (qui n'a jamais été transféré à *Vertebrata*) des îles Britanniques étaient identiques, et nous proposons de réduire la seconde à un synonyme de la première. Enfin, nous avons trouvé deux espèces pseudo-cryptiques représentées dans des spécimens morphologiquement attribués à *V. fruticulosa* (Wulfen) Kuntze, et nous proposons la résurrection de *V. martensiana* (Kützinger) Piñeiro-Corbeira, Maggs & Díaz-Tapia. Ce travail démontre encore une fois la pertinence de réévaluer la diversité des espèces d'algues rouges à l'aide d'outils moléculaires, même dans des régions où les flores sont considérées comme bien connues.

MOTS CLÉS
Vertebrata,
Polysiphonia,
 îles Britanniques,
cox1,
rbcl,
 distribution,
 espèces introduites,
 diversité pseudo-
 cryptique,
 nouvelle occurrence,
 combinaison nouvelle.

INTRODUCTION

The British Isles host a high seaweed diversity explained, in part, by their wide latitudinal span encompassing the cold-temperate northeast Atlantic region and the warm temperate northeast Atlantic subregion 1 (van den Hoek & Breeman 1990; fig. 1). The study of this diversity has a long and distinguished history that includes the publication of foundational macroalgal accounts (e.g. Stackhouse 1795, 1797, 1802; Greville 1830; Harvey 1857; Newton 1931). More recently, the seaweed flora of the British Isles has been revised, and detailed morphological descriptions provided according to contemporary standards (e.g. Maggs & Hommersand 1993; Brodie *et al.* 2007). As a result, the marine benthic flora of the British Isles is among the best characterized worldwide and recent additions generally consist of newly introduced species (e.g. Fletcher & Manfredi 1995; Bunker 2014) or the discovery of new cryptic or pseudo-cryptic species

morphologically similar to known species (e.g. Brodie & Irvine 1997; Leliaert *et al.* 2009; Walker *et al.* 2009). Despite the long history of study, some distinctive species have apparently been overlooked, such as the green alga *Flabellia petiolata* (Turra) Nizamuddin (Díaz-Tapia *et al.* 2020). Although the British Isles seaweed flora has been continuously updated, only some groups have been examined using an integrative approach that combines molecular tools and morphological studies. The use of this approach in seaweed diversity surveys often results in taxonomic discoveries including new cryptic or pseudo-cryptic species or new introductions, or the converse, placing recognized species into taxonomic synonymy (e.g. Díaz-Tapia *et al.* 2013a; Saunders *et al.* 2017; Pezozolesi *et al.* 2019; Savoie & Saunders 2019; Wolf *et al.* 2019).

The Rhodomelaceae is the largest family of the red algae with more than 1000 recognized species and, within this family, the Polysiphonieae and Strebloladidae are among the most speciose tribes (Guiry & Guiry 2019; Díaz-Tapia *et al.* 2017a).

In the British Isles they are represented by 22 species assigned to the genera *Carradoriella* P.C.Silva, *Leptosiphonia* Kylin, *Melanothamnus* Bornet & Falkenberg, *Polysiphonia* Greville and *Vertebrata* S.F.Gray (Maggs & Hommersand 1993 after taxonomic revision at the genus level by Díaz-Tapia *et al.* 2017a, b and Savoie & Saunders 2019). The Polysiphonieae and Streblocladiae are characterized by terete branched axes with a polysiphonous structure formed by an axial filament surrounded by pericentral cells and an attachment system that consists of unicellular rhizoids (Díaz-Tapia *et al.* 2017a). They exhibit a wide range of morphological characters that can be used for delineation of species (Maggs & Hommersand 1993; Stuercke & Freshwater 2008). In the British Isles, species are morphologically distinguishable by the study of a combination of some macroscopic and numerous microscopic characters (Maggs & Hommersand 1993). The microscopic nature of most relevant taxonomic characters and the large number of described species that are often small (<5 cm in length) and confined to particular habitats means that members of these tribes are good candidates for hiding cryptic diversity or remaining unnoticed.

During the 1990s, molecular data were determined for the tribes Streblocladiae and Polysiphonieae from the British Isles. This dataset generally included only one or two sequenced specimens of each species and it was used to infer phylogenetic relationships among taxa (McIvor *et al.* 2001; Díaz-Tapia *et al.* 2017b). In addition, a more extensive dataset was produced for the species *Melanothamnus harveyi* (Bailey) Díaz-Tapia & Maggs in order to investigate the relationships among populations of this introduced species (McIvor *et al.* 2001, as *Polysiphonia*). More recently, in 2014–2017, we obtained further sequences for species from the British Isles and other European regions. The objective of this present work is to reassess the diversity and taxonomy of selected groups in the tribes Streblocladiae and Polysiphonieae in the British Isles using an integrative approach that combines morphological observations and molecular data. The groups selected are those that exhibited anomalies when comparing the two approaches of morphological and molecular analyses. Various solutions are proposed here to deal with these challenges.

MATERIAL AND METHODS

Material was collected during sampling surveys in the British Isles, the Atlantic Iberian Peninsula and the Mediterranean Sea (Fig. 1; Annexe 1). Materials for DNA extraction were preserved in silica gel desiccant. Samples for morphological study were preserved in 4% formalin seawater at 4°C and stored in the dark. Some specimens were mounted in 20% Karo® Corn Syrup (ACH Foods, Memphis, TN, United States) and 80% distilled water. Sections for microscopic observations were made by hand using a razor blade. Voucher specimens were deposited in the SANT herbarium of the University of Santiago de Compostela (Spain). Morphological study focused on the assessment of diagnostic characters for the studied species. Detailed morphological descriptions

of these species in the study area and nearby locations were provided in Lauret (1970), Maggs & Hommersand (1993) and Díaz-Tapia *et al.* (2017c).

DNA was extracted from silica gel-dried material using the Qiagen DNeasy Plant mini Kit (Qiagen GmbH, Hilden, Germany), or the Promega Wizard Magnetic 96 DNA Plant System kit, following the manufacturer's instructions, or an adapted cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle 1987). PCR amplification was carried out for *rbcL* using the primers F7/RrbcStart, F7/R893, F57/rbcLrevNEW or F2/R1462 (Freshwater & Rueness 1994; Mamoozadeh & Freshwater 2011; Saunders & Moore 2013; Díaz-Tapia *et al.* 2018b) and for *cox1* using the forward primer GazF1 (Saunders 2005) and the newly designed reverse primer 1069R (5' GTTCTTCAAAGTATGYTAKGCWGG 3'). Reactions were performed in a total volume of 25 µl, consisting of 5 µl 5 × MyTaq™ reaction buffer, 0.7 µl 10 µM forward and reverse primers, 0.125 µl 1 U/µl MyTaq™ DNA Polymerase (Bioline), 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 Belfast on an AB3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, United States) or commercially by Macrogen or the sequencing service of the University of A Coruña.

A total of 27 new *rbcL* and four new *cox1* gene sequences were generated in this study for the target groups. They were compared with previously available sequences in GenBank using the BLAST tool. The four *cox1* sequences were aligned and compared; the total length of the alignment was 1414 bp. We downloaded from GenBank all publicly available *rbcL* sequences with <0.8% divergence from target species. In addition, we included in our *rbcL* phylogenetic analyses 51 sequences for representative species of the major lineages in the Streblocladiae and Polysiphonieae according to previous phylogenetic studies (Díaz-Tapia *et al.* 2017a). The sequences, their length and their corresponding GenBank accession numbers are listed in Annexe 1. Sequences were aligned using Muscle in Geneious 6.1.8 (Kearse *et al.* 2012). The total length of the *rbcL* alignment was 1467 bp. We included in our analyses a single sequence per species, except for the focal species of this study, for which all haplotypes were represented. The sequences included in the final alignment were selected considering their quality in terms of both length (the longest sequences) and the presence of ambiguous bases. With the aim of analysing the species assignment of newly sequenced specimens, phylogenetic trees for *rbcL* were estimated with Maximum Likelihood (ML) using RAxML 8.1.X (Stamatakis 2014). GTR-Gamma was used as the nucleotide model; branch support was estimated with 1000 bootstrap replicates. Three species of *Pterosiphonia* Falkenberg 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 (Díaz-Tapia *et al.* 2017a).

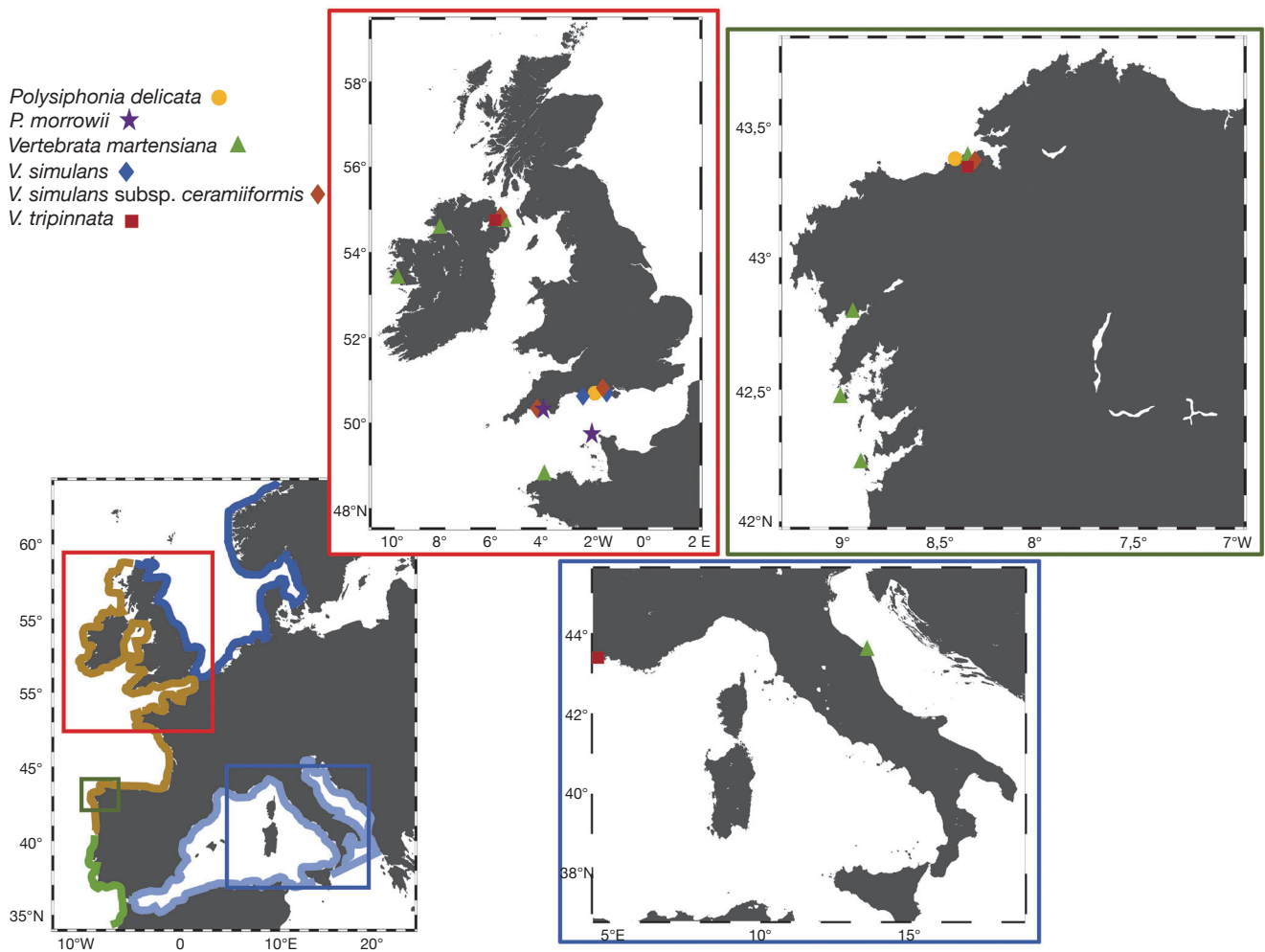


FIG. 1. — Collection sites of the species used in this study with indication of the European biogeographic regions according to van den Hoek and Breeman (1990): dark blue, cold-temperate northeast Atlantic Region; brown, warm-temperate northeast Atlantic subregion 1; green, warm-temperate northeast Atlantic subregion 2; light blue, east Mediterranean subregion.

RESULTS

Vertebrata simulans (Harvey) Kuntze and *Polysiphonia ceramiiformis* P.Crouan & H.Crouan

MOLECULAR IDENTIFICATION

Two *rbcL* sequences for *Vertebrata simulans* and three for *Polysiphonia ceramiiformis* from the British Isles (four of which were newly determined) were identical, and also identical to a newly determined sequence of *P. ceramiiformis* from Spain. These sequences were resolved in the phylogeny in a highly supported clade including eight other members of the genus *Vertebrata* (Fig. 2). Relationships among members of the clade including *V. simulans* were unresolved. The most closely related species in the phylogeny is *V. byssoides* (Goodenough & Woodward) Kuntze with 5.3% divergence in the *rbcL* gene.

Two *cox1* sequences were generated for both *V. simulans* and *P. ceramiiformis*. The three sequences from the United Kingdom including specimens morphologically identified as *V. simulans* and *P. ceramiiformis* were identical, while the sequence of *P. ceramiiformis* from Spain differed by 1 bp from the British specimens.

MORPHOLOGICAL OBSERVATIONS

Vertebrata simulans and *Polysiphonia ceramiiformis* are predominantly erect (Fig. 3A, D), attached to their substratum by short prostrate axes with unicellular rhizoids that are cut off from the pericentral cells. They have ecorticate axes with 10-13 pericentral cells. Axes are 100-300 µm in diameter, with segments 0.3-0.8 L/D (length/diameter). Branches are formed mostly every 2-9 segments, replacing trichoblasts.

Polysiphonia ceramiiformis has a soft succulent texture and branches form corymbose, characteristically forcipate apices (Fig. 3A-C). *Vertebrata simulans* is more slender and, in addition to indeterminate branches, it has abundant branchlets that remain short and spine-like (Fig. 3D-F).

Reproductive structures were not observed in specimens of *P. ceramiiformis* studied here, but tetrasporangia, spermatangia and cystocarps were described in Maggs & Hommersand (1993). *Vertebrata simulans* has tetrasporangia 40-70 µm in diameter, formed in short lateral branchlets, in short spiral series (Fig. 3G). Sexual reproductive structures are unknown except for the observation of spermatangia on a tetrasporophyte (Maggs & Hommersand 1993).

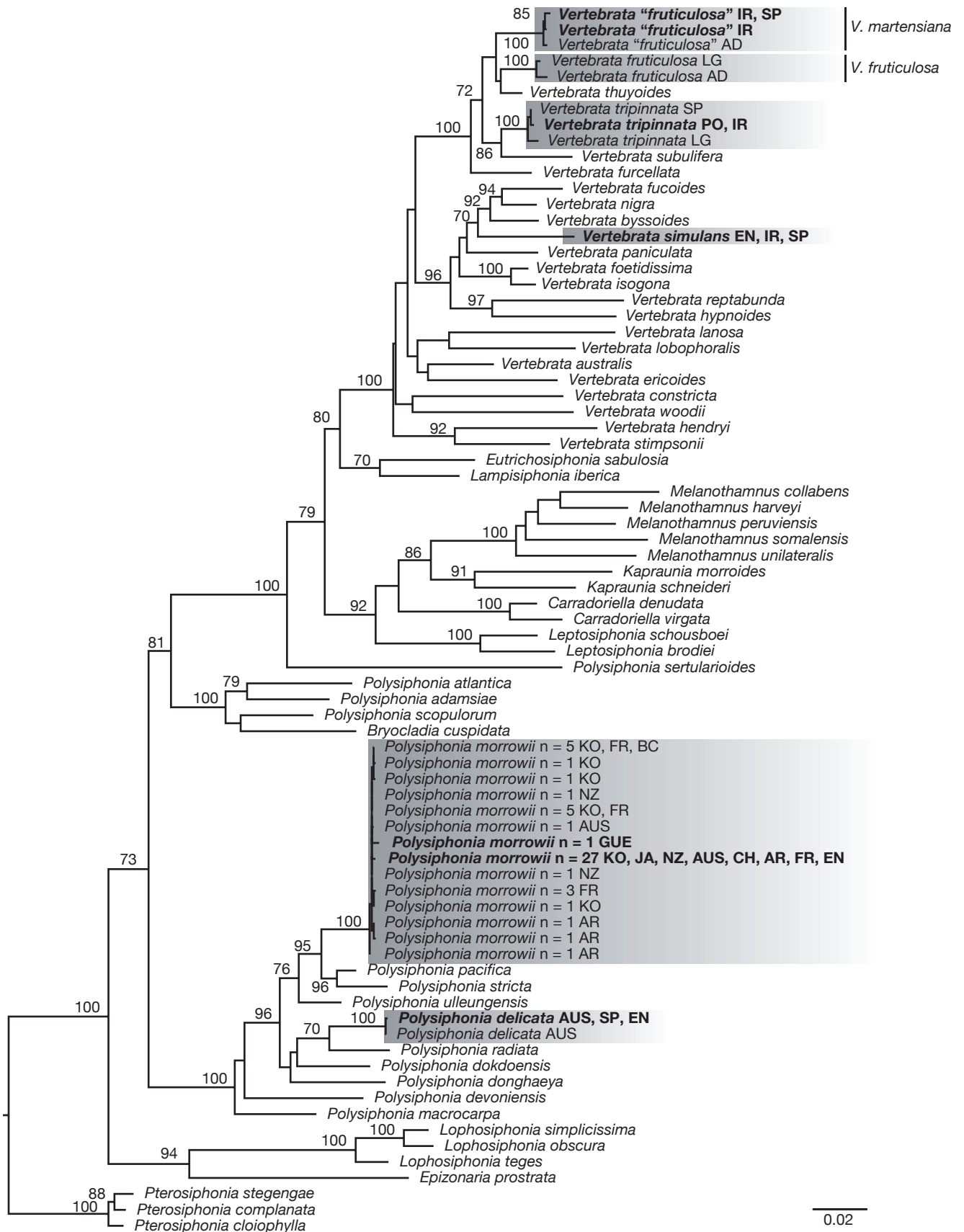


FIG. 2. — Phylogenetic tree estimated with ML analysis of *rbcL* sequences. Values at nodes indicate bootstrap support (BP) (only shown if ≥ 70). Species analysed in this study are grey-shaded and species or haplotypes found in the British Isles are in bold. For *P. morrowii* Harvey, the number (n) of sequences available for each haplotype is indicated. Codes for countries/regions: AD, Adriatic Sea; AR, Argentina; AUS, Australia; BC, British Columbia; CH, Chile; EN, England; FR, France; GUE, Guernsey; IR, Ireland; JA, Japan; KO, Korea; LG, Ligurian Sea; NZ, New Zealand; PO, Portugal; SP, Spain.

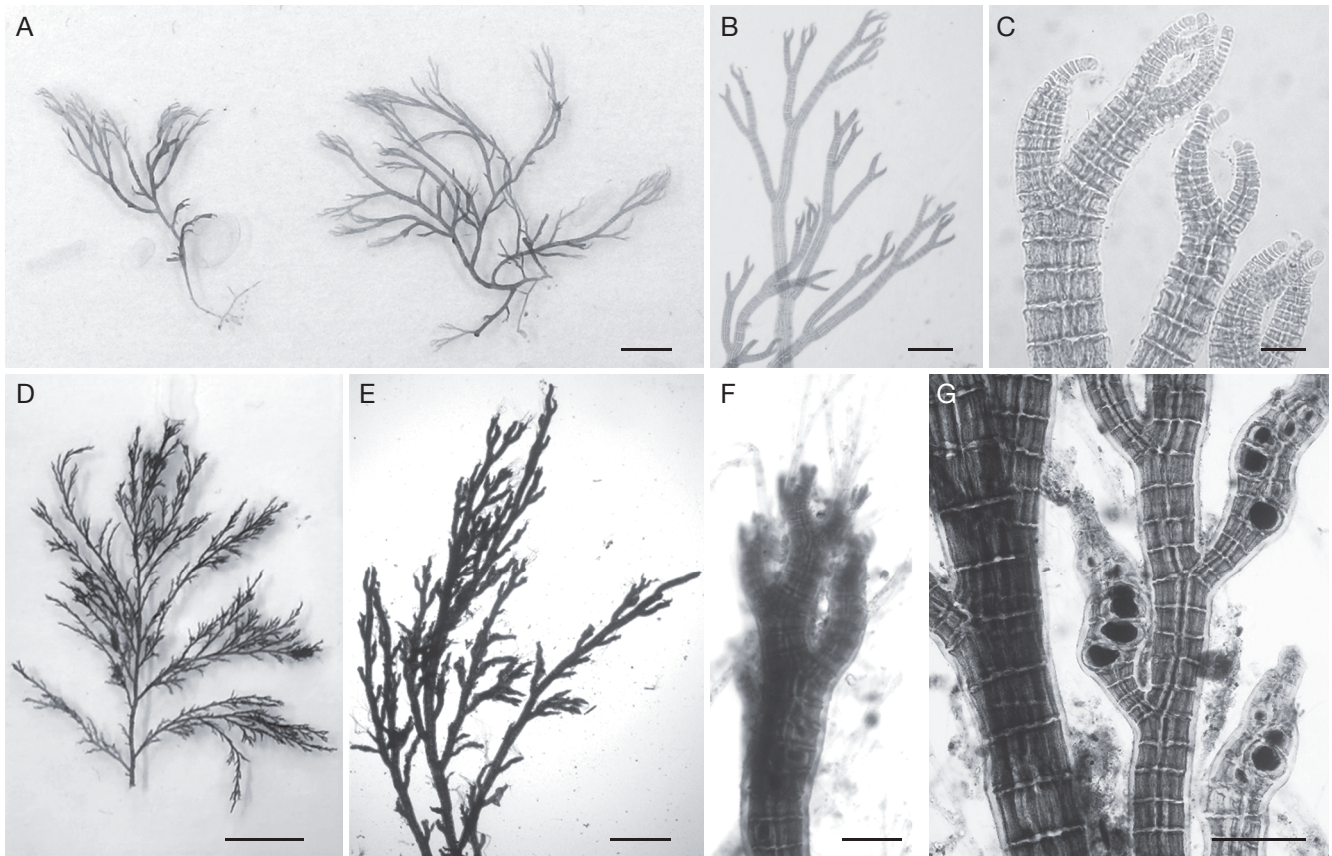


FIG. 3. — *Vertebrata simulans* (Harvey) Kuntze, *P. ceramiiformis* P.Crouan & H.Crouan morphotype: **A**, habit; **B**, upper part of a thallus; **C**, forcipate young branches. *Vertebrata simulans*: **D**, habit; **E**, upper part of a thallus; **F**, straight young branches; **G**, lateral short branches with mature tetrasporangia. Scale bars: A, 2 mm; B, 500 µm; C, F, G, 100 µm; D, 5 mm; E, 1000 µm.

Vertebrata fruticulosa (Wulfen) Kuntze

MOLECULAR IDENTIFICATION

In total 18 *rbcL* sequences, 16 newly determined, are available for specimens morphologically identified as *Vertebrata fruticulosa* from the United Kingdom, the Atlantic Iberian Peninsula and the Mediterranean. They were resolved in the *rbcL* phylogeny (Fig. 2) in a fully supported clade that also included sequences of *V. thuyoides* (Harvey) Kuntze, *V. tripinnata* (Harvey) Kuntze, *V. subulifera* (C.Agardh) Kuntze and *V. furcellata* (C.Agardh) Kuntze. Within this clade, specimens assigned to *V. fruticulosa* formed two clades that diverged by 3-3.3% (21-24 bp) and that are labelled in Figure 2 as *V. fruticulosa* and *V. martensiana*. Two haplotypes of *V. fruticulosa* were 0.6% (4 bp) divergent. Specimens of the *V. fruticulosa* clade were only found in the Mediterranean, including the Adriatic Sea, its type locality. *Vertebrata martensiana* has a wider distribution including the Adriatic Sea, the Atlantic Iberian Peninsula and the United Kingdom. Two haplotypes were found in the Atlantic and a third one in the Mediterranean and they diverged by 0.3-0.4% (4-6 bp).

MORPHOLOGICAL OBSERVATIONS

Specimens that were initially identified as *Vertebrata fruticulosa* (Fig. 4) were predominantly erect, attached

to the substratum by short prostrate axes that bear rhizoids cut off from the pericentral cells. Thalli were composed of several main axes that bear branches pseudodichotomously at wide angles (>30°) (Fig. 4A, E, I). They were 250-900 in diameter in mid-parts of the thallus. Trichoblasts were absent or scarcely developed. Axes had 8-12 pericentral cells and were corticate (Fig. 4D, H, L). Specimens of the *V. fruticulosa* and *V. martensiana* clades can be distinguished by the morphological characters indicated below.

Specimens placed in the *V. fruticulosa* clade had main axes of indeterminate growth that were alternately branched at regular intervals and bore alternate branches of determinate growth (Fig. 4A, B). Determinate branches, in turn, bore 2-3 orders of branches mostly every 3 segments (Fig. 4C) and were 7-9 mm in length. Axes had 11-12 pericentral cells (Fig. 4D).

The specimens in the *V. martensiana* clade had main axes branched at irregular intervals and bore branches of determinate growth (Fig. 4E, F, I, J). Mediterranean specimens had simple or once-branched determinate branches that were 1.5 mm in length (Fig. 4G). Axes had 8-10 pericentral cells (Fig. 4H). Atlantic specimens had determinate branches 3-5 mm in length that were simple or bore up to 2 branching orders (Fig. 4K) and axes with 8-12 pericentral cells (Fig. 4L).

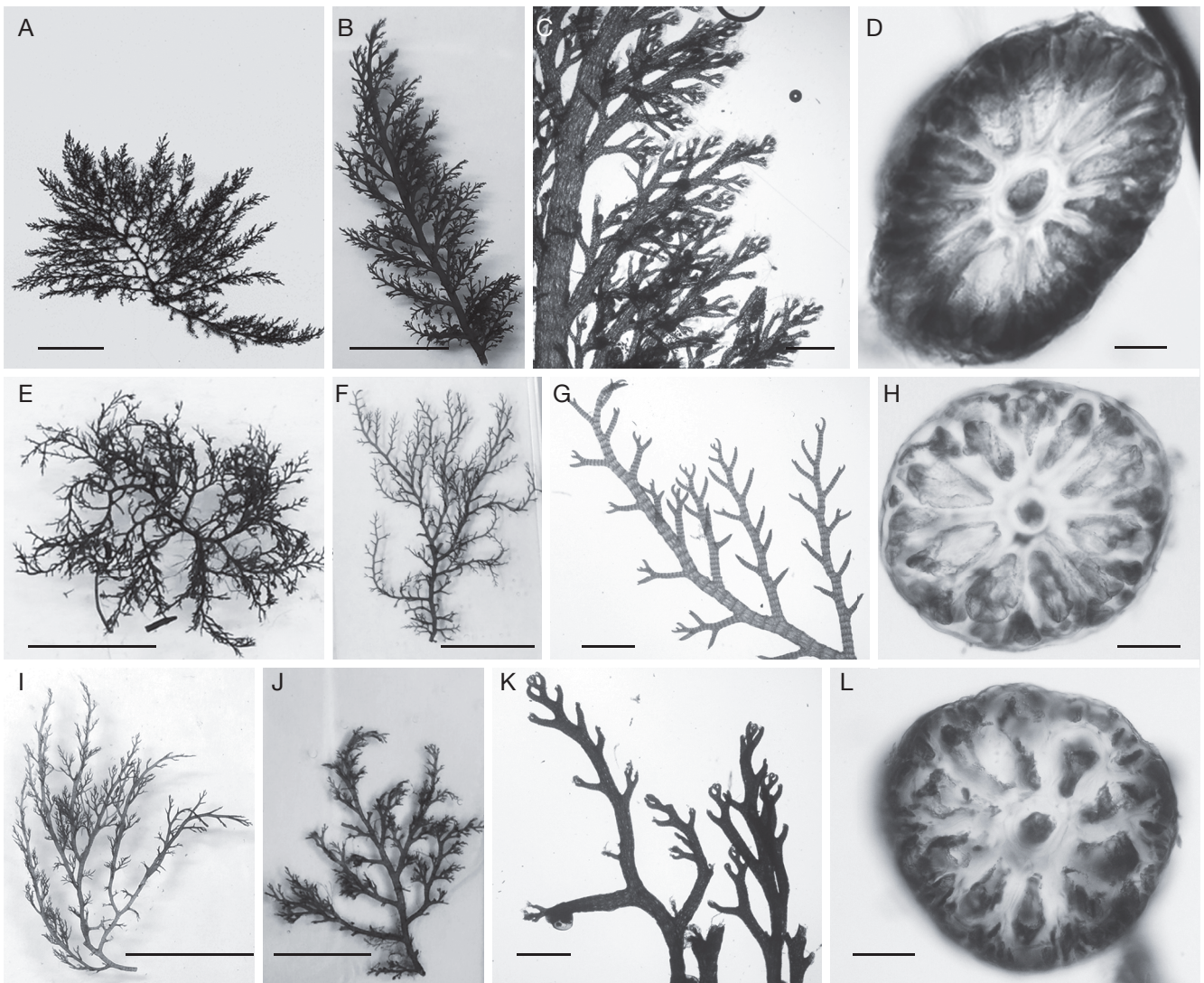


FIG. 4. — *Vertebrata fruticulosa* (Wulfen) Kuntze: **A**, habit; **B**, lateral branch bearing determinate branches; **C**, determinate branches bearing two-three branching orders; **D**, cross-section of an axis with 11 pericentral cells. *Vertebrata martensiana* (Kützinger) Piñeiro-Corbeira, Maggs & Díaz-Tapia from the Mediterranean Sea: **E**, **F**, habit; **G**, upper part of an erect axis with alternate determinate branches that are once branched; **H**, cross-section of an axis with eight pericentral cells. *Vertebrata martensiana* from the British Isles: **I**, **J**, habit; **K**, upper parts of erect axes with alternate determinate branches that are once or twice branched; **L**, cross-section of an axis with ten pericentral cells. Scale bars: **A**, **B**, **E**, **F**, **I**, **J**, 10 mm; **C**, **D**, **H**, **K**, **L**, 100 μ m; **G**, 1000 μ m.

Vertebrata tripinnata (Harvey) Kuntze

MOLECULAR IDENTIFICATION

Two new sequences were obtained for specimens from Donegal (Ireland) which were identical to a sequence from Portugal. They diverged by only 0.2–0.8% (1–5 bp) from sequences from northwestern Spain and the Mediterranean. In the *rbcl* phylogeny *Vertebrata tripinnata* was placed as sister to *V. subulifera* with moderate support. Sequence divergence between the two species was 3.8% (Fig. 2).

MORPHOLOGICAL OBSERVATIONS

Thalli forming dense tufts up to 3 cm high, dark red to brown in colour, covering rocky surfaces to ca. 20 cm² in extent. Thalli radially organized with indeterminate erect axes becoming decumbent when developing rhizoids in their basal parts (Fig. 5A), forming an extensive system of prostrate interwoven

axes and bearing unicellular rhizoids cut off from pericentral cells (Fig. 5B, C). Axes ecorticate with 17–19 pericentral cells (Fig. 5D). Erect axes rigid in texture, alternately branched up to three orders and mostly every six segments. Erect axes 150–400 (–500) μ m in diameter, with segments 0.4–0.8 (–1.75) L/D. Trichoblasts abundantly formed on every segment at the apices of erect axes and branches, dichotomously branched up to five orders (Fig. 5E), with multinucleate cells (Fig. 5F). Trichoblasts deciduous and leaving conspicuous scar cells when shed (Fig. 5G). Reproductive structures not observed.

Polysiphonia morrowii Harvey

MOLECULAR IDENTIFICATION

In total, 51 *rbcl* sequences have been published for *Polysiphonia morrowii* including data from both regions where the species

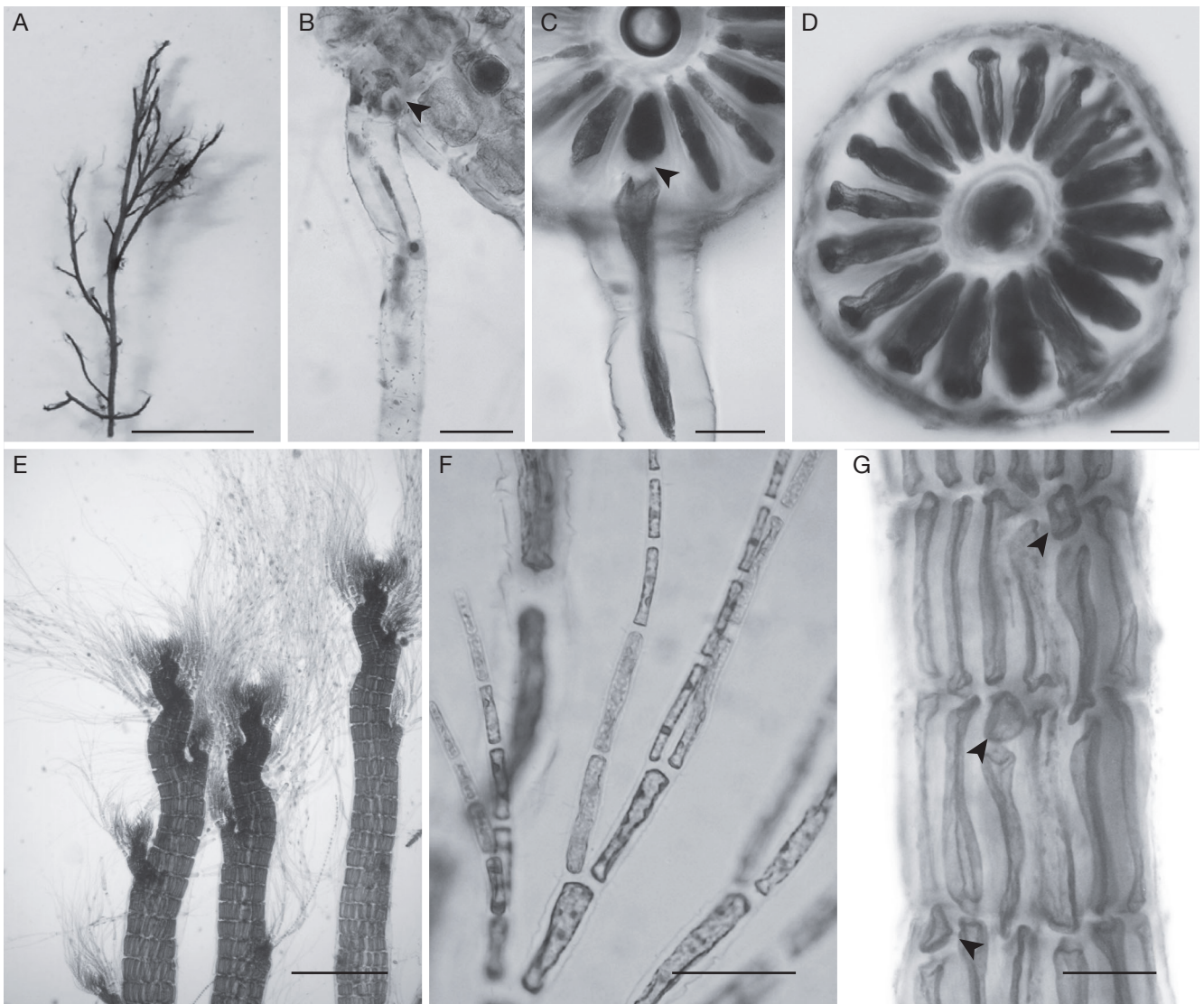


FIG. 5. — *Vertebrata tripinnata* (J.Agardh) Kuntze; **A**, habit; **B**, rhizoid cut off from pericentral cells (**arrowhead**); **C**, cross-section of a prostrate axes with a rhizoid cut off from pericentral cells (**arrowhead**); **D**, cross-section of an axis with 18 pericentral cells; **E**, upper parts of erect axes with short branches and long apical trichoblasts; **F**, trichoblast with multinucleate cells; **G**, scar cells of trichoblasts spirally arranged on every segment (**arrowheads**). Scale bars: A, 10 mm; B, 100 μ m; C, 100 μ m; D, 50 μ m; E, 500 μ m; F, 50 μ m; G, 100 μ m.

is considered native (eastern Asia) and introduced. Two new sequences have been obtained for specimens from the British Isles: one from Plymouth (England) and one from Alderney (Channel Islands). Overall, the sequences available represent 14 *rbcl* haplotypes (Fig. 2), with a sequence divergence of up to 0.5% (9 bp). The sequence from Plymouth was identical to specimens from western Asia, Australasia, Pacific and Atlantic South America and Atlantic France, while the sequence from Alderney represents a new haplotype. *Polysiphonia morrowii* was placed in the phylogeny in a highly supported clade with *P. stricta*, the generitype (Fig. 2). The nearest neighbour of *P. morrowii* was *P. pacifica* (AY958162) from which it diverged by 2.9-3.2%.

MORPHOLOGICAL OBSERVATIONS

Thalli forming tufts, up to 10 cm high, with interwoven prostrate axes and erect axes spirally branched mostly every five

segments (Fig. 6A, B). Axes ecorticate and with four pericentral cells. Erect axes growing from sharply pointed apical cells (Fig. 6C), increasing to 40-65 μ m in diameter in mid parts; segments 1.2-3.6 L/D. Trichoblasts not observed. Tetrasporangia formed in the last 2-3 branching orders in straight series (Fig. 6D), 20-30 μ m in diameter. Sexual reproductive structures were not observed.

Polysiphonia delicata Díaz-Tapia

MOLECULAR IDENTIFICATION

In total, 11 sequences have previously been published for *Polysiphonia delicata* from Spain and Australia, which represent two haplotypes, one found only in Australia and the other in both countries (Fig. 2). The newly determined sequence for an English specimen was identical to sequences from Spain and Tasmania.

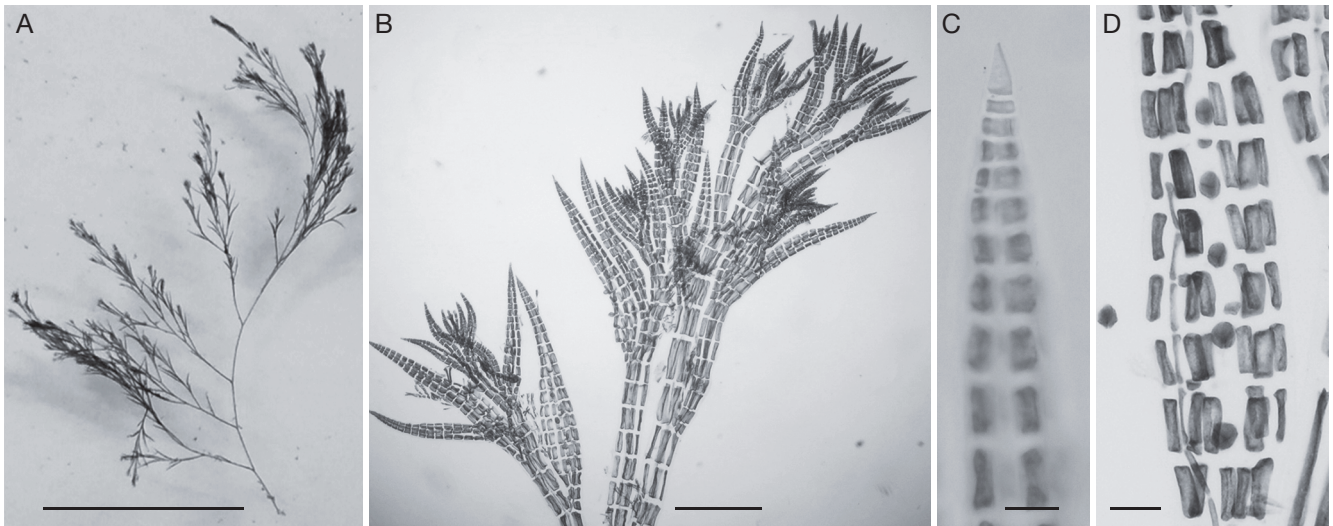


FIG. 6. — *Polysiphonia morrowii* Harvey: **A**, habit; **B**, upper part of an erect axis; **C**, apex of a branch with a pointed apical cell; **D**, young tetrasporangia in straight series. Scale bars: A, 10 mm; B, 100 μ m; C, D, 50 μ m.

Polysiphonia delicata was placed in the *rbcL* phylogeny in a highly supported clade with *P. stricta* (Mertens ex Dillwyn) Greville, the generitype, and eight other species. *Polysiphonia delicata* was sister to *P. radiata* Díaz-Tapia, but this relationship received low support (Fig. 2). Divergence between this pair of species was 4.1–4.2%.

MORPHOLOGICAL OBSERVATIONS

Thalli forming discrete tufts, 2 cm high (Fig. 7A), pink in colour and flaccid in texture. Axes ecorticate, with four pericentral cells (Fig. 7B). Prostrate axes bearing unicellular rhizoids in open connection with the pericentral cells (Fig. 7C). Erect axes 80–120 μ m in diameter, composed of segments 1.5–3.5 L/D. Trichoblasts absent. Plants dioecious. Mature cystocarps slightly urceolate, 430–650 μ m high and 260–400 μ m in diameter (Fig. 7D).

DISCUSSION

RELATIONSHIP BETWEEN *VERTEBRATA SIMULANS* (HARVEY) KUNTZE AND *POLYSIPHONIA CERAMIIFORMIS* P.CROUAN & H.CROUAN

There were no differences between *rbcL* and *cox1* sequences obtained from specimens morphologically identified as *Vertebrata simulans* and *Polysiphonia ceramiiformis*, despite the clear morphological differences. Both species have ecorticate axes with 10–13 pericentral cells, branches replacing trichoblasts and tetrasporangia in spiral series, but they differ markedly in habit (Maggs & Hommersand 1993). In the British Isles *Polysiphonia ceramiiformis* – which is morphologically assigned to *Vertebrata* here but has never formally been transferred – appears to be an annual with a soft succulent texture and branches arranged forming corymbose, characteristically forcipate apices. It grows directly on bare substrata. In contrast, *V. simulans* is apparently perennial, more slender, and branchlets often remain short and spine-like (Maggs & Hommersand 1993);

it is usually epiphytic. These differences in habit and habitat are always obvious and intermediate morphotypes were never observed. Both species have overlapping distributions in the British Isles, being restricted to a few sites on southern English coasts and also occurring in northwest France (Maggs & Hommersand 1993). *Polysiphonia ceramiiformis* also extends to northwestern Spain (this is the first record from the Atlantic Iberian Peninsula), the Canary Islands and the Mediterranean (Lauret 1970; Rojas-González & Afonso-Carrillo 2008; Rindi & Cinelli 2000). Despite their morphological differences, the identical *rbcL* and *cox1* sequences indicate that *V. simulans* and *P. ceramiiformis* correspond to a single species and accordingly we propose their synonymy, *V. simulans* having taxonomic priority.

REASSESSING THE DIVERSITY OF SPECIMENS MORPHOLOGICALLY IDENTIFIED AS *VERTEBRATA FRUTICULOSA* (WULFEN) KUNTZE

Our results evidence the existence of two pseudo-cryptic species within the previously recognized *Vertebrata fruticulosa*. These two species were 3–3.3% divergent in the *rbcL* gene and were not resolved as monophyletic in our phylogeny (Fig. 1). Moreover, they can be morphologically distinguished considering the branching pattern, length of determinate branches and the number of pericentral cells (Table 1). The type locality of *V. fruticulosa* is Trieste (Adriatic Sea) and the illustration provided in its original description shows a species with main axes bearing regular branches and long determinate branches that in turn bear up to three orders of short branches (Jacquin 1791). This morphology matches our specimens from the Adriatic and Ligurian Seas, in the Mediterranean, labelled in our Figure 1 as *V. fruticulosa*, and differs from specimens from the Atlantic and the Adriatic labelled in Figure 1 as *V. martensiana*. Consequently, we assigned the former specimens to *V. fruticulosa* so the second clade including specimens initially assigned to *V. fruticulosa* based on their morphology requires taxonomic revision.

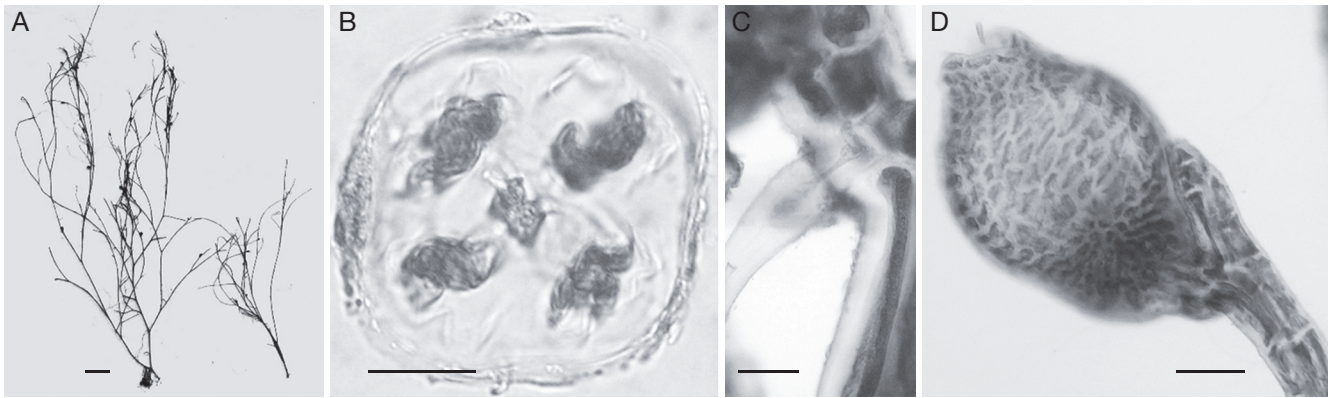


FIG. 7. — *Polysiphonia delicata* Díaz-Tapia: **A**, habit; **B**, cross-section of an axis with four pericentral cells; **C**, rhizoid in open connection with a pericentral cell; **D**, cystocarp. Scale bars: A, 10 mm; B, 20 µm; C, 200 µm; D, 50 µm.

TABLE 1. — Key morphological characters for distinguishing *Vertebrata fruticulosa* (Wulfen) Kuntze and *V. martensiana* (Kützting) Piñeiro-Corbeira, Maggs & Díaz-Tapia.

	<i>V. fruticulosa</i> (Wulfen) Kuntze	<i>V. martensiana</i> (Kützting) Piñeiro-Corbeira, Maggs & Díaz-Tapia (Atlantic)	<i>V. martensiana</i> (Kützting) Piñeiro- Corbeira, Maggs & Díaz-Tapia (Mediterranean)
Pericentral cells	11-12	8-12	8-10
Arrangement of indeterminate branches	Regular	Irregular	Irregular
Determinate branches			
Length (mm)	7-9	3-5	1.5
Branching	2-3 orders	Simple, once or twice branched	Simple or once branched

Five heterotypic synonyms of *Vertebrata fruticulosa* (basonym *Fucus fruticulosus* Wulfen in Jacquin 1791) are available: *Polysiphonia martensiana* Kützting (1843), *P. cymosa* Kützting (1849), *P. pycnophloea* Kützting (1849), *P. comatula* Kützting (1849) and *P. humilis* Kützting (1849). Two other species names listed as synonyms (Guiry & Guiry 2019) are *P. wulfenii* (Roth) J. Agardh (1842) and *P. comosa* Kützting (1843). *Polysiphonia wulfenii* is based on *Ceramium wulfenii* (Roth 1806) which cited *Fucus fruticulosus* as a synonym, so it is an illegitimate name. Similarly, *P. comosa* Kützting (1843) was later replaced by *P. comatula* (Kützting 1849), as the name *P. comosa* (Agardh) Endlicher (1843) had previously been applied to a species with six pericentral cells. All the heterotypic synonyms of *V. fruticulosa* have type localities in the Mediterranean except *P. martensiana* which was originally described from Biarritz, in southwestern France. Original descriptions of these species consist of a brief Latin diagnosis, but all were illustrated by Kützting (1864). *Polysiphonia pycnophloea*, *P. comatula* and *P. cymosa* have main axes that are unbranched or regularly branched, long determinate branches, and bear several orders of smaller branches (Kützting 1864). Therefore, we conclude that these three entities agree with *V. fruticulosa*. By contrast, *P. martensiana* and *P. humilis* have main axes with irregular branching and shorter determinate branches (Kützting 1864), similar to the species that requires revision. Of these two names, *P. martensiana* has priority and we propose the resurrection of this name. We observed

consistent differences in the branching pattern between *V. fruticulosa* and *P. martensiana*, and they are in agreement with the illustrations provided by Kützting (1864). However, the number of pericentral cells differs between our observations (11-12 in *V. fruticulosa* and 8-12 in *V. martensiana*) and descriptions in previous works. Kützting's illustrations show uniformly 9 pericentral cells (*P. fruticulosa*, *P. cymosa* and *P. pycnophloea*) and Öztig (1959) reported 9-10 and 11-12 pericentral cells in specimens assigned to *V. fruticulosa* from the Mediterranean and the Atlantic, respectively. It is likely that the number of pericentral cells in *V. fruticulosa* from the Mediterranean is more variable than in the specimens we examined, ranging from the nine pericentral cells reported in the literature to the 12 pericentral cells that we observed (FR, pers. obs.). Differences among Mediterranean and Atlantic specimens of *V. fruticulosa* have been discussed (Öztig 1959; Ardré 1970) and Ardré (1970) proposed the resurrection of *P. martensiana* for the Atlantic specimens assigned to *V. fruticulosa*, although this was not followed by subsequent researchers. Our work suggests that *V. fruticulosa* is restricted to the Mediterranean, but *V. martensiana* is certainly present in both the Mediterranean and the Atlantic.

The circumscription of the genera *Polysiphonia* and *Vertebrata* has recently been reviewed (Díaz-Tapia et al. 2017a, b). According to our phylogeny and in agreement with the morphological characters, *P. martensiana* clearly matches the concept of the genus *Vertebrata* and we propose the following new combination:

Family RHODOMELACEAE Horaninow
Genus *Vertebrata* S.F.Gray

Vertebrata martensiana (Kützting) Piñeiro-Corbeira,
Maggs & Díaz-Tapia, comb. nov.

Polysiphonia martensiana Kützting, *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange. Mit 80 farbig gedruckten Tafeln, gezeichnet und gravirt vom Verfasser*: 432 (1843) (basionym). — *Boergeseniella martensiana* (Kützting) Ardré, *Portugaliae Acta Biologica, Série B* 10: 198 (1970).

TYPE LOCALITY. — **Biarritz**, France (Kützting 1843).

EXPANDING THE DISTRIBUTION OF *VERTEBRATA TRIPINNATA* (J.AGARDB) KUNTZE TO THE NORTH OF IRELAND

Material of *Vertebrata tripinnata* was collected at a site in north Donegal, Ireland in July 2014 and 2017. This site at Island Reagh has unusual environmental conditions, as it is in part of Mulroy Bay that at low tide is covered by only 20 cm water depth, with much warmer temperatures in summer than on the surrounding open coast. Molecular data and morphological characters confirm that Irish specimens correspond to *V. tripinnata*. Sequences of Irish specimens were identical to or had a low divergence compared to sequences from Portugal, Spain and the Mediterranean. Other similar species previously reported in the British Isles are *Polysiphonia opaca* (C.Agardh) Moris & De Notaris, *V. simulans* and *V. subulifera*, but *P. opaca* has a greater number of pericentral cells (20–24) than *V. tripinnata* (17–19) and the two last species have fewer (10–13 and 12–15, respectively) (Maggs & Hommersand 1993; Díaz-Tapia *et al.* 2013b). Some British material from Swanage (Dorset) collected by Holmes in 1894 and sent to J.Agardh was identified as “*P.*” *tripinnata*, but an examination of cross-sections of these herbarium materials show that they have 10 pericentral cells and probably correspond to *V. simulans* (CAM, pers. obs.). *Vertebrata tripinnata* was originally described from the Mediterranean Sea and subsequently reported in the Canary Islands (Agardh 1842; Viera-Rodríguez *et al.* 1987). More recently it was recorded in Atlantic Europe, in northwest Spain (Díaz-Tapia *et al.* 2013b). Therefore, the record of *V. tripinnata* in the north of Ireland considerably extends the northern distribution limit for this species. The absence of previous verified records of this species in the British Isles can be explained because it is a rare species that only grows under particular environmental conditions in this latitude. Its distribution in the British Isles is probably wider, but considering its relatively small size and its similarity in appearance with other *Vertebrata* species it could easily have been misidentified. Although the possibility of its being an introduced species cannot be ruled out, Mulroy Bay supports other native species with disjunct distributions, such as *Codium bursa* (Oliv) C.Agardh.

NEW RECORDS OF INTRODUCED OR CRYPTOGENIC SPECIES

Polysiphonia morrowii and *P. delicata* are here recorded for the first time in the British Isles based on morphological and molecular evidence. Molecular data show that British specimens can be

unequivocally identified, as sequences from British specimens were identical or highly similar (sequence divergence <0.5%) to previously published *rbcL* sequences for these two species. Also, their morphological characters agree with the respective descriptions of the species. Other species in the British Isles that also have four pericentral cells and rhizoids in open connection with the pericentral cells are *P. devoniensis* Maggs & Hommersand, *P. atlantica* Kapraun & J.N.Norris and *P. stricta*. These species were molecularly characterized in previous studies and both *P. delicata* and *P. morrowii* were clearly placed in different positions in the *rbcL* phylogeny relative to these three species (Fig. 2). Moreover, they can be morphologically distinguished from native species. *Polysiphonia morrowii* and *P. stricta* share the majority of morphological characters but can be clearly distinguished because *P. morrowii* has pointed apical cells while *P. stricta*, like other species from the British Isles, has domed apical cells (Maggs & Hommersand 1993; Kim *et al.* 1994). *Polysiphonia delicata* differs from previously known species in the British Isles because it is pink in colour, thalli are up to 3 cm in length and 60–140 µm in diameter, branches are exogenous and cystocarps are slightly urceolate (British Isles) or globose (Australia, Piñeiro-Corbeira *et al.* 2019). Moreover *P. delicata* has spermatangial branches (not observed in British specimens) replacing trichoblasts and bearing sterile apical filaments (Díaz-Tapia *et al.* 2017c). *Polysiphonia devoniensis*, by contrast, is brown in colour, has spermatangial branches growing on one branch of fertile trichoblasts and cystocarps are globose. *Polysiphonia atlantica* has endogenous branches and spermatangial branches that lack sterile apical cells (Maggs & Hommersand 1993; Díaz-Tapia *et al.* 2017c). *Polysiphonia stricta* and *P. delicata* share most morphological characters, but *P. stricta* thalli are usually longer (up to 25 cm) and axes are thicker (50–300 µm).

The finding of *Polysiphonia morrowii* in the British Isles is not surprising as this species native to eastern Asia has been reported as introduced in Australia, New Zealand, Argentina, Chile, France and Italy (Curiel *et al.* 2002; Kim *et al.* 2004; Geoffroy *et al.* 2012; D’Archino *et al.* 2013; Croce & Parodi 2014; Piñeiro-Corbeira *et al.* 2019). The known distribution of *P. delicata* is more restricted and the species was recently reported for the first time from Spain and Australia, where it was considered cryptogenic as the habitat where it grows, mainly artificial substrata, suggests that it is an introduced species, although its native area is uncertain (Díaz-Tapia *et al.* 2017c; Piñeiro-Corbeira *et al.* 2019). The finding of *P. delicata* in the British Isles expands its known northward distribution in Europe, suggesting that it is probably more widespread than records suggest, but its small size and its morphological similarity to native species make it easily overlooked and a good candidate species for a cryptic introduction. Probable introduction vectors for both species, as for many introduced seaweeds, are aquaculture and shipping (Mineur *et al.* 2007, 2008). A large population of *P. morrowii* at Plymouth was found at Queen Anne’s Battery, a yacht harbour, implicating recreational vessels, but Plymouth has heavy maritime traffic including a wide variety of vessels. It was also growing in rock pools on the open coast at Plymouth Hoe. At Alderney, the site is also close to a busy harbour.

CONCLUSIONS

In this work we updated the flora of the British Isles for the rhodomelacean tribes Streblocladiae and Polysiphoniae based on molecular data and morphological observations. This approach led us to make several discoveries that required different taxonomic solutions, so that *Polysiphonia ceramiiformis* was reduced to a synonym of *Vertebrata simulans*, cryptic diversity was found in *V. fruticulosa* and consequently *V. martensiana* was resurrected for one of the discovered entities, and the known distribution of three species was expanded (*V. tripinnata*, *P. delicata* and *P. morrowii*). This work provides further support for the relevance of using molecular tools to understand red algal species diversity, even in regions where the seaweed flora is considered well-known, such as the British Isles. Our results suggest that the routine use of DNA sequence datasets for species diversity assessments in the British Isles and other European regions would almost certainly result in further taxonomic discoveries, especially in highly diverse and taxonomically complex algal groups. This approach is essential for improving our understanding of marine species diversity and it would provide basic information to detect shifts in species distributions as a consequence of global change as well as to detect new introduced species.

Acknowledgements

We thank Ignacio Bárbara for providing samples. We thank two anonymous reviewers for their suggestions and comments. Field work by PDT in the Mediterranean was funded by a grant awarded by the British Phycological Society. Funding support was provided by the programs “Axudas para a consolidación e estruturación de unidades de investigación competitivas do SUG” to PDT and CPC (grants GPC2015/025, ED431D 2017/20, ED431B 2018/49) and “Talento Senior” (grant 03IN858A2019-1630129) to PDT (Xunta de Galicia).

REFERENCES

- AGARDH J. G. 1842. — *Algae Maris Mediterranei et Adriatici, Observationes in Diagnosin Specierum et Dispositionem Generum*. Apud Fortin, Masson et Cie, Paris, 164 p.
- ARDRE F. 1970. — Contribution à l'étude des algues marines du Portugal. I. La Flore. *Portugaliae Acta Biologica, Série B* 10: 137-555.
- BÁRBARA I., CHOI H. G., SECILLA A., DÍAZ-TAPIA P., GOROSTIAGA J. M., SEO T. K., JUNG M. Y. & BERECIBAR E. 2013. — *Lampisiphonia iberica* gen. et sp. nov. (Ceramiaceae, Rhodophyta) based on morphology and molecular evidence. *Phycologia* 52: 137-155. <https://doi.org/10.2216/12-009.1>
- BRODIE J. & IRVINE L. M. 1997. — A comparison of *Porphyra dioica* sp. nov. and *P. purpurea* (Roth) C. Ag. (Rhodophyta: Bangiophycidae) in Europe. *Cryptogamie, Algologie* 18: 283-297.
- BRODIE J., MAGGS C. A. & JOHN D. M. 2007. — *Green Seaweeds of Britain and Ireland*. British Phycological Society, London, 242 p.
- BUNKER F. 2014. — *Chrysiomenia wrightii* (Wrights golden membrane weed) a new non-native to southwest England. *Phycologist* 87: 20.
- BUSTAMANTE D. E., WON B. Y., RAMIREZ M. E. & CHO T. O. 2012. — *Neosiphonia peruviana* sp. nov. (Rhodomelaceae, Rhodophyta) from the Pacific coast of South America. *Botanica Marina* 55: 359-366. <https://doi.org/10.1515/bot-2012-0146>
- BUSTAMANTE D. E., WON B. Y. & CHO T. O. 2014a. — *Polysiphonia dokdoensis* sp. nov. (Rhodomelaceae, Ceramiaceae) based on a population previously known as *Polysiphonia atlantica* sensu Kim & Lee from Korea. *Botanica Marina* 57: 281-289. <https://doi.org/10.1515/bot-2014-0011>
- BUSTAMANTE D. E., WON B. Y. & CHO T. O. 2014b. — *Polysiphonia ulleungensis* sp. nov. (Rhodomelaceae, Rhodophyta): a new diminutive species from Korea belonging to *Polysiphonia sensu stricto*. *Algae* 29: 111-120. <https://doi.org/10.4490/algae.2014.29.2.111>
- BUSTAMANTE D. E., WON B. Y. & CHO T. O. 2015. — *Polysiphonia freshwateri* sp. nov. and *Polysiphonia koreana* sp. nov.: two new species of *Polysiphonia* (Rhodomelaceae, Rhodophyta) from Korea. *European Journal of Phycology* 50: 330-342. <https://doi.org/10.1080/09670262.2015.1060633>
- BUSTAMANTE D. E., WON B. Y., MILLER K. A. & CHO T. O. 2017. — *Wilsonosiphonia* gen. nov. (Rhodomelaceae, Rhodophyta) based on molecular and morpho-anatomical characters. *Journal of Phycology* 53: 368-380. <https://doi.org/10.1111/jpy.12512>
- CARLILE A. L. 2009. — *Molecular systematics of North Pacific Ceramiaceae (Rhodophyta): Phylogeny, taxonomy, and phylogeography*. PhD, University of Washington, 296 p.
- CROCE M. E. & PARODI E. R. 2014. — The Japanese alga *Polysiphonia morrowii* (Rhodomelaceae, Rhodophyta) on the South Atlantic Ocean: first report of an invasive macroalga inhabiting oyster reefs. *Helgoland Marine Research* 68: 241-252. <https://doi.org/10.1007/s10152-014-0384-5>
- CURIEL D., BELLEMO G., LA ROCCA B., SCATTOLIN M. & MARZOCCHI M. 2002. — First report of *Polysiphonia morrowii* Harvey (Ceramiaceae, Rhodophyta) in the Mediterranean Sea. *Botanica Marina* 45: 66-70. <https://doi.org/10.1515/BOT.2002.008>
- D'ARCHINO R., NEILL K. F. & NELSON W. A. 2013. — Recognition and distribution of *Polysiphonia morrowii* (Rhodomelaceae, Rhodophyta) in New Zealand. *Botanica Marina* 56: 41-47. <https://doi.org/10.1515/bot-2012-0183>
- DÍAZ-TAPIA P., KIM M. S., SECILLA A., BÁRBARA I. & CREMADES J. 2013a. — Taxonomic reassessment of *Polysiphonia foetidissima* (Rhodomelaceae, Rhodophyta) and similar species, including *P. schneideri*, a newly introduced species in Europe. *European Journal of Phycology* 48: 345-362. <https://doi.org/10.1080/09670262.2013.842655>
- DÍAZ-TAPIA P., BÁRBARA I. & BERECIBAR E. 2013b. — Vegetative and reproductive morphology of *Polysiphonia tripinnata* (Rhodomelaceae, Rhodophyta): a new record from the European Atlantic coast. *Botanica Marina* 56: 151-160.
- DÍAZ-TAPIA P., MAGGS C. A., WEST J. A. & VERBRUGGEN H. 2017a. — Analysis of chloroplast genomes and a supermatrix inform reclassification of the Rhodomelaceae (Rhodophyta). *Journal of Phycology* 53: 920-937. <https://doi.org/10.1111/jpy.12553>
- DÍAZ-TAPIA P., MCLIVOR L., FRESHWATER D. W., VERBRUGGEN H., WYNNE M. J. & MAGGS C. A. 2017b. — The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata* S.F. Gray constitute well-defined clades of the red algal tribe Polysiphoniae (Rhodomelaceae, Ceramiaceae). *European Journal of Phycology* 52: 1-30. <https://doi.org/10.1080/09670262.2016.1256436>
- DÍAZ-TAPIA P., BÁRBARA I., CREMADES J., VERBRUGGEN H. & MAGGS C. A. 2017c. — Three new cryptogenic species in the tribes Polysiphoniae and Streblocladiae (Rhodomelaceae, Rhodophyta). *Phycologia* 56: 605-623. <https://doi.org/10.2216/17-17.1>
- DÍAZ-TAPIA P., PASELLA M. & VERBRUGGEN H. 2018a. — Molecular analyses resolve the phylogenetic position of *Polysiphonia adamsiae* (Rhodomelaceae, Rhodophyta) and reveal a strong phylogeographic structure in Australia. *Phycologia* 57: 593-600. <https://doi.org/10.2216/18-36.1>
- DÍAZ-TAPIA P., MAGGS C. A., MACAYA E. C. & VERBRUGGEN H. 2018b. — Widely distributed red algae often represent hidden introductions, complexes of cryptic species or species with strong

- phylogeographic structure. *Journal of Phycology* 54: 829-839. <https://doi.org/10.1111/jpy.12778>
- DÍAZ-TAPIA P., BALDOCK L. & MAGGS C. A. 2020. — Discovery of *Flabellia petiolata* (Halimedaceae, Chlorophyta) in the southern British Isles: A relict population or a new introduction? *Aquatic Botany* 160: 103-160. <https://doi.org/10.1016/j.aquabot.2019.103160>
- DOYLE J. J. & DOYLE J. L. 1987. — A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15. https://doi.org/10.1007/978-3-642-60441-6_4
- ENDLICHER S. L. 1843. — *Mantissa botanica altera. Sistens genera plantarum supplementum tertium.* apud Fridericum Beck, Universitatis Bibliopolam, Viena, 111 p.
- FLETCHER R. L. & MANFREDI C. 1995. — The occurrence of *Undaria pinnatifida* (Phaeophyceae, Laminariales) on the south coast of England. *Botanica Marina* 38: 355-358. <https://doi.org/10.1515/botm.1995.38.1-6.355>
- FRESHWATER D. W. & RUENESS J. 1994. — Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species based upon *rbcL* nucleotide sequence analysis. *Phycologia* 33: 187-194. <https://doi.org/10.2216/i0031-8884-33-3-187.1>
- GARCÍA-SOTO G. & LÓPEZ-BAUTISTA J. 2018. — Taxonomic notes on the genus *Alsidium* C. Agardh, including the merging of *Bryothamnion* Kützinger (Rhodomelaceae). *Algae* 33: 215-229. <https://doi.org/10.4490/algae.2018.33.6.25>
- GEOFFROY A., LE GALL L. & DESTOMBE C. 2012. — Cryptic introduction of the red alga *Polysiphonia morrowii* Harvey (Rhodomelaceae, Rhodophyta) in the north atlantic ocean highlighted by a DNA barcoding approach. *Aquatic Botany* 100: 67-71. <https://doi.org/10.1016/j.aquabot.2012.03.002>
- GEOFFROY A., DESTOMBE C., KIM B., MAUGER S., RAFFO M. P., KIM M. S. & LE GALL L. 2016. — Patterns of genetic diversity of the cryptogenic red alga *Polysiphonia morrowii* (Ceramiales, Rhodophyta) suggest multiple origins of the Atlantic populations. *Ecology and Evolution* 6: 5635-5647. <https://doi.org/10.1002/ece3.2135>
- GREVILLE R. K. 1830. — *Algae Britannicae, or Descriptions of the Marine and Other Inarticulated Plants of the British Islands, Belonging to the Order Algae; With Plates Illustrative of the Genera.* McLachlan & Stewart; Baldwin & Cradock, Edinburgh & London, 218 p.
- GUIRY M. D. & GUIRY G. M. 2019. — AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org> (Accessed on November 13, 2019).
- HARVEY W. H. 1857. — *Synopsis of British Seaweeds Compiled From Professor Harvey's Phycologia Britannica.* Lovell Reeve, London, 219 p.
- HUISMAN J. M., KIM B. & KIM M. S. 2017. — The phylogenetic position of *Polysiphonia scopulorum* (Rhodomelaceae, Rhodophyta) based on molecular analyses and morphological observations of specimens from the type locality in Western Australia. *Phytotaxa* 324: 51-62. <https://doi.org/10.11646/phytotaxa.324.1.3>
- JACQUIN N. J. 1791. — *Collectanea ad botanicam, chemiam, et historiam naturalem, spectantia cum figuris. Vol. III.* Ex Officina Wappleriana, Vindobonae, 306 p.
- KEARSE M., MOIR R., WILSON A., STONES-HAVAS S., CHEUNG M., STURROCK S., BUXTON S., COOPER A., MARKOWITZ S., DURAN C., THIERER T., ASHTON B., MEINTJES P. & DRUMMOND A. 2012. — Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649. <https://doi.org/10.1093/bioinformatics/bts199>
- KIM B. & KIM M. S. 2014. — Three new species of *Polysiphonia sensu lato* (Rhodophyta) based on the morphology and molecular evidence. *Algae* 29: 183-195. <https://doi.org/10.4490/algae.2014.29.3.183>
- KIM M. S., LEE I. K. & BOO S. M. 1994. — Morphological studies of the red alga *Polysiphonia morrowii* Harvey on the Korean Coast. *Korean Journal of Phycology* 9: 185-192.
- KIM M.-S., YANG E. C., MANSILLA A. & BOO S. M. 2004. — Recent introduction of *Polysiphonia morrowii* (Ceramiales, Rhodophyta) to Punta Arenas, Chile. *Botanica Marina* 47: 389-394. <https://doi.org/10.1515/BOT.2004.053>
- KIM M. S., KIM S. Y. & NELSON W. 2010. — *Symphyclocladia lithophila* sp. nov. (Rhodomelaceae, Ceramiales), a new Korean red algal species based on morphology and *rbcL* sequences. *Botanica Marina* 53: 233-241. <https://doi.org/10.1515/BOT.2010.031>
- KÜTZING F. T. 1843. — *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange. Mit 80 farbig gedruckten Tafeln, gezeichnet und gravirt vom Verfasser.* F. A. Brockhaus, Leipzig, 143-458 p.
- KÜTZING F. T. 1849. — *Species algarum.* F. A. Brockhaus, Leipzig, 922 p.
- KÜTZING F. T. 1864. — *Tabulae phycologicae; oder, Abbildungen der Tange. Vol. XIV.* Gedruckt auf kosten des Verfassers (in commission bei W. Köhne), Nordhausen, 35 p.
- LAURET M. 1970. — Morphologie, phenologie, répartition des *Polysiphonia* marins du littoral languedocien. II. Section *Polysiphonia*. *Naturalia Monspeliensia, Botanique* 21: 121-163.
- LELIAERT F., BOEDEKER C., PEÑA V., BUNKER F., VERBRUGGEN H. & DE CLERCK O. 2009. — *Cladophora rhodolithicola* sp. nov. (Cladophorales, Chlorophyta), a diminutive species from European maerl beds. *European Journal of Phycology* 44: 155-169. <https://doi.org/10.1080/09670260802573113>
- LIN S.-M., FREDERICQ S. & HOMMERSAND M. H. 2001. — Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbcL* sequences, including the phycodryoidae, subfam. nov. *Journal of Phycology* 37: 881-899. <https://doi.org/10.1046/j.1529-8817.2001.01012.x>
- MAGGS C. A. & HOMMERSAND M. H. 1993. — *Seaweeds of the British Isles. Volume 1 Rhodophyta. Part 3A Ceramiales.* The Natural History Museum, London, 464 p.
- MAMOOZADEH N. R. & FRESHWATER D. W. 2011. — Taxonomic notes on Caribbean *Neosiphonia* and *Polysiphonia* (Ceramiales, Florideophyceae): five species from Florida, USA and Mexico. *Botanica Marina* 54: 269-292. <https://doi.org/10.1515/bot.2011.036>
- MCIVOR L., MAGGS C. A., PROVAN J. & STANHOPE M. J. 2001. — *rbcL* sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Molecular Ecology* 10: 911-919. <https://doi.org/10.1046/j.1365-294X.2001.01240.x>
- MINEUR F., BELSHER T., JOHNSON M. P., MAGGS C. A. & VERLAQUE M. 2007. — Experimental assessment of oyster transfers as a vector for macroalgal introductions. *Biological Conservation* 137: 237-247. <https://doi.org/10.1016/j.biocon.2007.02.001>
- MINEUR F., JOHNSON M. P. & MAGGS C. A. 2008. — Macroalgal introductions by hull fouling on recreational vessels: seaweeds and sailors. *Environmental Management* 42: 667-676. <https://doi.org/10.1007/s00267-008-9185-4>
- NEWTON L. 1931. — *A Handbook of the British Seaweeds.* The Trustees of the British Museum, British Museum (Natural History), London, 478 p.
- ÖZTIG F. 1959. — Étude comparée de la structure morphologique et anatomique de *Boergesenella fruticulosa* (Wulf.) Kylin de la Méditerranée et de l'Océan Atlantique. *Vie Milieu* 10: 280-295.
- PEZZOLESI L., PEÑA V., LE GALL L., GABRIELSON P. W., KALEB S., HUGHEY J. R., RODONDI G., HERNANDEZ-KANTUN, J. J., FALACE A., BASSO D., CERRANO C. & RINDI F. 2019. — Mediterranean *Lithophyllum stictiforme* is a genetically diverse species complex x: implications for species circumscription, biogeography and conservation of coralligenous habitats. *Journal of Phycology* 55: 473-492. <https://doi.org/10.1111/jpy.12837>
- PIÑEIRO-CORBEIRA C., VERBRUGGEN H. & DÍAZ-TAPIA P. 2019. — Molecular survey of the red algal family Rhodomelaceae (Ceramiales, Rhodophyta) in Australia reveals new introduced species. *Journal of Applied Phycology*. <https://doi.org/10.1007/s10811-019-01932-4>

- RAFFO M. P., GEOFFROY A., DESTOMBE C. & SCHWINDT E. 2014. — First record of the invasive red alga *Polysiphonia morrowii* Harvey (Rhodomelaceae, Rhodophyta) on the Patagonian shores of the Southwestern Atlantic. *Botanica Marina* 57: 21-26.
- RINDI F. & CINELLI F. 2000. — Phenology and small-scale distribution of some rhodomelacean red algae on a western Mediterranean rocky shore. *European Journal of Phycology* 35: 115-125. <https://doi.org/10.1080/09670260010001735701>
- ROJAS-GONZÁLEZ B. & AFONSO-CARRILLO J. 2008. — Morfología y distribución de las especies de *Polysiphonia* en las Islas Canarias. 3. *Polysiphonia ceramiaeformis*, *P. denudata*, *P. furcellata* y *P. tepida* (Rhodophyta, Rhodomelaceae). *Vieraea* 36: 55-71.
- ROTH A. G. 1806. — *Catalecta botanica quibus plantae novae et minus cognitae describuntur atque illustrantur. Fasciculus tertius cum tabulis aenaeis XII*. Bibliopolio Io. Fr. Gleditschiano, Lipsiae, 350 p.
- SALOMAKI E. D., NICKLES K. R. & LANE C. E. 2015. — The ghost plastid of *Choreocolax polysiphoniae*. *Journal of Phycology* 51: 217-221. <https://doi.org/10.1111/jpy.12283>
- SAUNDERS G. W. 2005. — Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 360: 1879-1888. <https://doi.org/10.1098/rstb.2005.1719>
- SAUNDERS G. W. & MOORE T. E. 2013. — Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae* 28: 31-43. <https://doi.org/10.4490/algae.2013.28.1.031>
- SAUNDERS G. W., HUISMAN J. M., VERGÉS A., KRAFT G. T. & LE GALL L. 2017. — Phylogenetic analyses support recognition of ten new genera, ten new species and 16 new combinations in the family Kallymeniaceae (Gigartinales, Rhodophyta). *Cryptogamie, Algologie* 38: 79-132. <https://doi.org/10.7872/crya/v38.iss2.2017.79>
- SAVOIE A. M. & SAUNDERS G. W. 2016. — A molecular phylogenetic and DNA barcode assessment of the tribe Pterosiphonieae (Ceramiales, Rhodophyta) emphasizing the Northeast Pacific. *Botany* 94: 917-939. <https://doi.org/10.1139/cjb-2016-0083>
- SAVOIE A. M. & SAUNDERS G. W. 2019. — A molecular assessment of species diversity and generic boundaries in the red algal tribes Polysiphonieae and Strebloladiaceae (Rhodomelaceae, Rhodophyta) in Canada. *European Journal of Phycology* 54: 1-25. <https://doi.org/10.1080/09670262.2018.1483531>
- STACKHOUSE J. 1795. — *Nereis britannica; Continens Species Omnes fucorum in Insulis Britannicis Crescentium: Descriptione Latine Et Anglico, Necnon Iconibus Ad Vivum Depictis... Fasc. 1*, Hazard S. & White J., Bath & London, 30 p.
- STACKHOUSE J. 1797. — *Nereis britannica; Continens Species Omnes fucorum in Insulis Britannicis Crescentium: Descriptione Latine Et Anglico, Necnon Iconibus Ad Vivum Depictis... Fasc. 2*, Hazard S. & White J., Bath & London, 31-70 p.
- STACKHOUSE J. 1802. — *Nereis britannica; Continens Species Omnes fucorum in Insulis Britannicis Crescentium: Descriptione Latine Et Anglico, Necnon Iconibus Ad Vivum Depictis... Fasc. 3*, Hazard S. & White J., Bath & London, 71-112 p.
- STAMATAKIS A. 2014. — RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313. <https://doi.org/10.1093/bioinformatics/btu033>
- STUERCKE B. & FRESHWATER D. W. 2008. — Consistency of morphological characters used to delimit *Polysiphonia sensu lato* species (Ceramiales, Florideophyceae): analyses of North Carolina, USA specimens. *Phycologia* 47: 541-559. <https://doi.org/10.2216/08-09.1>
- STUERCKE B. & FRESHWATER D. W. 2010. — Two new species of *Polysiphonia* (Ceramiales, Florideophyceae) from the western Atlantic. *Botanica Marina* 53: 301-311. <https://doi.org/10.1515/BOT.2010.036>
- VAN DEN HOEK C. & BREEMAN A. M. 1990. — Seaweed biogeography of the North Atlantic: where are we now?, in GARBARY D. J. & SOUTH G. R. (eds), *Evolutionary Biogeography of the Marine Algae of the North Atlantic*. Springer-Verlag, Berlin Heidelberg: 55-86. <https://doi.org/10.1007/978-3-642-75115-8>
- VIERA-RODRÍGUEZ M. A., AUDIFFRED P. A. J., GIL-RODRÍGUEZ M. C., PRUD'HOMME VAN REINE W. F. & AFONSO-CARRILLO J. 1987. — Adiciones al catálogo de algas marinas bentónicas para el Archipiélago Canario. III. *Vieraea* 17: 227-35.
- WALKER R. H., BRODIE J., RUSSELL S., IRVINE L. M. & ORFANIDIS S. 2009. — Biodiversity of coralline algae in the northeastern Atlantic including *Corallina caespitosa* sp. nov. (Corallinoideae, Rhodophyta). *Journal of Phycology* 45: 287-297. <https://doi.org/10.1111/j.1529-8817.2008.00637.x>
- WOLF M. A., BUOSI A. & SFRISO A. 2019. — First record of *Acanthosiphonia echinata* (Rhodomelaceae, Rhodophyta) in the Mediterranean Sea, molecular and morphological characterization. *Botanica Marina* in press. <https://doi.org/10.1515/bot-2019-0072>
- YANG E., BOO S., BHATTACHARYA D., SAUNDERS G., KNOLL A. H., FREDERICQ S. GRAF L. & YOON H. S. 2016. — Divergence time estimates and the evolution of major lineages in the florideophyte red algae. *Scientific Reports* 6: 21361. <https://doi.org/10.1038/srep21361>

Submitted on 16 December 2019;
accepted on 23 April 2020;
published on 5 June 2020.

ANNEXE 1. — GenBank accession numbers and collection information of the sequences used in phylogenetic analyses. Collectors: **CAM**, Christine Maggs; **CP**, Cristina Piñeiro-Corbeira; **FB**, Francis Bunker; **FR**, Fabio Rindi; **IB**, Ignacio Bárbara; **LB**, Lin Baldock; **PD**, Pilar Díaz-Tapia.

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number	
				<i>rbcL</i>	<i>cox1</i>
<i>Bryocladia cuspidata</i> (J.Agardh) De Toni	–	Lin <i>et al.</i> 2001	1435	AF259498	
<i>Carradoriella virgata</i> (C. Agardh) Kylin	CH1671	Cape Province, South Africa; Bárbara <i>et al.</i> 2013	1467	JX828152	
<i>Carradoriella denudata</i> (Dillwyn) A.M.Savoie & G.W.Saunders	CH1954	Pontevedra, Spain; Bárbara <i>et al.</i> 2013 (as <i>Polysiphonia</i>)	1467	JX828143	
<i>Epizonaria prostrata</i> (Harvey) Díaz- Tapia & Maggs	PD1549	Western Australia; Díaz-Tapia <i>et al.</i> 2017a	1279	MF094087	
<i>Eutrichosiphonia sabulosia</i> (B.Kim & M.S.Kim) A.M.Savoie & G.W.Saunders	12sp1-01	Jeju, Korea; Kim & Kim 2014 (as <i>Polysiphonia</i>)	1250	KF479248	
<i>Kapraunia morroides</i> (B.Kim & M.S.Kim) A.M.Savoie & G.W.Saunders	12sp5-02	Jeju, Korea; Kim & Kim 2014 (as <i>Polysiphonia</i>)	1250	KF479255	
<i>Kapraunia schneideri</i> (Stuercke & Freshwater) A.M.Savoie & G.W.Saunders	PHYKOS-2454	Colón, Panama; Mamoozadeh & Freshwater 2011 (as <i>Polysiphonia</i>)	1346	HM573566	
<i>Lampisiphonia iberica</i> Bárbara, Secilla, Díaz & H.-G.Choi	CH1533	A Coruña, Spain; Bárbara <i>et al.</i> 2013	1467	JX828131	
<i>Leptosiphonia brodiei</i> (Dillwyn) Sprengel	PD516	Doaghbeg, Ireland; Díaz-Tapia <i>et al.</i> 2017a (as <i>Polysiphonia</i>)	1467	MF101425	
<i>Leptosiphonia schousboei</i> (Thuret) Kylin	CH826	Lugo, Spain; Bárbara <i>et al.</i> 2013	1467	JX828133	
<i>Lophosiphonia obscura</i> (C.Agardh) Falkenberg	CUK11188	Adelaide, Australia; Bustamante <i>et al.</i> 2017	1435	KT825865	
<i>Lophosiphonia simplicissima</i> Díaz- Tapia	SANT-A.24157	Asturias, Spain; Díaz-Tapia <i>et al.</i> 2017a	1448	MF094088	
<i>Lophosiphonia teges</i> (Womersley) Díaz-Tapia & Maggs	PD1823	Western Australia; Díaz-Tapia <i>et al.</i> 2017a	1296	MF094089	
<i>Melanothamnus collabens</i> (C.Agardh) Díaz-Tapia & Maggs	CH2526	Galicia, Spain; Bárbara <i>et al.</i> 2013 (as <i>Streblocladia</i>)	1467	JX828157	
<i>Melanothamnus japonicus</i> (Harvey) Díaz-Tapia & Maggs	CH2530	Galicia, Spain; Bárbara <i>et al.</i> 2013 (as <i>Neosiphonia</i>)	1467	JX828135	
<i>Melanothamnus peruviansis</i> (D.E.Bustamante, B.Y.Won, M.E.Ramirez & T.O.Cho) Díaz- Tapia & Maggs	TC6474	Peru; Bustamante <i>et al.</i> 2012 (as <i>Neosiphonia</i>)	1442	JN989968	
<i>Melanothamnus somalensis</i> Bornet & Falkenberg	2774	Oman; Díaz-Tapia <i>et al.</i> 2017b	1364	KX499555	
<i>Melanothamnus unilateralis</i> (Levring) Díaz-Tapia & Maggs	JF0192	Juan Fernández, Chile; Díaz-Tapia <i>et al.</i> 2017b	1282	KX499549	
<i>Polysiphonia adamsiae</i> Womersley	PD3590	Tasmania, Australia; Díaz-Tapia <i>et al.</i> 2018a	1349	MH101821	
<i>Polysiphonia scopulorum</i> Harvey	15Psc101	Western Australia; Huisman <i>et al.</i> 2017	1250	MF139308	
<i>Polysiphonia atlantica</i> Kapraun & J.N. Norris	CH1285	Lugo, Spain; Bárbara <i>et al.</i> 2013	1467	JX828142	
<i>Polysiphonia delicata</i> Díaz-Tapia	SANT31245	A Coruña, Spain; Díaz-Tapia <i>et al.</i> 2017c	795	KY620059	
	SANT31087	A Coruña, Spain; Díaz-Tapia <i>et al.</i> 2017c	1390	KY620062	
	SANT31247	Victoria, Australia; Díaz-Tapia <i>et al.</i> 2017c	1301	KY620060	
	SANT31248	Victoria, Australia; Díaz-Tapia <i>et al.</i> 2017c	1279	KY620061	
	PD3069	Tasmania, Australia; Piñeiro-Corbeira <i>et al.</i> 2019	1355	MN419248	
	PD3165	Tasmania, Australia; Piñeiro-Corbeira <i>et al.</i> 2019	1315	MN419247	
	PD3169	Tasmania, Australia; Piñeiro-Corbeira <i>et al.</i> 2019	855	MN419249	
	PD3181	Tasmania, Australia; Piñeiro-Corbeira <i>et al.</i> 2019	1316	MN419246	
	PD3336	Tasmania, Australia; Piñeiro-Corbeira <i>et al.</i> 2019	1326	MN419250	
	PD3514	Tasmania, Australia; Piñeiro-Corbeira <i>et al.</i> 2019	979	MN419244	
PD3519	Tasmania, Australia; Piñeiro-Corbeira <i>et al.</i> 2019	1319	MN419245		
PD3005	Studdland, England; drift; 5.V.2017; CAM	1304	MN867914		

ANNEXE 1. — Continuation.

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number	
				<i>rbcL</i>	<i>cox1</i>
<i>Polysiphonia devoniensis</i> Maggs & Hommersand	PD301	Wales; Díaz-Tapia <i>et al.</i> 2018b	1348	MG975690	
<i>Polysiphonia dokdoensis</i> D.E.Bustamante, B.Y.Won & T.O.Cho	TC9524	Dokdo Island, Korea; Bustamante <i>et al.</i> 2014a	1433	KJ407267	
<i>Polysiphonia donghaeya</i> B.Kim & M.S.Kim	CUK9556-H1	Korea; Bustamante <i>et al.</i> 2015 (as <i>P. koreana</i>)	1395	KJ957811	
<i>Polysiphonia macrocarpa</i> (C.Agardh) Sprengel	PHYKOS2627	Colón, Panama; Mamoozadeh & Freshwater 2011	1333	HM573545	
<i>Polysiphonia morrowii</i> Harvey	–	Korea; Kim <i>et al.</i> 2004	1379	AY396031	
	P1010	Korea; Geoffroy <i>et al.</i> 2016	1225	KP729450	
	CUK5035	Korea; Bustamante <i>et al.</i> 2015	1395	KJ957815	
	P1344	Korea; Geoffroy <i>et al.</i> 2016	1225	KP729457	
	AC229	Korea; Carlile 2009	1467	GQ252569	
	–	Korea; Kim <i>et al.</i> 2004	1379	AY396034	
	–	Korea; Kim <i>et al.</i> 2004	1379	AY396032	
	–	Korea; Kim <i>et al.</i> 2004	1379	AY396033	
	P1349	Korea; Geoffroy <i>et al.</i> 2016	1225	KP729456	
	mo004	Korea; Kim & Kim 2014	1250	KF479258	
	–	Korea; Kim <i>et al.</i> 2004	1379	AY396027	
	P48	Korea; Kim <i>et al.</i> 2004	1379	AY958161	
	–	Korea; Kim <i>et al.</i> 2004	1379	AY396030	
	P1300	Japan; Geoffroy <i>et al.</i> 2016	1225	KP729448	
	GWS008179	British Columbia; Savoie & Saunders 2019	1305	MF120896	
	SS0023	New Zealand; D'Archino <i>et al.</i> 2013	1387	KC152488	
	NZ04.5	New Zealand; Mamoozadeh & Freshwater 2011	1294	HM573583	
	NZ04.130	New Zealand; Mamoozadeh & Freshwater 2011	1373	HM573579	
	PD3068	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1305	MN419258	
	PD3073	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1012	MN419259	
	PD3074	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1301	MN419257	
	PD3076	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1311	MN419256	
	PD3094	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1313	MN419255	
	PD3095	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1315	MN419254	
	PD3135	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1318	MN419260	
	PD3136	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1317	MN419251	
	PD3176	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1314	MN419253	
	PD3522	Tasmania; Piñeiro-Corbeira <i>et al.</i> 2019	1295	MN419252	
	–	Chile; Kim <i>et al.</i> 2004	1379	AY396028	
	–	Chile; Kim <i>et al.</i> 2004	1379	AY396029	
	P762	Argentina; Raffo <i>et al.</i> 2014	1225	KF468807	
	P625	Argentina; Raffo <i>et al.</i> 2014	1225	KF468806	
	P764	Argentina; Raffo <i>et al.</i> 2014	1225	KP729452	
	P750	Argentina; Geoffroy <i>et al.</i> 2016	1225	KP729453	
	P727	Argentina; Geoffroy <i>et al.</i> 2016	1225	KP729454	
	P753	Argentina; Geoffroy <i>et al.</i> 2016	1225	KP729455	
	P754	Argentina; Raffo <i>et al.</i> 2014	1225	KF468805	
P720	Argentina; Raffo <i>et al.</i> 2014	1225	KF468804		
P619	Argentina; Raffo <i>et al.</i> 2014	1225	KF468803		
P406	France; Geoffroy <i>et al.</i> 2012	1225	JQ679026		
P329	France; Geoffroy <i>et al.</i> 2012	1225	JN097796		
P633	France; Geoffroy <i>et al.</i> 2016	1225	KP729451		
P660	France; Geoffroy <i>et al.</i> 2016	1225	KP729449		
P413	France; Geoffroy <i>et al.</i> 2012	1225	JF706223		
P322	France; Geoffroy <i>et al.</i> 2012	1225	JF706220		
P513	France; Geoffroy <i>et al.</i> 2012	1225	JF706226		
P459	France; Geoffroy <i>et al.</i> 2012	1225	JF706225		
P446	France; Geoffroy <i>et al.</i> 2012	1225	JF706224		
P407	France; Geoffroy <i>et al.</i> 2012	1225	JF706222		
P361	France; Geoffroy <i>et al.</i> 2012	1225	JF706221		
P317	France; Geoffroy <i>et al.</i> 2012	1225	JF706219		
PD3008	Plymouth, England; 25.IV.2017; on natural substrata in marina; CAM	1330	MN867912		
PD4472	Longis Bay, Alderney, Channel Islands; mid-shore pools; 30.V.2018; CAM	1359	MN867913		

ANNEXE 1. — Continuation.

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number	
				<i>rbcl</i>	<i>cox1</i>
<i>Polysiphonia pacifica</i> Hollenberg	P194	Seal Rock, Oregon, USA; Kim <i>et al.</i> 2004	1401	AY958162	
<i>Polysiphonia radiata</i> Díaz-Tapia	SANT-A.31131	Dique de Abrigo, A Coruña, Spain; Díaz-Tapia <i>et al.</i> 2017c	1391	KY620084	
<i>Polysiphonia sertularioides</i> (Grateloup) J.Agardh	PD001	Cabo Huertas, Alicante, Spain; Díaz-Tapia <i>et al.</i> 2017a	1467	MF101423	
<i>Polysiphonia stricta</i> (Dillwyn) Greville	PD550	Fanad Head, Donegal, Ireland; Díaz-Tapia <i>et al.</i> 2017a	1467	MF101428	
<i>Polysiphonia ulleungensis</i> D.E.Bustamante, B.Y.Won & T.O.Cho	TC9483	Ulleung-gun (Ulleung Island), Gyeongsangbuk-do, Korea; Bustamante <i>et al.</i> 2014b	1438	KJ028026	
<i>Pterosiphonia cloiophylla</i> (C.Agardh) Falkenberg	GWS036443	Beacon Island, Western Cape, South Africa; Savoie & Saunders 2016	1363	KU564509	
<i>Pterosiphonia complanata</i> (Clemente) Falkenberg	P732	Beganfry, France; Kim <i>et al.</i> 2010	1154	GQ867079	
<i>Pterosiphonia stegengae</i> Savoie & G.W.Saunders	GWS036449	Eastern Cape, South Africa; Savoie & Saunders 2016	1363	KU564497	
<i>Vertebrata australis</i> (C.Agardh) Kuntze	PD931	Victoria, Australia; Díaz-Tapia <i>et al.</i> 2017a	1467	MF101439	
<i>Vertebrata byssoides</i> (Goodenough & Woodward) Kuntze	–	Norway; Yang <i>et al.</i> 2016 (as <i>Brongniartella</i>)	1435	DQ787584	
<i>Vertebrata constricta</i> (Womersley) Díaz-Tapia & Maggs	NZ04.256	New Zealand; Stuercke & Freshwater 2010 (as <i>Polysiphonia</i>)	1353	GU385832	
<i>Vertebrata ericoides</i> (Harvey) Kuntze	GWS16590	Tasmania; Savoie & Saunders 2019	1363	MF120855	
<i>Vertebrata lanosa</i> (Linnaeus) T.Christensen	–	Beavertail State Park, Jamestown, Rhode Island, United States; Salomaki <i>et al.</i> 2015	1467	NC026523	
<i>Vertebrata isogona</i> (Harvey) Díaz- Tapia & Maggs	PD831	Frankston, Dave's Bay, Port Phillip Bay, Victoria, Australia; Díaz-Tapia <i>et al.</i> 2017a	1467	MF101433	
<i>Vertebrata foetidissima</i> (Cocks ex Bornet) Díaz-Tapia & Maggs	SANT25470	Portugal; Díaz-Tapia <i>et al.</i> 2013a (as <i>Polysiphonia</i>)	1241	JQ653293	
<i>Vertebrata fruticulosa</i> (Wulfen) Kuntze	PD2020	Ancona, Italy; intertidal; 8.V.2013; FR	756	MN867924	
	SANT25830	Anse de Malmousqui, French Mediterranean; intertidal; 9.V.2011; IB	706	MN867925	
<i>Vertebrata fucooides</i> (Hudson) Kuntze	CH1359	Spain; Bárbara <i>et al.</i> 2013 (as <i>Polysiphonia</i>)	1467	JX828146	
<i>Vertebrata furcellata</i> (C.Agardh) Kuntze	LMI469	Wales; Díaz-Tapia <i>et al.</i> 2017b	1245	KX499559	
<i>Vertebrata hendryi</i> (N.L.Gardner) A.M.Savoie & G.W.Saunders	GWS6441	British Columbia, Canadá; Savoie & Saunders 2019	1363	MF120848	
<i>Vertebrata hypnoides</i> (Welwitsch) Kuntze	SANT24410	Atlantic Spain; Díaz-Tapia <i>et al.</i> 2017b	1446	KX499547	
<i>Vertebrata lobophoralis</i> (Mamoozadeh & Freshwater) Freshwater	PHYKOS3537	Panama; Mamoozadeh & Freshwater 2011 (as <i>Polysiphonia</i>)	1340	HM573551	
<i>Vertebrata martensiana</i> (Suhr) Piñeiro- Corbeira, Maggs & Díaz-Tapia	CH049	Ireland; Bárbara <i>et al.</i> 2013 (as <i>Boergesenella thuyoides</i>)	1467	JX828161	
	PD556	Portaferry, Northern Ireland; intertidal; 19.VI.2014; PD & CAM	784	MN867935	
	LMI445	Doaghbeg, Donegal, Ireland; intertidal; 31.V.1999; CAM	1254	MN867932	
	PD571	Carna, Galway, Ireland; intertidal; 29.VI.2014; PD & CAM	725	MN867930	
	UA0268	Roscoff, France; García-Soto & López- Bautista 2018	554	MH388576	
	PD4870	Ártabra, A Coruña, Spain; intertidal; 05.VII.2019; PD	1349	MN867938	
	PD4872	Ártabra, A Coruña, Spain; intertidal; 05.VII.2019; PD	1007	MN867931	
	PD3064	Sálvora, A Coruña, Spain; intertidal; 06.VIII.2017; CP	1356	MN867937	
	PD4451	A Cova, Muros, A Coruña, Spain; intertidal; 16.VI.18; CP	907	MN867939	
	PD3041	Cies, Pontevedra, Spain; intertidal; 20.VII.2017; PD	1354	MN867934	
	PD3042	Cies, Pontevedra, Spain; intertidal; 20.VII.2017; PD	1353	MN867933	
	PD3038	Cies, Pontevedra, Spain; intertidal; 20.VII.2017; PD	1351	MN867936	

ANNEXE 1. — Continuation.

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number	
				<i>rbcL</i>	<i>cox1</i>
	PD4949	Ancona, Italy; shallow subtidal, epiphytic on <i>Treptacantha barbata</i> ; 18.IX.2019; FR	1234	MN867926	
	PD4950	Ancona, Italy; shallow subtidal, epiphytic on <i>Treptacantha barbata</i> ; 18.IX.2019; FR	1164	MN867927	
	PD4951	Ancona, Italy; shallow subtidal, epiphytic on <i>Treptacantha barbata</i> ; 18.IX.2019; FR	1303	MN867928	
	PD4952	Ancona, Italy; shallow subtidal, epiphytic on <i>Treptacantha barbata</i> ; 18.IX.2019; FR	1349	MN867929	
<i>Vertebrata nigra</i> (Hudson) Díaz-Tapia & Maggs	CH048	Ireland; Bárbara <i>et al.</i> 2013 (as <i>Polysiphonia</i>)	1463	JX828164	
<i>Vertebrata "paniculata"</i> (J.Agardh) Kuntze	–	Chile, Kim <i>et al.</i> 2004 (as <i>Polysiphonia</i>)	1379	AY396040	
<i>Vertebrata reptabunda</i> (Suhr) Díaz-Tapia & Maggs	SANT25139	Atlantic Spain; Díaz-Tapia <i>et al.</i> 2017b	1448	KX499554	
<i>Vertebrata simulans</i> (Harvey) Kuntze	PD3006	The Fleet, Dorset, England; intertidal; <i>V. simulans</i> morphology; 10.V.2017; CAM	1305	MN867920	MT125667
	PD2112	Kimmeridge, England; intertidal; <i>V. simulans</i> morphology; 6.VI.2015; PD & CAM	734	MN867921	MT125665
	PD3031	Ireland; Díaz-Tapia <i>et al.</i> 2017b (as <i>V. simulans</i>); <i>P. ceramiiformis</i> morphology.	1307	MN867919	
	PD2192	Wembury Point, Devon, England; intertidal; <i>P. ceramiiformis</i> morphology; 23.III.2016; PD, CAM & FB	1281	KX499568	MT125666
	C49	Chapman's Pool, Dorset, England; intertidal; <i>P. ceramiiformis</i> morphology; 2.V.2004; LB	553	MN867923	
	SANT28756	Margaritas, A Coruña, Spain; intertidal; <i>P. ceramiiformis</i> morphology; 4.XII.2013; PD	807	MN867922	MT125664
<i>Vertebrata subulifera</i> (C.Agardh) Kuntze	LMI1017	Ireland; Díaz-Tapia <i>et al.</i> 2017b	1245	KX499564	
<i>Vertebrata stimpsonii</i> (Harvey) Kuntze	CH073	Japan; Bárbara <i>et al.</i> 2013 (as <i>Enelittosiphonia</i>)	975	JX828126	
<i>Vertebrata thuyoides</i> (Harvey) Kuntze	PD546	Fanad Head, Ireland; Díaz-Tapia <i>et al.</i> 2017a	1467	MF101426	
<i>Vertebrata tripinnata</i> (Harvey) Kuntze	PD0584	Island Reagh, Carrigart, Donegal, Ireland; intertidal; 13.VII.2014; PD & CAM	–	–	
	PD3032A	Island Reagh, Carrigart, Donegal, Ireland; intertidal; 10.VII.2017; CAM	1296	MN867916	
	PD3032B	Island Reagh, Carrigart, Donegal, Ireland; intertidal; 10.VII.2017; CAM	1304	MN867917	
	SANT22246	Atlantic Spain; Díaz-Tapia <i>et al.</i> 2017b (as <i>Polysiphonia</i>)	613	KX499566	
	SANT24256	Baleal, Portugal; 13.VI.2010; intertidal; PD & IB	608	MN867918	
	PD2633	Seté, French Mediterranean; subtidal (–1 m); 11.VI.2016; PD	711	MN867915	
<i>Vertebrata woodii</i> (Harvey) Kuntze	GWS22313	California, United States; Savoie & Saunders 2016 (as <i>Pterochondria</i>)	1363	KU564507	