

1 This is an Accepted Manuscript of an article published by Taylor & Francis in the *European*
2 *journal of Phycology* on 18th February 2021, available at:
3 <https://doi.org/10.1080/09670262.2021.1871969>

**Phylogenetic analyses of Macaronesian turf-forming species reveal cryptic
diversity and resolve *Stichothamnion* in the *Vertebrata* clade (Rhodomelaceae,
Rhodophyta)**

Pilar Díaz-Tapia^{a,b}, Laura Muñoz-Luque^a, Cristina Piñeiro-Corbeira^a and Christine A.
Maggs^c

^a BioCost Research Group, Facultade de Ciencias, Universidade da Coruña, Campus da
Zapateira s/n, 15071, A Coruña, Spain

^b Instituto Español de Oceanografía (IEO), Centro Oceanográfico de A Coruña, Aptdo.
130, 15080, A Coruña, Spain

^c Portaferry Marine Laboratory, Queen's University Belfast, Belfast BT22 1PF,
Northern Ireland

CONTACT: Pilar Díaz-Tapia (e-mail: pdiaz@udc.es)

ABSTRACT

Algal turfs are ecosystem engineers receiving steadily growing attention in recent years in relation to their expansion on temperate reefs due to global change. However, their species diversity and taxonomy are still poorly understood, mostly based solely on morphological information. Algal turfs are the dominant type of assemblage in Macaronesia, but molecular diversity surveys have barely been applied in this region. We aim to show how molecular tools can assist us to improve our understanding of the diversity and taxonomy of natural algal turfs by studying the turf-forming rhodomelacean genus *Stichothamnion* which has only one recognized species in Macaronesia, the generitype *S. cymatophilum*. *Stichothamnion* resembles other members of the tribe Strebloladiaceae, but it has unbranched trichoblasts, an unusual character in the family. Here, we show using phylogenetic analyses of the *rbcL* gene that specimens collected in the Canary Islands and the Azores identifiable morphologically as *S. cymatophilum* exhibit cryptic diversity, and these analyses position *Stichothamnion* in the *Vertebrata* clade with full support. We place *Stichothamnion* in synonymy with *Vertebrata*, transfer the existing species, and describe a newly discovered cryptic species as *V. barbarae* Muñoz-Luque & Díaz-Tapia, sp. nov., found exclusively in the Azores. The new species was morphologically indistinguishable from *V. cymatophila* but diverged by 2.1-2.3% in the *rbcL* gene. This study illustrates the work required to better understand the extraordinary species diversity in algal turfs such as those in Macaronesia which remain taxonomically understudied.

KEYWORDS: algal turfs, Azores, Canary Islands, morphology, new combination, new species, new record, *rbcL*, taxonomy, Strebloladiaceae

Introduction

Global change is triggering profound changes in shallow benthic ecosystems in temperate areas worldwide – kelp forests and canopy algae are being replaced by algal turfs (Filbee-Dexter & Wernberg, 2018; O’Brien & Scheibling, 2018; Filbee-Dexter *et al.*, 2020). Whereas brown algae form kelp and large canopies, turfs are composed of small, often filamentous, densely entangled red, brown and green algae (Connell *et al.*, 2014). Observed distribution shifts on temperate coasts have led to the current view that temperate algal turfs are a consequence of the degradation of reef assemblages (Connell *et al.*, 2008; Filbee-Dexter & Wernberg, 2018; O’Brien & Scheibling, 2018; Filbee-Dexter *et al.*, 2020). However, in temperate regions, algal turfs are dominant in habitats subject to a wide variety of natural stresses such as desiccation, wave-exposure or high sediment loads including sand scour and burial (Littler, 1980; Stewart, 1982; Akioka *et al.*, 1999; Kelaher *et al.*, 2001; Mei & Schiel, 2007; Díaz-Tapia *et al.*, 2013a, b). Despite the significance of algal turfs as ecosystem engineers, their species diversity is still poorly understood, mostly characterized by morpho-anatomical identifications without molecular studies.

Molecular data have been successfully used in seaweed diversity surveys for the last 20 years leading to the discovery of cryptic diversity (Zuccarello & West, 2003; Saunders & Lehmkuhl, 2005; Jesus *et al.*, 2019). This approach has rarely been applied to turf-forming species, even when they are good candidates for cryptic diversity. Morphological identifications are challenging because the small thalli are easily overlooked and numerous species have convergent habits, prostrate systems bearing erect axes (Price & Scott, 1992; Díaz-Tapia & Bárbara, 2013, 2014). Consequently, many species can only be distinguished, if at all, by detailed microscopic study of anatomical characters. Molecular tools are therefore essential for assessing species

diversity. The few recent molecular studies that included algal turfs have led to the discovery of new regional records and cryptic or pseudo-cryptic diversity (Díaz-Tapia & Bárbara, 2013; Savoie & Saunders, 2016; Bustamante *et al.*, 2017; Freshwater *et al.*, 2017; Piñeiro-Corbeira *et al.*, 2020a, b; Díaz-Tapia *et al.*, 2020a, b).

Macaronesia comprises a group of Atlantic archipelagos in which algal turfs (Fig. S1) are the most common type of assemblage (Tuya & Haroun, 2006; Wallenstein *et al.*, 2009). These archipelagos host an exceptional algal diversity (949 species; Freitas *et al.*, 2019) characterized by early phycologists (e.g. Bory de Saint-Vincent, 1803; Børgesen, 1930; Schmidt, 1929) and updated in checklists or floras (e.g. Sansón, 1991; Neto, 1994; Rojas-González, 1997; Haroun *et al.*, 2002). The Rhodomelaceae is a major component of algal turfs in these archipelagos and it has been morphologically studied in detail (Rojas-González, 1997). Thus, the rhodomelacean flora of this region offers an exceptional opportunity to analyse the link between morphological and molecular identification of turf-forming species.

We selected *Stichothamnion* as an example of turf-forming algae in Macaronesia. It was described by Børgesen (1930) based on *S. cymatophilum*, a turf-forming species from the Canary Islands that has been also recorded in the Mediterranean (Sartoni, 1992). This species has endogenous branches, eight pericentral cells without cortication and one tetrasporangium per segment, characters that agree with *Vertebrata* (Díaz-Tapia *et al.* 2017b). Conversely, it is markedly dorsiventral, with erect and prostrate axes, leading Børgesen (1930) to discuss its similarities with other genera. Moreover, *S. cymatophilum* has unbranched trichoblasts, a very unusual character that contrasts with the typical dichotomously branched trichoblasts of most Rhodomelaceae, leading Børgesen (1930) to propose a new genus. A second species, *S. antillarum*, is only known from Saint Eustatius, Lesser Antilles (Vroman, 1967). The

two species share the key character of unbranched trichoblasts, but differ in the number of pericentral cells (8 in *S. cymatophilum* vs. 10-11 in *S. antillarum*) and the diameter of the axes (70 vs. 85-110 μm).

Affinities of the genus *Stichothamnion* within the Rhodomelaceae were discussed by Hommersand (1963), who placed it in the *Lophosiphonia* group, erected by Falkenberg (1901) for four dorsiventral genera not easily assignable to established tribes. The *Lophosiphonia* group is now known to include a morphologically diverse group of genera with divergent phylogenetic affinities (Díaz-Tapia *et al.*, 2017b). Some of the genera are recognized as independent tribes (*Ophidocladus*, *Pleurostichidium*) or are members of other tribes (*Lophosiphonia*), while others were placed in synonymy with the genus *Vertebrata* in the tribe Strebloladidae (*Ctenosiphonia*, some species assigned to *Lophosiphonia*) (Phillips, 2000; Díaz-Tapia *et al.*, 2017a, b; Pasella *et al.*, 2019). In the absence of molecular data for *Stichothamnion*, its phylogenetic relationships are enigmatic.

Our surveys of turf-forming species of the Rhodomelaceae in the Canary Islands and Azores in 2018 enabled us to obtain and sequence c. 400 specimens. Among them, we found six samples morphologically assignable to *Stichothamnion*. The objective of this work is to reassess the species diversity of the genus *Stichothamnion* in Macaronesia and analyse its phylogenetic relationships with other members of the Rhodomelaceae.

Materials and methods

Algal turfs were collected during sampling surveys in 2018 from Lanzarote (Canary Islands, Spain; 10 sites) and São Miguel (Azores, Portugal; 12 sites) (Fig. S2).

Rhodomelacean species of the turfs were morphologically recognized and carefully isolated using a stereomicroscope. As turf forming species in Macaronesia are very small in size (< 1 cm), each sample of each species consisted of several specimens. Part of each sample was preserved in silica gel for DNA extraction, while the remaining material was preserved for morphological study in 4% formalin seawater at 4°C and stored in the dark. For morphological observations, specimens were mounted in 20% Karo® Syrup (ACH Foods, Memphis, TN, USA) and 80% distilled water. Sections for microscopic observations were made by hand using a razor blade. Morpho-anatomical characters were studied by light microscopy. Voucher specimens were deposited in the herbarium of the University of Santiago de Compostela (SANT).

DNA was extracted from silica gel-dried material using an adapted cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1987). PCR amplification was carried out for *rbcL* using the primers F2 or F8/R1462 (Díaz-Tapia *et al.*, 2018). 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), 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 commercially by Macrogen Inc. (Madrid, Spain).

Six new *rbcL* gene sequences were generated in this study for the target group. We also included in our phylogenetic analyses 64 sequences representative of all *Vertebrata* spp. for which molecular data were available, and representative species of the major lineages in the Strebloladidae according to previous phylogenetic studies (Díaz-Tapia *et al.*, 2017a). To test the effect of species selection in the topology of the phylogenetic

145 trees, we analysed a second dataset using the same selection of sequences but excluding
146 three randomly chosen species (*V. reptabunda*, *V. fruticulosa* and *Polysiphonia*
147 *paniculata*). Sequences data and GenBank accession numbers are listed in Table S1.
148 Sequences were aligned using Muscle in Geneious 6.1.8 (Kearse *et al.*, 2012). We
149 included in our analyses a single sequence per species, except for the focal species of
150 this study, for which all haplotypes were represented. The sequences included in the
151 final alignment were selected considering their quality in terms of both length (the
152 longest sequences) and excluding those with ambiguous bases. Phylogenetic trees for
153 *rbcL* were estimated with Maximum Likelihood (ML) using RAxML 8.1.X
154 (Stamatakis, 2014). GTR-Gamma was used as the nucleotide model; branch support
155 was estimated with 1000 bootstrap replicates. Four species of the tribe Polysiphonieae
156 were selected as the outgroup based on our phylogenomic analyses of the major
157 lineages of the Rhodomelaceae which resolve this tribe as sister to the Streblocladiaceae
158 (Díaz-Tapia *et al.*, 2017a).

160 **Results**

161 ***Molecular identification and phylogeny***

162 Of the six *rbcL* sequences newly determined for specimens morphologically identified
163 as *Stichothamnion*, four were from the Azores and two from the Canary Islands. They
164 corresponded to three haplotypes that were resolved in a monophyletic, fully supported
165 clade in our phylogeny (Fig. 1). Only one haplotype was found in the Canary Islands,
166 the type locality of *S. cymatophilum*, while two haplotypes were found in the Azores.
167 One of the Azorean haplotypes was resolved as sister to the Canarian haplotype with
168 full support and divergence among them was only 0.5% (6 bp). Therefore, we assigned

the Azorean specimens of this haplotype to *S. cymatophilum*. The second haplotype found in the Azores diverged by 2.1-2.3 % (28-30 bp) from *S. cymatophilum* and we propose below a new species for these specimens.

The species morphologically assigned to *Stichothamnion* were resolved in a fully supported clade containing the species of *Vertebrata* (Fig. 1), leading us to propose placing the genus *Stichothamnion* in synonymy with *Vertebrata*. Within *Vertebrata*, relationships of the *Stichothamnion* lineage with other members of the clade were unresolved. The current tree topology positioned the *Stichothamnion* lineage as sister of other *Vertebrata* species, but this placement was unsupported (Fig. 1). Trees constructed using different species selections of the tribe Streblocladieae (e.g. Fig. S3) placed the *Stichothamnion* lineage among other *Vertebrata* spp., again without support.

According to the phylogenetic analyses, *S. cymatophilum* and a new species are respectively transferred or assigned to *Vertebrata*. In the absence of molecular data for *S. antillarum*, we propose its transfer to *Vertebrata*, as its unusual unbranched trichoblasts suggest that it is highly likely to be closely related to *S. cymatophilum* and the new species.

Vertebrata barbarae Muñoz-Luque & Díaz-Tapia, sp. nov. (Figs 3, 5, 6, 8, 9, 11, 15, 16, 19-21, 23)

DIAGNOSIS: Thalli forming turfs, decumbent, composed of an extensive system of prostrate axes that ventrally produce rhizoids and dorsally bear erect axes, up to 3 mm in length. Rhizoids unicellular, cut off from the pericentral cells. Axes ecorticate, with 8-9 pericentral cells. Erect axes 60-125 µm in diameter, pseudodichotomously branched, branches arising in the axils of trichoblasts. Trichoblasts unbranched, spirally

193 arranged on every segment or several segments apart. Spermatangial branches replacing
 194 trichoblasts. Procarps with 4-celled carpogonial branches. Cystocarps globose.
 195 Tetrasporangia forming slightly spiral series, one per segment. *RbcL* sequence of the
 196 holotype: MW246788.
 197 HOLOTYPE: SANT-Algae 33461, 14.iv.2018, leg. P. Díaz-Tapia.
 198 TYPE LOCALITY: Lagoa, São Miguel, Azores (37°44'31"N 25°34'23"W).
 199 ETYMOLOGY: Named in honour of Dr Ignacio Bárbara for his significant
 200 contributions to the knowledge of the seaweed flora of Spain and Portugal.
 201 HABITAT: Intertidal algal turfs.
 202 DISTRIBUTION: São Miguel, Azores, Portugal (Fig. S2).
 203 *Vertebrata cymatophila* (Børgesen) Muñoz-Luque & Díaz-Tapia, *comb. nov.* (Figs 2, 4,
 204 7, 10, 12-14, 17, 18, 22, 24)
 205 BASIONYM: *Stichothamnion cymatophilum* Børgesen (1930: 119, Marine algae from
 206 the Canary Islands especially from Tenerife and Gran Canaria III. Rhodophyceae. Part
 207 III. Ceramiales. *Kongelige Danske Videnskabernes Selskab, Biologiske Meddelelser* 9:
 208 1-159).
 209 TYPE: Herbarium of the University of Copenhagen (Afonso Carrillo & Sansón, 1999).
 210 TYPE LOCALITY: Las Palmas de Gran Canaria (Børgesen, 1930).
 211 HABITAT: Intertidal algal turfs. DISTRIBUTION: Canary Islands, Spain; São Miguel,
 212 Azores, Portugal (Fig. S2).
 213
 214 *Vertebrata antillarum* (Vroman) Muñoz-Luque & Díaz-Tapia, *comb. nov.*

BASIONYM: *Stichothamnion antillarum* Vroman (1967: 557, A new species of *Stichothamnion* (Rhodophyta) from the West Indies. *Acta Botanica Neerlandica* 15: 557-561).

HOLOTYPE: Herbarium of the Vrije Universiteit (Vroman, 1967).

TYPE LOCALITY: St. Eustatius, Back off Bay (Vroman, 1967).

DISTRIBUTION: Only known from its type locality.

Morphological observations of Macaronesian specimens

Morphological observations revealed that *Vertebrata cymatophila* and *V. barbarae* were indistinguishable. A description of their characters with the measurements for each species is presented in Table 1.

Vegetative morphology

Specimens forming densely interwoven turfs, mixed with other species with similar habit. Thalli decumbent, consisting of extensive prostrate systems that attach to the substratum by dorsally formed rhizoids and produce branches laterally that extend the prostrate axes (Figs 2, 3). Erect axes irregularly arranged throughout prostrate axes, pseudodichotomously branched up to five orders. Thalli pink to dark red in colour, with a fairly rigid texture.

Prostrate and erect axes with an axial cell surrounded by 8-9 pericentral cells, all similar in size; ecorticate (Figs 4, 5). Walls of the axes often thick in prostrate axes and basal parts of erect axes (Fig. 6). Prostrate axes expanding as erect axes produce

rhizoids at their basal parts (Fig. 7), also producing lateral branches at irregular intervals with apical cells that lack trichoblasts (Fig. 8). Rhizoids abundant, one or two per segment and often formed on every segment (Figs 6-9), unicellular, cut off from the pericentral cells, terminating in a rounded or digitate discoid pad (Figs 6, 9).

Erect axes with dome-shaped apical cells. Branches arising exogenously at the apices, in the axils of trichoblasts (Fig. 10), at intervals mostly of 4-8 segments (Figs 2, 3, 11); adventitious branches occasional. Trichoblasts almost always present, scarce to abundant, short to well-developed at the apices of erect axes, spirally arranged on every segment or several segments apart, unbranched and with multinucleate basal cells, deciduous and leaving conspicuous scar cells when shed (Figs 12-15).

Reproductive morphology

Spermatangial branches formed at the apices, on the second, third or fourth basal cells of fertile trichoblasts, cylindrical, with 0-2 sterile apical cells and, in *V. cymatophila*, sometimes with an apical filament (Figs 16-19).

Female reproductive structures observed only in *V. Barbarae* sp. nov. Procarps formed on modified trichoblasts at the apices of erect axes, consisting of a supporting cell, a four-celled carpogonial branch and two groups of sterile cells (Fig. 20). Cystocarps globose (Fig. 21) and carpospores clavate. Tetrasporangia subspherical, one per segment, forming slightly spiral series in apical branches (Figs 22-24).

Discussion

Cryptic diversity in the “Stichothamnion” lineage

Our molecular and morphological study of algal turfs in Azores and the Canary Islands revealed the existence of two closely related species that matched the morphological characters proposed for delineating *Stichothamnion cymatophilum* (Børgesen, 1930), the only member of the genus recognized in the northeastern Atlantic. The two species detected in our study were morphologically indistinguishable and, among the characters provided in the detailed original description of *S. cymatophilum*, it is particularly relevant that both are markedly dorsiventral, have 8-9 pericentral cells without cortication and unbranched trichoblasts. Other morphological characters in our specimens also agree with the description of *S. cymatophilum* (Børgesen, 1930), i.e. abundance of rhizoids, presence of thick prostrate axes walls, diameter of axes and reproductive structures, erect axes pseudodichotomously branched and trichoblasts growing on every segment or several segments apart. We observed a considerable variation in the anatomy of spermatangial branches in the Canary Islands specimens, as we found in a single specimen spermatangial branches with different numbers of sterile basal and apical cells. The original description of *S. cymatophilum* also shows some variability in spermatangial branch anatomy, as some of them lack or have a sterile apical cell, but there was uniformly one sterile basal cell (fig. 54b in Børgesen, 1930). The presence/absence of sterile apical cells is often considered a key character for species delineation in the Polysiphonieae and Streblocladieae as it is uniform in many species, but can vary within some species (Maggs & Hommersand, 1993; Stuercke & Freshwater, 2008; Díaz-Tapia & Bárbara, 2013; Díaz-Tapia *et al.*, 2017b). The presence of several basal sterile cells in spermatangial branches is rarer among the Streblocladieae and Polysiphonieae. Thus, our observations suggest that variability in the anatomy of spermatangial branches has no diagnostic value at the species level in the *Stichothamnion* lineage.

Only one of the two species detected in this study was found in the Canary Islands, the type locality of *S. cymatophilum* (Børgesen, 1930), and consequently we concluded that the Canarian specimens belong to this species that is reported here for the first time in the Azores. The second species, proposed here as *V. barbarae* sp. nov., was exclusively found in the Azores and can be clearly distinguished from the only other member of the genus, *S. antillarum* from the Caribbean, by the number of pericentral cells (10-11 vs. 8-9) (Vroman, 1967). *Stichothamnion cymatophilum* has also been reported from Alborán Island, in the Mediterranean (Sartoni, 1992), but whether this record actually corresponds to *V. cymatophila*, *V. barbarae*, or might represent another cryptic species cannot be ascertained in the absence of molecular data.

The two species identified in our study differed by 2.1-2.3% sequence divergence in the *rbcL* gene, evidence of the existence of cryptic diversity. Diversity surveys using molecular tools often lead to the discovery of new species that have remained hidden under a single taxon name (Savoie & Saunders, 2016, 2019; Jesus *et al.*, 2019; Díaz-Tapia *et al.*, 2020a). In most cases, detailed morphological studies reveal that newly discovered species can be morphologically distinguished and therefore represent pseudo-cryptic species (Walker *et al.*, 2009; Piñeiro-Corbeira *et al.*, 2020a). True cryptic diversity is less common in the red algae, but recognition of new species that cannot be distinguished morphologically at the resolution of light microscopy is becoming more frequent (Schneider *et al.*, 2017; Díaz-Tapia *et al.*, 2020b; Soares *et al.*, 2020).

Among our collection of 400 turf-forming red algae in the Macaronesia, only six specimens corresponded to *V. cymatophila* or *V. barbarae*, found at six sampling sites. This low collection rate shows that both species are of low abundance but must be relatively frequent in the study area. Both species were found growing intermixed with

other species and a detailed inspection of turfs under the stereomicroscope was required to detect them. This finding is unsurprising, as rare species are commonly encountered during molecular diversity surveys that analyse large specimen numbers (Pardo *et al.*, 2014; Savoie & Saunders, 2019; Díaz-Tapia *et al.*, 2020a).

Our data suggest that at least one of the species studied here is endemic to Macaronesia. The archipelagos that compose this bioregion are of recent volcanic origin and host a rich flora and fauna including a large number of endemic species in both the terrestrial and marine realms (Kim *et al.*, 2008; Freitas *et al.*, 2019). Unlike many plant and animal groups, most seaweeds reported in Macaronesia have their type localities in mainland Europe and only 17 species are considered endemic (Freitas *et al.*, 2019; Guiry & Guiry, 2020). Our study showed that more detailed seaweed diversity surveys in this bioregion using molecular tools reveal a more diverse endemic seaweed flora, especially relevant for algal turfs. A detailed study of species composition of algal turfs from the Azores based on morphological identifications found 139 species (Wallenstein *et al.*, 2019) corresponding to 34% of the 405 macroalgal species known in the Azores (Freitas *et al.*, 2019). To our knowledge no detailed reports of species composition of algal turfs in the Canary Islands are available, but this assemblage is mostly composed of members of the Ceramiales (Tuya & Haroun, 2006), which represents 38% of the 488 red algae known in this archipelago. Thus, algal turfs in Macaronesia are composed of an extraordinarily diverse set of species, and our finding of cryptic diversity in turf-forming species shows that much work is still needed to understand their diversity and taxonomy.

Phylogenetic relationships of “Stichothamnion”

334

335 The placement of *Stichothamnion* in the *Vertebrata* clade is in agreement with the
336 morphological characters proposed for the delineation of the genus *Vertebrata*,
337 including the synapomorphic character of multinucleate trichoblasts (Díaz-Tapia *et al.*,
338 2017b). Having more than five pericentral cells is another uniform character in
339 *Vertebrata* and is also observed in the *Stichothamnion* lineage (Choi *et al.*, 2001; Díaz-
340 Tapia *et al.*, 2017b). By contrast, spermatangial branches replacing trichoblasts in the
341 *Stichothamnion* lineage is unusual in *Vertebrata*, and most species form spermatangia
342 on one of the trichoblast branches (Díaz-Tapia *et al.*, 2017b). The only other known
343 exception is the morphologically unusual obligate epiphyte *V. lanosa* that lacks
344 vegetative trichoblasts (Maggs & Hommersand, 1993; Díaz-Tapia *et al.*, 2017b).
345 Trichoblasts in the *Stichothamnion* lineage are unbranched and consequently it is not
346 surprising that spermatangial branches replace them.

347 The new placement of these species formerly assigned to *Stichothamnion*
348 expands known morphological diversity in *Vertebrata*. This is the first time that
349 unbranched trichoblasts have been reported in this genus, differing from the typical
350 dichotomously branched trichoblasts in other species of *Vertebrata* and the family
351 Rhodomelaceae (Hommersand, 1963; Maggs & Hommersand, 1993; Stuercke &
352 Freshwater, 2008). Likewise, phylogenetic studies showed that five genera
353 (*Boergeseniella*, *Brongniartella*, *Ctenosiphonia*, *Enelittosiphonia*, *Pterochondria*)
354 distinguished in the past by peculiar morphological characters, including having two
355 tetrasporangia per segment, disc-like spermatangial branches or pigmented trichoblasts
356 at maturity, are actually members of *Vertebrata* (Díaz-Tapia *et al.*, 2017b; Savoie &
357 Saunders, 2019). Moreover, placement of the *Stichothamnion* lineage in *Vertebrata*
358 provides further examples in *Vertebrata* of species with clear differentiation between

erect axes and an extensive prostrate system. The only dorsiventrally branched species previously included in *Vertebrata* are *V. reptabunda* and *V. hypnoides* (Díaz-Tapia & Bárbara, 2013, as *Lophosiphonia* and *Ctenosiphonia*, respectively, Díaz-Tapia *et al.*, 2017b), while the radial branching pattern is more common (Díaz-Tapia *et al.*, 2017b). These five dorsiventral species (three of the *Stichithamnion* lineage, *V. reptabunda* and *V. hypnoides*) all form part of low-growing (< 20 mm in height) intertidal algal turfs and their dorsiventral branching pattern is probably an adaptation to this type of growth. Similarly, the habit of many other turf-forming species typically consists of prostrate and erect axes (Price & Scott, 1992; Díaz-Tapia *et al.*, 2013b; Connell *et al.*, 2014; Díaz-Tapia & Bárbara, 2013, 2014).

The synonymy of *Stichothamnion* and *Vertebrata* contributes to clarifying the phylogenetic relationships of the seven genera that have been placed at one time or another in the *Lophosiphonia* group in Falkenberg's (1901) and Hommersand's (1963) classifications of the Rhodomelaceae. At present, *Oligocladella* and *Falkenbergiella* are the only genera of this group whose affinities with other Rhodomelaceae have not yet been studied using molecular data. Both genera are characterized by having four pericentral cells, endogenous branches and unicellular rhizoids (Weber-van Bosse, 1911, 1913; as *Oligocladus*; Stegenga *et al.*, 1997). Considering the number of pericentral cells in these genera, it is very unlikely that they belong to *Vertebrata*. By contrast, these species resemble other members of the tribe Polysiphonieae, but molecular data are required to confirm their taxonomic placement.

The *Stichothamnion* lineage forms, within *Vertebrata*, a distinct clade with respect to other Atlantic clades of this genus (Fig. 1). This lineage has an amphi-Atlantic distribution including the Caribbean (*V. antillarum*), the western Mediterranean and Macaronesia (*V. barbarae*, *V. cymatophila*). Such distributions are often interpreted as

indicative of lineages that are potential remnants of the former Tethys Sea (Haroun & Prud'homme van Reine, 1993; Bauzà-Ribot *et al.*, 2012; Machín-Sánchez *et al.*, 2018). Phylogeographic analyses suggest that the opening of the Atlantic 90 Mya promoted vicariant speciation processes (Bauzà-Ribot *et al.*, 2012; Hou & Li, 2018) that might have led to the diversification of the *Stichothamnion* lineage in the eastern and western Atlantic. Alternatively, the diversification of this red algal lineage could be more recent, as the result of occasional dispersal events and subsequent speciation. Similar hypotheses have been proposed to explain the distribution of amphi-Atlantic lineages (Botello *et al.*, 2013; Hou & Li, 2018). Further studies including molecular data for *V. antillarum* are required to test these hypotheses.

Acknowledgements

We warmly thank Ana Neto for providing assistance during field work. This research was supported by computational facilities of Centro de Supercomputación de Galicia (CESGA).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Xunta de Galicia “Axudas de apoio á etapa de formación posdoutoral” (grant ED481D/2017/011) and “Talento Senior” (grant 03IN858A2019-1630129) to PDT.

Supplementary information

Fig. S1. Intertidal algal turfs from the Canary Islands (Spain).

Fig. S2. Sampling sites in São Miguel and Lanzarote. Distribution of *Vertebrata barbarae* sp. nov. and *V. cymatophila* are indicated by green and brown circles, respectively.

Fig. S3. Maximum likelihood phylogeny of the tribe Streblocladieae based on the alignment of 61 *rbcL* sequences (excluding three species of the *Vertebrata* lineage compared with Fig. 1). Lineage that consists of samples originally identified as *Stichothamnion* indicated in bold. Values at the nodes represent bootstrap support, only shown if ≥ 70 .

Table S1. GenBank accession numbers and collection information of the sequences used in phylogenetic analyses.

Author contributions

P. Díaz-Tapia: original concept, data collection, drafting and editing manuscript; L. Muñoz-Luque: data collection and curation, phylogenetic analyses; C. Piñeiro-Corbeira: conceptualization, editing manuscript; C.A. Maggs: conceptualization, editing manuscript.

References

- 428 Afonso Carrillo, J. & Sansón, M. (1999). *Algas, hongos y fanerógamas marinas de las*
 429 *Islas Canarias. Clave analítica*. Servicio de Publicaciones Universidad de la
 430 Laguna, La Laguna.
- 431 Akioka, H., Baba, M., Masaki, T. & Johansen, H.W. (1999). Rocky shore turfs
 432 dominated by *Corallina* (Corallinales Rhodophyta) in northern Japan.
 433 *Phycological Research*, **47**: 199-206.
- 434 Bauzà-Ribot, M.M., Francesco, Nardi C.J., Oromí, P., Pons, J. & Jaume, D. (2012).
 435 Mitogenomic phylogenetic analysis supports continental-scale vicariance in
 436 subterranean thalassoid crustaceans. *Current Biology*, **22**: 2069-2074.
- 437 Børgesen, F. (1930). Marine algae from the Canary Islands especially from Tenerife and
 438 Gran Canaria III. Rhodophyceae. Part III. Ceramiales. *Kongelige Danske*
 439 *Videnskabernes Selskab, Biologiske Meddelelser*, **9**: 1-159.
- 440 Bory de Saint-Vincent, J.B.G.M. (1803). *Essais sur les Isles Fortunées et l'antique*
 441 *Atlantide, ou précis de l'histoire générale de l'Archipel des Canaries*. Baudouin,
 442 Imprimeur de l'Institut National, Paris.
- 443 Botello, A., Iliffe, T. M., Alvarez, F., Juan, C., Pons, J. & Jaume, D. (2013). Historical
 444 biogeography and phylogeny of *Typhlatya* cave shrimps (Decapoda: Atyidae)
 445 based on mitochondrial and nuclear data. *Journal of Biogeography*, **40**: 594–607.
- 446 Bustamante, D.E., Won, B.Y., Miller, K.A. & Cho, T.O. (2017). *Wilsonosiphonia* gen.
 447 nov. (Rhodomelaceae, Rhodophyta) based on molecular and morpho-anatomical
 448 characters. *Journal of Phycology*, **53**: 368–380.
- 449 Choi, H.-G., Kim, M.-S., Guiry, M.D. & Saunders, G.W. (2001). Phylogenetic
 450 relationships of *Polysiphonia* (Rhodomelaceae, Rhodophyta) and its relatives

451 based on anatomical and nuclear small-subunit rDNA sequence data. *Canadian*
 452 *Journal of Botany*, **79**: 1465-1476.

453 Connell, S.D., Russell, B.D., Turner, D.J., Shepherd, S.A. Kildea, T., Miller, D.,
 454 Airoidi, L. & Cheshire, A. (2008). Recovering a lost baseline: missing kelp forests
 455 from a metropolitan coast. *Marine Ecology Progress Series*, **360**: 63-72.

456 Connell, S.D., Foster, M.S. & Airoidi, L. (2014). What are algal turfs? Towards a better
 457 description of turfs. *Marine Ecology Progress Series*, **495**: 299-307.

458 Díaz-Tapia, P. & Bárbara, I. (2013). Seaweeds from sand-covered rocks of the Atlantic
 459 Iberian Peninsula. Part 1. The Rhodomelaceae (Ceramiales, Rhodophyta).
 460 *Cryptogamie, Algologie*, **34**: 325-422.

461 Díaz-Tapia, P. & Bárbara, I. (2014). Seaweeds from sand-covered rocks of the Atlantic
 462 Iberian Peninsula. Part 2. Palmariales, Ceramiales (excluding Rhodomelaceae),
 463 Gelidiales, Gigartinales, Plocamiales, Rhodymeniales and Scytothamniales.
 464 *Cryptogamie Algologie*, **35**: 157-199.

465 Díaz-Tapia, P., Bárbara, I. & Díez, I. (2013a). Multi-scale spatial variability in intertidal
 466 benthic assemblages: differences between sand-free and sand-covered rocky
 467 habitats. *Estuarine, Coastal and Shelf Science*, **133**: 97-108,

468 Díaz-Tapia, P., Boo, S.M., Geraldino, P.J.L., Maneiro, I., Bárbara, I. & Hommersand,
 469 M.H. (2013b). Morphology and systematics of *Calliblepharis hypneoides*, sp.
 470 nov. (Cystocloniaceae, Rhodophyta) from the Atlantic Iberian Peninsula.
 471 *European Journal of Phycology*, **48**: 380-397.

472 Díaz-Tapia, P., Maggs, C.A., West, J.A. & Verbruggen, H. (2017a). Analysis of
 473 chloroplast genomes and a supermatrix inform reclassification of the
 474 Rhodomelaceae (Rhodophyta). *Journal of Phycology*, **53**: 920-937.

475 Díaz-Tapia, P., McIvor, L., Freshwater, D.W., Verbruggen, H., Wynne, M.J. & Maggs,
 476 C.A. (2017b). The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata*
 477 S.F. Gray constitute well-defined clades of the red algal tribe Polysiphonieae
 478 (Rhodomelaceae, Ceramiales). *European Journal of Phycology*, **52**: 1-20.

479 Díaz-Tapia, P., Maggs, C.A., Macaya, E.C. & Verbruggen, H. (2018). Widely
 480 distributed red algae often represent hidden introductions, complexes of cryptic
 481 species or species with strong phylogeographic structure. *Journal of Phycology*,
 482 **54**: 829-839.

483 Díaz-Tapia, P., Ly, M. & Verbruggen, H. (2020a). Extensive cryptic diversity in the
 484 widely distributed *Polysiphonia scopulorum* (Rhodomelaceae, Rhodophyta):
 485 molecular species delimitation and morphometric analyses. *Molecular*
 486 *Phylogenetics and Evolution*, **152**: 106909.

487 Díaz-Tapia, P., Maggs, C.A., Nelson, W., Macaya, E.C. & Verbruggen, H. (2020b).
 488 Reassessment of the genus *Lophurella* (Rhodomelaceae, Rhodophyta) from
 489 Australia and New Zealand reveals four cryptic species. *European Journal of*
 490 *Phycology*, **55**: 113-128.

491 Falkenberg, P. (1901). *Die Rhodomelaceen des Golfes von Neapel und der*
 492 *angrenzenden Meeres-Abschnitte. Fauna und Flora des Golfes von Neapel,*
 493 *Monographie 26.* Berlin.

494 Filbee-Dexter, K. & Wernberg, T. (2018). Rise of turfs: a new battlefront for globally
 495 declining kelp forests. *BioScience*, **68**: 64-76.

496 Filbee-Dexter, K., Wernberg, T., Grace, S.P., Thormar, J., Fredriksen, S., Narvaez,
 497 C.N., Feehan, C.J. & Norderhaug, K.M. (2020). Marine heatwaves and the
 498 collapse of marginal North Atlantic kelp forests. *Scientific Reports*, **10**: 13388.

499 Freitas, R., Romeiras, M., Silva, L., Cordeiro, R., Madeira, P., González, J.A., Wirtz, P.,
 500 Falcón, J.M., Brito, A., Floeter, S.R., Afonso, P., Porteiro, F., Viera-Rodríguez,
 501 M.A., Neto, A.I., Haroun, R., Farminhao, J.N.M., Reblelo, A.C., Baptista, L.,
 502 Melo, C.S., Martínez, A., Núñez, J., Berning, B., Johnson, M.E. & Ávila, S.P.
 503 (2019). Restructuring of the 'Macaronesia' biogeographic unit: A marine multi-
 504 taxon biogeographical approach. *Scientific Reports*, **9**: 15792.

505 Freshwater, D.W., Idol, J.N., Parham, S.L., Fernández-García, C., León, N., Gabrielson,
 506 P.W. & Wysor, B. (2017). Molecular assisted identification reveals hidden red
 507 algae diversity from the Burica Peninsula, Pacific Panama. *Diversity*, **9**: 19.

508 Guiry M. D. & Guiry G. M. (2020). AlgaeBase. World-wide electronic publication,
 509 National University of Ireland, Galway. <http://www.algaebase.org> searched on 22
 510 June 2020.

511 Haroun, R. J. & Prud'homme van Reine, W. F. (1993). A biogeographical study of
 512 *Laurencia* and *Hypnea* species of the Macaronesian region. *Courier*
 513 *Forschunsinstitut Senckenberg*, **159**: 119-125.

514 Haroun, R.J., Gil-Rodríguez, M.C., Díaz de Castro, J. & Prud'homme van Reine, W.F.
 515 (2002). A checklist of the marine plants from the Canary Islands (central eastern
 516 Atlantic Ocean). *Botanica Marina*, **45**: 139-169.

517 Hommersand, M.H. (1963). The morphology and classification of some Ceramiaceae
 518 and Rhodomelaceae. *University of California Publications in Botany*, **35**: 165-
 519 366.

- Jesus, P.B., Costa, A.L., Nunes, J.M.C, Manghisi, A., Genovese, G., Morabito, M. & Schnadelbach, S. (2019). Species delimitation methods reveal cryptic diversity in the *Hypnea cornuta* complex (Cystocloniaceae, Rhodophyta). *European Journal of Phycology*, **54**: 135-153.
- 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.
- Kelaher, B.P., Chapman, M.G. & Underwood, A.J. (2001). Spatial patterns of diverse macrofaunal assemblages in coralline turf and their associations with environmental variables. *Journal of the Marine Biological Association of the United Kingdom*, **81**: 917e930.
- Kim, S.-C., McGowen, M.R., Lubinsky, P., Barber, J.C., Mort, M.E. & Santos-Guerra, A. (2008). Timing and tempo of early and successive adaptive radiations in Macaronesia. *PLoS ONE*, **3**: e2139.
- Littler, M.M. (1980). Overview of the rocky intertidal systems of southern California. In *The California Islands: Proceedings of a multidisciplinary symposium* (Power, D. M., editor), 265-306. Santa Barbara Museum of Natural History, Santa Barbara.
- Machín-Sánchez, M., Gil-Rodríguez, M.C. & Haroun, R. (2018). Phylogeography of the red algal *Laurencia* complex in the Macaronesia region and nearby coastal areas: recent advances and future perspectives. *Diversity*, **10**: 10.
- Maggs, C.A. & Hommersand, M.H. (1993). *Seaweeds of the British Isles. Volume 1. Rhodophyta. Part 3A. Ceramiales*. HMSO, London.

544 Mei, J. & Schiel, D.R. (2007). Survival strategies in *Polysiphonia adamsiae* and *P.*
545 *strictissima* (Rhodophyta, Rhodomelaceae) subjected to sediment deposition and
546 grazing pressure. *New Zealand Journal of Marine and Freshwater Research*, **41**:
547 325e334.

548 Neto, A.I. (1994). Checklist of the benthic marine macroalgae of the Azores.
549 *Arquipélago*, **12A**: 15-34.

550 Pardo, C., Lopez, L., Peña, V., Hernández-Kantún, J., Le Gall, L., Bárbara, I. &
551 Barreiro, R. (2014). A multilocus species delimitation reveals a striking number of
552 species of coralline algae forming maerl in the OSPAR maritime area. *PLoS ONE*,
553 **9**: e104073.

554 Pasella, M.M., Verbruggen, H., Nelson W.A. & Díaz-Tapia, P. (2019) The phylogenetic
555 position of the morphologically unusual *Pleurostichidium falkenbergii*
556 (Rhodomelaceae, Rhodophyta) based on plastid phylogenomics. *Phycologia*, **58**:
557 319-325.

558 Phillips, L.E. (2000). Taxonomy of the New Zealand-endemic genus *Pleurostichidium*
559 (Rhodomelaceae, Rhodophyta). *Journal of Phycology*, **36**: 773-786.

560 Piñeiro-Corbeira, C., Maggs, C.A., Rindi, F., Bunker, F., Baldock, L. & Díaz-Tapia, P.
561 (2020a). Molecular assessment of the tribes Streblocladieae and Polysiphonieae
562 (Rhodomelaceae, Rhodophyta) in the British Isles reveals new records and species
563 that require taxonomic revision. *Cryptogamie, Algologie*, **41**: 55-72.

564 Piñeiro-Corbeira, C., Verbruggen, H. & Díaz-Tapia, P. (2020b). Molecular survey of
565 the red algal family Rhodomelaceae (Ceramiales, Rhodophyta) in Australia
566 reveals new introduced species. *Journal of Applied Phycology*, **32**: 2535-2547.

- 567 Price, I.R. & Scott, F.J. (1992). *The turf algal flora of the Great Barrier Reef. Part I.*
568 *Rhodophyta*. Botany Department, James Cook University, Townsville.
- 569 Rojas-González, B. (1997). *Estudio de las especies de la familia Rhodomelaceae*
570 *(Rhodophyta), con exclusión de las tribus Chondrieae y Laurencieae, en las Islas*
571 *Canarias*. Universidad de La Laguna, La Laguna.
- 572 Sansón, M. (1991). *Estudio de las especies de la familia Ceramiaceae (Rhodophyta) en*
573 *las Islas Canarias*. Universidad de La Laguna, La Laguna.
- 574 Sartoni, G. (1992). *Stichothamnion cymatophilum* (Rhodomelaceae, Rhodophyta) a new
575 record for Mediterranean algal flora. *Cryptogamie, Algologie*, **13**: 39-43.
- 576 Saunders, G.W. & Lehmkuhl, K.V. (2005). Molecular divergence and morphological
577 diversity among four cryptic species of *Plocamium* (Plocamiales,
578 Florideophyceae) in northern Europe. *European Journal of Phycology*, **40**: 293-
579 312.
- 580 Savoie, A.M. & Saunders, G.W. (2016). A molecular phylogenetic and DNA barcode
581 assessment of the tribe Pterosiphonieae (Ceramiales, Rhodophyta) emphasizing
582 the Northeast Pacific. *Botany*, **94**: 917-939.
- 583 Savoie, A.M. & Saunders, G.W. (2019). A molecular assessment of species diversity
584 and generic boundaries in the red algal tribes Polysiphonieae and Streblocladiae
585 (Rhodomelaceae, Rhodophyta) in Canada. *European Journal of Phycology*, **54**: 1-
586 25.
- 587 Schmidt, O.C. (1929). Beiträge zur Kenntnis der Meeresalgen der Azoren. II. *Hedwigia*,
588 **69**: 165-72.

589 Schneider, C.W., Quach P.K. & Lane C.E. (2017). A case for true morphological
590 cryptsis: Pacific *Dasya anastomosans* and Atlantic *D. cryptica* sp. nov.
591 (Dasyaceae, Rhodophyta). *Phycologia*, **56**: 359–368.

592 Soares, L.P., Guimarães S. M.P. de B., Toyota Fujii M., Yoneshigue-Valentin, Y.,
593 Sousa Batista, M.G. & Yokoya, N.S. (2020). *Rhodachlya westii* sp. nov.
594 (Rhodachlyales, Rhodophyta), a new species from Brazil, revealed by an
595 integrative taxonomic approach. *Phycologia*, **59**: 346-354.

596 Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-
597 analysis of large phylogenies. *Bioinformatics*, **30**: 1312–1313.

598 Stegenga, H., Bolton, J.J. & Anderson, R.J. (1997). *Seaweeds of the South African west*
599 *coast*. Bolus Herbarium, University of Cape Town, Cape Town.

600 Stewart, J.G. (1982). Anchor species and epiphytes in intertidal algal turf. *Pacific*
601 *Science*, **36**: 45–59.

602 Stuercke, B. & Freshwater, D.W. (2008). Consistency of morphological characters used
603 to delimit *Polysiphonia sensu lato* species (Ceramiales, Florideophyceae):
604 analyses of North Carolina, USA specimens. *Phycologia*, **47**: 541-559.

605 Tuya, F. & Haroun, R. (2006). Spatial patterns and response to wave exposure of
606 shallow water algal assemblages across the Canarian Archipelago: a multi-scaled
607 approach. *Marine Ecology Progress Series*, **311**: 15-28.

608 Vroman, M. (1967). A new species of *Stichothamnion* (Rhodophyta) from the West
609 Indies. *Acta Botanica Neerlandica*, **15**: 557-561.

610 Wallenstein, F.M., Terra, M.R., Pombo, J. & Neto, A.I. (2009). Macroalgal turfs in the
611 Azores. *Marine Ecology*, **30**: 113-117.

- 612 Walker, R.H., Brodie, J., Russell, S., Irvine, L.M. & Orfanidis, S. (2009). Biodiversity
613 of coralline algae in the northeastern Atlantic including *Corallina caespitosa* sp.
614 nov. (Corallinoideae, Rhodophyta). *Journal of Phycology*, **45**: 287-297.
- 615 Weber-van Bosse, A. (1911). Notice sur quelques genres nouveaux d'algues de
616 l'Archipel Malaisien. *Annales du Jardin Botanique de Buitenzorg*, **24**: 25-33.
- 617 Weber-van Bosse, A. (1913). Marine algae. Rhodophyceae, of the "Sealark" Expedition,
618 collected by Mr. J. Stanley Gardiner, M.A. *Transactions of the Linnean Society of*
619 *London, Second Series, Botany*, **8**: 105-142.
- 620

621 Table 1. Measurements of morphological characters for *Vertebrata cymatophila* and *V.*
622 *barbarae* sp. nov.

	<i>V. cymatophila</i>	<i>V. barbarae</i>
Pericentral cells	8-9 (-10)	8-9
Prostrate axes		
Diameter (µm)	90-120	60-125
Segments L/D	0.4-0.83	0.3-0.8
Rhizoids		
Maximum length (µm)	700	790
Filament diameter (µm)	15-35	10-50
Erect axes		
Length (µm)	3500	3000
Apical cell diameter (µm)	10-17	10-17
Diameter (µm)	55-145	60-125
Segments L/D	0.3-1.1	0.3-1.3
Trichoblasts		
Maximum length (µm)	1100	313
Diameter of basal cell (µm)	5-13	2.5-10
Spermatangial branches (µm)	25-40 × 150-235	22-65 × 112-173
Procarys	-	4-celled
Cystocarys (µm)	-	300 × 350
Tetrasporangia (µm)	22-40 × 25-40	17-43 × 17-48

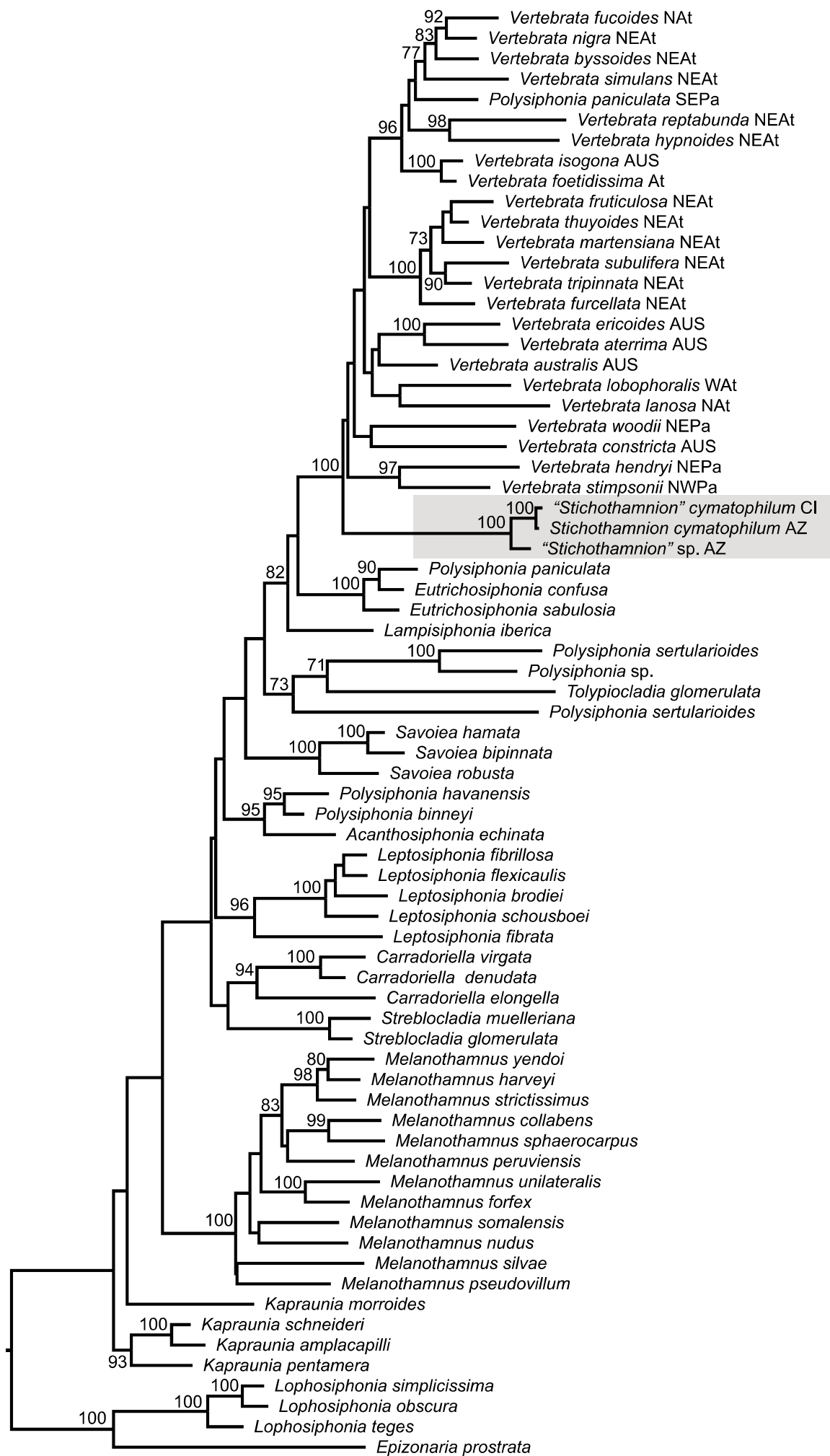
623 L/D = Length/Diameter

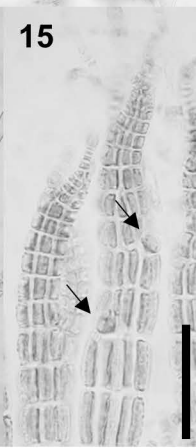
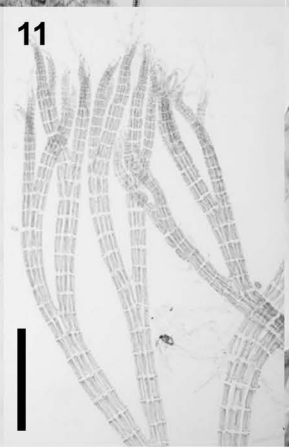
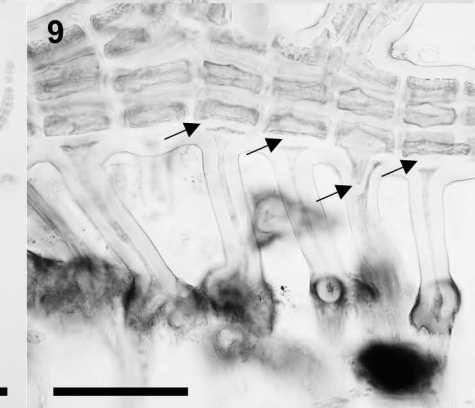
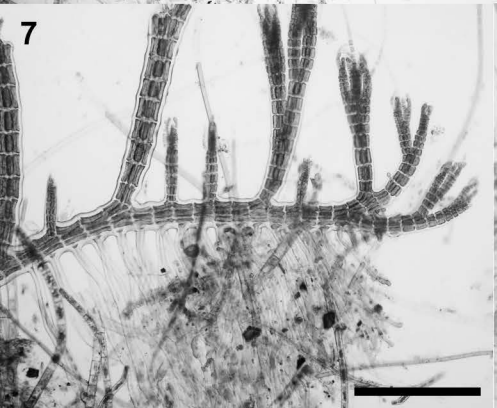
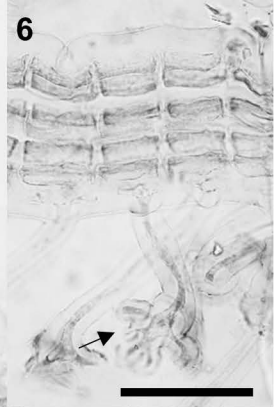
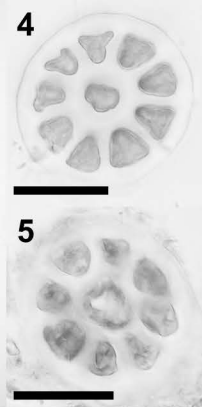
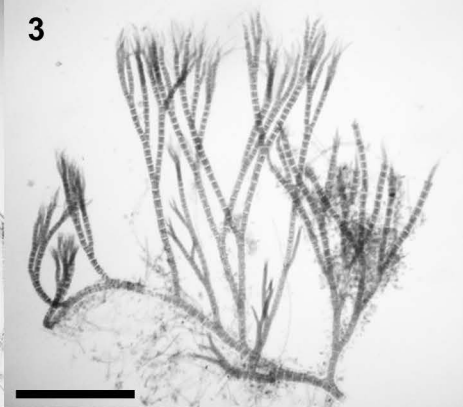
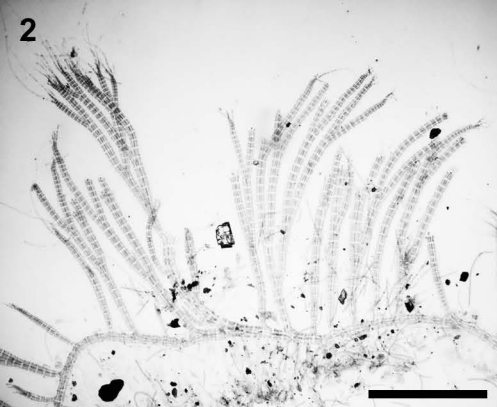
Figure legends

Fig. 1. Maximum likelihood phylogeny of the tribe Streblocladieae based on *rbcL* sequences. Lineage that consists of samples originally identified as *Stichothamnion* is shaded. Values at the nodes represent bootstrap support, only shown if ≥ 70 . Distribution of species of the genus *Vertebrata* is indicated: AUS = Australasia, AZ = Azores, CI = Canary Islands, At = Atlantic, NAt = North Atlantic, NEAt = Northeastern Atlantic, WAt=Western Atlantic, NEPa = Northeastern Pacific, NWPa = Northwestern Pacific; SEPa = Southeastern Pacific.

Figs 2-15. *Vertebrata cymatophila* (2, 4, 7, 10, 12-14) and *V. barbarae* sp. nov. (3, 5, 6, 8, 9, 11, 15): Vegetative morphology. **Figs 2-3.** Habit of a specimen with prostrate and erect axes. **Figs 4-5.** Cross section of an axis with nine (Fig. 4) and eight (Fig. 5) pericentral cells. **Fig 6.** Prostrate axis with a rhizoid terminated in a digitate discoid pad (arrow) and a thick wall. **Fig. 7.** Prostrate axis with abundant rhizoids on every segment. **Fig. 8.** Apexes of prostrate axes lacking trichoblasts. **Fig. 9.** Rhizoids cut off from pericentral cells (arrows). **Fig. 10.** Branch formed in the axil of a trichoblasts (arrow). **Fig. 11.** Erect axes pseudodichotomously branched. **Fig. 12.** Trichoblast cell with multiple nuclei (arrows). **Fig. 13.** Apices of erect axes with long unbranched trichoblasts. **Fig. 14.** Apices of erect axes with spirally arranged trichoblasts. **Fig. 15.** Apices of erect axes with scar cells of trichoblasts (arrows). Scale bars: Figs 2-3 = 700 μm ; Figs 4-5, 12 = 30 μm ; Figs 6, 9, 10, 14, 15 = 80 μm ; Figs 7, 11, 13 = 300 μm ; Fig. 8 = 160 μm .

Figs 16-24. *Vertebrata cymatophila* (17, 18, 22, 24) and *V. barbarae* sp. nov. (16, 19-21, 23): Reproductive morphology. **Fig. 16.** Apical branches bearing abundant spermatangial branches. **Figs 17-19.** Spermatangial branches with 1-5 basal (arrowheads) and 0-4 apical (arrows) sterile cells. **Fig. 20.** Procarp showing the supporting cell (su), the sterile basal cell (st) and the four-celled carpogonial branch (1-4). **Fig. 21.** Cystocarp. **Figs 22-24.** Apical branches with tetrasporangia. Scale bars: Figs 16, 21, 24 = 150 μm ; Figs 17-19 = 70 μm ; Fig. 20 = 20 μm ; Figs 22-23 = 200 μm .





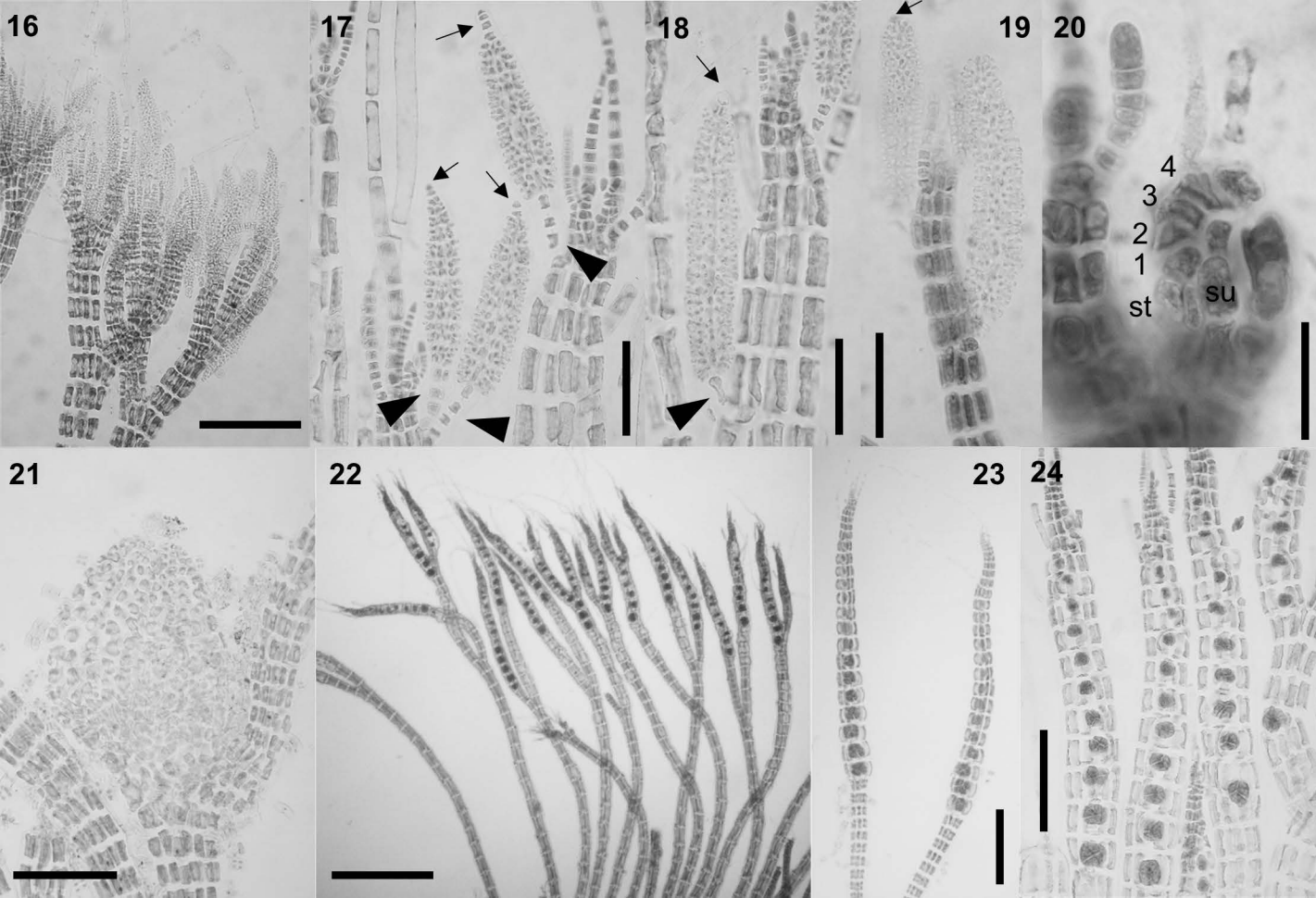




Fig. S1. Intertidal algal turfs from the Canary Islands (Spain).

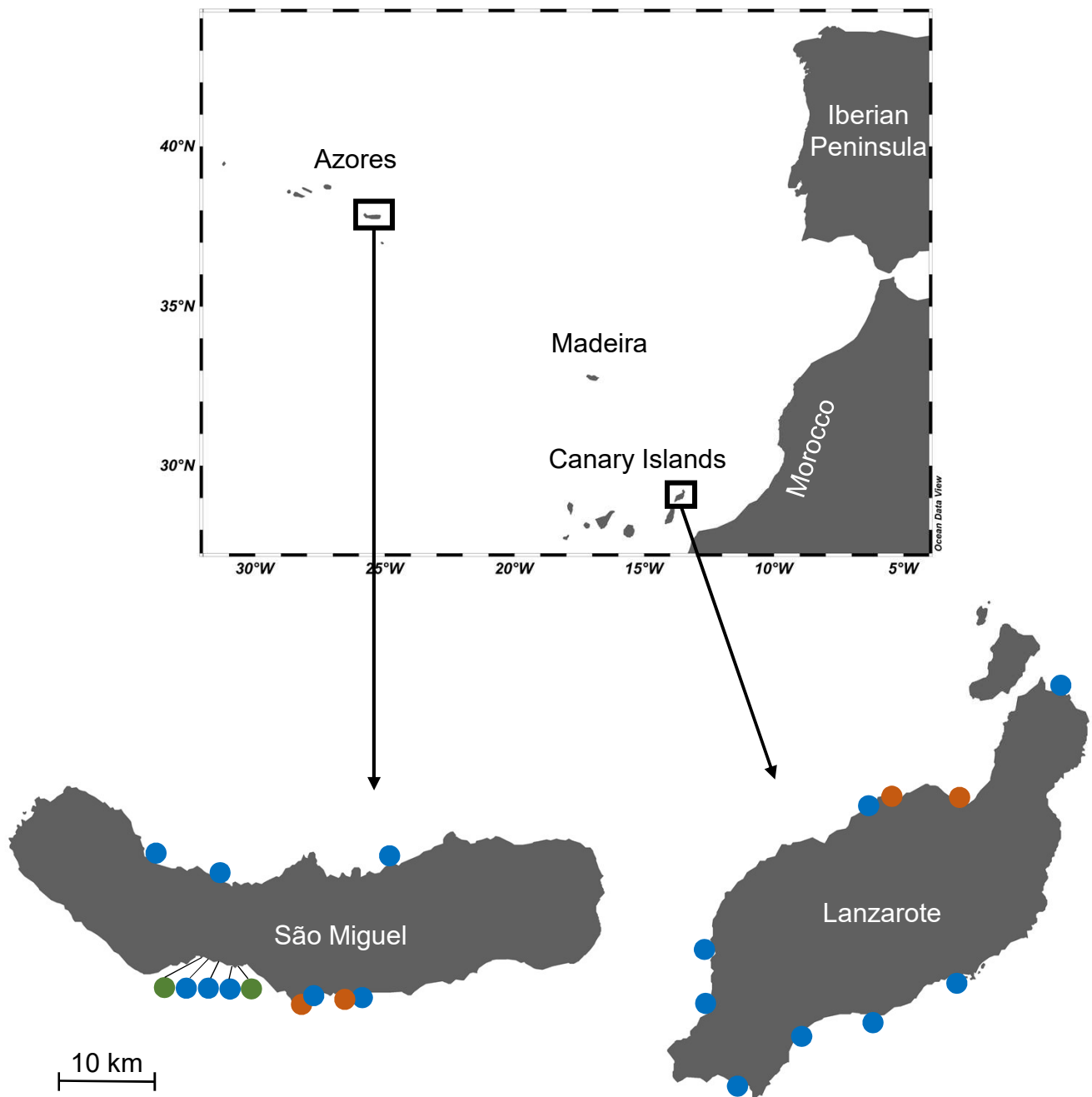


Fig. S2. Sampling sites in São Miguel and Lanzarote. Distribution of *Vertebrata barbarae* sp. nov. and *V. cymatophila* are indicated by green and brown circles, respectively.

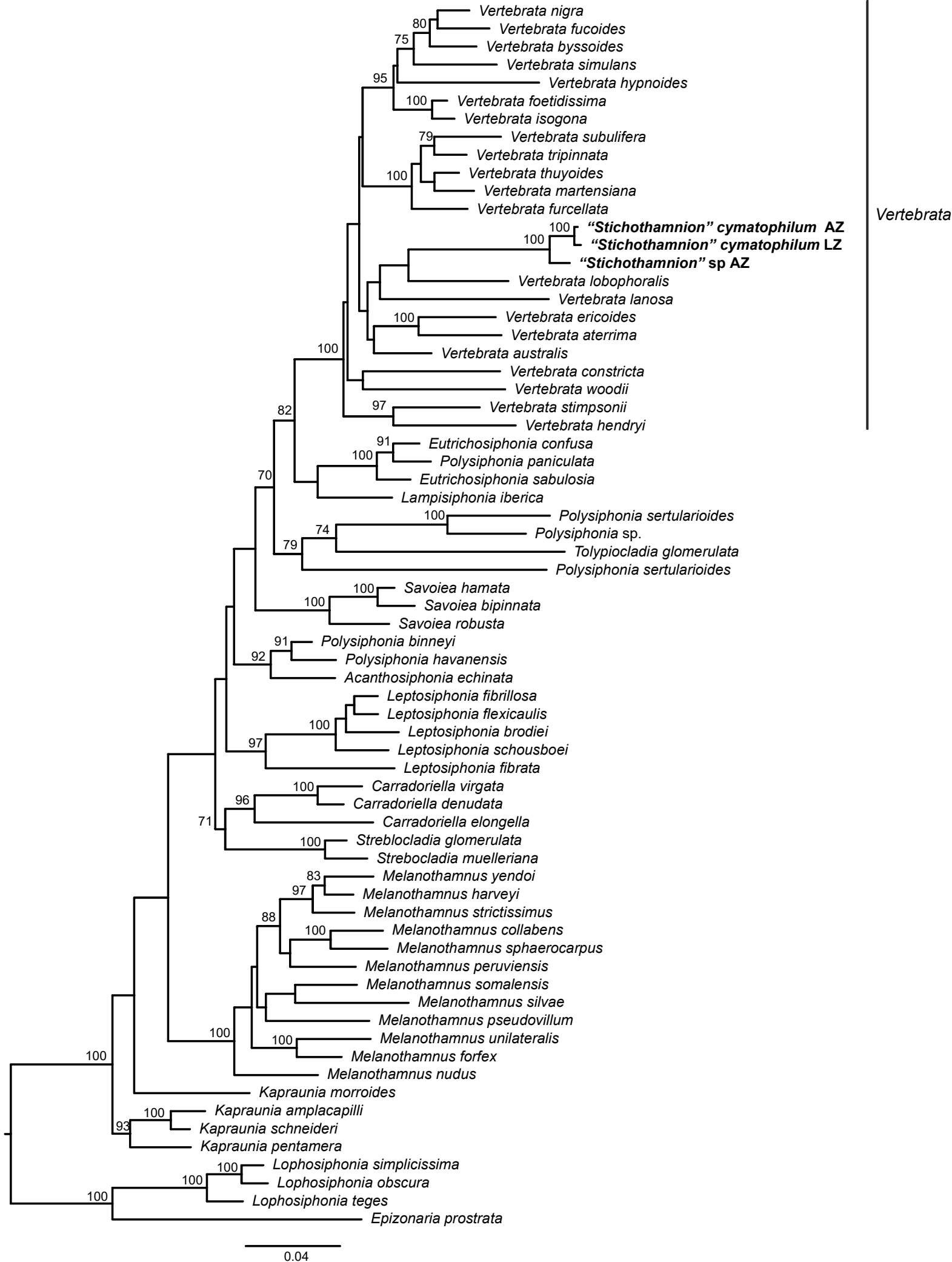


Fig. S3. Maximum likelihood phylogeny of the tribe Strebloladiaceae based on the alignment of 61 *rbcL* sequences (excluding three species of the *Vertebrata* lineage compared with Fig. 1). Lineage that consists of samples originally identified as *Stichothamnion* indicated in bold.

Values at the nodes represent bootstrap support, only shown if ≥ 70.

Table S1. GenBank accession numbers and collection information of the sequences used in phylogenetic analyses.

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number
<i>Acanthosiphonia echinata</i> (Harvey) Savoie & G.W.Saunders	TC8872	Indonesia; Bustamante et al. (2013, as <i>Neosiphonia</i>)	1345	KM609434
<i>Carradoriella virgata</i> (C. Agardh) Kylin	CH1671	Cape Province, South Africa; Bárbara et al. (2013)	1467	JX828152
<i>Carradoriella denudata</i> (Dillwyn) Savoie & G.W.Saunders	CH1954	Pontevedra, Spain; Bárbara et al. (2013, as <i>Polysiphonia</i>)	1467	JX828143
<i>Carradoriella elongella</i> (Harvey) Savoie & G.W.Saunders	DNA-ID 468	Wales, United Kingdom; McIvor et al. (2001, as <i>Polysiphonia</i>)	1245	AF342913
<i>Epizonaria prostrata</i> (Harvey) Díaz-Tapia & Maggs	PD1549	Western Australia; Díaz-Tapia et al. (2017a)	1279	MF094087
<i>Eutrichosiphonia confusa</i> (Hollenberg) A.M.Savoie & G.W.Saunders	TC607	-	1440	KR090584
<i>Eutrichosiphonia sabulosia</i> (B.Kim & M.S.Kim) A.M.Savoie & G.W.Saunders	12sp1-01	Jeju, Korea; Kim and Kim (2014, as <i>Polysiphonia</i>)	1250	KF479248
<i>Kapraunia amplacapilli</i> (B.Kim & M.S.Kim) Savoie & G.W.Saunders	12sp403	Jeju, Korea; Kim and Kim (2014, as <i>Polysiphonia</i>)	1250	KF479252
<i>Kapraunia morroides</i> (B.Kim & M.S.Kim) A.M.Savoie & G.W.Saunders	12sp5-02	Jeju, Korea; Kim and Kim (2014, as <i>Polysiphonia</i>)	1250	KF479255
<i>Kapraunia pentamera</i> (Hollenberg) Savoie & G.W.Saunders	PHYKOS.3529	Panama; Mamoozadeh & Freshwater (2012, as <i>Polysiphonia</i>)	1387	HM573564
<i>Kapraunia schneideri</i> (Stuercke & Freshwater) Savoie & G.W.Saunders	PHYCOS.2454	Panama; Mamoozadeh & Freshwater (2012, as <i>Polysiphonia</i>)	1346	HM573566
<i>Lampisiphonia iberica</i> Bárbara, Secilla, Díaz-Tapia & H.-G.Choi	CH1533	A Coruña, Spain; Bárbara et al. (2013)	1467	JX828131
<i>Leptosiphonia brodiei</i> (Dillwyn) Sprengel	PD516	Doaghbeg, Ireland; Díaz-Tapia et al. (2017a, as <i>Polysiphonia</i>)	1467	MF101425
<i>Leptosiphonia fibrata</i> (C.Agardh) Savoie & G.W.Saunders	DNA-ID 257	Ireland; McIvor et al. (2001, as <i>Polysiphonia</i>)	1245	AF342915
<i>Leptosiphonia fibrillosa</i> (Agardh) Savoie & G.W.Saunders	DNA-ID 255	Ireland; McIvor et al. (2001, as <i>Polysiphonia</i>)	1245	AF342912
<i>Leptosiphonia flexicaulis</i> (Harvey) Savoie & G.W.Saunders	GWS007393	Newfoundland and Labrador, Canada; Savoie & Saunders (2019)	1363	MF120877
<i>Leptosiphonia schousboei</i> (Thuret) Kylin	CH826	Lugo, Spain; Bárbara et al. (2013)	1467	JX828133

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number
<i>Lophosiphonia obscura</i> (C.Agardh) Falkenberg	CUK11188	Australia; Bustamante et al. (2017)	1435	KT825865
<i>Lophosiphonia simplicissima</i> Díaz-Tapia	SANT-A.24157	Spain; Díaz-Tapia et al. (2017a)	1448	MF094088
<i>Lophosiphonia teges</i> (Womersley) Díaz-Tapia & Maggs	PD1823	Western Australia; Díaz-Tapia et al. (2017a)	1296	MF094089
<i>Melanothamnus collabens</i> (C.Agardh) Díaz-Tapia & Maggs	CH2526	Spain; Bárbara et al. (2013, as <i>Streblocladia</i>)	1467	JX828157
<i>Melanothamnus forfex</i> (Harvey) Díaz-Tapia & Maggs	PD1571	Western Australia; Díaz-Tapia et al. (2017b)	1324	KX499565
<i>Melanothamnus harveyi</i> (Bailey) Díaz-Tapia & Maggs	PD890	Victoria, Australia; Díaz-Tapia et al. (2017a)	1467	MF101437
<i>Melanothamnus nudus</i> (Mamoozadeh & Freshwater) Freshwater	PHYKOS-2613	Panama; Mamoozadeh & Freshwater (2012, as <i>Polysiphonia</i>)	1337	HM573571
<i>Melanothamnus peruviansis</i> (Bustamante, B.Y.Won, M.E.Ramirez & T.O.Cho) Díaz-Tapia & Maggs	TC6474	Peru; Bustamante et al. (2012, as <i>Neosiphonia</i>)	1442	JN989968
<i>Melanothamnus pseudovillum</i> (Hollenberg) Díaz-Tapia & Maggs	PHYCOS.3533	Panama; Mamoozadeh & Freshwater (2012, as <i>Neosiphonia</i>)	1284	HM573568
<i>Melanothamnus silvae</i> (Bustamante, B.Y.Won & T.O.Cho) Díaz-Tapia & Maggs	-	Indonesia; Bustamante et al. (2013, as <i>Neosiphonia</i>)	1452	KF146878
<i>Melanothamnus somalensis</i> Bornet & Falkenberg	2774	Oman; Díaz-Tapia et al. (2017b)	1364	KX499555
<i>Melanothamnus sphaerocarpus</i> (Børgesen) Díaz-Tapia & Maggs	FL05-6	Florida, USA; Mamoozadeh & Freshwater (2012, as <i>Neosiphonia</i>)	1170	HM573569
<i>Melanothamnus strictissimus</i> (J.D.Hooker & Harvey) Díaz-Tapia & Maggs	PD3448	Tasmania, Australia; Piñeiro-Corbeira et al. (2020b)	1312	MN419269
<i>Melanothamnus unilateralis</i> (Levring) Díaz-Tapia & Maggs	JF0192	Juan Fernández, Chile; Díaz-Tapia et al. (2017b)	1282	KX499549
<i>Melanothamnus yendoi</i> (T.Segi) Díaz-Tapia & Maggs	21N_yend_Gijang	Korea; Kim & Yang (2006, as <i>Neosiphonia</i>)	1245	DQ787495
<i>Polysiphonia binneyi</i> Harvey	PHYKOS-2517	Panama; Mamoozadeh & Freshwater (2011)	1336	HM573555
<i>Polysiphonia havanensis</i> Montagne	PHYKOS-2628	Panama; Mamoozadeh & Freshwater (2011, as <i>Polysiphonia</i>)	1294	HM573554
<i>Polysiphonia paniculata</i> Montagne	TC6524	-	1440	KR090582
	-	Chile; Kim et al. (2004)	1379	AY396040

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number
<i>Polysiphonia sertularioides</i> (Grateloup) J.Agardh	PD001	Cabo Huertas, Alicante, Spain; Díaz-Tapia et al. (2017a)	1467	MF101423
	PD863	Victoria, Australia; Díaz-Tapia et al. (2017a)	1467	MF101423
<i>Polysiphonia</i> sp.	WAR62	Western Australia; Huisman et al. (2017)	1250	MF139314 KU564490
<i>Savoiea bipinnata</i> (Postels & Ruprecht) M.J.Wynne	KAM0009	Kamchatka, Russia; Savoie & Saunders (2016, as <i>Polyostea</i>)		
<i>Savoiea hamata</i> (E.S.Sinova) M.J.Wynne	GWS027925	British Columbia, Canadá; Savoie & Saunders (2016, as <i>Polyostea</i>)	1363	KU564512
<i>Savoiea robusta</i> (N.L.Gardner) M.J.Wynne	GWS022432	British Columbia, Canadá; Savoie & Saunders (2016, as <i>Polyostea</i>)	1363	KU564477
<i>Streblocladia glomerulata</i> (Montagne) Papenfuss	NZ	New Zealand; Díaz-Tapia et al. (2017b)	1367	KX499579
<i>Streblocladia muelleriana</i> (J.Agardh) L.E.Phillips, Hommersand, N.M.Adams & W.A.Nelson	-	New Zealand; Fujii et al. (2006, as <i>Polysiphonia</i>)	1419	AY588412
<i>Tolypocladia glomerulata</i> (C.Agardh) F.Schmitz	PD1825	Western Australia; Díaz-Tapia et al. (2017a)	1467	MF101467
<i>Vertebrata australis</i> (C.Agardh) Kuntze	PD931	Victoria, Australia; Díaz-Tapia et al. (2017a)	1467	MF101439
<i>Vertebrata aterrima</i> (J.D.Hooker & Harvey) Kuntze	NZ04-525	New Zealand; Mamoozadeh & Freshwater (2012, as <i>Polysiphonia</i>)	1350	HM573577
<i>Vertebrata barbarae</i> Díaz-Tapia & Muñoz-Luque, sp. nov.	PD3791	Lagoa, São Miguel, Azores; 14.iv.2018; intertidal	1394	MW246788
	PD3815	Ilhéu São Roque, São Miguel, Azores; 14.iv.2018; intertidal	1374	MW246789
<i>Vertebrata byssoides</i> (Goodenough & Woodward) Kuntze	-	Norway; Yang et al. (2016, as <i>Brongniartella</i>)	1435	DQ787584
<i>Vertebrata cymatophila</i> (Børgesen) Díaz-Tapia & Muñoz-Luque, comb. nov.	PD3872	Caloura, São Miguel, Azores; 16.iv.2018; intertidal	1300	MW246790
	PD3911	Vila Franca, São Miguel, Azores; 18.iv.2018; intertidal	1312	MW246791
	PD4323	Caleta de Famara, Lanzarote, Canary Islands; 13.vi.2018; intertidal	1289	MW246792
	PD4373	Caleta de Caballo Lanzarote, Canary Islands; 15.vi.2018; intertidal	1289	MW246793
<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 foetidissima</i> (Cocks ex Bornet) Díaz-Tapia & Maggs	SANT25470	Portugal; Díaz-Tapia et al. (2013, as <i>Polysiphonia</i>)	1241	JQ653293
<i>Vertebrata fruticulosa</i> (Wulfen) Kuntze	PD2020	Italy; Piñeiro-Corbeira et al. (2020a)	756	MN867924

Species	Specimen	Collection site; habitat; date; collectors / Publication	Sequence length (bp)	GenBank accession number
<i>Vertebrata fucoides</i> (Hudson) Kuntze	CH1359	Spain; Bárbara et al. (2013, as <i>Polysiphonia</i>)	1467	JX828146
<i>Vertebrata furcellata</i> (C.Agardh) Kuntze	LMI469	Wales; Díaz-Tapia et al. (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 et al. (2017b)	1446	KX499547
<i>Vertebrata isogona</i> (Harvey) Díaz-Tapia & Maggs	PD831	Frankston, Dave's Bay, Port Phillip Bay, Victoria, Australia; Díaz-Tapia et al. (2017a)	1467	MF101433
<i>Vertebrata lanosa</i> (Linnaeus) T.Christensen	-	Beavertail State Park, Jamestown, Rhode Island, USA; Salomaki et al. (2015)	1467	NC026523
<i>Vertebrata lobophoralis</i> (Mamoozadeh & Freshwater) Freshwater	PHYKOS3537	Panama; Mamoozadeh & Freshwater (2012, as <i>Polysiphonia</i>)	1340	HM573551
<i>Vertebrata martensiana</i> (Suhr) Piñeiro-Corbeira, Maggs & Díaz-Tapia	CH049	Ireland; Bárbara et al. (2013, as <i>Boergeseniella fruticulosa</i>)	1467	JX828161
<i>Vertebrata nigra</i> (Hudson) Díaz-Tapia & Maggs	CH048	Ireland; Bárbara et al. (2013, as <i>Polysiphonia</i>)	1463	JX828164
<i>Vertebrata reptabunda</i> (Suhr) Díaz-Tapia & Maggs	SANT25139	Atlantic Spain; Díaz-Tapia et al. (2017b)	1448	KX499554
<i>Vertebrata simulans</i> (Harvey) Kuntze	PD3031	Ireland; Díaz-Tapia et al. (2017b)	1307	MN867919
<i>Vertebrata subulifera</i> (C.Agardh) Kuntze	LMI1017	Ireland; Díaz-Tapia et al. (2017b)	1245	KX499564
<i>Vertebrata stimpsonii</i> (Harvey) Kuntze	CH073	Japan; Bárbara et al. (2013, as <i>Enelittosiphonia</i>)	975	JX828126
<i>Vertebrata thuyoides</i> (Harvey) Kuntze	PD546	Fanad Head, Ireland; Díaz-Tapia et al. (2017a)	1467	MF101426
<i>Vertebrata tripinnata</i> (Harvey) Kuntze	PD3032B	Ireland; Piñeiro et al. (2020a)	1304	MN867917
<i>Vertebrata woodii</i> (Harvey) Kuntze	GWS22313	California, USA; Savoie & Saunders (2016, as <i>Pterochondria</i>)	1363	KU564507

References

- Bárbara, I., Choi, H.G., Secilla, A., Díaz-Tapia, P., Gorostiaga, J.M., Seo, T.K., Jung, M.Y. & Bercibar, E. (2013). *Lampisiphonia iberica* gen. et sp. nov. (Ceramiales, Rhodophyta) based on morphology and molecular evidence. *Phycologia*, **52**: 137-155.
- Bustamante, D.E., Won, B.Y., Ramirez, M.E. & Cho, T.O. (2012). *Neosiphonia peruviansis* sp. nov. (Rhodomelaceae, Rhodophyta) from the Pacific coast of South America. *Botanica Marina*, **55**: 359-366.
- Bustamante, D.E., Won, B.Y. & Cho, T.O. (2013). *Neosiphonia baliana* sp. nov. and *N. silvae* sp. nov. (Rhodomelaceae, Rhodophyta) from Bali, Indonesia. *Botanica Marina*, **56**: 515-524.
- 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
- Díaz-Tapia, P., Kim, M.S., Secilla, A., Bárbara, I. & Cremades, J. (2013). 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.
- 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.
- Díaz-Tapia, P., McIvor, 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 Polysiphonieae (Rhodomelaceae, Ceramiales). *European Journal of Phycology*, **52**: 1-20
- Fujii, M.T., Guimarães, S.M.P.B., Gurgel, C.D.F. & Fredericq, S. (2006). Characterization and phylogenetic affinities of the red alga *Chondrophycus flagelliferus* (Rhodomelaceae, Ceramiales) from Brazil on the basis of morphological and molecular evidence. *Phycologia*, **45**: 432-441.
- 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.
- Kim, M.-S., Chan Yang, E., Mansilla, A. & Min Boo, S. (2004). Recent introduction of *Polysiphonia morrowii* (Ceramiales, Rhodophyta) to Punta Arenas, Chile. *Botanica Marina*, **47**: 389-394.
- 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.
- Kim, M.S. & Yang, E.C. (2006). Taxonomy and phylogeny of *Neosiphonia japonica* (Rhodomelaceae, Rhodophyta) based on *rbcL* and *cpeA/B* gene sequences. *Algae*, **21**: 287-294
- 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.
- Mamoozadeh, N.R. & Freshwater, D.W. (2012). *Polysiphonia sensu lato* (Ceramiales, Florideophyceae) species of Caribbean Panama including *Polysiphonia lobophoralis* sp. nov. and *Polysiphonia nuda* sp. nov. *Botanica Marina*, **55**: 317-347.

- McIvor, L., Maggs, C.A., Provan, J. & Stanhope, M.J. (2001). *rbc* L sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Molecular Ecology*, **10**: 911-919.
- Piñeiro-Corbeira, C., Maggs, C. A., Rindi, F., Bunker, F., Baldock, L. & Díaz-Tapia, P. (2020a). Molecular assessment of the tribes Streblocladieae and Polysiphonieae (Rhodomelaceae, Rhodophyta) in the British Isles reveals new records and species that require taxonomic revision. *Cryptogamie, Algologie*, **41**: 55-72
- Piñeiro-Corbeira, C., Verbruggen, H. & Díaz-Tapia, P. (2020b). Molecular survey of the red algal family Rhodomelaceae (Ceramiales, Rhodophyta) in Australia reveals new introduced species. *Journal of Applied Phycology*, **32**: 2535-2547.
- Salomaki, E.D., Nickles, K.R. & Lane, C.E. (2015). The ghost plastid of *Choreocolax polysiphoniae*. *Journal of Phycology*, **51**: 217–221.
- 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.
- Savoie, A.M. & Saunders, G.W. (2019). A molecular assessment of species diversity and generic boundaries in the red algal tribes Polysiphonieae and Streblocladieae (Rhodomelaceae, Rhodophyta) in Canada. *European Journal of Phycology*, **54**: 1-25.
- Stuercke, B., & Freshwater, D.W. (2010). Two new species of *Polysiphonia* (Ceramiales, Florideophyceae) from the western Atlantic. *Botanica Marina*, **53**: 301-311.
- 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.