WIDELY DISTRIBUTED RED ALGAE OFTEN REPRESENT HIDDEN INTRODUCTIONS, COMPLEXES OF CRYPTIC SPECIES OR SPECIES WITH STRONG PHYLOGEOGRAPHIC STRUCTURE

Pilar Díaz-Tapia[2]

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

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

Christine A. Maggs

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

Erasmo C. Macaya

Laboratorio de Estudios Algales (ALGALAB), Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile

Millennium Nucleus Ecology and Sustainable Management of Oceanic Island (ESMOI)

Centro FONDAP de Investigaciones en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL)

Heroen Verbruggen

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

[2]Author for correspondence: e-mail pdiaz@udc.es

Running title: Red algae with wide geographical distributions

ABSTRACT

Despite studies suggesting that most seaweeds are poor dispersers, many red algal species are reported to have circumglobal distributions. Such distributions have mostly been based on morphological identifications, but molecular data have revealed a range of issues with morphologically defined species boundaries. Consequently, the real distribution of such reportedly circumglobal species must be questioned. In this study, we analysed molecular datasets (*rbc*L gene) of nine species in the Rhodomelaceae for which samples were available from widely spaced geographical locations. Three overall patterns were identified: 1) species showing strong phylogeographic structure (i.e. phylogenetic similarity correlates with geographical provenance), often to the point that populations from different locations could be considered as different species (*Lophosiphonia obscura, Ophidocladus simpliciusculus*, *Polysiphonia villum* and *Xiphosiphonia pinnulata*); 2) species with a broad distribution that is explained, in part, by putative human-mediated transport (*Symphyocladia dendroidea* and *Polysiphonia devoniensis*); and 3) non-monophyletic complexes of cryptic species, most with a more restricted distribution than previously thought (*Herposiphonia tenella, S. dendroidea* and the *X. pennata* complex that includes the species *X. pinnulata* and *S. spinifera*). This study shows that widely distributed species are the exception in marine red algae, unless they have been spread by humans.

Key words: introductions, new record, phylogeography, Rhodomelaceae, Polysiphonieae, Pterosiphonieae, Herposiphonieae, *rbc*L, species boundaries, species complexes

59 INTRODUCTION

60 Phylogeography of marine organisms is influenced by barriers to dispersal and
61 geographical distance, as well as by aspects of their life-history, physiology and ecology
62 (Jackson 1974, Palumbi 1994, Riginos et al. 2011). The dispersal ability of seaweeds is
63 generally very limited, of the order of tens of meters or less (Santelices 1990, Kinlan
64 and Gaines 2003, Destombe et al. 2009). However, long-distance dispersal is known in
65 brown seaweeds with buoyant structures (Fraser et al. 2009, Macaya and Zuccarello
66 2010), which can act as rafts promoting in turn the dispersal of the epiphytic species
67 that they host (Fraser et al. 2013, Macaya et al. 2016, López et al. 2017, 2018). Still, a
68 large proportion of macroalgae are epilithic, so their expected dispersal ability is very
69 limited and consequently their distribution range is expected to be relatively small.
70 Paradoxically, many macroalgal species are reported to be very widely or even globally
71 distributed.

72 Records are usually based only on morphological identification, which can be
73 inaccurate due to morphological plasticity within species as well as similarity between
74 cryptic species (e.g. Verbruggen 2014, Schneider et al. 2017). Closer investigation of
75 material from distant regions using DNA data commonly leads to the discovery of
76 cryptic species (e.g. Won et al. 2009, Bustamante et al. 2014, Schneider et al. 2017).
77 Even though studies combining morphological and molecular data are increasing, DNA
78 databases are still very limited for most algal groups and molecular data are often
79 available only for some regions. As a consequence, the true distribution of many
80 seaweed species should be regarded as uncertain. Few studies have reassessed the
81 distribution of widely reported red algal species using molecular data from a broad
82 sampling area. Complexes of look-alike species, as well as widely distributed species,
83 have been detected (Zuccarello et al. 1999, Zuccarello et al. 2002a, Zuccarello & West

84 2003, Won et al. 2009). Among the widely distributed species, some exhibit high
85 genetic variability and strong phylogeographic structure that often distinguishes
86 populations from different basins (Zuccarello et al. 2002a, b, Won et al. 2009). Other
87 widely distributed species lack phylogeographic signal, suggesting long-distance
88 dispersal processes by unknown mechanisms (Zuccarello et al. 2002a, Fraser et al.
89 2013). Therefore, red algal phylogeographic patterns are highly heterogeneous and
90 depend on evolutionary histories and dispersal abilities.

91 In addition to natural dispersal mechanisms, human-mediated vectors can transport
92 seaweeds from native areas to other world regions and rapidly alter distribution patterns
93 (Straub et al. 2016). More than 208 red algal species have been considered as introduced
94 or cryptogenic in one or several regions (Thomsen et al. 2016). Cryptic introductions
95 are common in the red algae and non-native species often remain unnoticed until
96 diversity surveys use molecular tools (McIvor et al. 2001, Zuccarello et al. 2002b, Díaz-
97 Tapia et al. 2013b, 2017a). Considering the low dispersal ability of non-buoyant
98 epilithic red algae, we hypothesize that the distribution of most truly cosmopolitan
99 species can be explained by human-mediated transport – which is frequently provided
100 as a potential explanation for wide distributions of species (Zuccarello et al. 2002a,b,
101 Fraser et al. 2013).

102 The Rhodomelaceae, with >1,000 recognized species, is the most diverse red algal
103 family (Guiry and Guiry 2018). It includes numerous examples of widely reported
104 species and, as in most red algal groups, cryptic diversity is common (e.g. Zuccarello et
105 al. 2002a, Díaz-Tapia and Bárbara 2013, Bustamante et al. 2014, Savoie and Saunders
106 2016, Zuccarello et al. 2018). Among the red algae the Rhodomelaceae accounts for the
107 largest number of introduced species (Williams and Smith 2007). Members of this
108 family are often major components of algal turfs where canopy-forming brown algae

with buoyant structures are rare as a consequence of the stressful conditions imposed by the presence of sediment (Airoldi 1998, Díaz-Tapia et al. 2013a). This makes the family a good candidate to test hypotheses about species distributions and phylogeographic patterns.

The objective of this paper is to reassess the wide reported distributions of nine turf-forming species of the family Rhodomelaceae using DNA sequences. Using molecular data from distant locations within each species' reported distribution range, we evaluate whether these are indeed widely distributed species, analyse the observed phylogeographic patterns, and consider whether these species may have been introduced into one or several regions by human activities.

MATERIALS AND METHODS

Material of *Herposiphonia tenella*, *Lophosiphonia obscura*, *Ophidocladus simpliciusculus*, *Polysiphonia villum*, *P. devoniensis*, *Symphyocladia spinifera*, *S. dendroidea*, *Xiphosiphonia pennata* and *X. pinnulata* was collected in Norway, United Kingdom, France, Spain, Portugal, Italy, Brazil, Chile, Australia and South Africa during general sampling surveys of the family Rhodomelaceae (Table S1). All these species form epilithic turfs, most of them on intertidal sand-covered rocks (Womersley 2003, Díaz-Tapia & Bárbara 2013). *Lophosiphonia obscura* was found in North Atlantic brackish water coastal lagoons or estuaries, and *Symphyocladia dendroidea* was collected in Australian and Chilean shallow subtidal turfs. Distribution maps of records for these species (Figs 1-3, lines) were drawn up based on information available in AlgaeBase and references therein (Guiry and Guiry 2018).

DNA was extracted from silica gel-dried material following Saunders and McDevit (2012), using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) or the Promega Wizard Magnetic 96 DNA Plant System kit (Promega Corporation, Madison, USA), following the manufacturer's instructions. PCR amplification was carried out for the *rbc*L gene using primers F7/RrbcStart, F7/R893 or F57/rbcLrevNEW (Freshwater and Rueness 1994, Mamoozadeh and Freshwater 2011, Saunders and Moore 2013), as well as the newly designed primers F2 (TGTCTAACTCTGTAGAACAACGGA), F8 (ACTCTGTAGAASAACGGACAMG), R1008 (AACTACTACAGTACCAGCATG), R1464 (AACATTAGCTGTTGGAGTTTCYAC) and R1452 (TGGAGTTTCYACRAAGTCAGCTGT). Names of these primers indicate their position in the *rbc*L gene (e.g. first base of F2 primer corresponds with the second base of the *rbc*L gene). PCR reactions were performed in a total volume of 25 µl containing 1× MyTaqTM reaction buffer, 0.28 µM of forward and reverse primers, 0.125 units My TaqTM DNA Polymerase (Bioline, London, UK) and 1 µl template DNA. The PCR profile consisted of initial denaturation (93ºC for 3 min), 35 cycles of denaturation (94ºC for 30 s), primer annealing (45ºC for 30 s), and extension (74ºC for 90 s) and final extension (74ºC for 5 min). The PCR products were purified and sequenced at Queen's University of Belfast on an AB3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) or commercially by Macrogen or the sequencing service of the University of A Coruña.

A total of 128 new *rbc*L sequences were generated in this study and an additional 91 sequences were downloaded from GenBank (Table S1). Length of sequences ranged from 585 to 1467 (Table S1). Sequences were aligned using Muscle in Geneious 6.1.8 (Kearse et al. 2012). As a first stage, we analysed these sequences in taxon-rich datasets

for the tribes Herposiphonieae, Pterosiphonieae, Polysiphonieae and Streblocladieae to verify that the target species were monophyletic. Based on the resulting trees, we selected all sequences corresponding to the species (or group of closely related species) that are the focus of this paper. These datasets were analyzed species by species using the unweighted pair group method with arithmetic mean (UPGMA). For two complexes of non-sister species in our initial taxon-rich trees (*Symphyocladia dendroidea* and *Herposiphonia tenella*), we included wider species sampling considering the available data for the respective genera (Table S1). We performed maximum likelihood (ML) analyses separately for each of the two genera using RAxML 8.1.X (Stamatakis 2014). GTR-Gamma was used as the nucleotide model and branch support was estimated with 1000 bootstrap replicates. Three species of *Xiphosiphonia* and *Dipterosiphonia* were selected as the respective outgroups for the *Symphyocladia* and *Herposiphonia* trees based on our phylogenomic analyses of the major lineages of the Rhodomelaceae (Díaz-Tapia et al. 2017b).

RESULTS

The taxonomy of several of the studied species is complex and details are provided in Supplement 1.

Ophidocladus simpliciusculus

Ophidocladus simpliciusculus was collected in four out of the six world regions where it has been reported (Fig. 1a). The UPGMA analyses included 15 newly determined *rbc*L sequences and two downloaded from GenBank (Table S1). Sequences comprised four haplotypes (Fig. 1b): haplotype 1, seven samples from Europe (Atlantic and Mediterranean); haplotype 2, six samples from Australia; haplotype 3, one sample from South Africa; and haplotype 4, three samples from Brazil. The South African sample

182 differed by only 0.1% (1 bp) from the Australian samples, while Brazilian samples were
183 the most divergent (up to 0.8% and 11 bp) from samples from other regions. Our results
184 indicate that *O. simpliciusculus* has a unique *rbc*L haplotype in each region, but it has a
185 strong phylogeographic structure.

186

187 *Lophosiphonia obscura*

188 *Lophosiphonia obscura* has been reported in the Atlantic and Indo-Pacific and
189 sequences are available from Europe and Australia (Fig. 1c). Furthermore, our dataset
190 also included *Polysiphonia hemisphaerica* from Norway and *P. boldii* from Texas, USA
191 which may be conspecific as suggested by the low *rbc*L divergence with *L. obscura* (see
192 Supplement 1). We analysed six newly determined *rbc*L sequences and two
193 downloaded from GenBank for *Lophosiphonia obscura, Polysiphonia hemisphaerica*
194 and *P. boldii* (Table S1). Four haplotypes were found (Fig. 1d): 1) four samples from
195 Spain (Atlantic and Mediterranean) and Norway; 2) a sample from the United
196 Kingdom; 3) a sample from USA; and 4) two samples from Australia. Atlantic samples
197 differed by 0.1-0.2% (1-2 bp), while Australian samples were 0.7-0.9% (8-11) divergent
198 from the Atlantic samples. Our results indicate that the lineage formed by these three
199 taxa is moderately variable in the North Atlantic, and is clearly separated from the
200 Australian populations.

201

202 *Polysiphonia villum*

203 Molecular data were obtained from two regions where *Polysiphonia villum* (as *P.*
204 *scopulorum* var. *villum*, see Supplement 1) had previously been reported (Fig. 1e).
205 Furthermore, it was also sampled in Spain, the French Mediterranean and Australia,

where it is here newly recorded. The 13 sequences determined for *P. villum* and the two downloaded from GenBank (Table S1) belong to three haplotypes (Fig. 1f): 1) four samples from Australia, 2) three samples from Brazil and 3) eight samples from the North Atlantic (North Carolina, Spain and France). Australian samples were 0.5-0.6% (4-7 bp) divergent from the Atlantic samples and the Brazilian sequences differed by 0.2% (2 bp) from the North Atlantic samples. Thus, *P. villum* shows a clear phylogeographic structure.

Polysiphonia devoniensis

Our dataset included samples from the previously recorded distribution in Atlantic Europe, as well as from the northwestern Mediterranean (Italy and France), the Adriatic Sea (Italy) and Victoria (Australia), from where *P. devoniensis* is here recorded for the first time (Fig. 2a, Table S1). Furthermore, sequences of *P. kapraunii* from North Carolina were also included in our dataset (see Supplement 1). Analyses including an *rbc*L sequence of *P. kapraunii* from GenBank and 21 newly determined sequences of *P. devoniensis* (Table S1) showed eight haplotypes (Fig. 2b). One haplotype was found in the northwestern Mediterranean, Atlantic Spain and Australia; one occurred in the Adriatic Sea and the northwestern Mediterranean; and six haplotypes were each represented by a single sample (two from Wales, two from the Adriatic Sea, one from the northwestern Mediterranean and one from North Carolina). The North Carolina sample identified as *P. kapraunii* was 0.2-0.3% (3-4 bp) divergent from two of the European samples (PD301 and PD2430). These three samples differ from the others by sequence divergences of 1-1.4% (12-18 bp), while divergence between the other five haplotypes is 0.1-1% (1-9 bp). The lineage formed by samples assigned to *P.*

devoniensis and *P. kapraunii* has a high genetic diversity and the distribution of haplotypes lacks geographic structure.

Symphyocladia dendroidea complex

Sequences of *Symphyocladia dendroidea* are available from most of the previously known distribution (British Columbia, California, Chile, Peru, Japan and the Mediterranean). Some of these sequences were labelled as *Pterosiphonia tanakae* (see Supplement 1). Furthermore, we collected this species in a Galician marina (northwestern Spain) and in Australia (Victoria), where it is here recorded for the first time (Fig. 2c).

The *rbc*L data for *Symphyocladia dendroidea* reveal cryptic diversity in the Americas, as specimens from Peru and Chile and specimens from British Columbia (here referred as *S. dendroidea* 2) do not constitute a clade (Fig. S1, Table S1). In addition to these regions, both were recorded in California. *S. dendroidea* is resolved as sister to *S. parasitica* with high support, while *S. dendroidea* 2 is placed in a moderately supported clade together with *S. brevicaulis* and *S. baileyi* (Fig. S1). Molecular data show that *S. dendroidea* has a wide distribution in the Pacific and occurs in some European locations, while *S. dendroidea* 2 is apparently restricted to Pacific North America.

In total, 28 *rbc*L sequences were analysed for *Symphocladia dendroidea* (some sequences labelled as *S. tanakae*, see Supplement 1) including 13 newly determined and 15 downloaded from GenBank (Table S1). The UPGMA dendrogram shows seven haplotypes (Fig. 2d) of which five comprise samples from Pacific South America, one includes samples from Australia and Japan, and the other consists of samples from

California and Europe. Maximum variability between South American haplotypes is 0.6% (8 bp), and sequence divergence between them and the two other clades is 0.4-1% (3-9 bp). These levels of *rbc*L variation suggest that this entity may consist of multiple species or highly differentiated populations.

Xiphosiphonia pennata complex*,* including *X. pinnulata* and *Symphyocladia spinifera*

Xiphosiphonia pennata has been reported in the Atlantic and Indo-Pacific (Fig 2e) and this morphological species is a complex of at least three non-sister species. Their taxonomy has been resolved with the clarification of the identity of *X. pinnulata* and *S. spinifera* that have been misidentified as *X. pennata* (see Supplement 1).

At present, 39 *rbc*L sequences (16 newly determined and 23 downloaded from GenBank) are available for *Symphyocladia spinifera* from California, Pacific South America, Australia and Korea (Fig. 2f). The UPGMA shows 10 haplotypes of which four correspond to Korean samples, four to Peruvian samples, one to Australian samples and one to a Washingtonian sample (Fig. 2f). Sequence divergence among haplotypes is up to 0.9% (7 bp). Australian samples match the morphological concept of *Xiphosiphonia pennata*, but our molecular data reveal that none of them grouped with the European *X. pennata* but instead are mostly closely related to *S. spinifera*. Therefore, *X. pennata* should be excluded from the recorded Australian flora and replaced by *S. spinifera*. Interestingly, all the Australian samples belong to a single haplotype, which contrasts with the four haplotypes found in both Peru and Korea.

Xiphosiphonia pinnulata sequences were resolved as three haplotypes of which two were found in European samples and one in Brazilian samples (Fig. 2g). Sequence divergence among them is up to 0.7% (9 bp) and between the two European clades is up

278 to 0.3% (3 bp). *X. pennata* was only found in the Atlantic Iberian Peninsula. Therefore,
279 the widely reported *X. pennata* (as *Pterosiphonia pennata*) is apparently restricted to
280 European shores. *X. pinnulata* is restricted to the Atlantic, where it has a strong
281 phylogeographic structure with divergences that may even suggest they are separate
282 species. *S. spinifera* is restricted to the Pacific and it has a high genetic variability
283 between regions and within regions in Korea and South America.

284

285 *Herposiphonia tenella* complex

286 In total, 27 *rbc*L sequences were obtained for samples morphologically identified as
287 *Herposiphonia tenella* from Europe, North America and Queensland (Australia) (Fig.
288 3). They were analysed together with the available *rbc*L data for the genus (15 species).
289 The phylogeny resolved *H. tenella* in seven lineages, four from the Atlantic and three
290 from Queensland (Fig. 4). Sequence divergence among the lineages was at least 1.9%,
291 while divergence within them was up to 0.7%. Only two of these lineages were resolved
292 as sisters (1.9-2.1% sequence divergence), while the others, despite morphological
293 similarities, were more closely related to other lineages. Thus, *Herposiphonia tenella* is
294 a large species complex that requires taxonomic revision to better understand its cryptic
295 diversity and the distribution of the resulting new species. Its type locality is in the
296 Mediterranean, where three of the four European lineages were collected.

297

298 DISCUSSION

299 *Species complexes*

300 In this work we detected several complexes of non-sister species (*Xiphosiphonia*
301 *pennata*, *Symphyocladia dendroidea* and *Herposiphonia tenella*). Also, we found

302 species-level taxa that represent monophyletic lineages containing several haplotypes
303 that in most cases are distributed in accordance with geographic regions. They could
304 also be classified as species complexes, as sequence divergences between haplotypes
305 are often large (up to 1.4%), possible evidence for multiple species. Interpretations of
306 genetic divergences when delineating species boundaries vary among authors. For
307 example, *Melanothamnus harveyi/japonicus* and other closely related species have been
308 interpreted as a single species with an intraspecific variability in the *rbc*L gene ≤ 2.1 %
309 (McIvor et al. 2001, as *Polysiphonia*) or as a species complex in which interspecific
310 variability in the *rbc*L gene is 0.3-0.7 % (Savoie and Saunders 2015, as *Neosiphonia*).
311 The species concept has been hotly debated, but there is a general consensus that
312 speciation is a process that takes place when gene flow is interrupted as a consequence
313 of isolation of populations (Coyne et al. 1988, Leliaert et al. 2014). In the present work,
314 assessing species boundaries was not always straightforward, and we used information
315 based on genetic divergences, species distribution and, in one lineage, interbreeding
316 experiments described by Rueness (1973). The first scenario we encountered consists of
317 species with a variety of haplotypes found in distant locations. Genetic isolation by
318 distance seems obvious considering our data and, in some cases, where the divergences
319 between distant populations are relatively large (≤ 0.9%), one might consider them
320 different species (*Ophidocladus simpliciusculus* from Europe *vs.* Brazil *vs.*
321 Australia/South Africa, *Lophosiphonia obscura* from the North Atlantic *vs.* Australia,
322 *Polysiphonia villum* from the Atlantic *vs.* Australia, and *Xiphosiphonia pinnulata* from
323 Brazil *vs.* Europe). However, the low number of samples in some regions or species, as
324 well as the lack of sampling in other regions where these species were recorded or may
325 be still unknown precludes a definitive conclusion. Perhaps the observed large sequence
326 divergences between the lineages within these species would be less evident with larger

datasets. A second scenario is similar to the former, as it consists of species with a variety of haplotypes, but in this case several haplotypes share the same distribution (*P. devoniensis*, *Symphyocladia dendroidea, S. spinifera*). Thus, despite *rbc*L divergences among some haplotypes (≤ 1.4%) being even larger than in the previous group (≤ 0.9%), whether they are at present reproductively isolated and should be considered as distinct species is uncertain. Interbreeding experiments may assist to clarify if these species should be considered as distinct or not. While successful reproduction may have multiple interpretations (Leliaert et al. 2014), unsuccessful reproduction indicates reproductive incompatibility. The third scenario we found in this work is represented by *Lophosiphonia obscura* whose eastern and western Atlantic populations have low genetic distances (0.1-0.2 %) in the *rbc*L gene, and also in the more variable *cox*1 marker (0.6-1.2 %, HQ412544-5 as *P. hemisphaerica* and *P. boldii*, MF094025). Despite this, crossing experiments demonstrate that isolates from Texas and from Norway fail to produce fully fertile progeny (Rueness 1973, as *P. hemisphaerica* and *P. boldii*). This suggests that these two populations are reproductively isolated, and that divergent selection may be acting on these populations but *rbc*L and *cox*1 gene sequences do not reflect this isolation (Nosil et al. 2009). These three scenarios show different evolutionary patterns even among closely related species (e.g. *P. villum vs. P. devoniensis*). Therefore, application of genetic distances in delineating species boundaries should be evaluated on a case by case basis. While these are very interesting issues from a taxonomic perspective, they are not the focus of this paper. From a phylogeographic point of view, whether these closely related monophyletic lineages are different species or not is of minor importance, because either way they share a common ancestor from which several genetic entities evolved.

352 *Phylogeographic patterns*

353 The paradox between expected dispersal limitation (Santelices 1990, Kilan and Gaines
354 2003) and wide reported species distributions led us to hypothesize that such widely
355 distributed species would either have strong phylogeographic structure or were spread
356 by humans. Our results confirmed these hypotheses and exposed a third scenario, where
357 the morphologically defined species was in fact a complex of non-sister cryptic species.

358 Three of the species exhibit genetic variability with clear phylogeographic structure
359 in Australia, and the North and South Atlantic (*Ophidocladus simpliciusculus*,
360 *Lophosiphonia obscura* and *Polysiphonia villum*). This result is not unexpected
361 considering that genetic divergence is promoted by the isolation among populations
362 separated by large geographic distances (Palumbi 1994). However, the observed genetic
363 divergence is relatively low (≤ 0.9%) considering that Australia and the North and
364 South Atlantic have been separated since about 80 My (Jordan et al. 2016). Therefore,
365 rather than this genetic divergence resulting from an 80 My old vicariant evolution,
366 long-distance dispersal processes acting on a common ancestor and subsequent
367 divergence into differentiated populations are invoked to explain the observed patterns.
368 Mechanisms responsible for this long-distance dispersal are obscure considering that
369 these species either occur in coastal lagoons/estuaries or on sand-covered rocks where
370 buoyant macroalgae that can act as rafts are rare (Airoldi 1998, Díaz-Tapia et al.
371 2013a). Molecular data have provided evidence for long-distance dispersal in other red
372 algal species but mechanisms remain unknown (Zuccarello et al. 2002a, Fraser et al.
373 2013). The genetic separation among geographically distant lineages may indicate that
374 long-distance dispersal occurs at a low rate. Alternatively, density-dependent processes
375 are involved and once a population colonizes a new region it prevents the establishment
376 of latecomers (Waters et al. 2013). Furthermore, available data for the three species

377 mentioned above indicate different evolutionary histories and/or dispersal paths. For
378 instance, in *L. obscura* and *P. villum* the largest sequence divergences are between
379 Australian and Atlantic populations, whereas in *O. simpliciusculus* the Australian
380 haplotype is relatively close to South African and European haplotypes but the
381 divergence across the Atlantic (Brazil *vs.* Europe) is much larger.

382 Several species showed a diversity of haplotypes sharing the same region: the
383 Pacific *Symphyocladia spinifera* and *S. dendroidea*, as well as the Atlantic *Polysiphonia*
384 *devoniensis*. The origin of this diversity must be related to processes of isolation that led
385 to genetic differentiation, followed by local dispersal events. As for *Ophidocladus*
386 *simpliciusculus*, *Lophosiphonia obscura* and *Polysiphonia villum*, dispersal mechanisms
387 for *S. spinifera* and *P. devoniensis* are unknown. In contrast, *S. dendroidea* has been
388 reported growing on stranded holdfasts of the floating alga *Durvillaea antarctica*
389 (Macaya et al. 2016, López et al. 2017, 2018), which could contribute to dispersal after
390 genetic differentiation influenced its genetic structure.

391 The disjunct distribution of a second group of species (*Polysiphonia devoniensis*
392 and *Symphyocladia dendroidea*) can be explained by human-mediated introduction
393 events. The human transport of species from native (donor) to introduction (recipient)
394 regions causes the rapid expansion of species' distribution and alters natural
395 phylogeographic patterns (Straub et al. 2016). The discovery of *P. devoniensis* in
396 Victoria (Australia), exhibiting a single haplotype that is also present in Europe,
397 suggests that this species has been introduced into this country from Europe, possibly
398 Atlantic Spain or the NW Mediterranean. *Symphyocladia dendroidea* was recorded as
399 an introduced species in the French Mediterranean in 2005 (Boudouresque and
400 Verlaque 2008, as *P. tanakae*) and our recent discovery of the same haplotype in a
401 marina in Atlantic Spain probably represents a secondary introduction and suggests that

402 the species is spreading in Europe via hull fouling. The presence of several genetically
403 separated lineages of *S. dendroidea* in Pacific South America contrasts with the
404 occurrence of a single haplotype in Japan, Australia and California. Japan and Australia
405 have the same haplotype, suggesting that one or both populations could be introduced.
406 Genetic diversity of seaweeds in the introduced regions is either similar or reduced
407 relative to the native area (McIvor et al. 2001, Provan et al. 2008, Voisin et al. 2005,
408 Geoffroy et al. 2016). The finding of diverse haplotypes in the introduced region is
409 indicative of an introduction involving several haplotypes or multiple introductions,
410 depending on the phylogeographic structure in the native area (McIvor et al. 2001,
411 Voisin et al. 2005, Geoffroy et al. 2016). A single haplotype of both *S. dendroidea* and
412 *P. devoniensis* has been detected in the areas where the introduction of these species is
413 certain, suggesting that their introduction is the result of a single event in which a single
414 haplotype was involved. However, much more complex scenarios could explain the
415 observed patterns and a better understanding of the phylogeographic patterns in native
416 and introduced areas would be needed to elucidate the introduction dynamics.

417 The third group of species analyzed here involved species complexes of non-sister
418 cryptic species (*Xiphosiphonia pennata* including *X. pinnulata* and *Symphyocladia*
419 *spinifera*; *S. dendroidea;* and *Herposiphonia tenella*). In both cases species found in the
420 Atlantic and Pacific basins differ, but in addition several species were found with
421 overlapping distributions in some regions of each basin. Therefore, the distribution of
422 these widely reported species is much narrower than previously thought. Cryptic algal
423 species often involve a group of morphologically similar species that are genetically
424 differentiated, but resolve as a monophyletic group (Zuccarello et al. 2002a, Won et al.
425 2009, Payo et al. 2013). However, examples of non-monophyletic cryptic "species"
426 have also been documented in the red algae (Zuccarello et al. 2018). Morphological

similarity among non-monophyletic groups of cryptic species can be explained by evolutionary convergence, morphological stasis or developmental constraints (Leliaert et al. 2014, Zuccarello et al. 2018). *X. pennata, S. spinifera* and *S. dendroidea* are placed in a tribe (Pterosiphonieae) with high morphological variation ranging from filiform to foliose species (Díaz-Tapia et al. 2017b). The body plan of both species is filiform, among the simplest observed in the tribe, and morphological stasis is a plausible explanation for their similarity. In the tribe Herposiphonieae all species are very similar in morphology, with limited differences in their body plans (Díaz-Tapia et al. 2017b) and the cryptic diversity in *Herposiphonia tenella* might result from morphological stasis or developmental constraints.

Understanding the processes underlying phylogeographic patterns requires the study of numerous specimens from across the entire distribution of the species. In this regard, we recognize important limitations in our work that prevent us from fully elucidating causes of the observed phylogeographic patterns, leading to some tentative conclusions about the potentially introduced status of some of the analysed populations. However, most of the species treated here are rare in all or part of their known distribution range so improving the datasets would be very difficult. For example, *Lophosiphonia obscura*, despite being widely reported, is very rare in the regions here studied: the sample from the UK used in this study is the first one collected since 1970 (Maggs and Hommersand 1993). In Spain, we found it only once in the Atlantic and once in the Mediterranean, and the species is here recorded for only the third time in Australia. Our work should thus be interpreted as one of the first attempts to understand phylogeographic patterns of widely distributed red algal species. Even though their evolutionary history is not well known, our analyses provide clear examples of 1) species with wide distributions and strong phylogeographic structure that reflects the

geographical distance; 2) species with a broad distribution that can be only explained by human-mediated transport; and 3) species complexes in which non-monophyletic cryptic diversity has been found . This study indicates that widely distributed species are the exception in red algae, except when they have been spread by humans.

Acknowledgements

We thank Francis Bunker, Fabio Rindi, Iara Chapuis, Jan Rueness and Max Hommersand for providing samples and Joana Costa, as well as other researchers included in Table S1, for assistance in the field. We thank Marisa Pasella for carrying out part of the molecular work. PDT acknowledges support by the postdoctoral program "Axudas de apoio á etapa de formación posdoutoral", as well as the funding program "Axudas para a consolidación e estruturación de unidades de investigación competitivas do SUG", grant GPC2015/025 (Xunta de Galicia). Field work in the Mediterranean Sea was funded by a grant awarded to PDT by the British Phycological Society. Part of the research presented here was funded through the Australian Biological Resources Study (ABRS), including participation in a Bush Blitz expedition, a Bush Blitz Strategic Taxonomy Grant (TTC216-03) and a National Taxonomy Research Grant (RFL213-08). Sampling in Western Australia, Queensland and Tasmania was made possible through funding from the Holsworth Wildlife Research Endowment. ECM acknowledges support by the Chilean Millennium Initiative (NC120030) grant.

References

Airoldi, L. 1998. Roles of disturbance, sediment stress, and substratum retention on spatial dominance in algal turf. *Ecology* 79:2759–70.

476 Boudouresque, C.-F. & Verlaque, M. 2008. Biological pollution in the Mediterranean
477 Sea: invasive versus introduced macrophytes. *Mar. Pollut. Bull.* 44:32-8.

478 Bustamante, D.E., Won, B.Y. & Cho, T.O. 2014. *Polysiphonia dokdoensis* sp. nov.
479 (Rhodomelaceae, Ceramiales) based on a population previously known as *Polysiphonia*
480 *atlantica sensu* Kim and Lee from Korea. *Bot. Mar.* 57:281-9.

481 Coyne, J.A., Orr, H.A., & Futuyma, D.J. 1988. Do we need a new species concept?
482 *Syst. Biol.* 37:190–200.

483 Destombe, C., Godin, J., Lefebvre, C., Dehorter, O., Vernet, Ph. 2009. Differences in
484 dispersal abilities of haploid and diploid spores of *Gracilaria verrucosa* (Gracilariales,
485 Rhodophyta). *Bot. Mar.* 35:93-8.

486 Díaz-Tapia, P. & Bárbara, I. 2013. Seaweeds from sand-covered rocks of the Atlantic
487 Iberian Peninsula. Part. 1. The Rhodomelaceae (Ceramiales, Rhodophyta). *Cryptog.*
488 *Algol.* 34:325-422.

489 Díaz-Tapia, P., Bárbara, I. & Díez, I. 2013a. Multi-scale spatial variability in intertidal
490 benthic assemblages: Differences between sand-free and sand-covered rocky habitats,
491 *Estuar. Coast. Shelf Sci.* 133:97-108.

492 Díaz-Tapia, P., Kim, M.S., Secilla, A., Bárbara, I., Cremades, J. 2013b. Taxonomic
493 reassessment of *Polysiphonia foetidissima* (Rhodomelaceae, Rhodophyta) and similar
494 species, including *P. schneideri*, a newly introduced species in Europe. *Eur. J. Phycol.*
495 48:345-62.

496 Díaz-Tapia, P., Bárbara, I., Cremades, J., Verbruggen, H. & Maggs C.A. 2017a. Three
497 new cryptogenic species in the tribes Polysiphonieae and Streblocladieae
498 (Rhodomelaceae, Rhodophyta). *Phycologia* 56:605-23.

499 Díaz-Tapia, P., Maggs, C. A., West, J. A. & Verbruggen, H. 2017b. Analysis of
500 chloroplast genomes and a supermatrix inform reclassification of the Rhodomelaceae
501 (Rhodophyta). *J. Phycol.* 53:920–37.

502 Fraser, C.I., Nikula, R., Spencer, H.G., & Waters, J.M. 2009. Kelp genes reveal effects
503 of subantarctic sea ice during the Last Glacial Maximum. *Proc. Natl. Acad. Sci. U.S.A.*
504 106:3249-53.

505 Fraser, C.I., Zuccarello, G.C., Spencer, H.G., Salvatore, L.C., Garcia, G.R. & Waters,
506 J.M. 2013. Genetic affinities between trans-oceanic populations of non-buoyant
507 macroalgae in the high latitudes of the southern hemisphere. *PLoS ONE* 8:e69138.

508 Freshwater, D.W. & Rueness, J. 1994. Phylogenetic relationships of some European
509 *Gelidium* (Gelidiales, Rhodophyta) species based upon *rbc*L nucleotide sequence
510 analysis. *Phycologia* 33:187-94.

511 Geoffroy, A., Destombe, C., Kim, B, Mauger, S., Raffo, M.P., Kim, M.S. & Le Gall, L.
512 2016. Patterns of genetic diversity of the cryptogenic red alga *Polysiphonia morrowii*
513 (Ceramiales, Rhodophyta) suggest multiple origins of the Atlantic populations. *Ecol.*
514 *Evol.* 6:5635-47.

515 Guiry, M.D. & Guiry, G.M. 2018. *AlgaeBase*; World-Wide Electronic Publication,
516 National University of Ireland: Galway, UK, 2018; Available online:
517 http://www.algaebase.org (accessed on 6 January 2018).

518 Jackson, J.B.C. 1974. Biogeographic consequences of eurytopy and stenotopy among
519 marine bivalves and their evolutionary significance. *Amer. Nat.* 108:541–60.

520 Jordan, S.M.R., Barraclough T.G. & Rosindell, J. 2016. Quantifying the effects of the
521 break up of Pangaea on global terrestrial diversification with neutral theory. *Phil. Trans.*
522 *R. Soc. B* 371:20150221.

523 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton,
524 S, Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. &
525 Drummond, A. 2012. Geneious Basic: An integrated and extendable desktop software
526 platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–9.

527 Kinlan, B.P. & Gaines, S.D. 2003. Propagule dispersal in marine and terrestrial
528 environments: a community perspective. *Ecology* 84:2007–20.

529 Leliaert F., Verbruggen H., Vanormelingen P., Steen, F., López-Bautista J.M.,
530 Zuccarello, G.C. & De Clerck O. 2014. DNA based species delimitation in algae. *Eur.*
531 *J. Phycol.* 49: 179–96.

532 López, B.A., Tellier, F., Retamal-Alarcón, J.C., Pérez-Araneda, K., Fierro, A.O.,
533 Macaya, E.C., Tala, F. & Thiel, M. 2017. Phylogeography of two intertidal seaweeds,
534 *Gelidium lingulatum* and *G. rex* (Rhodophyta: Gelidiales), along the South East Pacific:
535 patterns explained by rafting dispersal? *Mar. Biol*. 164:188.

536 López, B.A., Macaya E. C., Rivadeneira M., Tala F., Tellier F. & Thiel M. 2018.
537 Epibiont communities on stranded kelp rafts of *Durvillaea Antarctica* (Fucales,
538 Phaeophyceae) – do positive interactions facilitate range extensions? *J. Biogeogr,* in
539 press.

540 Macaya, E.C. & Zuccarello, G.C. 2010. Genetic structure of the giant kelp *Macrocystis*
541 *pyrifera* along the southeastern Pacific. *Mar Ecol Prog Ser* 420:103–12.

542 Macaya, E.C., López, B., Tala, F., Tellier, F. & Thiel, M. 2016. Float and raft: role of
543 buoyant seaweeds in the phylogeography and genetic structure of non-buoyant
544 associated flora. *In* Hu, Z.M. & Fraser, C.I. [Eds.] *Seaweed phylogeography*. Springer,
545 Dordrecht, pp. 97–130.

546 Maggs, C.A. & Hommersand, M.H. 1993. *Seaweeds of the British Isles. Volume 1.*
547 *Rhodophyta. Part 3A. Ceramiales*. HMSO, London, 444 pp.

548 Mamoozadeh, N.R. & Freshwater, D.W. 2011. Taxonomic notes on Caribbean
549 *Neosiphonia* and *Polysiphonia* (Ceramiales, Florideophyceae): five species from
550 Florida, USA and Mexico. *Bot. Mar.* 54:269-92.

551 McIvor, L., Maggs, C.A., Provan, J. & Stanhope, M.J. 2001. *rbc*L sequences reveal
552 multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Mol. Ecol.*
553 10: 911–9.

554 Nosil, P., Funk, D.J. & Ortiz-Barrientos, D. 2009. Divergent selection and
555 heterogeneous genomic divergence. *Molec. Ecol.* 18:375–402.

556 Palumbi, S.R. 1994. Genetic divergence, reproductive isolation, and marine speciation.
557 *Annu. Rev. Ecol Syst.* 25:547-72.

558 Payo, D.A., Leliaert, F., Verbruggen, H., D'hondt, S., Calumpong, H. P. & De Clerck,
559 O. 2013. Extensive cryptic species diversity and fine-scale endemism in the marine red
560 alga *Portieria* in the Philippines. *Proc. R. Soc. Lond. B Biol. Sci.* 280: 20122660.

561 Provan, J., Booth, D., Todd, N.P., Beatty, G.E. & Maggs, C.A. 2008. Tracking
562 biological invasions in space and time: elucidating the invasive history of the green alga
563 *Codium fragile* using old DNA. *Divers. Distributions* 14:343–54.

564 Riginos, C., Douglas, K. E., Jin, Y., Shanahan, D. F. & Treml, E.A. 2011. Effects of
565 geography and life history traits on genetic differentiation in benthic marine fishes.
566 *Ecography* 34:566-75.

567 Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds.
568 *Oceanogr. Mar. Biol. Ann. Rev.* 28:177-276.

569 Saunders, G.W. & McDevit, D.C. 2012. Methods for DNA Barcoding Photosynthetic
570 Protists Emphasizing the Macroalgae and Diatoms. *In* Kress, W. & Erickson, D. [Eds.]
571 *DNA Barcodes. Methods in Molecular Biology (Methods and Protocols)*. Humana
572 Press, Totowa, USA, pp. 207-22

573 Saunders, G.W. & Moore, T.E. 2013. Refinements for the amplification and sequencing
574 of red algal DNA barcode and RedToL phylogenetic markers: a summary of current
575 primers, profiles and strategies. *Algae* 28:31-43.

576 Savoie, A. M. & Saunders, G. W. 2015. Evidence for the introduction of the Asian red
577 alga *Neosiphonia japonica* and its introgression with *Neosiphonia harveyi* (Ceramiales,
578 Rhodophyta) in the Northwest Atlantic. *Mol. Ecol*. 24:5927–37.

579 Savoie, A.M. & Saunders, G.W. 2016. A molecular phylogenetic and DNA barcode
580 assessment of the tribe Pterosiphonieae (Ceramiales, Rhodophyta) emphasizing the
581 Northeast Pacific. *Botany* 94: 917-39.

582 Schneider, C.W., Quach, P.K. & Lane, C.E. 2017. A case for true morphological
583 crypsis: Pacific *Dasya anastomosans* and Atlantic *D. cryptica* sp. nov. (Dasyaceae,
584 Rhodophyta). *Phycologia* 56:359-68.

585 Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-
586 analysis of large phylogenies. *Bioinformatics* 30:1312–13.

587 Straub, S.C., Thomsen, M.S. & Wernberg, T. 2016. The dynamic biogeography of the
588 anthropocene: the speed of recent range shifts in seaweeds. *In* Hu, Z.-M. & Fraser, C.
589 [Eds.] *Seaweed Phylogeography*. Springer, Amsterdam, pp. 63–93.

590 Thomsen M.S., Wernberg T., South P.M. & Schiel D.R. 2016. Non-native seaweeds
591 drive changes in marine coastal communities around the world. In: *In* Hu, Z.M. &
592 Fraser, C.I. [Eds.] *Seaweed phylogeography*. Springer, Dordrecht, pp. 147-185.

593 Verbruggen, H. 2014. Morphological complexity, plasticity, and species diagnosability
594 in the application of old species names in DNA-based taxonomies. *J. Phycol.* 50:26-31.

595 Voisin, M., Engel, C.R. & Viard. F. 2005. Differential shuffling of native genetic
596 diversity across introduced regions in a brown alga: Aquaculture *vs*. maritime traffic
597 effects. *Proc. Natl. Acad. Sci. USA*, 102:5432–7.

598 Waters, J.M., Fraser, C.I. & Hewitt, G.M. 2013. Founder takes all: density-dependent
599 processes structure biodiversity. *Trends Ecol. Evol.* 28:78-85.

600 Williams, S.L. & Smith, J.E. 2007. A global review of the distribution, taxonomy, and
601 impacts of introduced seaweeds. *Annu. Rev. Ecol. Evol. Syst.* 38:327–59.

602 Womersley, H.B.S. 2003. *The marine benthic flora of southern Australia - Part IIID*
603 *Ceramiales - Delesseriaceae, Sarcomeniaceae, Rhodomelaceae.* Australian Biological
604 Resources Study and State Herbarium of South Australia, Canberra and Adelaide, 533
605 pp.

606 Won, B.Y., Cho, T.O. & Fredericq, S. 2009. Morphological and molecular
607 characterization of species of the genus *Centroceras* (Ceramiaceae, Ceramiales),
608 including two new species. *J. Phycol.* 45:227-50.

609 Zuccarello, G., West, J. & King, R. 1999. Evolutionary divergence in the *Bostrychia*
610 *moritziana*/*B. radicans* complex (Rhodomelaceae, Rhodophyta): molecular and
611 hybridization data. *Phycologia* 38:234–44.

612 Zuccarello, G.C., Sandercock, B. & West, J.A. 2002a. Diversity within red algal
613 species: variation in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and
614 *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies. *Eur.*
615 *J. Phycol.* 37:403-18.

616 Zuccarello, G. C., West, J. & Rueness, J. 2002b. Phylogeography of the cosmopolitan
617 red alga *Caulacanthus ustulatus* (Caulacanthaceae, Gigartinales). *Phycol. Res.* 50:163-
618 72.

619 Zuccarello, G.C. & West, J. 2003. Multiple cryptic species: molecular diversity and
620 reproductive isolation in the *Bostrychia radicans*/*B. moritziana* complex
621 (Rhodomelaceae, Rhodophyta) with focus on North American isolates. *J. Phycol.*
622 39:948–59.

623 Zuccarello, G.C., West J. A. & Kamiya, M. 2018. Non-monophyly of *Bostrychia*
624 *simpliciuscula* (Ceramiales, Rhodophyta): Multiple species with very similar
625 morphologies, a revised taxonomy of cryptic species. *Phycological Res.*, 66:100-7.

626

627 Figure legends

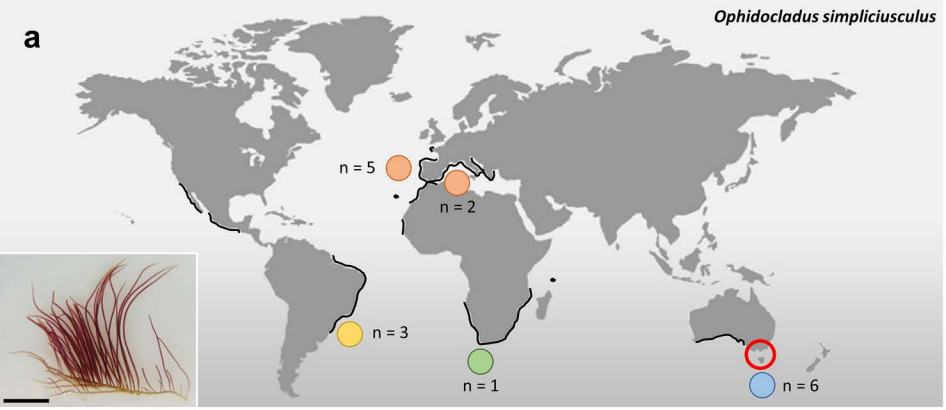
628 **Figure 1** Distribution and UPGMA unrooted distance phylogram based on *rbc*L
629 sequences of *Ophidocladus simpliciusculus* (a, b), *Lophosiphonia obscura* (as
630 *Polysiphonia hemisphaerica* and *P. boldii* in Norway and Texas, respectively, see
631 Supplement 1) (c, d) and *P. villum* (e, f). In figures a, c and e, circles indicate the
632 regions from which sequences are available and their colors indicate the distribution of
633 haplotypes. Areas outlined in red are regions where the species is recorded for the first
634 time. Coastline in black shows the reported distribution (Guiry and Guiry 2018). In
635 figure c, black coastlines represent the recorded distribution of *Lophosiphonia obscura,*
636 red line *P. hemisphaerica* and yellow line *P. boldii*. Scale bars: 5 mm in (a), 8 mm in
637 (c), 6 mm in (e).

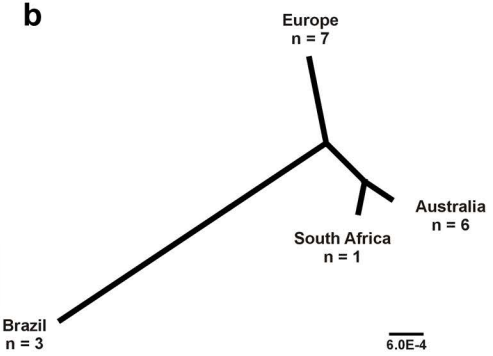
638 **Figure 2** Distribution and UPGMA unrooted distance phylogram based on *rbc*L
639 sequences of *Polysiphonia devoniensis* (as *P. kaprauni* in North Carolina) (a, b),
640 *Symphyocladia dendroidea* (c, d) and *S. spinifera/Xiphosiphonia pennata/X. pinnulata*
641 (e, f, g). Symbols are as in Figure 1, and pie divisions in Figures a, c and e indicate
642 proportions of each haplotype when multiple haplotypes were present. In figure e,
643 circles with white border correspond to *S. spinifera* and the ones with black border to *X.*
644 *pinnulata*. Encircled areas marked with red color are regions where the species are here
645 recorded for the first time. In figure a, black lines represent the recorded distribution of
646 *P. devoniensis* and red line the distribution of *P. kapraunii*. In figure c, black lines
647 represent the recorded distribution of *S. dendroidea*, blue lines the regions where it was
648 recorded as *S. tanakae*, red line the region where molecular data demonstrated that *S.*
649 *dendroidea* 2 is present, the asterisk indicates the area where both *S. dendroidea* 2 and
650 *S. tanakae* were reported based on molecular data, and plus symbols the regions from
651 which sequences of *S. dendroidea* 2 are available. In figure e, black lines represent the

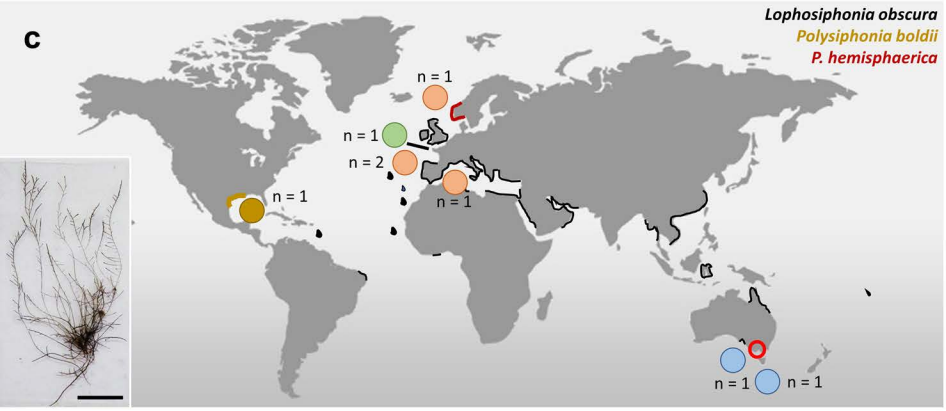
652 recorded distribution of *X. pennata*; yellow lines the regions where molecular data
653 showed the presence of *S. spinifera* instead *X. pennata*, red lines regions where only *X.*
654 *pinnulata* has been recorded based on molecular data and blue line the region where
655 both *X. pinnulata* and *X. pennata* have been recorded based on molecular data. Scale
656 bars: 6 mm in (a), 7 mm in (c), 4 mm in (e).

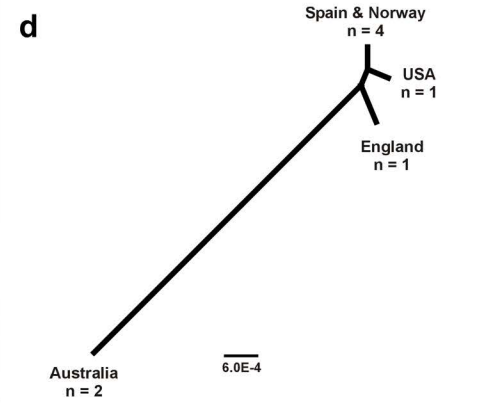
657 **Figure 3** Distribution of *Herposiphonia tenella*. Asterisks indicate the regions from
658 which sequences are available. Scale bar: 1 mm.

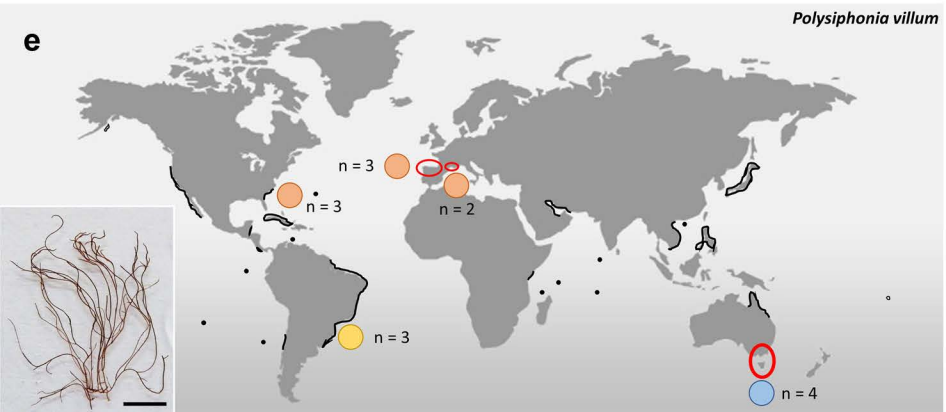
659 **Figure 4** RAxML tree based on *rbc*L sequences of the genus *Herposiphonia*. Samples
660 that morphologically correspond with *Herposiphonia tenella* are in bold. Bootstrap
661 values are indicated on the nodes when > 80. BE (Belize), CA (Canada), CI (Canary
662 Islands), FR (France), IN (India), IT (Italy), KO (Korea), NC (North Carolina), PO
663 (Portugal), QL (Queensland), SP (Spain), WA (Western Australia), VIC (Victoria).

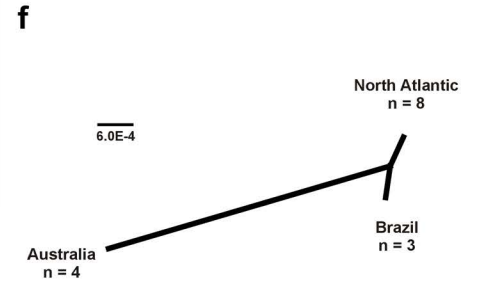

a
Ophidocladus simpliciusculus
n = 5
n = 2
n = 3
n = 1
n = 6

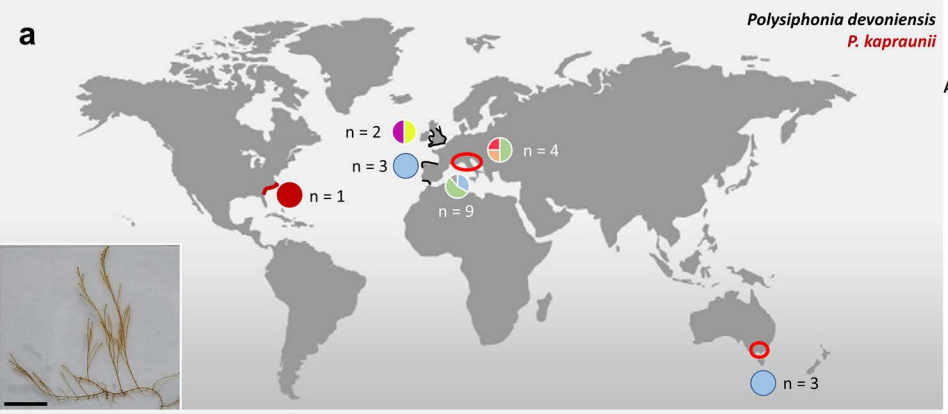

b
Europe
n = 7
Australia
n = 6
South Africa
n = 1
Brazil
n = 3
6.0E-4

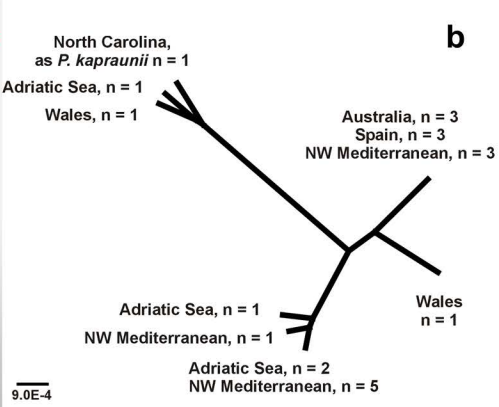

c
Lophosiphonia obscura
Polysiphonia boldii
P. hemisphaerica
n = 1
n = 1
n = 2
n = 1
n = 1
n = 1
n = 1


d
Spain & Norway
n = 4
USA
n = 1
England
n = 1
Australia
n = 2
6.0E-4

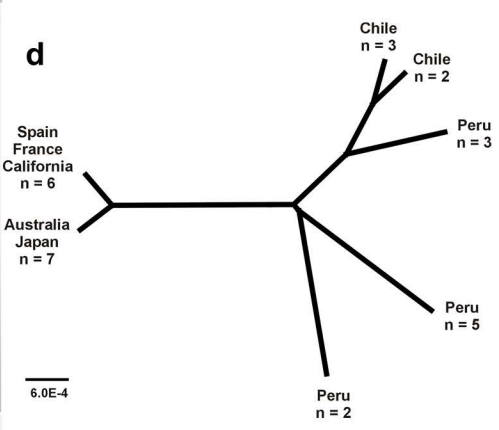

e
Polysiphonia villum
n = 3
n = 3
n = 2
n = 3
n = 4

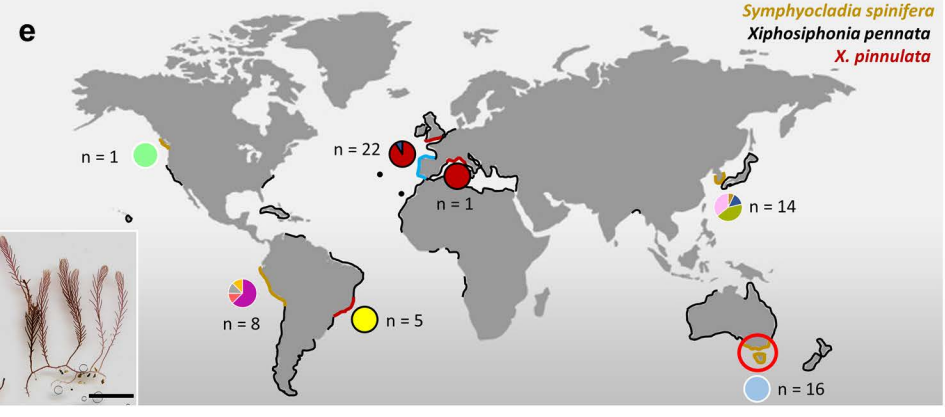

f
North Atlantic
n = 8
6.0E-4
Brazil
n = 3
Australia
n = 4

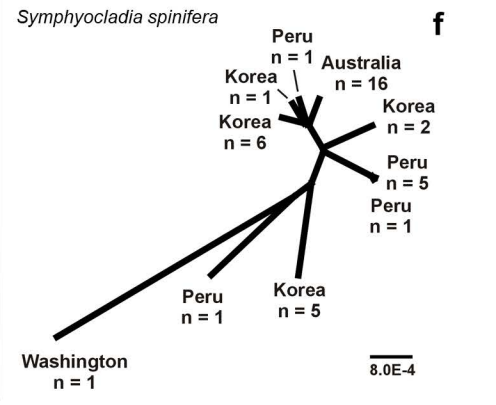

a
Polysiphonia devoniensis
P. kapraunii
n = 2
n = 3
n = 4
n = 1
n = 9
n = 3

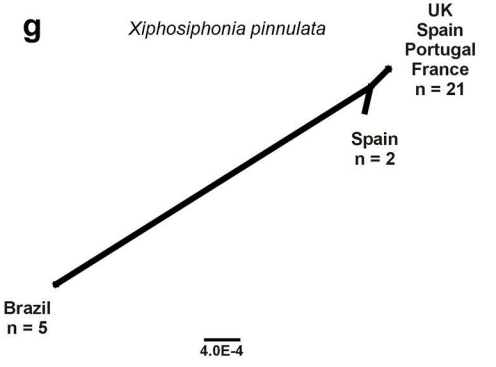

b
North Carolina, as P. kapraunii n = 1
Adriatic Sea, n = 1
Wales, n = 1
Australia, n = 3
Spain, n = 3
NW Mediterranean, n = 3
Wales n = 1
Adriatic Sea, n = 1
NW Mediterranean, n = 1
Adriatic Sea, n = 2
NW Mediterranean, n = 5
9.0E-4


c
Symphyocladia dendroidea
n = 3
n = 1
n = 2
n = 2
n = 10
n = 5
n = 5

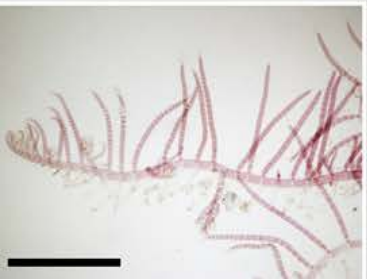

d
Chile n = 3
Chile n = 2
Peru n = 3
Spain France California n = 6
Australia Japan n = 7
Peru n = 5
Peru n = 2
6.0E-4

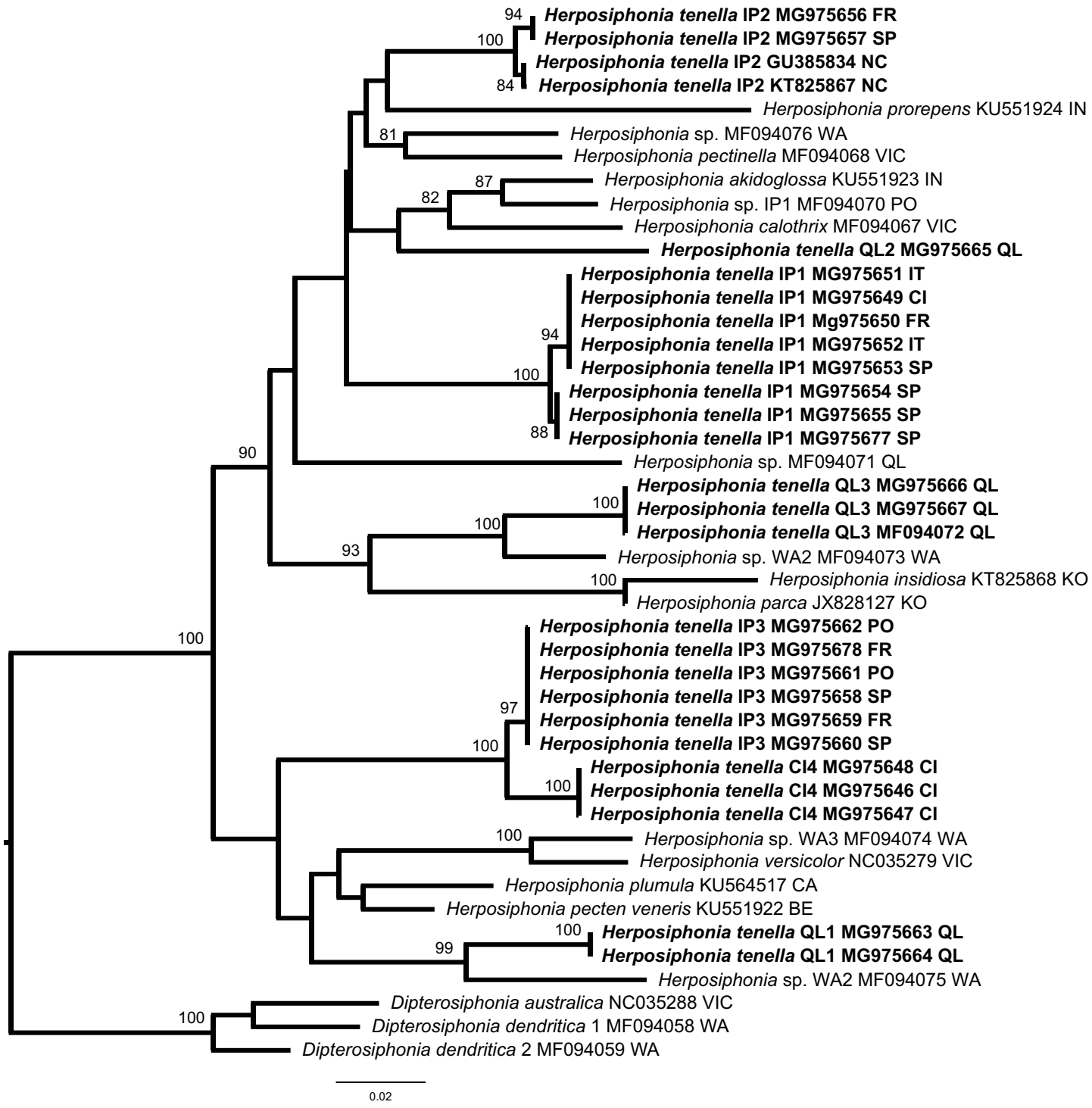

e
Symphyocladia spinifera
Xiphosiphonia pennata
X. pinnulata
n = 1
n = 22
n = 1
n = 14
n = 8
n = 5
n = 16


Symphyocladia spinifera
f
Peru n = 1
Australia n = 16
Korea n = 1
Korea n = 6
Korea n = 2
Peru n = 5
Peru n = 1
Peru n = 1
Korea n = 5
Washington n = 1
8.0E-4


g
Xiphosiphonia pinnulata
UK Spain Portugal France n = 21
Spain n = 2
Brazil n = 5
4.0E-4

Herposiphonia tenella




94
Herposiphonia tenella IP2 MG975656 FR
100
Herposiphonia tenella IP2 MG975657 SP
Herposiphonia tenella IP2 GU385834 NC
84
Herposiphonia tenella IP2 KT825867 NC
Herposiphonia prorepens KU551924 IN
81
Herposiphonia sp. MF094076 WA
Herposiphonia pectinella MF094068 VIC
87
Herposiphonia akidoglossa KU551923 IN
82
Herposiphonia sp. IP1 MF094070 PO
Herposiphonia calothrix MF094067 VIC
Herposiphonia tenella QL2 MG975665 QL
Herposiphonia tenella IP1 MG975651 IT
Herposiphonia tenella IP1 MG975649 CI
Herposiphonia tenella IP1 Mg975650 FR
94
Herposiphonia tenella IP1 MG975652 IT
100
Herposiphonia tenella IP1 MG975653 SP
Herposiphonia tenella IP1 MG975654 SP
Herposiphonia tenella IP1 MG975655 SP
88
Herposiphonia tenella IP1 MG975677 SP
90
Herposiphonia sp. MF094071 QL
Herposiphonia tenella QL3 MG975666 QL
100
Herposiphonia tenella QL3 MG975667 QL
100
Herposiphonia tenella QL3 MF094072 QL
93
Herposiphonia sp. WA2 MF094073 WA
100
Herposiphonia insidiosa KT825868 KO
Herposiphonia parca JX828127 KO
100
Herposiphonia tenella IP3 MG975662 PO
Herposiphonia tenella IP3 MG975678 FR
Herposiphonia tenella IP3 MG975661 PO
Herposiphonia tenella IP3 MG975658 SP
97
Herposiphonia tenella IP3 MG975659 FR
100
Herposiphonia tenella IP3 MG975660 SP
Herposiphonia tenella CI4 MG975648 CI
100
Herposiphonia tenella CI4 MG975646 CI
Herposiphonia tenella CI4 MG975647 CI
100
Herposiphonia sp. WA3 MF094074 WA
Herposiphonia versicolor NC035279 VIC
Herposiphonia plumula KU564517 CA
Herposiphonia pecten veneris KU551922 BE
100
Herposiphonia tenella QL1 MG975663 QL
99
Herposiphonia tenella QL1 MG975664 QL
Herposiphonia sp. WA2 MF094075 WA
Dipterosiphonia australica NC035288 VIC
100
Dipterosiphonia dendritica 1 MF094058 WA
Dipterosiphonia dendritica 2 MF094059 WA
0.02

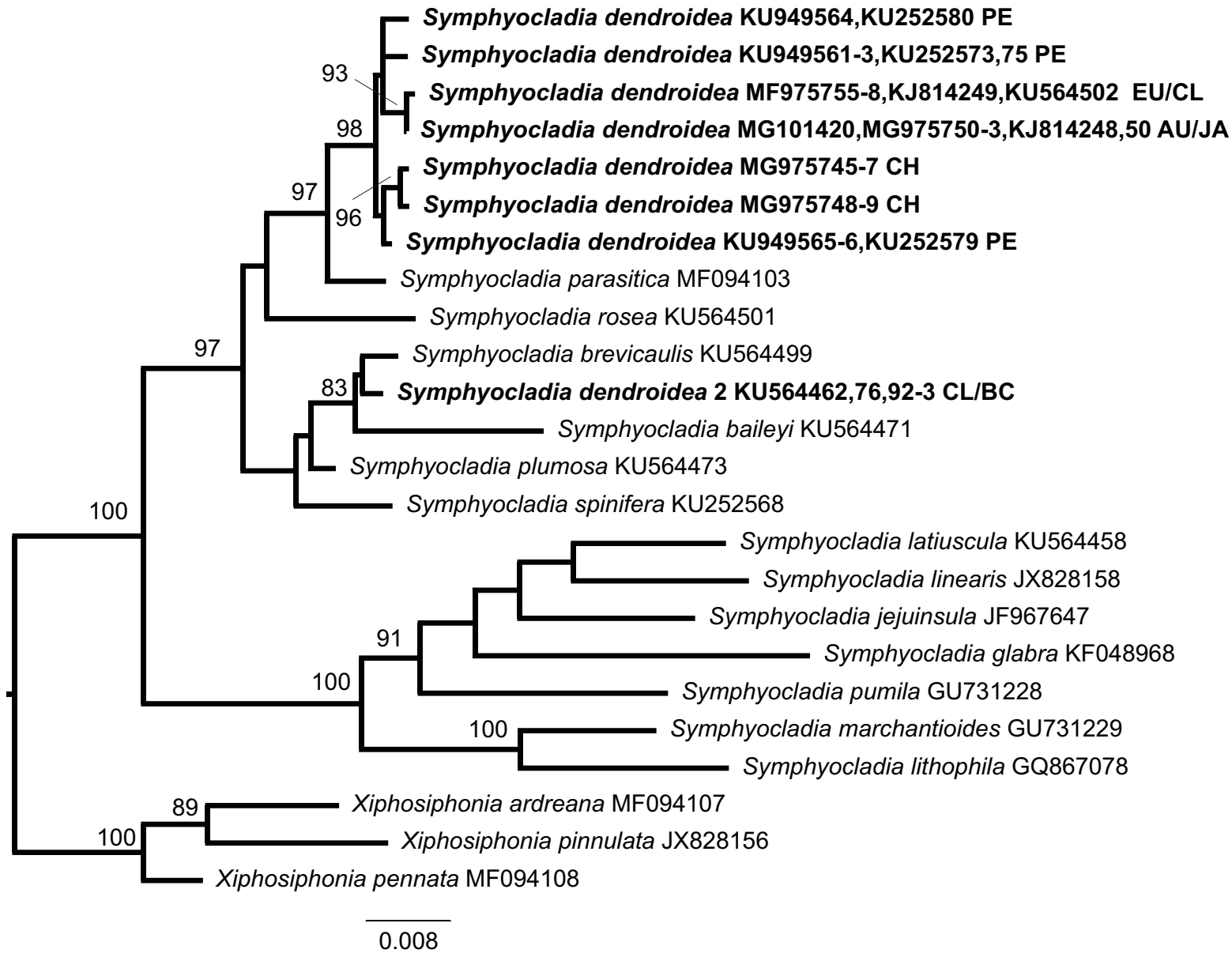


Figure S1 RAxML tree based on *rbc*L sequences of the genus *Symphyocladia. S. dendroidea* and *S. dendroidea* 2 are marked in bold. Bootstrap values are indicated on the nodes when > 80. AU (Australia), BC (British Columbia), CL (California), CH (Chile), EU (Europe), JA (Japan), PE (Peru).

Supplement S1. Taxonomic notes.

Lophosiphonia obscura, Polysiphonia hemisphaerica and P. boldii

The taxonomic history of *Lophosiphonia obscura* is complex (Silva et al. 1996, Díaz-Tapia and Bárbara 2013), involving several names that have been proposed in different regions. Among these names are *Polysiphonia hemisphaerica* from Scandinavia (Rueness 1971) and *P. boldii* from USA (Wynne and Edwards 1970). Rueness (1973) showed that male and female gametophytes of these two species successfully interbreed, producing carpospores from which tetrasporophytes germinate. However, the viability of tetraspores obtained from these tetrasporophytes was reduced. Thus, despite their high *cox*1 similarity, *P. boldii* and *P. hemisphaerica* failed to produce fully fertile progeny and the taxonomic status of these two species remained unresolved (Rueness 1973, 2010). We obtained *rbc*L sequences for the three taxa and our phylogenetic analysis revealed that they form a clade, are closely related (tree not shown), and whether they should be considered a single species is unclear. For simplicity, we are including these three currently recognized taxonomic species under the heading "*Lophosiphonia obscura*" but a taxonomic revision is required to clarify their taxonomic status.

Polysiphonia villum

Polysiphonia villum was originally described based on Mexican material (Agardh 1863) and its taxonomic status with regard to the Australian *P. scopulorum* was questioned (Cribb 1956). Based on its morphology, Hollenberg (1968) proposed to retain *P. villum* as a variety of *P. scopulorum*. However, molecular data showed that material attributed to these two taxa represents two different, non-monophyletic, molecular entities (Huisman et al. 2017). Consequently, here we apply the name *P. villum* to the molecular entity that matches American specimens (Stuercke and Freshwater 2008) and differs from Australian specimens (Huisman et al. 2017). Assessment of the correspondence of both *P. scopulorum* and *P. villum* with their respective type material has not been studied.

Polysiphonia devoniensis and P. kapraunii

Polysiphonia devoniensis was originally described from southern England (Maggs and Hommersand 1993) and subsequently recorded at other locations in Atlantic Europe (Díaz-Tapia and Bárbara 2013). More recently, *P. kapraunii* was described from North Carolina (Stuercke and Freshwater 2010). Our phylogenetic analysis revealed that these two entities form a clade, are closely related (tree not shown), and whether they should be considered a single species is unclear.

Symphyocladia dendroidea

Symphyocladia dendroidea was originally described from Peru and subsequently recorded from other locations in Pacific America, Argentina and Namibia (Fig. 2c). More recently, *S. tanakae* was described from Japan (Uwai and Masuda 1999, as *Pterosiphonia tanakae*) and it was recorded as an introduced species in the Mediterranean (Verlaque et al. 2015). The synonymy between these two species was proposed based on similarity of sequence data from their type localities (Bustamante et al. 2016a).

Xiphosiphonia pennata, X. pinnulata and Symphyocladia spinifera

Xiphosiphonia pennata was widely reported on Atlantic and Pacific coasts (Fig. 2e, as *Pterosiphonia pennata*) and successive publications have unmasked cryptic diversity in this taxon, resolving a Pacific and two Atlantic species. Maggs and Hommersand (1993) noted that the smaller, less robust European forms of this species should be assigned to *X. pinnulata* (as *P. pinnulata*). This distinction was later supported by molecular data (Díaz-Tapia and Bárbara 2013). Kim et al. (2012) revealed that Korean specimens previously regarded as *X. pennata* represented a distinct molecular species compared to European specimens and *P. arenosa* was described. Recently, the synonymy of *P. arenosa* with *Symphyocladia spinifera* (as *P. spinifera*; type locality Peru) was proposed (Bustamante et al. 2016b). A taxonomic revision of the tribe Pterosiphonieae demonstrated that, despite the high morphological similarity among *X. pennata*, *X. pinnulata* and *S. spinifera,* they do not form a clade (Savoie and Saunders 2016). In fact, they are placed in two distinct lineages and accordingly, the Pacific entities *P. arenosa* and *P. spinifera* were transferred to the genus *Symphyocladia*, while the Atlantic entities *P. pennata* and *P. pinnulata* were placed in the new genus *Xiphosiphonia* (Savoie and Saunders 2016).

References

Agardh, J.G. 1863. *Species genera et ordines algarum, seu descriptiones succinctae specierum, generum et ordinum, quibus algarum regnum constituitur. Volumen secundum: algas florideas complectens. Part 2, fasc. 3.* C.W.K. Gleerup, Lundae, 787-1138, 1158-1291 pp.

Bustamante, D.E., Won, B.Y. & Cho, T.O. 2016a. Morphology and phylogeny of *Pterosiphonia dendroidea* (Rhodomelaceae, Ceramiales) described as *Pterosiphonia tanakae* from Japan. *Bot. Mar.* 59:353-61.

Bustamante, D.E., Won, B.Y. & Cho, T.O. 2016b. The conspecificity of *Pterosiphonia spinifera* and *P. arenosa* (Rhodomelaceae, Ceramiales) inferred from morphological and molecular analyses. *Algae* 31:105-15.

Cribb, A.B. 1956. Records of marine algae from south-eastern Queensland. II. *Polysiphonia* and *Lophosiphonia*. *Pap. Dept. Biol. Univ. Queensland* 3:131-47.

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

Hollenberg, G.J. 1968. An account of the species of *Polysiphonia* of the central and western tropical Pacific Ocean. I. *Oligosiphonia*. *Pac. Sci.* 22:56-98.

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., Kim, S. Y., Yang, M.Y., Kim, B. & Díaz-Tapia, P. 2012. Morphology and molecular study of *Pterosiphonia arenosa* sp. nov. (Rhodomelaceae, Rhodophyta) from Jeju Island, Korea. *Algae* 27:259-68.

Maggs, C.A. & Hommersand, M.H. 1993. *Seaweeds of the British Isles. Volume 1. Rhodophyta. Part 3A. Ceramiales*. HMSO, London, 444 pp.

Rueness, J. 1971. *Polysiphonia hemisphaerica* Aresch. in Scandinavia. *Nor. J. Bot.* 18:65-74.

Rueness, J. 1973. Speciation in *Polysiphonia* (Rhodophyceae, Ceramiales) in view of hybridization experiments: *P. hemisphaerica* and *P. boldii*. *Phycologia* 12:107-9.

Rueness, J. 2010. DNA barcoding of select freshwater and marine red algae (Rhodophyta). *Cryptog. Algol.* 31: 377-86.

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: 917-39.

Silva, P.C., Basson, P.W. & Moe, R.L. 1996. Catalogue of the benthic marine algae of the Indian Ocean. *Univ. Calif. Publ. Bot.*, 79:1-1259.

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*, 47:541-59.

Stuercke, B. & Freshwater, D.W. 2010. Two new species of *Polysiphonia* (Ceramiales, Florideophyceae) from the western Atlantic. *Bot. Mar.* 53:301-11.

Uwai, S. & Masuda, M. 1999. *Pterosiphonia tanakae* (Rhodomelaceae, Ceramiales), a new red algal species from Japan. *Phycol. Res.* 47:241-50.

Verlaque, M., Ruitton, S., Mineur, F. & Boudouresque, C.-F. 2015. *CIESM atlas of exotic species of the Mediterranean. Macrophytes.* CIESM Publishers, Monaco, 362 pp.

Wynne, M.J. & Edwards, P. 1970. *Polysiphonia boldii* sp. nov. from Texas. *Phycologia* 9:11-6.