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Extensive cryptic diversity in the widely distributed *Polysiphonia scopulorum* (Rhodomelaceae, Rhodophyta): molecular species delimitation and morphometric analyses

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ABSTRACT

Our knowledge of seaweed diversity and biogeography still largely relies on information derived from morphological identifications, but the use of molecular tools is revealing that cryptic diversity is common among algae. *Polysiphonia scopulorum* is a turf-forming red alga widely reported in tropical and temperate coasts worldwide. The only study based on material collected from its Australian type locality and the Iberian Peninsula indicates that it is a species complex, but the extent of cryptic diversity across its geographical range is not known. To investigate the species diversity in *P. scopulorum*, the geographical distribution of species-level lineages and their morphological characterization, we collected 135 specimens from Australia, South Africa and southern Europe. Two gene datasets (*cox*1 and *rbc*L) were used to delimit species using three methods (GMYC, PTP, ABGD), leading to a consensus result that our collections of the *P. scopulorum* complex comprise 12 species. Five of these species were resolved in a highly supported clade, while the other seven species were related to other taxonomically accepted species or in unresolved parts of the tree. Morphometric and statistical analysis of a set of ten quantitative characters showed that there are no clear morphological correlates of species boundaries, demonstrating true cryptic diversity in the *P. scopulorum* complex. Distribution patterns of the 12 species were variable, ranging from species only known from a single site to species with a wide distribution spanning three continents. Our study indicates that a significant level of undiscovered cryptic diversity is likely to be found in algal turfs, a type of seaweed community formed by small entangled species.

Keywords: Algal turfs; Biogeography; *cox*1; Morphology; *Polysiphonia caespitosa*; *rbc*L

1. Introduction

Marine macroalgae show a wide morphological diversity ranging from tiny filamentous to highly complex morphologies (Coomans and Hommersand, 1990). Morphological species delineation in algae is often problematic due to a lack of useful characters and phenotypic plasticity, and this problem is more severe for algae with simple morphologies (Verbruggen, 2014). For this reason, it has become common practice to use molecular tools to delimit species in algae, leading to the discovery of substantial cryptic diversity (e.g. Guillemin et al., 2016; Leliaert et al., 2014, 2018; Yang et al., 2018; Pezzolesi et al., 2019; Camacho et al., 2019; Kang et al., 2019).

Algal turf is an assemblage that consists of densely entangled small macroalgae (Connell et al., 2014). It comprises of many species of red, brown and green algae, many of which evolved a similar body plan consisting of prostrate axes attached to the substratum by rhizoids and erect axes where the reproductive structures develop (Price and Scott, 1992; Díaz-Tapia and Bárbara, 2013, 2014). The small size, similar overall morphology and densely entangled nature of turf algae makes biodiversity surveys and taxonomic work particularly challenging and labour-intensive. Because of their structural simplicity and similar body plan, it seems likely that cryptic diversity will be prevalent among turf algae (e.g. Verbruggen et al., 2009; Díaz-Tapia and Bárbara, 2013; Díaz-Tapia et al., 2018b, 2020).

While the use of molecular data is well established as a primary source of information for algal species delineation, it remains important to characterise morphological traits of the discovered species. This information allows an understanding of whether and how morphological data can be used as a proxy trait to recognise species defined through DNA data, and to arrive at satisfactory nomenclatural decisions that reconcile the newly proposed taxonomies with previously described species names. Most taxonomists prefer to work with

discrete morphological traits, but those are not always available, particularly in structurally simple taxa. In such situations like that, morphometric analyses focusing on measurable traits can be used, and there are several examples of their application in algal taxonomy (Verbruggen et al., 2005a,b, 2017; Meynard et al., 2019).

The red algal genus *Polysiphonia* is a very commonly encountered member of algal turfs. It is the largest genus in the red algal family Rhodomelaceae, with 191 currently accepted species (Guiry and Guiry, 2020). The morphological complexity of *Polysiphonia* is relatively high, with many macroscopic and microscopic qualitative diagnostic characters for species delineation (Stuercke and Freshwater, 2008). However, the high diversity of species within this genus still makes many species share an identical set of qualitative characters, imposing limits to basing taxonomy on morphology (Díaz-Tapia et al., 2017a). *Polysiphonia scopulorum* Harvey is a turf-forming species originally described from Rottnest Island (Western Australia) that has since been reported in tropical and temperate regions of the Indian, Pacific and Atlantic oceans based on morphological identifications (Harvey, 1855; Guiry and Guiry, 2020). Specimens from South Africa were described as a separate species, *P. caespitosa* (Pocock) Hollenberg, that was later synonymised with *P. scopulorum* based on morphological studies (Stegenga et al., 1997; Rull Lluch, 2002). The taxonomy of these two names was never studied using molecular information and is still unclear. The *rbc*L sequences of *P. scopulorum* presently available on GenBank from Spain and Western Australia suggest that *P. scopulorum* might be a species complex with separate species in these two regions (Huisman et al., 2017).

Our goal in this study is to investigate species boundaries in the *Polysiphonia scopulorum* complex using a large collection of samples from the Indian, Pacific and Atlantic oceans. We aim to determine the number of species present in the *P. scopulorum* complex, their geographical distributions, and whether morphological information can be used to

differentiate among them. Our approach consists of analyses of *rbc*L and *cox*1 alignments using a range of species delimitation algorithms. Quantitative morphometric analyses are used to identify the morphological correlates of species boundaries.

2. Material and methods

2.1 Field collections and morphological identification

Samples of *Polysiphonia* were collected from Europe (Atlantic Iberian Peninsula, Azores, Canary Islands, Mediterranean), Australia (Western Australia, South Australia, Victoria, Tasmania) and South Africa (Table S1 in Supplementary Material). Samples were collected using a knife for scraping the algal turfs in the intertidal during low tide, or in the upper subtidal by snorkelling. Samples were placed in plastic bags and transported to the laboratory where they were carefully cleaned and isolated using a stereomicroscope. Materials for DNA extraction were dried in silica gel desiccant. Plants for morphological examination were preserved in 4% formalin seawater at 4°C and stored in the dark.

Specimens of *Polysiphonia scopulorum* were morphologically identified using the available references for each sampled region (Rojas-González, 1997; Stegenga et al., 1997; Womersley, 1979, 2003; Díaz-Tapia and Bárbara, 2013). Specimens morphologically identified in this study as *P. scopulorum* presented the following key characters that are in agreement with previous descriptions of the species and differ from other recorded species in the respective regions: dorsiventral habit with extensive prostrate axes that lack trichoblasts in their apical cells and that bear short erect axes (up to 2 cm in length), thallus brownish-red to black in colour with a fairly rigid texture, rhizoids unicellular in open connection with the pericentral cells, axes with four pericentral cells and (35-) 50-100 (-110) μm in diameter, erect axes unbranched or bearing irregularly arranged branches that are often adventitious,

branches independent of trichoblasts, apical cells dome-shaped and trichoblasts from absent to well developed spirally arranged on every segment. According to this definition, we excluded from our analysis specimens referred to *P. villum* J.Agardh or *P. scopulorum* var. *villum* (J.Agardh) Hollenberg, that are red in colour and flaccid in texture (PD pers. obs. on our own collections and images available at http://www.boldsystems.org/), making them easily distinguishable from specimens here assigned to the *P. scopulorum* complex.

2.2 DNA extraction and sequencing

DNA was extracted from silica gel-dried material following Saunders and McDevit (2012) or an adapted cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). PCR amplification of *rbc*L was carried out using primers F57/rbcLrevNEW, F2/ R1008, F2/R1464 or F2/R1452 (Saunders and Moore, 2013; Díaz-Tapia et al., 2018b). PCR amplification of *cox*1 was carried out using the newly designed forward primer 97F (5' GCTTTTGCWGGWRTTTTAGGAGG 3'), and reverse primer 1023R (5' CCTTCCTCACATWGTWGCAATTCA 3'). Reactions were performed in a total volume of 25 µl, consisting of 5 µl 5× MyTaqTM reaction buffer, 0.7 µl 10 µM of forward and reverse primers, 0.125 µl 1U µl–1 My TaqTM DNA Polymerase (Bioline, London, UK), 17.475 µl MilliQ® water and 1 µl template DNA. The PCR profile consisted of initial denaturation (93°C for 3 min), 35 cycles of denaturation (94°C for 30 s), primer annealing (45°C for 30 s), and extension (74°C for 90 s) and final extension (74°C for 5 min). The PCR products were purified and sequenced by Macrogen (Korea) or the sequencing service of the University of A Coruña.

2.3 Molecular species delimitation

Sequences of the *rbc*L and *cox*1 genes of samples morphologically identified as *Polysiphonia scopulorum* collected from Australia, South Africa and Europe were used for the molecular analysis. We also included in our analyses sequences from 11 other species that were resolved in the same major clade in previous studies, as well as four species placed in a sister clade that were used as the outgroup (Díaz-Tapia et al., 2017b). GenBank accession numbers of the used sequences are provided in Table S1 (in Supplementary Material). The sequences were checked for quality, corrected and aligned in Geneious Prime using MUSCLE (Kearse et al., 2012). Two alignments were produced with 214 *rbc*L sequences (135 of *P. scopulorum* and 79 sequences of other species) and 158 *cox*1 sequences (128 of *P. scopulorum* and 30 sequences of other species). Identical sequences were removed from both alignments with the longest sequences retained, leaving 55 *rbc*L sequences (25 of *P. scopulorum* and 30 sequences of other species) and 42 *cox*1 sequences (33 of *P. scopulorum* and 9 sequences of other species). Ultrametric trees were built with the unique alignments in BEAST v1.8.4 (Suchard et al., 2018) using GTR and Gamma + Invariant Sites models, uncorrelated relaxed clock and Coalescent: Constant Size tree prior, run for the default number of states. Maximum Likelihood (ML) trees were also built for both unique alignments using RAxML 8.1 (Stamatakis, 2014). GTR-Gamma was used as the nucleotide substitution model and branch support was estimated with 1000 bootstrap replicates.

Three species delimitation algorithms were applied to both the *rbc*L and *cox*1 alignments; Automatic Barcode Gap Discovery (ABGD; Puillandre et al., 2012a), Generalized Mixed Yule Coalescent (GYMC; Fujisawa and Barraclough, 2013), and Poisson tree processes (PTP; Zhang et al., 2013). ABGD was run on the full alignments using default settings from an online server (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html), GMYC was run on the BEAST trees in R v3.5.2 and PTP on the RAxML trees in Python.

165 The results of each of the analyses were then compared and used to inform the samples used
166 for the morphometric analysis.

167 In order to visualise haplotype variability in the *cox*1 gene and the geographical
168 distribution of haplotypes for the resolved clade that included the largest number of samples,
169 we clustered them using the TCS method (Clement et al., 2000) in POPART (Leigh and
170 Bryant, 2015).

171

172 *2.4 Morphometric analysis and morphological observations*

173 For morphometric data collection, wet preserved samples were mounted in 20% Karo®
174 Syrup solution (ACH Foods, Memphis, Tennessee, USA) and photographs of all parts of the
175 samples were taken under a light microscope. Measurements of characters for each sample
176 were then made digitally using the photographs in ImageJ (Schneider et al., 2012).
177 Quantitative data was collected for 10 characters (Fig. 1), taken from non-branching areas of
178 mature, non-damaged tissue, with five replicates of each measurement per sample. Data was
179 collected from 28 samples in total, including representatives of each of the species inferred
180 by the molecular data analysis. The number of samples available for each inferred species
181 was unequal, ranging from 1 to 43 (Table S1 in Supplementary Material), and at least two
182 samples were selected when possible.

183 The morphometric data were analysed with multivariate statistics in R v3.5.2. The
184 measurements were averaged across replicates within a sample to produce a dataset with one
185 entry per sample. In order to visualise the overall morphospace of *P. scopulorum*, a principal
186 components analysis was carried out with the FactoMineR package (Lê et al. 2008).
187 Discriminant analysis was carried out to identify the morphological differences between the *a*
188 *priori* groups (i.e., the candidate species based on molecular data) using the candisc package.

189 A second DA was performed on samples from a subset of candidate species to further
190 distinguish between them.

191

192 **3. Results**

193 *3.1 Phylogeny and molecular species delimitation*

194 The summary of the results for the GMYC, PTP and ABGD analyses for the *rbc*L and *cox*1
195 datasets were mapped onto a RAxML tree constructed using the *rbc*L dataset (Fig. 2).

196 For the *rbc*L sequence data, the delimitation analyses were completely congruent and
197 suggest there are 12 separate molecular candidate species within the *Polysiphonia*
198 *scopulorum* complex, which are numbered 1-12 in the figure (Fig. 2). The relationships
199 among candidate species were resolved in some cases but not in others, and it is clear from
200 the RAxML phylogeny that the *P. scopulorum* complex is not monophyletic, with other
201 named species present in the lineage (Fig. 2). *Polysiphonia scopulorum* 2-6 were resolved in
202 a highly supported clade and *P. scopulorum* 10 and 11 were placed as sister taxa but this
203 relationship was unsupported. These two independent clades were placed as sister to clades
204 including other currently recognized species. *Polysiphonia scopulorum* 7 was placed with
205 high support as sister to *P. adamsiae* Womersley. *Polysiphonia scopulorum* 1, 7, 8, 9 and 12
206 were placed in the phylogeny among other currently recognized species and their
207 phylogenetic relationships were unresolved.

208 The delimitation analyses of the *cox*1 sequence data were fairly congruent, but they
209 differed slightly in the number of delimited molecular species hypothesis (11, 10 and 9 in
210 GMYC, PTP and ABGD, respectively) and the clades resolved as separate species (Fig. 2).
211 *Polysiphonia scopulorum* 7 was separated as two hypothetical species in GMYC and *P.*
212 *scopulorum* 11 was divided into two hypothetical species in GMYC and PTP. *Polysiphonia*

213 *scopulorum* 2, 10 and 12 were not represented in *cox*1 dataset due to PCR amplification
214 failure.

215 Divergences between the candidate species were higher than intraspecific divergences
216 for both *rbc*L and *cox*1 in the analysed dataset. Interspecific divergences ranged from 0.9% to
217 8.7% while intraspecific variability was ≤0.5% for the *rbc*L gene (Table S2 in Supplementary
218 Material). Interspecific divergences were higher in *cox*1, ranging from 3.1% to 11.7%, while
219 intraspecific divergence ≤2.6% (Table S3 in Supplementary Material).

220 Only four of the candidate species identified in this study matched with sequences
221 deposited in GenBank, while molecular data was not previously published for the other eight
222 candidate species. Four *rbc*L sequences of *Polysiphonia scopulorum* newly determined in this
223 study were identical to three available sequences in GenBank (MF139308-10, Huisman et al.,
224 2017). An *rbc*L sequence of *P. scopulorum* from GenBank (JX828149, Bárbara et al., 2013)
225 was identical to several of our sequences of *P. scopulorum* 4. An *rbc*L sequence extracted
226 from the complete plastid genome of a specimen identified as *P. scopulorum* (MF093999,
227 Díaz-Tapia et al., 2017b) was identical to several of the newly determined sequences
228 assigned to *P. scopulorum* 6. Finally, an *rbc*L sequence assigned in GenBank to *Polysiphonia*
229 sp. (MH101826, Díaz-Tapia et al., 2018a) matched *P. scopulorum* 7.

230

231 *3.2 Species distributions*

232 Four of the candidate species were abundant in the sampling sites and were collected in at
233 least four different localities (Fig. 3). *Polysiphonia scopulorum* 7 was abundantly collected in
234 South Australia, Victoria and Tasmania. Other three of the abundant candidate species (3, 4
235 and 6) were resolved in the same clade with high support (Fig. 2). *Polysiphonia scopulorum* 4
236 had the widest distribution, as it was found in several localities from South Africa, southern

237 Europe, the Azores, as well as a single site in South Australia. The haplotype network of *cox*1
238 sequences from three regions shows six haplotypes that differ among regions, one found in
239 Azores, one in South Australia and four in South Africa (Fig. 4). The closely related *P.*
240 *scopulorum* 3 was only found in the Canary Islands where it was common and a single *cox*1
241 haplotype was detected (Figs 3 and 4). *Polysiphonia scopulorum* 6 was abundantly collected
242 in the Australian states Victoria and Tasmania, as well as a single site in South Australia (Fig.
243 3). Six *cox*1 haplotypes were identified, two found in both Tasmania and eastern Victoria,
244 one exclusive to Tasmania, two only found in western Victoria and one in South Australia
245 (Fig. 4). In the same clade were also resolved *P. scopulorum* 5, a rare species collected only
246 in two sites from Western Australia, and *P. scopulorum* 2 that was found in a single site of
247 the French Riviera (Fig. 3).

248 Eight of the candidate species were rare, collected from one or two localities only
249 (Fig. 3). Among them, only *Polysiphonia scopulorum* 9 had a wide distribution in Australia
250 that included Western Australia and Queensland. However, the sample from Queensland was
251 collected on a pontoon in a marina, suggesting that this could be an introduced species in this
252 region. Most of the rare candidate species were found in Australia, except *P. scopulorum* 2
253 from the French Riviera.

254 In most sampling sites, only one candidate species was found, but some of the
255 Australian localities yielded up to three candidate species. On Rottnest Island, the type
256 locality of *Polysiphonia scopulorum,* we identified three different candidate species (Fig. 3).

257

258 *3.3 Morphometric analysis*

259 The principal components analysis of the morphometric data reveals the overall structure of
260 the morphospace of *Polysiphonia scopulorum* (Fig. 5). The variable structure (grey dotted

vectors) indicates that there are three major groups of correlated variables, the first composed of axes and rhizoid diameters (4th quadrant: PAXsegdia, EAXsegdia, RHdia), the second related to cell lengths of the erect axes (1st quadrant: EAXcytlen, EAXseglen), and the third related to overall cell size of prostrate axes (along x-axis: PAXcytlen, PAXcytdia, PAXseglen). Samples are spread continuously across the first two axes, indicating that no groupings clearly stand out and that there is morphological continuity across the various candidate species. Samples belonging to the same candidate species tend to cluster near one another on the biplot, but there is substantial overlap between the candidate species. Generally speaking, no clear separation of any candidate species is apparent on the biplot, except perhaps for some candidate species found on the extremity of the morphospace (e.g. *P. scopulorum* 10 and 11 on the left on the biplot), but only a single sample of each of these candidate species was available to us and the full range of morphological variability of these species is likely to be larger.

A discriminant analysis was carried out aiming to find those dimensions in the morphometric data with which the species are best separated. The biplot of this analysis (Fig. 6) shows wider separation of some candidate species, especially along the x-axis, but many species remain very close to one another and the polygons for the best represented candidate species (*Polysiphonia scopulorum* 4, 6, 7) showed clear overlap. The diameter of cells in the erect axis (EAXcytdia) is most strongly correlated with the x-axis, suggesting this is a key trait in which some species could be recognised. A second overall dimension along which some candidate species appear to be separated runs from the bottom left to the top right of the graph and is correlated with the diameters of axes and rhizoids (EAXsegdia, RHdia, PAXsegdia).

Because separation between *Polysiphonia scopulorum* 4, 6 and 7 appears particularly problematic, we ran a second discriminant analysis just on the samples of those three species.

The biplot of that analysis (Fig. 7 A) represents nearly all the variation in the dataset but still fails to separate *P. scopulorum* 4 (yellow) from *P. scopulorum* 6 (navy), indicating that these species are indistinguishable. *Polysiphonia scopulorum* 7 samples are separated from the other two species along the first axis, suggesting that characters with a high score against that axis may help in identifying that species. The positive correlation with EAXsegdia, RHdia suggests that *P. scopulorum* 7 has wider erect axes and rhizoids, and the negative correlation with EAXcytlen suggests shorter cells. However, density plots – a smoothed version of histograms – show significant overlap between the three species for all of these candidate variables, a clear testament that for practical taxonomic purposes, these candidate species are entirely indistinguishable based on these morphometric traits (Fig 7 B-D).

3.5. Tetrasporangial cover cells

Our examinations of the morphology of the studied specimens also revealed a qualitative character useful for distinguishing some groups of candidate species. *Polysiphonia scopulorum* 3-6, 8, 9 and 11 had tetrasporangia with three cover cells (Figs. S1 A-D, F-H), while *P. scopulorum* 7 had tetrasporangia with two cover cells (Fig. S1 E). Tetrasporangia were not observed in *P. scopulorum* 1, 2, 10, 12. According to Huisman et al. (2017), *P. scopulorum* 1 has tetrasporangia with two cover cells.

4. Discussion

This study revealed the existence of extensive cryptic diversity among specimens morphologically identified as *Polysiphonia scopulorum*. The combination of analyses of two molecular markers, *rbc*L and *cox*1, and three methods for species boundaries delineation led us to a consensus of 12 candidate species within the allegedly widely distributed species (Fig.

3A; Guiry and Guiry, 2020). Distribution patterns and frequency of these candidate species were highly variable, ranging from rare species only known from one site to widely distributed species including three continents. Morphometric analyses revealed that while some species may be distinguishable based on combinations of quantitative traits, morphological overlap between species is prevalent.

4.1 Species delimitation

The use of the *rbc*L molecular marker was more decisive for establishing boundaries between candidate species in our dataset than *cox*1. Results obtained with the analysis of the *rbc*L gene were totally congruent among the three used methods. By contrast, each of the three methods applied to the *cox*1 dataset resolved a different number of species and slightly differed in the clades resolved as candidate species. This is in line with the high variability observed in the performance between markers and methods in other red algal datasets (e.g. Payo et al., 2013; Liu et al., 2015; Guillemin et al., 2016; Jesús et al., 2019) and further supports the importance of using multi-locus approaches in species diversity assessments (Leliaert et al., 2014). An advantage of using *cox*1 is that sequence divergences among candidate species is higher, producing a clearer barcoding gap than *rbc*L (Guillemin et al., 2016). However, in our dataset, even though the intra- and interspecific divergences were lower in the *rbc*L gene, they did not overlap. A recurrent problem with the use of *cox*1 for algae is the failure of PCR reactions as a consequence of primer mismatches (Sherwood et al., 2010; Saunders and McDevit, 2012; Díaz-Tapia et al., 2017a) and large introns in the gene in green seaweeds (Repetti et al., 2020). In this study we used an alignment of complete *cox*1 sequences for representative species of the family Rhodomelaceae in order to design new primers for an amplicon of about 850 bp. This amplicon did not correspond to the

previously used COI5P region (Saunders and McDevit, 2012), but overlapped it by 540 bp. Even with these custom-designed primers in more conserved regions than previously used primer sets, we were unable to obtain PCR products for three of the studied species (*Polysiphonia scopulorum* 2, 10 and 12).

Among the 12 consensus candidate species resolved within the *Polysiphonia scopulorum* complex based on the molecular data, only five were resolved in a highly supported clade (species 2-6) while the other seven were interspersed between other taxonomically accepted species. Our *rbc*L phylogeny lacks resolution along the backbone of the tree, a common problem in algal phylogenies constructed based on a single molecular marker (Oliveira et al., 2018), and it is possible that a better resolved phylogeny would reveal that some of these candidate species would cluster together into larger lineages. But even if considering the possibility that some candidate species are sister lineages of one another, the high sequence divergence among them (≥ 5.4 and 4.4% in the *cox*1 and *rbc*L genes, respectively) leave no doubt that *P. scopulorum* 1, 7, 8-12 represent distinct species.

Five of the candidate species resolved by the molecular delineation of species boundaries were placed in a highly supported clade in the *rbc*L phylogenetic tree (*Polysiphonia scopulorum* 2-6). All the analyses support their separation based on molecular data, and even GMYC and PTP for the *cox*1 gene resolved one additional species. A consensus among methods and molecular markers is often used as the major argument for establishing species delineation in monophyletic groups. However, additional evidence provided by morphological or distributional information should be also critically considered before adopting taxonomic decisions (Puillandre et al., 2012b; Kekkonen and Hebert, 2014; Guillemin et al., 2016; Jesús et al., 2019). Two pairs of species were found to cohabit in the same sampling sites; *P. scopulorum* 2 and 4 in the French Riviera and *P. scopulorum* 4 and 6 in South Australia. The finding of two sympatric candidate species is an indication of genetic

isolation among them and used as an argument for supporting species separation (Kekkonen and Hebert, 2014).

4.2. Biogeographic patterns

Distribution patterns of the putative species were variable, ranging from species only known in a single site to species with a wide distribution that covers three continents. Such variability is unsurprising and similar results were observed in other red algal studies that included molecular data from sampling wide geographical areas (Zuccarello et al., 2002a, b; Won et al., 2009; Díaz-Tapia et al., 2018b).

Six species were only found once and each in a single locality, suggesting a high endemicity and rarity of many of the discovered species. High levels of endemicity have been found in other cryptic seaweed species (Tronholm et al., 2012; Payo et al., 2013; Pardo et al., 2014; Leliaert et al., 2018; Guillemin et al., 2016; Díaz-Tapia et al., 2018b) and these results are concordant with the limited dispersal ability of the red algae (Santelices, 1990; Kinlan and Gaines, 2003). However, the distribution of the rare species could be wider but was not detected in our collections because several regions where *Polysiphonia scopulorum* was reported remain unexplored (Fig. 3). Pacific North America was not sampled in this study, but species here included in the *P. scopulorum* complex were not found in a recent extensive survey of *Polysiphonia* spp in this region using molecular tools (Savoie and Saunders, 2019). Most probably, records of *P. scopulorum* from this region actually correspond to *P. villum*, congruent with results by Savoie and Saunders (2019, as *P. scopulorum* var. *villum*). By contrast, molecular data of *P. scopulorum* from tropical regions are completely absent and the potential presence of the putative species here identified is uncertain. Besides potentially broadening the distribution of some of the cryptic species detected here, it is highly likely that

383 a further sampling would result in the discovery of additional cryptic species. This is
384 particularly likely to be the case in tropical regions, where algal turfs are extremely common
385 (Price and Scott, 1992; Littler and Littler, 2013) and less intensively studied than in the
386 warm-temperate regions that form the focus of our work. Tropical turfs have scarcely been
387 studied using molecular tools and we predict that they will turn out to be a treasure trove of
388 new species and cryptic diversity, including but certainly not limited to the *P. scopulorum*
389 complex.

390 Interestingly, two of the three putative species identified in Rottnest Island, the type
391 locality of *Polysiphonia scopulorum*, were only found in Western Australia, while one of
392 them was also collected in a marina in Queensland and further collections are required to
393 clarify whether it is native or introduced in this state. Thus, according to our data the genuine
394 *P. scopulorum* is endemic to Australia, congruent with conclusions in previous studies
395 (Huisman et al., 2017), and *P. scopulorum* has a narrow distribution rather than the wide
396 distribution reported based on morphological identifications (Fig. 3A; Guiry and Guiry,
397 2020).

398 *Polysiphonia scopulorum* 4 has a wide distribution that comprises South Africa,
399 southern Europe and Australia and this is the only species of the complex that we detected in
400 South Africa, the type locality of *P. caespitosa*. This is one of the few examples in which the
401 wide distribution of a seaweed species, encompassing at least three continents without a clear
402 human-mediated dispersal, has been demonstrated using molecular data (but see Zuccarello et
403 al., 2002a; Won et al., 2009; Díaz-Tapia et al., 2018b). Within *P. scopulorum* 4 we identified
404 multiple *cox*1 and *rbc*L haplogroups with a strong phylogeographic structure, so that each
405 haplogroup was only present in a particular region (Fig. 4). This structure is congruent with
406 the occurrence of rare dispersal events followed by genetic divergence of the resulting
407 populations isolated by distance. Similar processes have been suggested to explain the

genetic structure of other macroalgae and marine organisms (Palumbi, 1994; Tronholm et al., 2012; Díaz-Tapia et al., 2018b). *Polysiphonia scopulorum* 4 was abundantly collected in several sites from South Africa and Europe, while a single specimen was detected in South Australia. This finding might correspond to a relatively recent dispersal event, and we cannot rule out that it could be human mediated. There are several examples of algal introductions in Australia, and *Polysiphonia* spp are among the most frequently introduced seaweeds (Williams and Smith, 2007; Piñeiro-Corbeira et al., 2019). However, the South Australian specimen was collected in a National Park, more than 60 km away from potential vectors for marine species introductions such as harbours or aquaculture facilities (PIRSA, 2017).

Polysiphonia scopulorum 3, 6 and 7 were regionally abundant and represent intermediate examples between rare species with narrow distributions and widely spread species. *Polysiphonia scopulorum* 6 and 7 share the same range and were abundantly found in South Australia, Victoria and Tasmania. Likewise, *P. scopulorum* 3 was abundantly collected in the Canary Islands and our results suggest that it is endemic to this volcanic archipelago. This is the most common scenario found when cryptic diversity is detected in seaweed species (Won et al., 2009; Pardo et al., 2014; Guillemin et al., 2016; Pezzolesi et al., 2019; Jesús et al., 2019).

4.3 A case of true cryptic diversity

The 12 species resolved based on molecular data share the full set of qualitative traits available for species identification and delimitation in *Polysiphonia* and related genera (Stuercke and Freshwater, 2008). However, a detailed study of additional morphological characters allowed us to find one qualitative trait that distinguishes two groups of species. *P. scopulorum* 7 and 1 have tetrasporangia with two cover cells (this work; Huisman et al.,

2017), while all the other species in which tetrasporangia were observed had three cover cells (*P. scopulorum* 3-6, 8, 9 and 11). This appears to be a consistent character that varies among species of the tribe Polysiphonieae and can be used for species identification (Díaz-Tapia and Bárbara, 2013; Díaz-Tapia et al., 2017c, 2018a), but its utility hinges on the studied plants being reproductive tetrasporophytes, which will not always be the case, and we were unable to score this trait for *P. scopulorum* 1, 2, 10 and 12 for this reason.

Considering that qualitative characters clearly cannot distinguish between the 12 species in the complex, morphometric analyses of quantitative traits might provide a proxy for morphological species recognition. This approach has been successfully applied in previous algal studies, allowing the delineation of morphologically similar species in genera of the green and red algae (Verbruggen et al. 2005a,b; Meynard et al. 2019). The term "cryptic diversity" has been often applied to groups of morphologically similar seaweed species unmasked when applying molecular tools. However, more detailed morphological investigation often finds previously overlooked traits in which they differ, making them pseudo-cryptic species (e.g. Camacho et al., 2019; Jesús et al., 2019; Meynard et al., 2019). In the case of the *Polysiphonia scopulorum* complex, however, morphometric analyses of cells, segments and rhizoids showed a morphological continuum across the candidate species, demonstrating that the complex is an example of true cryptic diversity, where species cannot be distinguished despite detailed morphometric and statistical analysis. While some of the species may be distinguishable using complex combinations of variables in discriminant analyses (e.g. *P. scopulorum* 7 in Fig. 7A), this does not enable us to identify these species in a practical taxonomic sense, as the species show significant overlap in all relevant variables (Fig. 7B-E).

Conclusions

This work demonstrates that the turf-forming algae *Polysiphonia scopulorum* is actually a complex in which 12 putative species were resolved based on molecular data. Most of them are morphologically indistinguishable and this is, to our knowledge, the first example of cryptic diversity of seaweeds demonstrated by a detailed study of qualitative characters and morphometric traits analysed statistically. Biogeographic patterns greatly differed among species, suggesting that they displayed different evolutionary histories despite they being highly similar in habitat and morphology. Our work evidenced that the *P. scopulorum* complex requires taxonomic revision, including an assessment of how the discovered molecular entities map to available names (i.e. *P. scopulorum* and *P. caespitosa*), the recognition of new species for the non-monophyletic lineages, and a critical assessment of the species delineation in the clade that groups *P. scopulorum* 2-6. Our study also is a clear testament to the need to improve our understanding of the species diversity in algal turfs, a type of community that is commonly neglected in general diversity surveys because working with intermixed small-sized species is tedious. But it is very timely to develop a better knowledge of the diversity in algal turfs, as this type of community is replacing kelp forests in temperate ecosystems and corals in tropical regions as a consequence of global change (Filbee-Dexter and Wernberg, 2018; O'Brien and Scheibling, 2018).

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Appendix A. Supplementary material

Supplementary material

Fig. S1. Tetrasporangia cover cells in the *Polysiphonia scopulorum* complex. A-G and H: *P. scopulorum* 3-9 and 11, respectively. Detail of erect axes showing tetrasporangia (t), with two presporangial cover cells (pr), a postsporangial cover cell (po) and a scar cells of trichoblasts (sc). Tetrasporangia not shown in A, E and H because they are in a lower focal plane. The postsporangial cover cell is absent in E, and scar cells are absent in G. Scale bars: 40 μm.

Table S1. Specimens collection information indicating the ones used in morphometric analyses (M), GenBank accesion numbers and lenght of the sequences.

503 **Table S2.** Percentage (above) and number (down) of bases that differ among species *rbc*L
504 sequences included in our alingment.

505 **Table S3.** Percentage (above) and number (down) of bases that differ among species *cox*1
506 sequences included in our alingment.

507

508 **References**

509 Bárbara, I., Choi, H.-G., Secilla, A., Díaz-Tapia, P., Gorostiaga, J.M., Seo, T.K., Jung, M.-Y.,
510 Berecibar, E., 2013. *Lampisiphonia iberica* gen. et sp. nov. (Ceramiales, Rhodophyta)
511 based on morphology and molecular evidence. Phycologia. 52, 137-155.

512 Camacho, O., Fernández-García, C., Vieira, C., Gurgel, C.F.D., Norris, J.N, Freshwater,
513 D.W., Fredericq, S., 2019. The systematics of *Lobophora* (Dictyotales,
514 Phaeophyceae) in the western Atlantic and eastern Pacific oceans: eight new species.
515 J. Phycol. 55, 611-624.

516 Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene
517 genealogies. Molec. Ecol. 9, 1657–1659.

518 Connell S.D., Foster M.S., Airoldi L., 2014. What are algal turfs? Towards a better
519 description of turfs. Mar. Ecol. Prog. Ser. 495, 299-307.

520 Coomans, R.J., Hommersand, M.H., 1990. Vegetative growth and organization, in: Cole,
521 K.M., Sheath, R.G. (Eds.), Biology of the red algae. Cambridge University Press,
522 Cambridge, pp. 275-304.

523 Díaz-Tapia, P., Bárbara, I., 2013. Seaweeds from sand-covered rocks of the Atlantic Iberian
524 Peninsula. Part 1. The Rhodomelaceae (Ceramiales, Rhodophyta). Cryptogamie
525 Algol. 34, 325-423.

526 Díaz-Tapia, P., Bárbara, I., 2014. Seaweeds from sand-covered rocks of the Atlantic Iberian
527 Peninsula. Part 2. Palmariales, Ceramiales (excluding Rhodomelaceae), Gelidiales,
528 Gigartinales, Plocamiales, Rhodymeniales and Scytothamniales. Cryptogamie Algol.
529 35, 157-199.
530 Díaz-Tapia, P., Bárbara, I., Cremades, J., Verbruggen, H., Maggs, C.A., 2017a. Three new
531 cryptogenic species in the tribes Polysiphonieae and Streblocladieae (Rhodomelaceae,
532 Rhodophyta). Phycologia. 56, 605-623.
533 Díaz-Tapia, P., Maggs, C.A., West, J.A., Verbruggen, H., 2017b. Analysis of chloroplast
534 genomes and a supermatrix inform reclassification of the Rhodomelaceae
535 (Rhodophyta). J. Phycol. 53, 920-937.
536 Díaz-Tapia, P., McIvor, L., Freshwater, D.W., Verbruggen, H., Wynne, M.J., Maggs, C.A.,
537 2017c. The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata* S.F. Gray
538 constitute well-defined clades of the red algal tribe Polysiphonieae (Rhodomelaceae,
539 Ceramiales). Eur. J. Phycol. 52, 1-20.
540 Díaz-Tapia, P., Pasella, M., Verbruggen, H., 2018a. Molecular analyses resolve the
541 phylogenetic position of *Polysiphonia adamsiae* (Rhodomelaceae, Rhodophyta) and
542 reveal a strong phylogeographic structure in Australia. Phycologia. 57, 593-600.
543 Díaz-Tapia, P., Maggs, C.A., Macaya, E.C., Verbruggen, H., 2018b. Widely distributed red
544 algae often represent hidden introductions, complexes of cryptic species or species
545 with strong phylogeographic structure. J. Phycol. 54, 829–839.
546 Díaz-Tapia, P., Maggs, C.A., Nelson, W., Macaya, E.C. & Verbruggen, H., 2020.
547 Reassessment of the genus *Lophurella* (Rhodomelaceae, Rhodophyta) from Australia
548 and New Zealand reveals four cryptic species. Eur. J. Phycol. 55, 113-128.
549 Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh
550 leaf tissue. Phytochem. Bull. 19, 11-15.

551 Filbee-Dexter, K., Wernberg, T., 2018. Rise of turfs: a new battlefront for globally declining
552 kelp forests. BioScience. 68, 64–76.
553 Fujisawa, T., Barraclough, T.G., 2013. Delimiting species using single-locus data and the
554 generalized mixed Yule coalescent approach: a revised method and evaluation on
555 simulated data sets. Syst. Biol. 62, 707-724.
556 Guillemin, M.L., Contreras-Porcia, L., Ramirez, M.E., Macaya, E.C., Contador, C.B.,
557 Woods, H., Wyatt C., Brodie, J., 2016. The bladed Bangiales (Rhodophyta) of the
558 South Eastern Pacific: molecular species delimitation reveals extensive diversity.
559 Molec. Phylogenet. Evol. 94, 814–826.
560 Guiry, M.D., Guiry, G.M., 2020. AlgaeBase, World-wide electronic publication, National
561 University of Ireland, Galway. http://www.algaebase.org (accessed 04 February
562 2020).
563 Harvey, W.H., 1855. Some account of the marine botany of the colony of western Australia.
564 Trans. Roy. Irish Acad. 22, 525-566.
565 Huisman, J. M., Kim, B., Kim, M.S., 2017. The phylogenetic position of *Polysiphonia*
566 *scopulorum* (Rhodomelaceae, Rhodophyta) based on molecular analyses and
567 morphological observations of specimens from the type locality in Western Australia.
568 Phytotaxa. 324, 51-61.
569 Jesús, P.B., Nauer, F., Lyra, G.M., Cassano, V., Oliveira, M.C., Nunes, J.M.C.,
570 Schnadelbach, A.S., 2019. Species delimitation methods reveal cryptic diversity in the
571 *Hypnea cornuta* complex (Cystocloniaceae, Rhodophyta). Eur. J. Phycol. 54, 135-
572 153.
573 Kang, J.H., Jang, J.E., Kim, J.H., Byeon, S.Y., Kim, S., Choi, S.K., Kang, Y.H., Park S.R.,
574 Lee, H.J., 2019. Species composition, diversity, and distribution of the genus *Ulva*

along the coast of Jeju Island, Korea based on molecular phylogenetic analysis. PLoS ONE. 14, e0219958.

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.

Kekkonen, M., Hebert, P.D., 2014. DNA barcode based delineation of putative species: efficient start for taxonomic workflows. Mol. Ecol. Resour. 14, 706-715.

Kinlan, B. P., Gaines, S. D., 2003. Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology. 84, 2007–2020.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR: An R Package for Multivariate Analysis. J. Stat. Softw. 25, 1-18.

Leigh J.W., Bryant, D., 2015. POPART: full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116.

Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., López-Bautista, J.M., Zuccarello, G.C., De Clerck, O., 2014 DNA-based species delimitation in algae. Eur. J.Phycol. 49, 179-196.

Leliaert, F., Payo, D.A., Gurgel, C.F.D., Schils, T., Draisma, S.G.A., Saunders, G.W., Kamiya, M., Sherwood, A.R., Lin, S.-M., Huisman, J.M., Le Gall, L., Anderson, R.J., Bolton, J.J., Mattio, L., Zubia, M., Spokes, T., Vieira, C., Payri, C.E., Coppejans, E., D'hondt, S., Verbruggen, H., De Clerck, O., 2018. Patterns and drivers of species diversity in the Indo-Pacific red seaweed *Portieria*. J Biogeogr. 45, 2299– 2313.

Littler, M.M., Littler, D.S., 2013. The nature of turf and boring algae and their interactions on reefs, in: Lang, M.A., Sayer, M.D.J. (Eds.), Proceedings of the 2013 AAUS/ESDP

Curaçao Joint International Scientific Diving Symposium, October 24-27, 2013, Curaçao. American Academy of Underwater Sciences, Dauphin Island, pp. 213-217.

Liu, S.-L., Lin, S.-M., Chen, P.-C., 2015. Phylogeny, species diversity and biogeographic patterns of the genus *Tricleocarpa* (Galaxauraceae, Rhodophyta) from the Indo-Pacific region, including *T.confertus* sp. nov. from Taiwan. Eur. J. Phycol. 50, 439-456.

Meynard, A., Zapata, J., Salas, N., Betancourtt, C., Pérez-Lara, G., Castañeda, F., Ramírez, M.E., Contador, C.B., Guillemin, M.L., Contreras-Porcia, L., 2019. Genetic and morphological differentiation of *Porphyra* and *Pyropia* species (Bangiales, Rhodophyta) coexisting in a rocky intertidal in central Chile. J. Phycol. 55, 297-313.

O'Brien, J.M., Scheibling, R.E., 2018. Turf wars: competition between foundation and turf-forming species on temperate and tropical reefs and its role in regime shifts. Mar. Ecol. Prog. Ser. 590, 1-17.

Oliveira, M.C., Repetti, S.I., Iha, C., Jackson, C.J., Díaz-Tapia, P., Lubiana, K.M.F., Cassano, V., Costa, J.F., Cremen, M.C.M., Marcelino, V.R., Verbruggen, H., 2018. High-throughput sequencing for alga systematics. Eur. J. Phycol. 53, 256-272.

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

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

Payo, D.A., Leliaert, F., Verbruggen, H., D'hondt, S., Calumpong, H.P., De Clerck, O., 2013. Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieria* in the Philippines. Proc. R. Soc. B. 28, 020122660.

624 Pezzolesi, L., Peña, V., Le Gall, L.,Gabrielson, P.W., Kaleb, S., Hughey, J.R., Rodondi, G.,
625 Hernandez-Kantun, J.J., Felace, A., Basso, D., Cerrano, C., Rindi, F., 2019.
626 *Mediterranean Lithophyllum stictiforme* (Corallinales, Rhodophyta) ia a genetically
627 diverse species complex: implications for species circumscription, biogeography and
628 conservation of coralligenous habitats. J. Phycol. 55, 473-492.
629 Piñeiro-Corbeira, C., Verbruggen, H., Diaz-Tapia P., 2019. Molecular survey of the red algal
630 family Rhodomelaceae (Ceramiales, Rhodophyta) in Australia reveals new introduced
631 species. J. Appl. Phycol. in press.
632 PIRSA (Primary Industries and Regions South Australia). 2017. ZONING IN: South
633 Australian Aquaculture Report 2015/16. Adelaide.
634 Price, I.R., Scott, F.J., 1992. The turf algal flora of the Great Barrier Reef. Part I.
635 Rhodophyta. Botany Department, James Cook University, Townsville, Australia.
636 Puillandre, N., Lambert, A., Brouillet, S., Achaz, G., 2012a. ABGD, Automatic Barcode Gap
637 Discovery for primary species delimitation. Molec. Ecol. 21, 1864-1877.
638 Puillandre, N., Modica, M.V., Zhang, Y., Sirovich, L., Boisselier, M.C., Cruaud, C., Holford,
639 M., Samadi, S., 2012b. Large-scale species delimitation method for hyperdiverse
640 groups. Mol. Ecol. 21, 2671-2691.
641 Repetti, S. I., Jackson, C. J., Judd, L. M., Wick, R. R., Holt, K. E., Verbruggen, H., 2020. The
642 inflated mitochondrial genomes of siphonous green algae reflect processes driving
643 expansion of noncoding DNA and proliferation of introns. PeerJ. 8, e8273.
644 Rojas-González, B., 1997. Estudio de las especies de la Familia Rhodomelaceae
645 (Rhodophyta), con exclusión de las Tribus Chondrieae y Laurencieae, en las Islas
646 Canarias. Universidad de La Laguna, La Laguna.
647 Rull Lluch, J., 2002. Marine benthic algae of Namibia. Sci. Mar. 66, 5-256.

Santelices, B., 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanogr. Mar. Biol. Ann. Rev. 28, 177–276.

Saunders G.W., McDevit D.C., 2012. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms, in: Kress, W., Erickson, D. (Eds.), DNA Barcodes. Methods in Molecular Biology (Methods and Protocols). Humana Press, Totowa, USA, pp. 207–222.

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

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. E. J. Phycol. 54, 1-25.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods. 9, 671.

Sherwood, A.R., Kurihara, A., Conklin, K.Y., Sauvage, T., Presting, G.G., 2010. The Hawaiian Rhodophyta Biodiversity Survey (2006-2010): a summary of principal findings. BMC Plant Biol. 10, 258.

Stamatakis, A., 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics. 30, 1312-1313.

Stegenga, H., Hall, A., Bolton, J. J., Anderson, R. J., 1997. Seaweeds of the South African west coast (Vol. 18). Bolus Herbarium, University of Cape Town, Cape Town.

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

672 Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018.
673 Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus
674 Evol. 4, vey016.
675 Tronholm, A., Leliaert, F., Sanson, M., Afonso-Carrillo, J., Tyberghein, L., Verbruggen, H.,
676 De Clerck, O., 2012. Contrasting geographical distributions as a result of thermal
677 tolerance and long-distance dispersal in two allegedly widespread tropical brown
678 algae. PLoS ONE. 7, e30813.
679 Verbruggen, H., 2014. Morphological complexity, plasticity, and species diagnosability in the
680 application of old species names in DNA-based taxonomies. J. Phycol. 50, 26-31.
681 Verbruggen, H., De Clerck, O., W.H.C.F., Coppejans, E., 2005a. Molecular and
682 morphometric data pinpoint species boundaries in *Halimeda* section Rhipsalis
683 (Bryopsidales, Chlorophyta). J. Phycol. 41, 606-621.
684 Verbruggen, H. De Clerck, O., Cocquyt, E., Kooistra, W.H.C.F., Coppejans, E., 2005b.
685 Morphometric taxonomy of siphonous green algae: a methodological study within the
686 genus *Halymeda* (Bryopsidales). J. Phycol. 41, 126-139.
687 Verbruggen, H., Vlaeminck, C., Sauvage , T., Sherwood , A.R., Leliaert, F., De Clerck, O.,
688 2009. Phylogenetic analysis of *Pseudochlorodesmis* strains reveals cryptic diversity
689 above the family level in the siphonous green algae (Bryopsidales, Chlorophyta). J.
690 Phycol. 45, 726-731.
691 Verbruggen, H., Brookes, M.J., Costa, J.F., 2017. DNA barcodes and morphometric data
692 indicate that *Codium fragile* may consist of two species (Bryopsidales, Chlorophyta).
693 Phycologia. 56, 54-62.
694 Williams, S.L., Smith, J.E., 2007. A global review of the distribution, taxonomy, and impacts
695 of introduced seaweeds. Annu. Rev. Ecol. Evol. Syst. 38, 327–359.

696 Womersley, H.B.S., 1979. Southern Australian species of *Polysiphonia* Greville
697 (Rhodophyta). Aust. J. Bot. 27, 459-528.
698 Womersley, H.B.S., 2003. The marine benthic flora of southern Australia. Part IIID:
699 Ceramiales-Delesseriaceae, Sarcomeniaceae, Rhodomelaceae. Australian Biological
700 Resources Study, Canberra.
701 Won, B.Y., Cho, T.O., Fredericq, S., 2009. Morphological and molecular characterization of
702 species of the genus *Centroceras* (Ceramiaceae, Ceramiales), including two new
703 species. J. Phycol. 45, 227–50.
704 Yang, L.E., Zhou, W., Hu, C.M., Deng, Y.Y., Xu, G.P., Zhang, T., Russell, S., Zhu, J.Y., Lu,
705 Q.Q., Brodie, J., 2018. A molecular phylogeny of the bladed Bangiales (Rhodophyta)
706 in China provides insights into biodiversity and biogeography of the genus *Pyropia*.
707 Mol. Phyl. Evol. 120, 94– 102.
708 Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method
709 with applications to phylogenetic placements. Bioinformatics. 29, 2869-2876.
710 Zuccarello, G. C., Sandercock, B., West, J. A., 2002a. Diversity within red algal species:
711 variation in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and
712 *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies.
713 Eur. J. Phycol. 37, 403–418.
714 Zuccarello, G. C., West, J., Rueness, J., 2002b. Phylogeography of the cosmopolitan red alga
715 *Caulacanthus ustulatus* (Caulacanthaceae, Gigartinales). Phycol. Res. 50, 163–172.
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717

718 **Figure legends**

719 **Fig. 1.** A: Thallus of *Polysiphonia scopulorum* consisting on prostrate axes (pa) with rhizoids
720 (r) and erect axes (ea). Quantitative variables measured for morphometric data analysis are
721 indicated on erect (B) and prostrate (C) axes. Their legend includes the abbreviations used in
722 Figs 5-7. Scale bars: 1 mm (A); 120 μm (B, C).

723 **Fig. 2.** RAxML tree inferred using *rbc*L sequence data. Results of analyses of molecular data
724 using GMYC, PTP and ABGD for the *Polysiphonia scopulorum* complex were mapped in
725 this tree and are indicated by vertical bars on the right. Values at nodes indicate bootstrap
726 support (BP) when >70.

727 **Fig. 3.** Distribution of *Polysiphonia scopulorum*. (A) Distribution of *P. scopulorum* according
728 to morphological identifications indicated with red lines (Guiry and Guiry, 2020; and
729 references therein). (B-D) Maps of southern Europe, South Africa and Australia and showing
730 distribution of the putative species in the *P. scopulorum* complex resolved in the analyses of
731 molecular data. Numbers in the legend correspond to the numbers of the 12 *P. scopulorum*
732 species identified in the tree (Fig. 2). The red and blue stars indicate the type localities of *P.*
733 *scopulorum* and *P. caespitosa*, respectively.

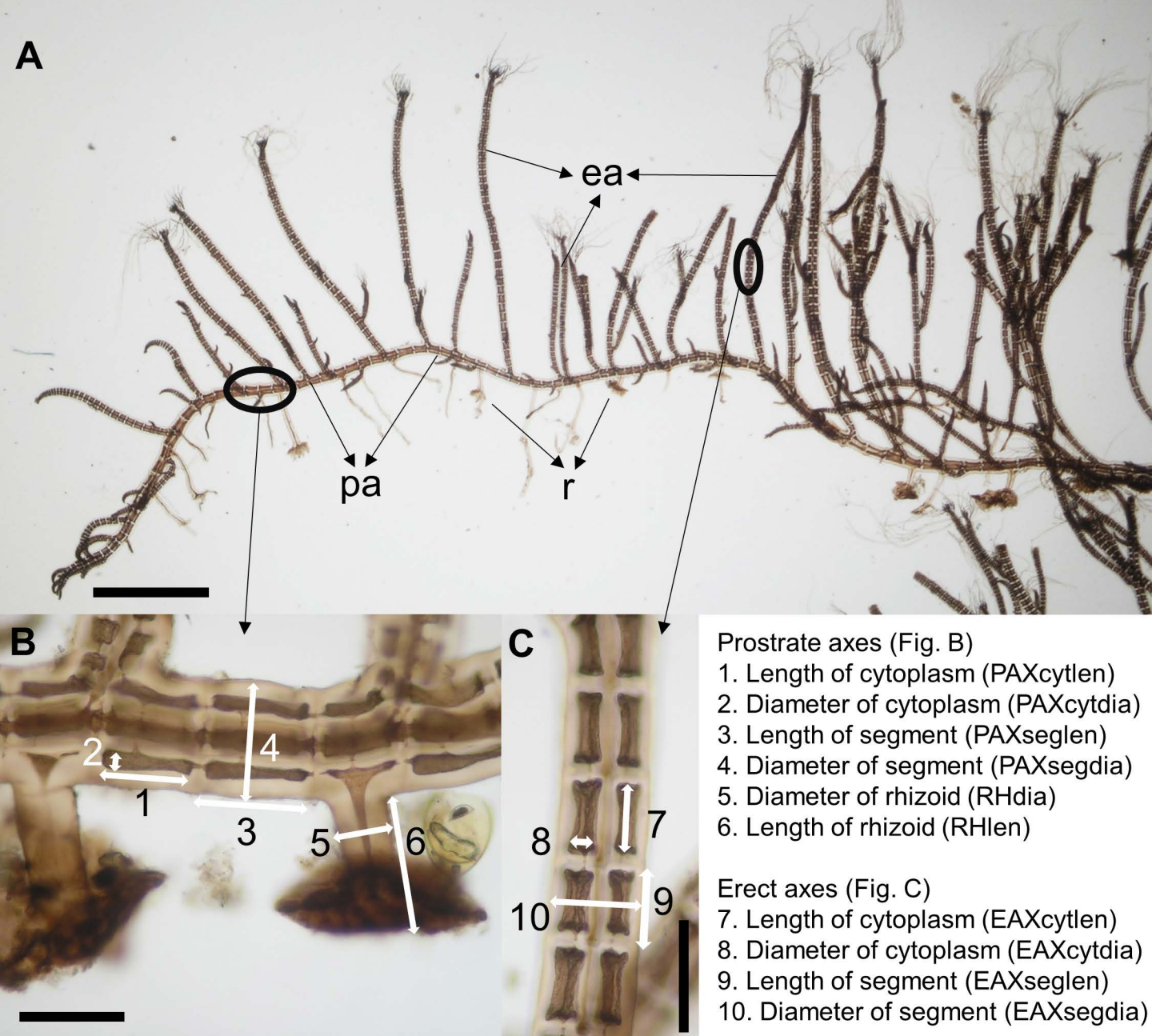
734 **Fig. 4.** Phylogenetic relationships among haplotypes of *Polysiphonia scopulorum* 3-6 and
735 their geographic distribution based on *cox*1 data set. Each circle represents a haplotype and
736 its size is proportional to the frequency in which the haplotype was found. Black circles
737 represent hypothetical un-sampled haplotypes. The number of base pares that differ among
738 haplotypes is indicated by a number when it is >1.

739 **Fig. 5.** Biplot of a Principal Components Analysis of morphometric data, representing ca.
740 70% of the variation between the first two dimensions. For species where multiple samples

were included in morphometric analyses, a polygon was drawn around them to better show their spread. Abbreviations follow Fig. 1.

Fig. 6. Biplot of the main discriminant analysis of morphometric data, representing ca. 70% of the variation between the first two dimensions. Annotations follow Fig. 1.

Fig. 7. Detailed analysis of morphological differences between *Polysiphonia scopulorum* 4 (yellow), 6 (navy) and 7 (brown). (A) Biplot of discriminant analysis, with annotations following Fig. 1. (B-E) Density plots of indicated morphometric variables.

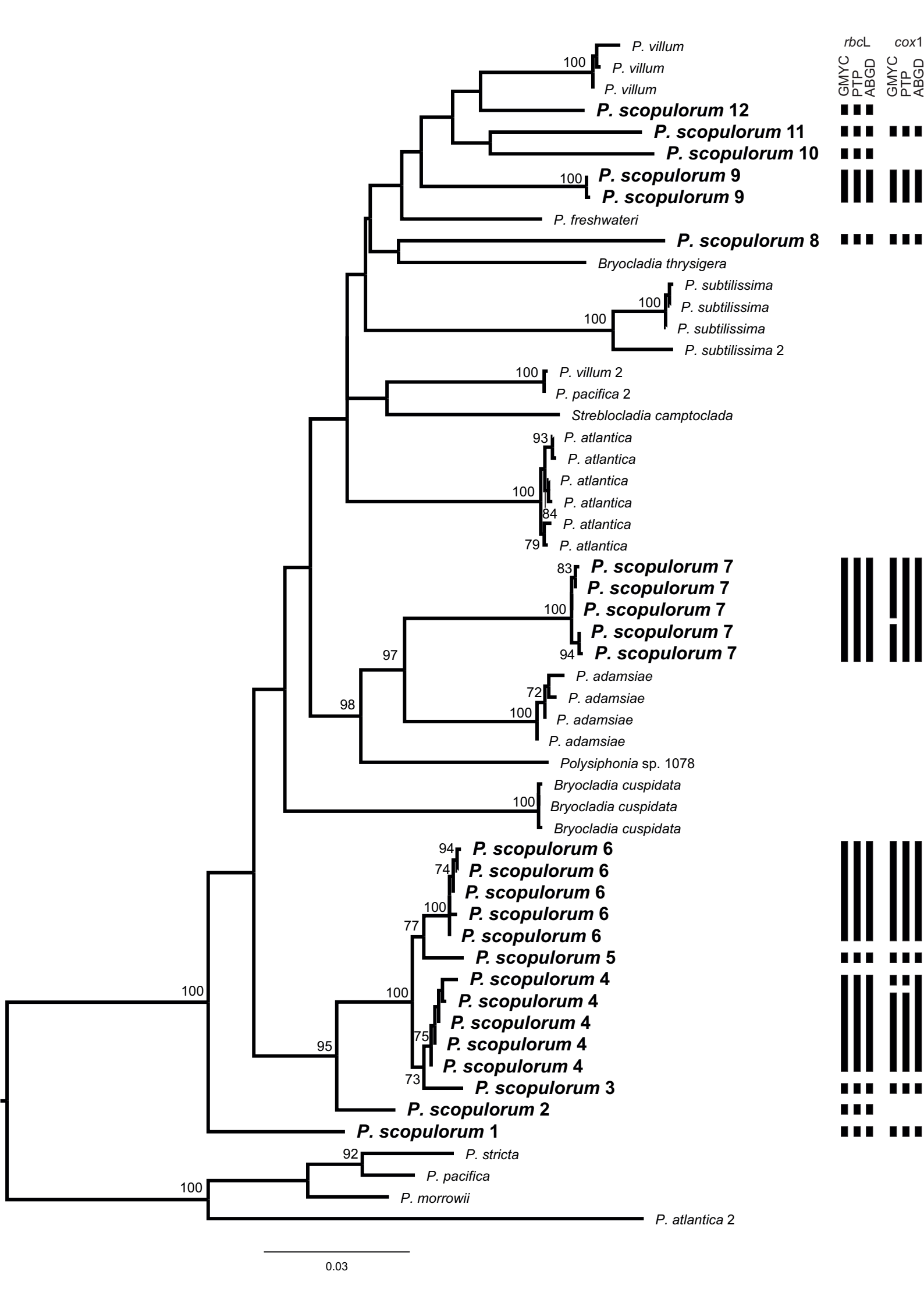


Prostrate axes (Fig. B)

1. Length of cytoplasm (PAXcytlen)
2. Diameter of cytoplasm (PAXcytdia)
3. Length of segment (PAXseglen)
4. Diameter of segment (PAXsegdia)
5. Diameter of rhizoid (RHdia)
6. Length of rhizoid (RHlen)

Erect axes (Fig. C)

7. Length of cytoplasm (EAXcytlen)
8. Diameter of cytoplasm (EAXcytdia)
9. Length of segment (EAXseglen)
10. Diameter of segment (EAXsegdia)


rbcL
cox1
GMYC
PTP
ABGD
GMYC
PTP
ABGD
P. villum
P. villum
P. villum
P. scopulorum 12
P. scopulorum 11
P. scopulorum 10
P. scopulorum 9
P. scopulorum 9
P. freshwateri
P. scopulorum 8
Bryocladia thrysigera
P. subtilissima
P. subtilissima
P. subtilissima
P. subtilissima 2
P. villum 2
P. pacifica 2
Streblocladia camptoclada
P. atlantica
P. atlantica
P. atlantica
P. atlantica
P. atlantica
P. atlantica
P. scopulorum 7
P. scopulorum 7
P. scopulorum 7
P. scopulorum 7
P. scopulorum 7
P. adamsiae
P. adamsiae
P. adamsiae
P. adamsiae
Polysiphonia sp. 1078
Bryocladia cuspidata
Bryocladia cuspidata
Bryocladia cuspidata
P. scopulorum 6
P. scopulorum 6
P. scopulorum 6
P. scopulorum 6
P. scopulorum 6
P. scopulorum 5
P. scopulorum 4
P. scopulorum 4
P. scopulorum 4
P. scopulorum 4
P. scopulorum 4
P. scopulorum 3
P. scopulorum 2
P. scopulorum 1
P. stricta
P. pacifica
P. morrowii
P. atlantica 2
100
100
100
100
100
100
94
74
100
77
100
95
75
73
92
100
93
100
84
79
83
100
94
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72
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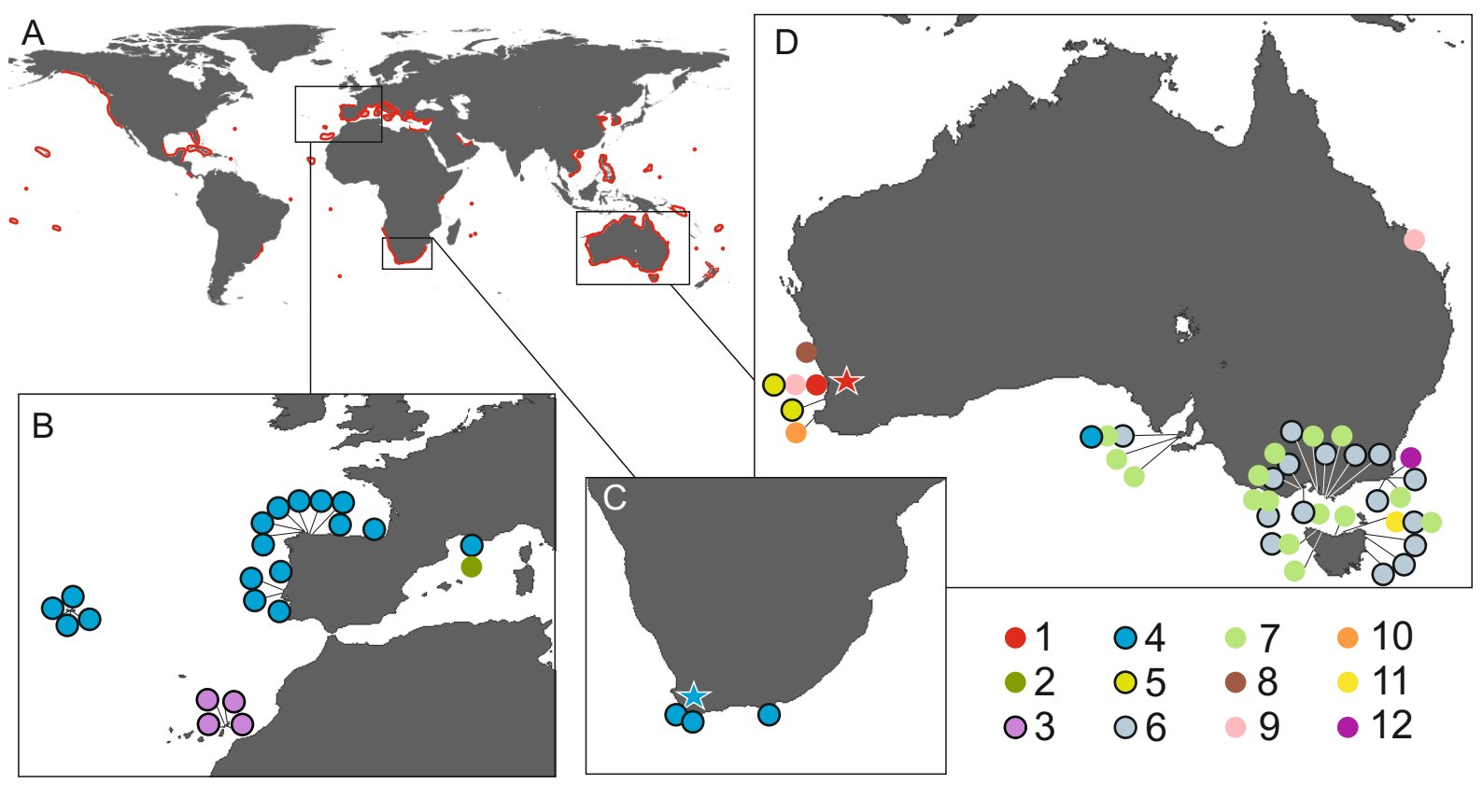
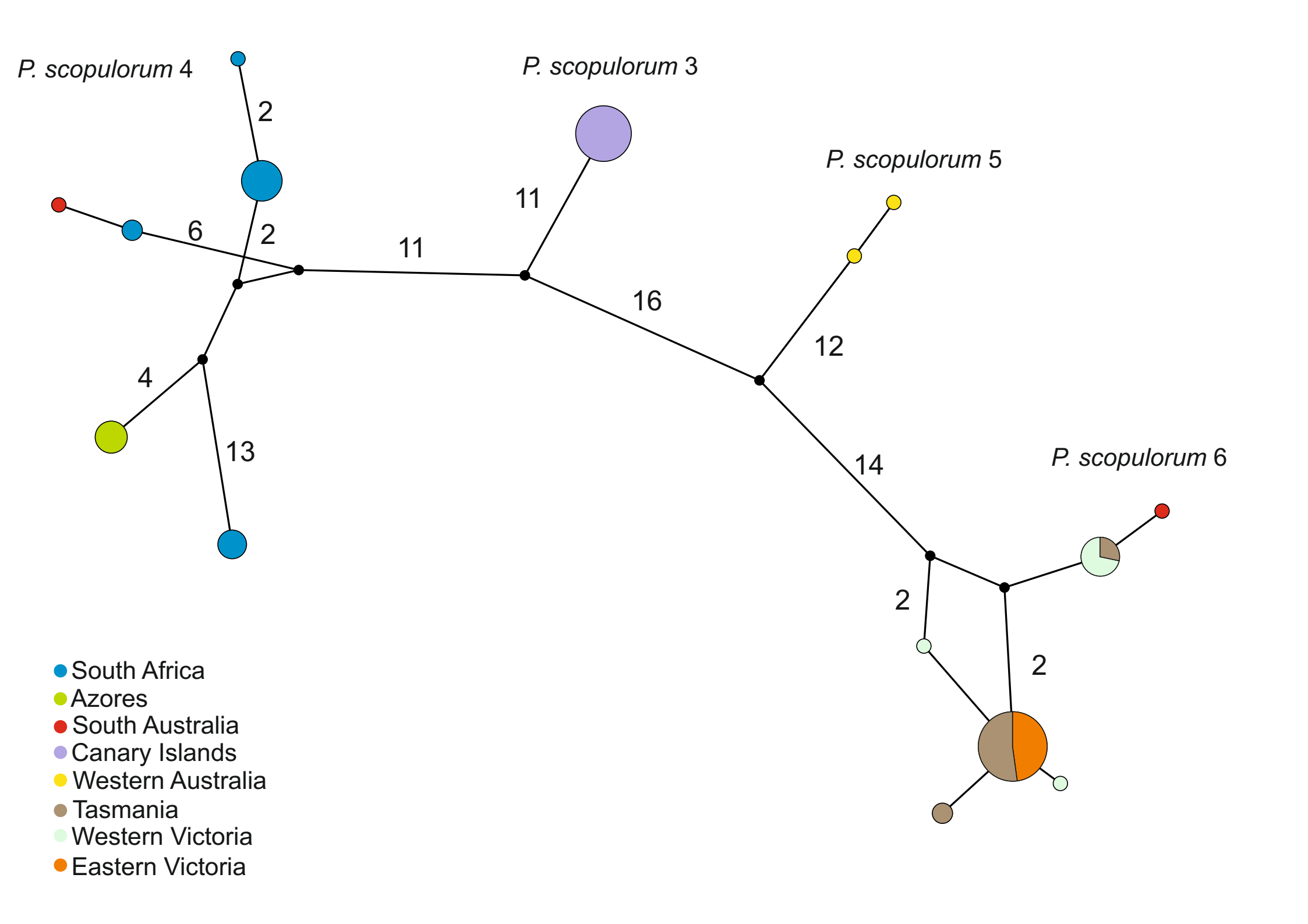
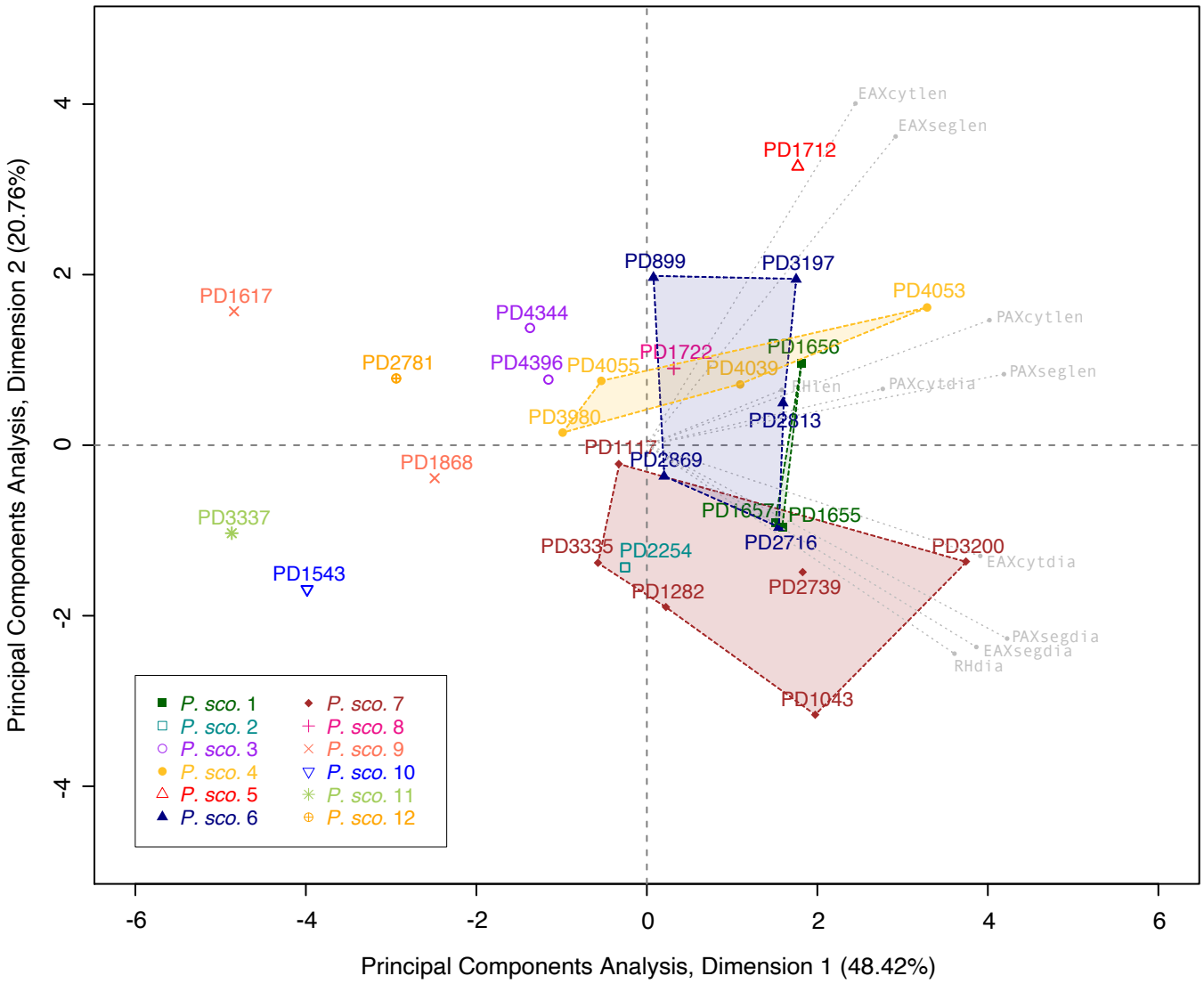
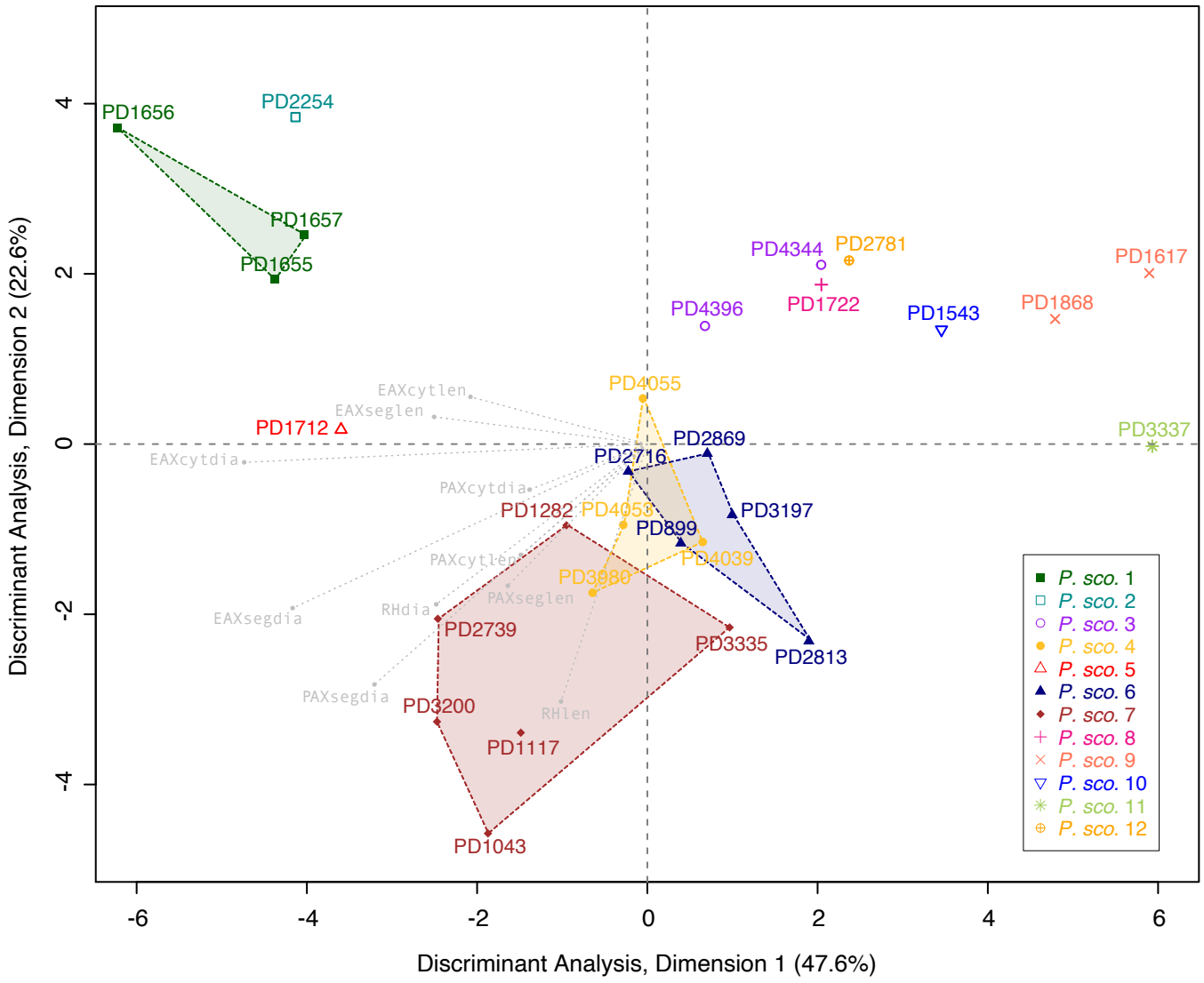
100
0.03

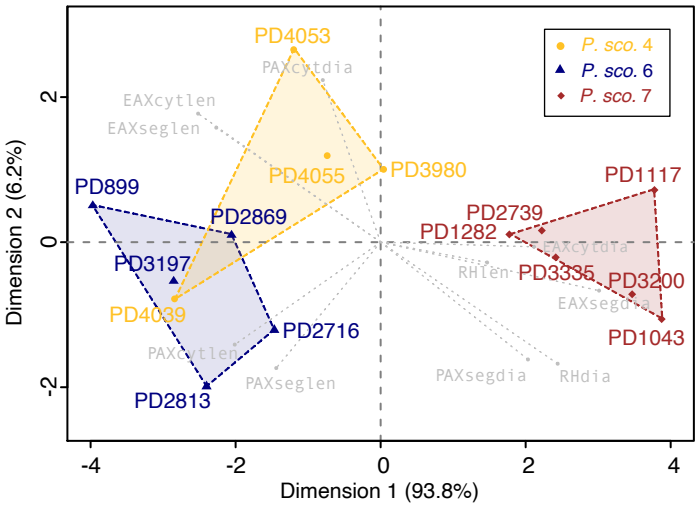

A
B
C
D
1
2
3
4
5
6
7
8
9
10
11
12


P. scopulorum 4
P. scopulorum 3
P. scopulorum 5
P. scopulorum 6
2
6
2
11
11
16
12
4
13
14
2
2
South Africa
Azores
South Australia
Canary Islands
Western Australia
Tasmania
Western Victoria
Eastern Victoria

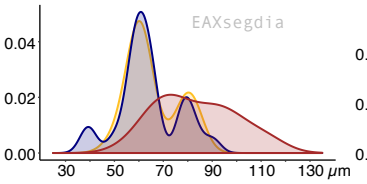

Principal Components Analysis, Dimension 2 (20.76%)
Principal Components Analysis, Dimension 1 (48.42%)
-6
-4
-2
0
2
4
6
EAXcytlen
EAXseglen
PD1712
PD899
PD3197
PD1617
PD4053
PD4344
PAXcytlen
PD2781
PD4396
PD4055
PD1722
PD1656
PD4039
PAXcytdia
PAXseglen
PD3980
PD2813
PD1117
PD1868
PD2869
PD3337
PD1657
PD1655
PD3335
PD2254
PD2716
PD3200
EAXcytdia
PD1543
PD1282
PD2739
PAXsegdia
EAXsegdia
RHdia
PD1043
P. sco. 1
P. sco. 2
P. sco. 3
P. sco. 4
P. sco. 5
P. sco. 6
P. sco. 7
P. sco. 8
P. sco. 9
P. sco. 10
P. sco. 11
P. sco. 12


PD1656
PD2254
PD1657
PD1655
PD4344
PD2781
PD1617
PD4396
PD1722
PD1543
PD1868
EAXcytlen
EAXseglen
PD4055
PD1712
PD3337
PD2869
EAXcytdia
PD2716
PAXcytdia
PD1282
PD4058
PD3197
PD899
PAXcytlen
PD4039
PD3980
EAXsegdia
RHdia
PAXseglen
PD2739
PD3335
PD2813
PAXsegdia
PD3200
RHlen
PD1117
PD1043
P. sco. 1
P. sco. 2
P. sco. 3
P. sco. 4
P. sco. 5
P. sco. 6
P. sco. 7
P. sco. 8
P. sco. 9
P. sco. 10
P. sco. 11
P. sco. 12
Discriminant Analysis, Dimension 2 (22.6%)
Discriminant Analysis, Dimension 1 (47.6%)
-6
-4
-2
0
2
4
6
-4
-2
0
2
4

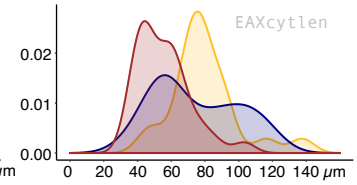
A. Discriminant analysis



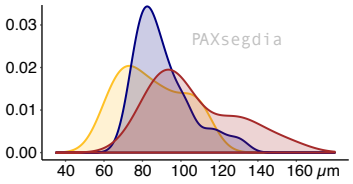
B. Erect axis diameter



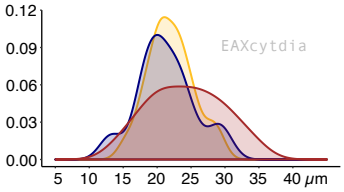
D. Erect axis cytoplasm length

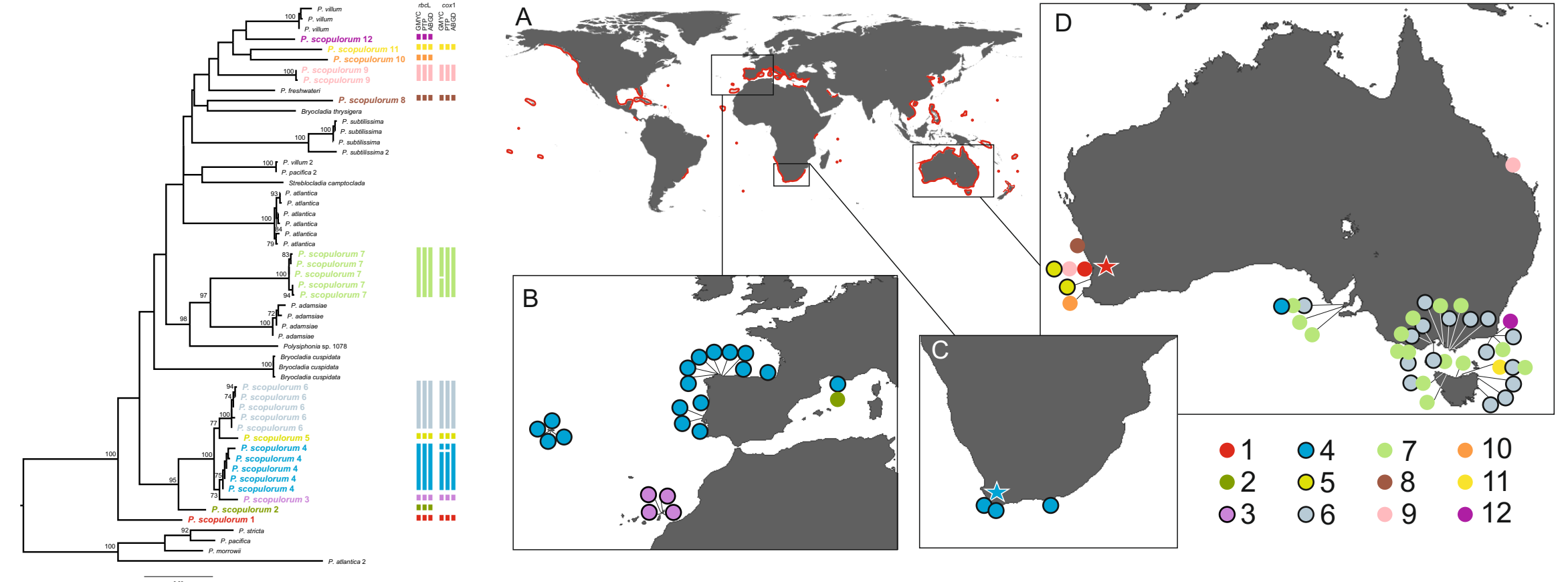


C. Prostrate axis diameter



E. Erect axis cytoplasm diameter




rbcL
cox1
GMYC
PTP
ABGD
P. villum
P. villum
P. villum
P. scopulorum 12
P. scopulorum 11
P. scopulorum 10
P. scopulorum 9
P. scopulorum 9
P. freshwateri
P. scopulorum 8
Bryocladia thrysigera
P. subtilissima
P. subtilissima
P. subtilissima
P. subtilissima 2
P. villum 2
P. pacifica 2
Streblocladia camptoclada
P. atlantica
P. atlantica
P. atlantica
P. atlantica
P. atlantica
P. atlantica
P. scopulorum 7
P. scopulorum 7
P. scopulorum 7
P. scopulorum 7
P. scopulorum 7
P. adamsiae
P. adamsiae
P. adamsiae
P. adamsiae
Polysiphonia sp. 1078
Bryocladia cuspidata
Bryocladia cuspidata
Bryocladia cuspidata
P. scopulorum 6
P. scopulorum 6
P. scopulorum 6
P. scopulorum 6
P. scopulorum 6
P. scopulorum 5
P. scopulorum 4
P. scopulorum 4
P. scopulorum 4
P. scopulorum 4
P. scopulorum 3
P. scopulorum 2
P. scopulorum 1
P. stricta
P. pacifica
P. morrowii
P. atlantica 2
A
B
C
D
1
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12

Fig. S1. Tetrasporangia cover cells in the *Polysiphonia scopulorum* complex. A-G and H: *P. scopulorum* 3-9 and 11, respectively. Detail of erect axes showing tetrasporangia (t), with two presporangial cover cells (pr), a postsporangial cover cell (po) and a scar cells of trichoblasts (sc). Tetrasporangia not shown in A, E and H because they are in a lower focal plane. The postsporangial cover cell is absent in E, and scar cells are absent in G. Scale bars = 40 µm.

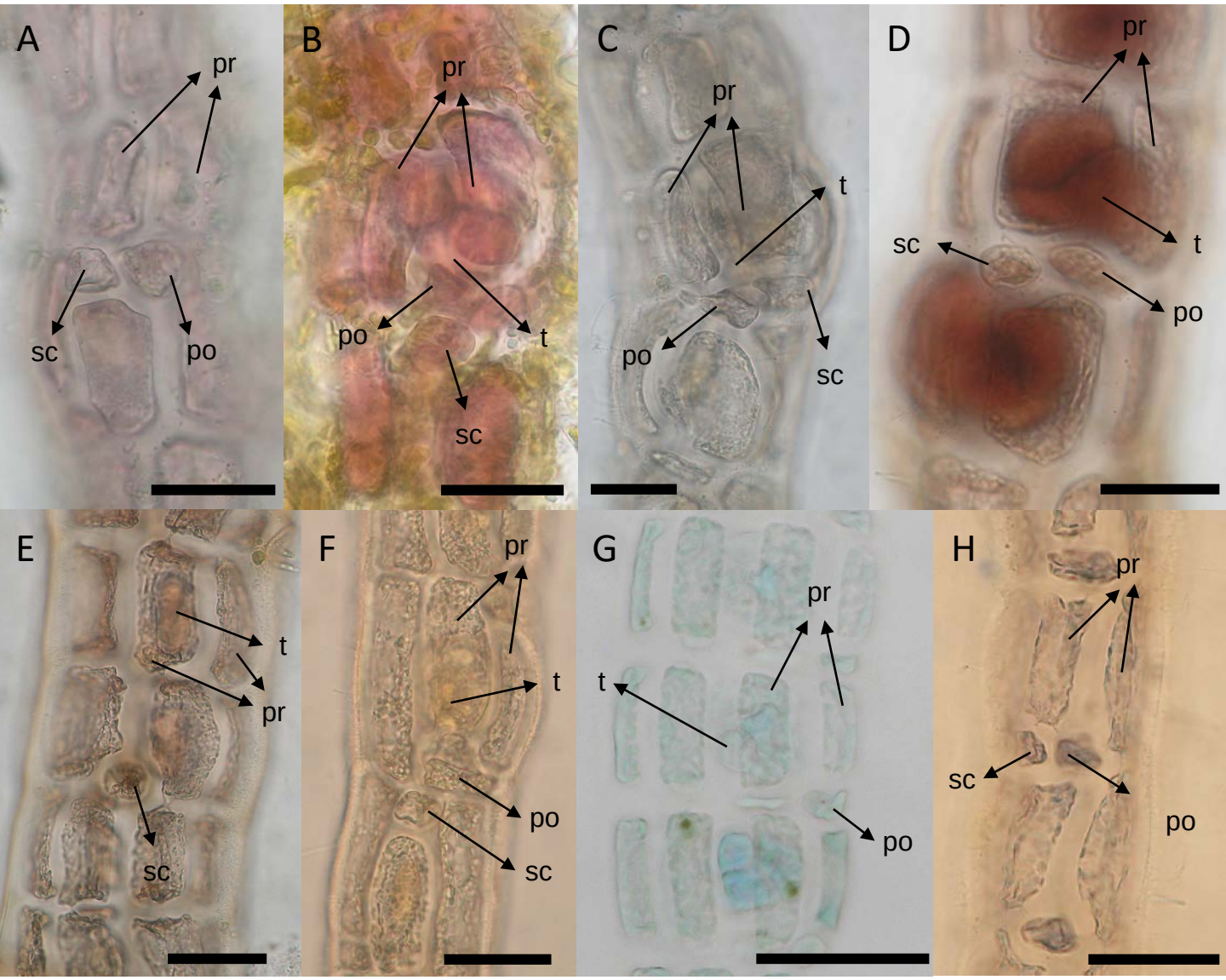

Table S1. Specimens collection information indicating the ones used in morphometric analyses (M), GenBank accesion numbers and length of sequences.

Species	Specimen	Collection information	M	*rbc* L	length	*cox* 1	length
P. scopulorum 1	PD1637	Parakeet Beach, Rottnest Island, Western Australia; 17.03.2015		MT676284	702	-	-
	PD1655	Parakeet Beach, Rottnest Island, Western Australia; 17.03.2015	1	MT676285	1367	MT676017	847
	PD1656	Parakeet Beach, Rottnest Island, Western Australia; 17.03.2015	1	MT676286	1096	MT676015	868
	PD1657	Parakeet Beach, Rottnest Island, Western Australia; 17.03.2015	1	MT676287	1379	MT676016	847
	15Psc101	Fays Bay, Rottnest Island, Western Australia (Huisman et al. 2017)		MF139308	1250	-	-
	15Psc102	Fays Bay, Rottnest Island, Western Australia (Huisman et al. 2017)		MF139309	1250	-	-
	15Psc103	Fays Bay, Rottnest Island, Western Australia (Huisman et al. 2017)		MF139310	1250	-	-
P. scopulorum 2	PD2254	Calanque du Sormiou, France; 23.05.2016	1	MT676277	1279	-	-
P. scopulorum 3	PD4124	Puerto del Carmen, Canary Islands; 11.06.2018		MT676159	1337	MT676018	819
	PD4136	Puerto del Carmen, Canary Islands; 11.06.2018		MT676160	1354	MT676019	819
	PD4233	El Golfo, Canary Islands; 12.06.2018		MT676161	1238	-	-
	PD4296	Caleta de Famara, Canary Islands; 13.06.2018		MT676162	1339	MT676020	819
	PD4300	Caleta de Famara, Canary Islands; 13.06.2018		MT676163	1339	MT676021	819
	PD4308	Caleta de Famara, Canary Islands; 13.06.2018		MT676164	1356	MT676022	819
	PD4322	Caleta de Famara, Canary Islands; 13.06.2018		MT676165	1349	MT676023	819
	PD4332	Caleta de Famara, Canary Islands; 13.06.2018		MT676166	1339	MT676024	819
	PD4334	Caleta de Famara, Canary Islands; 13.06.2018		MT676167	1363	MT676025	819
	PD4339	Castillo de Águila, Canary Islands; 14.06.2018		MT676168	1365	MT676026	819
	PD4344	Castillo de Águila, Canary Islands; 14.06.2018	1	MT676169	1346	MT676027	819
	PD4347	Castillo de Águila, Canary Islands; 14.06.2018		MT676170	1336	MT676028	819
	PD4349	Castillo de Águila, Canary Islands; 14.06.2018		MT676171	1346	-	-
	PD4354	Castillo de Águila, Canary Islands; 14.06.2018		MT676172	1362	MT676031	740
	PD4396	Puerto del Carmen, Canary Islands; 15.06.2018	1	MT676173	1369	MT676029	819
	PD4398	Puerto del Carmen, Canary Islands; 15.06.2018		MT676174	1358	MT676030	819
P. scopulorum 4	PD4553	Gym Beach, South Australia; 27.10.2018		MT676191	1363	MT676036	850
	CH1290	Spain (Bárbara et al. 2013)		JX828149	1467	-	-
	SANT.A.24676	Almograve, Portugal; 22.02.2011		MT676205	1457	KF648510	614
	SANT.A.25144	Zumaia, Spain; 18.03.2011		MT676206	1449	KF648509	614
	PD2245	Calanque du Sormiou, France; 23.05.2016		MT676207	744	-	-

PD3684	Praia do Populo, Azores; 12.04.2018		MT676193	1376	MT676039	833
PD3691	Praia do Populo, Azores; 12.04.2018		MT676194	1376	MT676040	833
PD3759	Maia, Azores; 13.04.2018		MT676195	1376	MT676044	752
PD3760	Maia, Azores; 13.04.2018		MT676196	1376	MT676046	712
PD3767	Maia, Azores; 13.04.2018		MT676203	1383	MT676045	752
PD3775	Maia, Azores; 13.04.2018		MT676197	1376	MT676047	698
PD3778	Maia, Azores; 13.04.2018		MT676204	1365	-	-
PD3820	Praia San Roque, Azores; 15.04.2018		MT676198	1376	MT676048	722
PD3822	Praia San Roque, Azores; 15.04.2018		MT676199	1376	MT676049	684
PD3824	Praia San Roque, Azores; 15.04.2018		MT676200	1376	MT676050	747
PD3963	Pau d´Agua, Azores; 19.04.2018		MT676201	1376	MT676041	833
PD3968	Pau d´Agua, Azores; 19.04.2018		MT676202	1376	MT676042	833
PD3980	Pau d´Agua, Azores; 19.04.2018	1	MT676192	1377	MT676043	833
PD4009	Hermanaus, South Africa		MT676189	1297	MT676037	850
PD4015	Hermanaus, South Africa		MT676190	1338	MT676038	849
PD4026	Jeffrey´s Bay, South Africa		MT676179	1323	MT676052	850
PD4030	Jeffrey´s Bay, South Africa		MT676180	1031	MT676051	850
PD4032	Jeffrey´s Bay, South Africa		MT676181	1337	MT676059	867
PD4033	Jeffrey´s Bay, South Africa		MT676182	1349	MT676053	850
PD4035	Jeffrey´s Bay, South Africa		MT676183	1354	-	-
PD4037	Jeffrey´s Bay, South Africa		MT676184	1328	MT676054	850
PD4039	Jeffrey´s Bay, South Africa	1	MT676185	1351	MT676055	850
PD4042	Jeffrey´s Bay, South Africa		MT676186	1351	MT676056	850
PD4044	Jeffrey´s Bay, South Africa		MT676187	1356	MT676057	850
PD4045	Jeffrey´s Bay, South Africa		MT676188	1345	MT676058	850
PD4048	Muizenberg Bay; South Africa		MT676175	1341	MT676032	850
PD4052	Muizenberg Bay; South Africa		MT676176	1353	MT676033	850
PD4053	Muizenberg Bay; South Africa	1	MT676177	1358	MT676035	869
PD4055	Muizenberg Bay; South Africa	1	MT676178	1222	MT676034	850
SANT.A.24420	Galicia, Spain (Díaz-Tapia & Bárbara 2013)		-	-	KF671181	614
SANT.A.24413	Galicia, Spain (Díaz-Tapia & Bárbara 2013)		-	-	KF671178	614
SANT.A.24214	Portugal (Díaz-Tapia & Bárbara 2013)		-	-	KF671177	614
SANT.A.24435	Galicia, Spain (Díaz-Tapia & Bárbara 2013)		-	-	KF671175	614

	SANT.A.24212	Portugal (Díaz-Tapia & Bárbara 2013)		-	-	KF671166	614
	SANT.A.24343	Asturias, Spain (Díaz-Tapia & Bárbara 2013)		-	-	KF671163	614
	SANT.A.24479	Galicia, Spain (Díaz-Tapia & Bárbara 2013)		-	-	KF671161	614
	SANT.A.24257	Portugal (Díaz-Tapia & Bárbara 2013)		-	-	KF671159	614
	SANT.A.24158	Asturias, Spain (Díaz-Tapia & Bárbara 2013)		-	-	KF671152	614
P. scopulorum 5	PD1636	Parakeet Beach, Rottnest Island, Western Australia; 17.03.2015		MT676278	1413	MT676060	850
	PD1712	Grey, Western Australia; 20.03.2015	1	MT676279	1249	MT676061	886
P. scopulorum 6	PD770	13th beach, Victoria, Australia; 9.11.2014		MT676239	1341	MT676069	850
	PD899	Queenscliff beach, Victoria, Australia; 01.12.2014	1	MF093999	1467	MF093999	1605
	PD984	Minya reef, Victoria, Australia; 10.12.2014		MT676233	1344	MT676065	850
	PD1120	Killornei beach, Victoria, Australia; 27.12.2014		MT676234	1407	MT676068	859
	PD1258	Inverloch, Victoria, Australia; 18.01.2015		MT676235	1375	-	-
	PD1295	Flat Rocks, Victoria, Australia; 19.01.2015		MT676241	705	MT676066	850
	PD1333	Walkerville, Victoria, Australia; 20.01.2015		MT676229	1406	MT676074	850
	PD1480	Back Beach, Sorento, Victoria, Australia; 28.02.2015		MT676232	1045	MT676064	848
	PD2716	Bastion point, Victoria, Australia; 08.11.2016	1	MT676227	1310	MT676076	849
	PD2813	Bastion point, Victoria, Australia; 11.11.2016	1	MT676226	1315	MT676078	850
	PD2814	Bastion point, Victoria, Australia; 11.11.2016		MT676225	1103	MT676079	850
	PD2816	Bastion point, Victoria, Australia; 11.11.2016		MT676224	1314	MT676080	850
	PD2823	Bastion point, Victoria, Australia; 11.11.2016		MT676221	1320	MT676077	849
	PD2824	Bastion point, Victoria, Australia; 11.11.2016		MT676220	1305	MT676081	850
	PD2863	Shipwreck Creek, Victoria, Australia; 13.11.2016		MT676218	1295	MT676082	850
	PD2869	Shipwreck Creek, Victoria, Australia; 13.11.2017	1	MT676238	1318	MT676070	850
	PD2879	Shipwreck Creek, Victoria, Australia; 13.11.2018		MT676222	1320	MT676075	848
	PD2885	Shipwreck Creek, Victoria, Australia; 13.11.2019		MT676208	1086	MT676083	848
	PD3195	Boat Harbour, Tasmania, Australia; 02.11.2017		MT676231	1308	MT676067	850
	PD3197	Boat Harbour, Tasmania, Australia; 02.11.2017	1	MT676230	1318	MT676063	867
	PD3326	Georgetown, Tasmania, Australia; 05.11.2017		MT676237	1309	MT676071	850
	PD3386	The Gardens, Tasmania, Australia; 07.11.2017		MT676217	1308	MT676084	850
	PD3422	Binaong Bay, Tasmania, Australia; 08.11.2017		MT676236	1015	MT676072	857
	PD3430	Binaong Bay, Tasmania, Australia; 08.11.2017		MT676223	1320	MT676085	850
	PD3433	Binaong Bay, Tasmania, Australia; 08.11.2017		MT676215	1308	MT676073	850
	PD3439	Binaong Bay, Tasmania, Australia; 08.11.2017		MT676214	1320	MT676086	850

	PD3442	Binaong Bay, Tasmania, Australia; 08.11.2017		MT676213	1321	MT676087	850
	PD3455	Binaong Bay, Tasmania, Australia; 08.11.2017		MT676219	1295	MT676088	850
	PD3458	Bicheno, Tasmania, Australia; 09.11.2017		MT676212	1290	MT676089	850
	PD3473	Bicheno, Tasmania, Australia; 09.11.2017		MT676211	1307	MT676090	850
	PD3479	Bicheno, Tasmania, Australia; 09.11.2017		MT676228	1310	MT676091	850
	PD3486	Bicheno, Tasmania, Australia; 09.11.2017		MT676210	1349	MT676092	850
	PD3489	Bicheno, Tasmania, Australia; 09.11.2017		MT676209	1298	MT676093	850
	PD3492	Bicheno, Tasmania, Australia; 09.11.2017		MT676216	1308	MT676094	850
	PD4550	Gym Beach, South Australia; 27.10.2018		MT676240	1361	MT676062	850
P. scopulorum 7	PD762	Lighthouse reef, Victoria, Australia; 08.11.2014		MT676260	1410	MT676108	850
	PD954	Galaneuse reef, Victoria, Australia; 02.12.2014		MT676261	1410	MT676109	850
	PD969	13th beach, Victoria, Australia; 09.11.2014		MT676259	1038	MT676110	850
	PD987	Minya reef, Victoria, Australia; 10.12.2014		MT676251	664	-	-
	PD1036	Minya reef, Victoria, Australia; 10.12.2014		MT676258	1340	-	-
	PD1043	Kilkunda, Victoria, Australia; 23.12.2014	1	MT676257	1409	MT676111	850
	PD1052	Kilkunda, Victoria, Australia; 23.12.2014		MH101826	1413	MT676114	850
	PD1117	Killornei beach, Victoria, Australia; 27.12.2014	1	MT676256	1327	MT676095	850
	PD1121	Pea Soup, Victoria, Australia; 28.12.2014		MT676265	1327	MT676107	850
	PD1282	Twen Reef, Victoria, Australia; 19.01.2015	1	MT676255	1411	MT676112	850
	PD1477	Back Beach, Sorento, Victoria, Australia; 28.02.2015		MT676254	1385	MT676113	850
	PD2739	Pebbly beach, Victoria, Australia; 09.11.2016	1	MT676253	1126	MT676115	850
	PD2758	Pebbly beach, Victoria, Australia; 09.11.2016		MT676252	1324	MT676116	850
	PD2765	Pebbly beach, Victoria, Australia; 09.11.2016		MT676262	1306	MT676117	850
	PD3200	Boat Harbour, Tasmania, Australia; 02.11.2017	1	MT676249	1305	MT676096	850
	PD3202	Boat Harbour, Tasmania, Australia; 02.11.2017		MT676243	1314	MT676097	850
	PD3210	Boat Harbour, Tasmania, Australia; 02.11.2017		MT676248	1308	MT676098	850
	PD3233	Devonport, Tasmania, Australia; 03.11.2017		MT676242	1038	MT676099	850
	PD3241	Devonport, Tasmania, Australia; 03.11.2017		MT676263	1308	MT676105	850
	PD3242	Devonport, Tasmania, Australia; 03.11.2017		MT676244	1315	MT676100	850
	PD3265	Devonport, Tasmania, Australia; 03.11.2017		MT676250	1318	MT676101	850
	PD3289	Wynyard, Tasmania, Australia; 04.11.2017		MT676264	1316	MT676106	850
	PD3335	Georgetown, Tasmania, Australia; 05.11.2017	1	MT676247	1323	MT676102	850
	PD3338	Georgetown, Tasmania, Australia; 05.11.2017		MT676245	1301	MT676103	850

	PD3339	Georgetown, Tasmania, Australia; 05.11.2017		MT676246	1313	MT676104	850
	PD4545	Gym Beach, South Australia; 27.10.2018		MT676269	1368	MT676118	850
	PD4547	Gym Beach, South Australia; 27.10.2018		MT676271	1361	MT676119	850
	PD4638	Chinaman Hut, South Australia; 29.10.2018		MT676270	1368	MT676121	850
	PD4640	Chinaman Hut, South Australia; 29.10.2018		MT676273	1366	MT676122	850
	PD4641	Chinaman Hut, South Australia; 29.10.2018		MT676266	1368	MT676125	850
	PD4646	Chinaman Hut, South Australia; 29.10.2018		MT676268	1361	-	-
	PD4652	Chinaman Hut, South Australia; 29.10.2018		MT676267	1363	MT676123	850
	PD4653	Chinaman Hut, South Australia; 29.10.2018		MT676274	1365	MT676124	850
	PD4690	Pondalowi Bay, South Australia; 30.10.2018		MT676272	1360	MT676120	850
P. scopulorum 8	PD1722	Green Head, Western Australia; 21.03.2015	1	MT676281	1361	MT676132	850
P. scopulorum 9	PD1617	Rottnest Island, Western Australia; 15.03.2015	1	MT676275	1410	MT676126	850
	PD1868	Gladstone, Queensland; 13.05.2015	1	MT676276	1412	MT676127	850
P. scopulorum 10	PD1543	Channel Rocks, Western Australia; 21.03.2015	1	MT676282	704	-	-
P. scopulorum 11	PD3337	Georgetown, Tasmania, Australia; 05.11.2017	1	MT676280	1307	MT676133	840
P. scopulorum 12	PD2781	South Gabo Harbour, Victoria, Australia; 10.11.2016	1	MT676283	1320	-	-
Bryocladia cuspidata	GGSCSR	Venezuela (García-Soto & López-Bautista 2018)		MH388522	1057	-	-
	-	-		AF259498	1435	-	-
	PD618	Brazil (Díaz-Tapia et al. 2018a)		MH395866	1342	-	-
Bryocladia thrysigera	PD662	Brazil (Díaz-Tapia et al. 2018a)		MF094040	1303	-	-
	PD619	Praia de Parati, Ubu; 08.09.2014		MT676134	1301	-	-
P. adamsiae	PD2751	Australia (Díaz-Tapia et al. 2018a)		MH101810	1315	-	-
	PD2752	Australia (Díaz-Tapia et al. 2018a)		MH101811	1305	-	-
	PD3080	Australia (Díaz-Tapia et al. 2018a)		MH101812	1316	-	-
	PD3377	Australia (Díaz-Tapia et al. 2018a)		MH101813	1322	-	-
	PD3380	Australia (Díaz-Tapia et al. 2018a)		MH101814	1311	-	-
	PD3382	Australia (Díaz-Tapia et al. 2018a)		MH101815	1321	-	-
	PD3384	Australia (Díaz-Tapia et al. 2018a)		MH101816	1311	-	-
	PD3414	Australia (Díaz-Tapia et al. 2018a)		MH101817	1316	-	-
	PD3456	Australia (Díaz-Tapia et al. 2018a)		MH101818	1314	-	-
	PD3461	Australia (Díaz-Tapia et al. 2018a)		MH101819	1320	-	-
	PD3589	Australia (Díaz-Tapia et al. 2018a)		MH101820	1325	-	-
	PD3590	Australia (Díaz-Tapia et al. 2018a)		MH101821	1349	-	-

	PD3591	Australia (Díaz-Tapia et al. 2018a)	MH101822	1304	-	-
	PD3595	Australia (Díaz-Tapia et al. 2018a)	MH101823	1309	-	-
	PD3602	Australia (Díaz-Tapia et al. 2018a)	MH101824	1316	-	-
	PD3606	Australia (Díaz-Tapia et al. 2018a)	MH101825	1293	-	-
P. atlantica	CH1268	Spain (Bárbara et al. 2013)	JX828141	1467	-	-
	CH1285	Spain (Bárbara et al. 2013)	JX828142	1467	-	-
	PD4105	Baleo, A Coruña, Spain; 27.05.2018	MT676137	1320	-	-
	PD4107	Baleo, A Coruña, Spain; 27.05.2018	MT676136	1339	-	-
	PD4108	Baleo, A Coruña, Spain; 27.05.2018	MT676135	1372	-	-
	PD3677	Praia do Populo, Azores; 12.04.2018	MT676145	1311	-	-
	PD3680	Praia do Populo, Azores; 12.04.2018	MT676138	1328	-	-
	PD3681	Praia do Populo, Azores; 12.04.2018	MT676146	1336	-	-
	PD3687	Praia do Populo, Azores; 12.04.2018	MT676147	1336	-	-
	PD3724	Calhetas, Azores; 13.04.2018	MT676153	1351	-	-
	PD3774	Maia, Azores; 13.04.2018	MT676154	1334	-	-
	PD3776	Maia, Azores; 13.04.2018	MT676155	1334	-	-
	PD3830	Praia San Roque, Azores; 15.04.2018	MT676148	1375	-	-
	PD3831	Praia San Roque, Azores; 15.04.2018	MT676149	1351	-	-
	PD3845	Praia San Roque, Azores; 15.04.2018	MT676150	1379	-	-
	PD3871	Caloura, Azores; 16.04.2018	MT676143	1370	-	-
	PD3877	Vila Franca, Azores; 17.04.2018	MT676156	1375	-	-
	PD3880	Vila Franca, Azores; 17.04.2018	MT676142	1359	-	-
	PD3878	Vila Franca, Azores; 17.04.2018	MT676157	1375	-	-
	PD3879	Vila Franca, Azores; 17.04.2018	MT676151	1377	-	-
	PD3883	Vila Franca, Azores; 17.04.2018	MT676144	1370	-	-
	PD3907	Vila Franca, Azores; 18.04.2018	MT676158	1383	-	-
	PD3920	Vila Franca, Azores; 18.04.2018	MT676152	1384	-	-
	PD3966	Pau d´Agua, Azores; 19.04.2018	MT676139	1374	-	-
	PD3977	Pau d´Agua, Azores; 19.04.2018	MT676140	1382	-	-
	PD3981	Pau d´Agua, Azores; 19.04.2018	MT676141	1383	-	-
	SANT.A.26230	Llas, Asturias, Spain; 19.iv.2011	-	-	MT676131	619
P. atlantica 2	NC.04	USA (Stuercke & Freshwater 2008)	EU492910	1341	-	-
P. freshwateri	CUK10427	Korea (Bustamante et al. 2015)	KJ957812	1395	-	-

P. morrowii	AC229	Korea (Carlile 2009, as *Polysiphonia* sp.)	GQ252569	1467	-	-
P. pacifica	P194	USA (Kim & Yang 2005)	AY958162	1401	-	-
Polysiphonia sp.	SMB-2003a	Chile (Kim et al. 2004)	AY396038	1379	-	-
	SMB-2003a	Chile (Kim et al. 2004)	AY396037	1379	-	-
P. scopulorum var *villum* sensu Savoie & Saunders (2019)	AC172	USA, (Carlile 2009, as *P. pacifica* var *delicatula*)	GQ252566	1467	-	-
	AC181	USA (Carlile 2009, as *P. pacifica*)	GQ252565	1467	-	-
	-	USA (Kim et al. 2004)	AY396039	1379	-	-
	GWS006329	USA (Savoie & Saunders 2019)	MF120852	1338	HM916746	664
	GWS006336	USA (Savoie & Saunders 2019)	MF120834	1338	HM916753	664
	GWS002902	Canada (Savoie & Saunders 2019)	-	-	HM918528	664
	GWS004788	Canada (Savoie & Saunders 2019)	-	-	HM918699	664
	GWS008217	Canada (Savoie & Saunders 2019)	-	-	HM917000	664
	GWS008218	Canada (Savoie & Saunders 2019)	-	-	HM917001	664
	GWS008278	Canada (Savoie & Saunders 2019)	-	-	HM916554	664
	GWS009051	Canada (Savoie & Saunders 2019)	-	-	HM916560	664
	GWS019608	Canada (Savoie & Saunders 2019)	-	-	HQ544523	664
	GWS019672	Canada (Savoie & Saunders 2019)	-	-	HQ544569	664
	GWS020743	Canada (Savoie & Saunders 2019)	-	-	HQ545054	664
	GWS020755	Canada (Savoie & Saunders 2019)	-	-	HQ545062	664
	GWS022212	USA (Savoie & Saunders 2019)	-	-	KM254519	661
	GWS022224	USA (Savoie & Saunders 2019)	-	-	KM254911	661
	GWS022244	USA (Savoie & Saunders 2019)	-	-	HQ544219	661
	GWS022266	USA (Savoie & Saunders 2019)	-	-	KM254964	604
	GWS022301	USA (Savoie & Saunders 2019)	-	-	KM254832	661
	GWS022390	USA (Savoie & Saunders 2019)	-	-	KM254236	664
	GWS028437	Canada (Savoie & Saunders 2019)	-	-	MF120785	664
	GWS034876	Canada (Savoie & Saunders 2019)	-	-	MF120768	664
	GWS036020	Canada (Savoie & Saunders 2019)	-	-	MF120627	664
P. stricta	PD550	UK (Díaz-Tapia et al. 2017)	MF101428	1467	-	-
P. subtilissima	NC.21	North Carolina, USA (Stuercke & Freshwater 2008)	EU492917	1341	-	-
	NC.24	Panama (Mamoozadeh & Freshwater 2012)	-	-	HM573529	639

	-	USA (Lam et al. 2013)	JX294917	1352	JX294915	617
	HI.01	Hawai, USA (Stuercke & Freshwater 2008)	EU492919	1341	-	-
	-	Spain (Lam et al. 2013)	JX294918	1352	JX294916	617
P. subtilissima 2	PHYKOS.3271	Panama (Mamoozadeh & Freshwater 2012)	HM573575	1284	-	-
P. villum sensu Díaz-Tapia et al. 2018b	SANT.A.25434	Spain (Díaz-Tapia et al. 2018b)	MG975710	1356	MT676128	617
	SANT.A.25665	Spain (Díaz-Tapia et al. 2018b)	MG975711	1350	MT676130	617
	SANT.A.28109	Spain (Díaz-Tapia et al. 2018b)	MG975716	1357	-	-
	SANT.A.26253	Ber, A Coruña, Spain; 17.04.2011	-	-	MT676129	617
	PD2249	France (Díaz-Tapia et al. 2018b)	MG975721	1231	-	-
	PD2250	France (Díaz-Tapia et al. 2018b)	MG975722	13281	-	-
	PD3058	North Carolina, USA (Díaz-Tapia et al. 2018b)	MG975712	1318	-	-
	NC.33	North Carolina, USA (Stuercke & Freshwater 2008, as *P. scopulorum* var. v*illum)*	EU492915	1341	-	-
	NC.09	North Carolina, USA (Stuercke & Freshwater 2008, as *P. scopulorum* var. v*illum)*	-	-	HM573535	673
	PD603	Brazil (Díaz-Tapia et al. 2018b)	MG975717	837	-	-
	PD614	Brazil (Díaz-Tapia et al. 2018b)	MG975718	1327	-	-
	PD631	Brazil (Díaz-Tapia et al. 2018b)	MG975719	1297	-	-
	PD967	Brazil (Díaz-Tapia et al. 2018b)	MG975720	1341	-	-
	PD3194	Australia (Díaz-Tapia et al. 2018b)	MG975715	1346	-	-
	PD3198	Australia (Díaz-Tapia et al. 2018b)	MG975713	1327	-	-
	PD3208	Australia (Díaz-Tapia et al. 2018b)	MG975714	1319	-	-
Streblocladia camptoclada	GWS036399	South Africa (Savoie & Saunders 2019)	MF120884	1363	MF120765	664

References

Bárbara, I., Choi, H.-G., Secilla, A., Diaz-Tapia, P., Gorostiaga, J.M., Seo, T.K., Jung, M.-Y. & Berecibar, 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. & Cho, T.O. (2015). *Polysiphonia freshwateri* sp. nov. and *Polysiphonia koreana* sp. nov.: two new species of *Polysiphonia* (Rhodomelaceae, Rhodophyta) from Korea. European Journal of Phycology 50: 330-342.

Carlile, A.L. 2009. Molecular Systematics of North Pacific Ceramiaceae (Rhodophyta): Phylogeny, Taxonomy, and Phylogeography. University of Washington, 296 pp.

Díaz-Tapia, P. & Bárbara, I. (2013). Seaweeds from sand-covered rocks of the Atlantic Iberian Peninsula. Part 1. The Rhodomelaceae (Ceramiales, Rhodophyta). Cryptogamie, Algologie, 34: 325-423.

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

Díaz-Tapia, P., Pasella, M. & Verbruggen, H. (2018a). Molecular analyses resolve the phylogenetic position of *Polysiphonia adamsiae* (Rhodomelaceae, Rhodophyta) and reveal a strong phylogeographic structure in Australia. *Phycologia* 57: 593-600.

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

García-Soto, G. & Lopez-Bautista, J. (2018). Taxonomic notes on the genus *Alsidium* C. Agardh, including the merging of *Bryothamnion* Kützing (Rhodomelaceae). Algae. 33: 215-229.

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, M.S. & Yang, E.C. (2005). Taxonomic note of *Polysiphonia pacifica* (Ceramiales, Rhodophyta) complex with focus on Pacific isolates. Algae 20: 15-23

Lam, D.W., Garcia-Fernández, M.E., Aboal, M. & Vis, M.L. (2013). *Polysiphonia subtilissima* (Ceramiales, Rhodophyta) from freshwater habitats in North America and Europe is confirmed as conspecific with marine collections. Phycologia 52: 156-160.

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.

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. (2008). Consistency of morphological characters used to delimit *Polysiphonia sensu lato species* (Ceramiales, Florideophyceae): analyses of North Carolina, USA specimens. Phycologia 47: 541-559.

Table S2. Percentage (above) and number (down) of bases that differ among species *rbc* L sequences included in our alingment.

	P. scopulorum 1	P. scopulorum 2	P. scopulorum 3	P. scopulorum 4	P. scopulorum 5	P. scopulorum 6	B. cuspidata	Polysiphonia sp.	P. adamsiae	P. scopulorum 7	P. atlantica	S. camptoclada	P. villum 2	P. subtilissima2	P. subtilissima	B. thrysigera	P. scopulorum 8	P. freshwateri	P. scopulorum 9	P. scopulorum 10	P. scopulorum 11	P. scopulorum 12	P. villum
P. scopulorum 1	0	5	7	7.1-7.8	5.1	6.4-7.1	5.9-6.2	6.7	6.2-6.5	6.1-6.4	6.8-6.9	6.9	6.7	7.9	7.5-7.8	6.3	7.8	5.4	5.5-5.6	6	5.7	4.9	4.9-5.2
P. scopulorum 2	64	-	3	2.7-2.9	3,4	3-3.1	5.9-6.1	6.2	5.7-6.2	6.5-6.7	5.7-5.9	6.4	5.8-5.9	7.2	6.8-6.9	6.3	7.7	5.5	5.8	5	6.6	5.5	4.7-5.1
P. scopulorum 3	68	39	0	0.9-1.1	1.6	1.7-2	6.7-7.1	7.1	6.7-7.2	7.3-7.7	6.9-7.1	7.4	7-7.1	8.6	7.9-8.2	6.5	8.4	6.8	6.7	6.5	8	6.7	6.9-7.3
P. scopulorum 4	66-68	34-37	12-15	0.5 (6)	1.3-1.6	1-1.5	6.2-6.9	6.2-7.1	6.2-7	6.7-7.3	6.2-6.8	6.6-7	6.1-6.9	8.3-8.7	7.7-8.3	6.3-6.8	8.1-8.6	6.3-6.8	6.2-6.7	6.1-6.4	7.8-8.3	6.4-6.7	6.7-7.4
P. scopulorum 5	70	43	22	18-21	0	1.2-1.4	6.8-7	6.7	6.9-7.3	7.5-7.8	6.8-7.2	7	7-7.1	8.3	7.8	6.8	8.5	6.2	6.3-6.4	6.1	8.2	6.6	6.9-7.2
P. scopulorum 6	69-73	38-40	23-25	15-19	17-19	0.4 (5)	6.3-7.1	6.5-6.6	6.4-7	7.1-7.6	6.1-6.7	6.8-7.1	6.4-7.2	8.3	7.7-8.1	6.5-6.9	8.2-8.5	6.1-6.5	5.9-6.3	6.5-7.2	7.7-7.8	6.4-6.6	6.9-7.4
B. cuspidata	62-83	66-77	75-92	71-90	74-93	71-90	0.1 (2)	7-7.2	7.1-7.7	6.4-6.9	6.5-7.7	7.4-7.8	6.3-6.7	8.2-8.3	7-8.2	5.7-6.3	7.4	5.7-6	6.3-6.5	5.3-5.6	7-7.2	5.5-5.9	6-6.4
Polysiphonia sp.	87	79	94	84-88	93	84-88	74-97	0	5.6-5.9	5.6-5.8	6.2-6.4	5.8	6.7	8.3	7.7-7.8	7.1	7.5	6	6.9	7.8	7.7	7.6	6.8-6.9
P. adamsiae	81-82	73-79	91-94	84-92	93-96	86-90	75-100	74-77	0.6 (8)	4.7-5.4	6.2-6.8	6,2-6,4	6.1-6.5	8.3-8.6	8.1-8.6	7.1-7.4	8-8.3	6.2-6.6	6.4-6.9	6.6-7.3	7.2-7.4	7.2-7.5	6.8-6.3
P. scopulorum 7	84-88	83-85	97-102	89-96	101-106	95-101	66-90	74-77	62-71	0.3 (4)	7.1-7.6	6.8-7.2	6-6.4	8.2-8.3	8-8.4	7.2-7.4	8.7-8.4	7-7.1	7.4-7.5	6.4-7	8-8.3	7.7-7.9	6.8-7.4
P. atlantica	93-95	73-75	93-97	86-93	94-98	81-91	69-109	84-86	83-89	95-102	0.5 (7)	5.6-5.9	4.9-5.3	6.9-7.2	6.8-7.3	6.3-6.5	6.8-7.1	5.9-6.2	6.1-6.5	6.1-6.7	6.4-6.7	6.2-6.3	5.8-6.3
S. camptoclada	93	82	98	90-93	96	91-94	78-105	78	82-84	91-95	75-78	-	5.3-5.4	7.2	7-7.2	5.9	6.9	5.4	6.5	7.1	7.1	6.4	6.3-6.7
P. villum 2	92-93	74-75	96-97	91-93	99-100	93-96	67-96	92-93	82-86	80-88	70-74	72-73	0.1 (1)	7-7.1	6.9-7.2	5.1	7.3-7.4	5.4-5.4	6.5-6.7	6.5-7.2	4.7-5.1	4.9-5	4.9-5.3
P. subtilissima 2	101	90	108	106-109	107	104-107	81-106	107	105-109	105-106	88-92	93	90-91	-	2-2.2	7.5	7.7	6.1	7.5	6.9	7.9	6.9	6.8-6.3
P. subtilissima	101-102	87-88	105-106	103-107	105-106	102-106	82-106	103-104	107-111	102-112	92-99	93-96	93-98	26-28	0.3 (4)	7.3-7.5	7.7-8	6.2-6.5	7.5-7.8	6.8-7	7.9-8	7.4-7.5	6.3-6.9
B. thrysigera	80	78	81	82-85	88	85-87	56-82	92	89-92	91-96	80-82	75	66-67	95	96-98	0	6.7	5.1	5.5	6,8	6.7	5.2	5,1-5,5
P. scopulorum 8	108	99	114	114-117	120	110-117	78-105	104	105-110	113-119	95-98	94	104-105	99	104-108	87	-	6.7	6.4-6.5	8,2	6.7	6.7	6.1-6.5
P. freshwateri	80	70	91	88-91	87	82-87	63-81	83	83-87	92-97	82-84	73	76-77	78	87	67	94	-	4,4-4,5	5,3	5.4	4.8	4.6-5.1
P. scopulorum 9	86-87	74	90-91	88-91	89-90	82-86	69-89	95	86-91	99-105	85-89	89	78-80	99	100-105	72	91-92	62-63	0.1 (1)	5.3-5.5	5.8	5.1	5.4-5.7
P. scopulorum 10	42	32	46	43-46	43	44-47	35-37	52	46-48	43-46	42-47	48	44-47	42	43-48	40	58	36	37-38	-	5.6	4.4	3.5-3.9
P. scopulorum 11	94	85	104	102-107	107	99-102	74-91	100	93-96	104-406	84-87	93	61-66	100	102-103	84	88	71	76	36	-	4.7	4.7-5.1
P. scopulorum 12	79	70	87	84-87	87	84-86	57-77	100	94-99	101-103	82-83	85	65-66	88	97	65	88	63	67	29	61	-	3.6-4.1
P. villum	82-84	60-65	93-97	91-97	93-97	91-96	61-68	90-91	89-95	91-97	79-82	71-76	65-71	81-85	83-92	65-68	81-87	61-68	71-76	36-41	61-66	47-53	0.6 (7)

Table S3. Percentage (above) and number (down) of bases that differ among species *cox1* sequences included in our alingment.

	P. scopulorum 1	P. scopulorum 3	P. scopulorum 4	P. scopulorum 5	P. scopulorum 6	P. scopulorum 7	P. atlantica	S. camptoclada	P. villum 2	P. subtilissima	P. scopulorum 8	P. scopulorum 9	P. scopulorum 11	P. villum
P. scopulorum 1	0	6.2	6.4-7.4	**5.4-5.5**	6.5-6.7	8-8.3	10.4	11.4	8.5-8.8	11.7	8.8	9.1	8.1	8.9
P. scopulorum 3	51	0	**3.1-3.5**	**4.8-4.9**	**5.3-5.7**	8.1-8.4	8.6	10.2	6.6-7	8.9	8.5	8.7-8.8	8.4	8
P. scopulorum 4	42-58	18-28	2.6 (22)	**5.1-5.9**	**5.5-6.8**	7.8-9.3	8.3-9	10.5-11.6	8.6-10.3	9.5-10.7	8.9-9.6	8.6-9.1	8-9.5	6.6-9
P. scopulorum 5	47	39-40	31-51	0.1 (1)	**3.5-3.9**	7.9-8.2	9-9.3	10.4-10.5	6.1-6.4	9.9-10	8.8-8.9	9.3-9.6	7.9-8	8.8-9
P. scopulorum 6	55,-57	43-47	36-57	30-33	0.7(6)	8.7-8.1	9.4-9.7	10.4-11.1	7.6-8.3	10.5-11	8.5-9	9-9.5	8.9-9.4	9.8-10.5
P. scopulorum 7	68-70	66-69	45-79	67-70	69-74	2.6(14)	9.5-9.8	10.8-11.1	7.6-8.4	10.3-10.8	8.5-8.8	9.4-9.8	9.5-9.8	8.8-9.4
P. atlantica	59	66	40-50	49-54	52-58	57-59	-	10	6.5-6.8	11,1	9.7	9.5-9.9	9.2	10.8-11
S. camptoclada	70	57	62-71	62-65	64-69	64-66	62	-	7.5-8	11	9.3	7.6	8.2	9.2-9.4
P. villum 2	52-54	37-39	47-63	36-43	45-51	45-50	40-42	57-58	0.6 (4)	8.3-8.6	6.8-7.1	6.8-7.1	5.9-6.2	7.3-7.7
P. subtilissima	66	51	50-66	55-58	57-67	56-59	69	68	51-53	0	10.3	10.1	9.7	10.1-10.5
P. scopulorum 8	75	70	49-82	75-76	81-85	81-85	53	55	40-42	56	-	8.4	7.4	7.5
P. scopulorum 9	77	71-72	47-77	79-82	85-89	80-83	52-54	45	40-42	55	71	0.2 (2)	8.3	8.4
P. scopulorum 11	68	66	45-78	66	74-77	70-72	52	50	36-38	55	61	68	-	6.5
P. villum	56	41	38-55	48-52	54-62	48-51	67-68	57-58	45-48	64-65	41	46	37	0.2 (1)