

RESEARCH ARTICLE

Molecular analyses of turf algae reveal a new species and an undetected introduction in the Pterosiphoniae (Rhodomelaceae, Rhodophyta)

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Abstract

Introduced seaweeds and undescribed species often remain undetected because marine regional floras are as yet poorly understood. DNA sequencing facilitates their detection, but databases are incomplete, so their improvement will continue to lead the discovery of these species. Here we aim to clarify the taxonomy of two turf-forming red algal Australian species that morphologically resemble the European *Aphanocladia stichidiosa*. We also aim to elucidate whether either of these species could have been introduced in Europe or Australia. We studied their morphology, analyzed 17 *rbcL* sequences of European and Australian specimens, examined their generic assignment using a phylogeny based on 24 plastid genomes, and investigated their biogeography using a taxon-rich phylogeny including 52 *rbcL* sequences of species in the Pterosiphoniae. The *rbcL* sequences of one of the Australian species were identical to *A. stichidiosa* from Europe, considerably expanding its known distribution. Unexpectedly, our phylogenetic analyses resolved this species in the *Lophurella* clade rather than in *Aphanocladia* and the new combination *L. stichidiosa* is proposed. The other Australian species is described as *L. pseudocorticata* sp. nov. Although *L. stichidiosa* was originally described in the Mediterranean ca. 70 years ago, our phylogenetic analyses placed it in a lineage restricted to the southern hemisphere, showing that it is native to Australia and introduced to Europe. This study confirms that further work using molecular tools is needed to characterize seaweed diversity, especially among the poorly explored algal turfs, and showcases the usefulness of phylogenetic approaches to uncover introduced species and to determine their native ranges.

KEYWORDS

algal turfs, *Aphanocladia*, Australia, Europe, *Lophurella*, new combination, new species, non-native species, plastid genomes, *rbcL*

Abbreviations: CTAB, cetyltrimethylammonium bromide; ML, maximum likelihood.

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INTRODUCTION

The introduction of species mediated by human activities is a major environmental problem in marine ecosystems (de Castro et al., 2017). Introduced species can become invasive, causing negative environmental impacts, such as the reduction of native biodiversity or the alteration of species interactions in marine communities, as well as disturbing human activities such as fishing or tourism (Katsanevakis et al., 2014; Molnar et al., 2008). As a consequence, government and international organizations have developed measures to prevent and manage introduced species (e.g., EU Regulation 1143/2014).

The implementation of management plans for introduced species depends on our ability to detect introductions in the first instance. This task can be challenging in seaweeds and marine organisms more broadly. One key gap is our incomplete knowledge of biodiversity, with estimates suggesting that between one-third and two-thirds of marine eukaryotes and one-half of red algae remain undescribed (Appeltans et al., 2012; Guiry, 2012). This affects the detection of introduced species, and cryptogenic species are common among seaweeds (van der Loos et al., 2023). The introduced status of these species often cannot be determined with certainty because their potential native areas are unknown (Díaz-Tapia, Bárbara, et al., 2017; Mineur et al., 2012; Sherwood et al., 2020).

The application of molecular data has facilitated major advances in seaweed species identification and has been a critical tool in detecting introduced species, leading to the identification of cryptic introduced species that had remained undetected, for instance, due to morphological similarities with native species (Saunders, 2009; Zuccarello et al., 2002). However, this approach requires that molecular data from both native and introduced regions are available, which is rarely the case for many seaweed groups. As a result, we can expect historical introductions will continue to be detected as molecular datasets for regional floras around the world become more complete (Nelson et al., 2021; Piñeiro-Corbeira et al., 2020; Rodríguez-Prieto et al., 2021; Verges et al., 2013; Wolf et al., 2011).

Once species with disjunct distributions, representing potential introductions, are detected, the native and the introduced ranges still need to be determined. Non-native regions can be easily inferred for newly introduced species if conspicuously different from native ones and/or if introduced species are seen in places with strong potential for introductions such as harbors, marinas, or aquaculture facilities. However, distinguishing between native and introduced distribution ranges can be difficult for old introduced

species that have had time to spread into natural habitats (Vieira et al., 2019). In such cases, phylogenetic analyses can assist us to elucidate the status of the species in the different regions based on the biogeographical affinities of lineages (Dijoux et al., 2014; Steen et al., 2017).

Members of the red algal family Rhodomelaceae are among the most common introduced species worldwide (Williams & Smith, 2007). Species identification in this family is often problematic as there are many species that are similar in morphology, and several introduced and cryptogenic species have been detected in recent years using molecular tools (Bustamante et al., 2015a; Díaz-Tapia, Bárbara, et al., 2017; Sherwood et al., 2020). In addition, many cryptic and semi-cryptic species have been detected in the family (Bustamante et al., 2015b; Mamoozadeh & Freshwater, 2012; Savoie & Saunders, 2016). Also, new species have been observed in poorly explored habitats or assemblages (Díaz-Tapia, Tüney-Kizilkaya, & Taskin, 2022; Kim & Kim, 2014), such as algal turfs that are composed of small, densely packed macroalgae (Connell et al., 2014; Díaz-Tapia & Bárbara, 2014). Turfs are among the dominant algal assemblages in tropical reefs, and their abundance is expanding in temperate regions as a consequence of the decline of kelp forests (Filbee-Dexter & Wernberg, 2018; O'Brien & Scheibling, 2018; Price & Scott, 1992). However, their biodiversity is poorly understood, and specific studies of these assemblages in several world regions have revealed significant numbers of undiscovered species (Díaz-Tapia et al., 2020, 2021; Rodríguez-Buján et al., 2021; Soares et al., 2022; Verbruggen et al., 2009).

Our diversity surveys of algal turfs from the south coasts of Australia revealed two entities morphologically similar to *Aphanocladia stichidiosa*, a species originally described from Naples, Italy (as *Polysiphonia stichidiosa*) and restricted to European shores, the Canary Islands and the Azores (Ardré, 1969; Cremades & Bárbara, 1990; Funk, 1955; León-Cisneros et al., 2012; Rojas-González & Afonso-Carrillo, 2003). In this work we aim to clarify the taxonomic identity of the two Australian species that morphologically resemble *A. stichidiosa*, analyzing their relationships with European specimens of this species, with the generitype *A. delicatula* as well as with other species of the tribe Pterosiphonieae. To determine if any of the studied species could have been introduced by human activities on the European or Australian coasts, we have studied the biogeographic distribution of Pterosiphonieae lineages. Phylogenies in this group are challenging when based on one or a few molecular markers, so we have used a phylogenomic approach that has been proven useful to address this problem in the red algae (Costa

et al., 2016; Díaz-Tapia, Maggs, et al., 2017; Díaz-Tapia, Pasella et al., 2019; Díaz-Tapia, Rodríguez-Buján, et al., 2022; Lyra et al., 2021).

MATERIALS AND METHODS

Collections and morphological observations

Six and three samples, respectively, of two entities that morphologically resemble the European *Aphanocladia stichidiosa* were collected in Australia during our sampling surveys of algal turfs (Table S1 in the Supporting Information). Nine samples of the species were collected in Europe, including the Mediterranean Sea (the type locality), the Atlantic Iberian Peninsula, the Canary Islands, and the Azores (Table S1). The taxonomic assignment of European specimens is unequivocal, as *A. stichidiosa* is the only species in this region with four pericentral cells and cortication formed near to the apical cells but remaining incomplete throughout the axes (Afonso-Carrillo & Sansón, 1999; Ardré, 1969, 1970; Coppejans, 1983; Cremades & Bárbara, 1990; Funk, 1955). In order to study the relationships of the two Australian entities with closely related taxa of the tribe Pterosiphonieae, samples of another nine species were collected, including the generitype of *Aphanocladia*, *A. delicatula*. Part of each sample was preserved in silica gel for DNA extraction, while the remaining material was preserved for morphological study in 4% formalin seawater at 4°C and stored in the dark.

For morphological observations, specimens were mounted in 20% Karo® Syrup (ACH Foods) and 80% distilled water. Sections for microscopic observations were made by hand using a razor blade. Morpho-anatomical studies were performed using light microscopy.

DNA extraction and preliminary analyses of the *rbcL* gene

DNA was extracted from silica gel-dried material using an adapted cetyltrimethylammonium bromide (CTAB) protocol (Cremen et al., 2016; Doyle & Doyle, 1987). PCR amplification was carried out for *rbcL*, the molecular marker for which more sequence data are available in the family Rhodomelaceae, using the primers F7/R753, F57/*rbcL*revNEW, and F2/R1464 or R1452 (Díaz-Tapia et al., 2018; Freshwater & Rueness, 1994; Gavio & Fredericq, 2002; Saunders & Moore, 2013). Reactions were performed in a total volume of 25 µL, consisting of 1× MyTaq™ reaction buffer, 0.28 µM of forward and reverse primers, 0.125 units MyTaq™ DNA Polymerase

(Bioline), 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), extension (74°C for 90 s), and final extension (74°C for 5 min). The PCR products were purified and sequenced commercially by MacroGen Inc. In total, 17 *rbcL* sequences were newly determined in this study (Table S1).

Newly determined sequences of the *rbcL* gene were aligned, and uncorrected p-distances were calculated. They were also aligned with previously published sequences of species of the tribe Pterosiphonieae to achieve an initial assessment of the relationships of the two Australian species (the focus of this study) and other members of this tribe using IQ-Tree phylogeny. Models of nucleotide evolution were selected based on the Bayesian Information Criterion using ModelFinder in IQ-Tree (Kalyaanamoorthy et al., 2017). Maximum likelihood (ML) phylogenetic trees were inferred in IQ-TREE v2.1.2 (Minh et al., 2020) using the model TN+I+G4 (Tamura & Nei, 1993), and branch support was determined using 1000 replicates for non-parametric bootstrap (Felsenstein, 1985) and ultrafast bootstrap (Hoang et al., 2018).

Organelar phylogenomics

Based on the preliminary *rbcL* tree (Figure S1 in the Supporting Information), we selected one to three species of each genus for phylogenomic analyses, using either previously published or newly determined organelar genomes using high-throughput sequencing (Table S2 in the Supporting Information). We also used *Pleurostichidium falckenbergii*, *Herposiphonia versicolor*, and *Dipterosiphonia australica* as outgroups based on Díaz-Tapia, Maggs, et al. (2017) and Pasella et al. (2019).

DNA, extracted as described in the previous section, was used to prepare barcode sequencing libraries (350 nt or 150 nt) with the NEBNext® DNA Library Prep Kit (NEB) or the VAHTS Universal DNA Library Prep Kit (Vazyme). Libraries were sequenced on Illumina SBS at Novogene or Illumina NovaSeq at Genewiz. Assembly and annotation of the genomes were performed as previously described (Marcelino et al., 2016; Verbruggen & Costa, 2015). GenBank accession numbers for annotated genomes are provided in Table S2.

We assembled a dataset consisting of the 10 newly determined plastid genomes and 14 genomes previously published for the family Rhodomelaceae (Table S2). All protein-coding genes were aligned at the amino acid level using MAFFT v7.245 (Katoh & Standley, 2013) with default settings and were checked visually in Geneious 7.0.6 (Biomatters). Nucleotide

alignments were constructed based on the inferred amino acid alignments using TranslatorX (Abascal et al., 2010), and the alignments were concatenated. Selection of models of nucleotide evolution, tree inference, and branch support estimation were carried out as explained in the previous section. The used model was GTR+F+R5 (Posada, 2003).

To obtain a phylogenetic tree including species for which full plastid genomes are not available, we also assembled a dataset containing 31 additional *rbcL* sequences of species of the tribe Pterosiphoniae that were downloaded from GenBank (Table S1). One sequence was included per species. When several sequences were available, we selected the longest one among those without ambiguous bases. Taxonomic assignment of these sequences followed Guiry & Guiry (2023; see Table S1). Sequences of the *rbcL* gene were aligned using Geneious 7.0.6. A phylogeny was constructed from the *rbcL* alignment (as for the plastid dataset) using the plastid genome phylogeny as a backbone constraint. This approach leverages the strong signal of the plastid genomes while adding into the phylogeny the extra sequences of 31 species based on the *rbcL* data available for them. Data were analyzed as previous datasets, using the TN+F+I+G4 model (Tamura & Nei, 1993).

RESULTS

Molecular identification and phylogeny

The six *rbcL* sequences of one of the Australian entities were identical to the nine European specimens, providing evidence that *Aphanocladia stichidiosa* is present in Australia. The three identical *rbcL* sequences of the second Australian entity diverged from those of *A. stichidiosa* by 3.7%–4.1% (24–53 base pairs). Thus, this entity clearly differs genetically from *A. stichidiosa* and a new species will be proposed for it below.

The aligned dataset of plastid genomes consisted of 197 concatenated genes amounting to 143,490 nucleotides from 24 rhodomelacean species, including 21 species of the tribe Pterosiphoniae and three other species in the family. The nodes of the phylogeny received full support except the one branch, joining *Deltalsia* and *Amplisiphonia*, that was 98%–99% supported (Figure 1). Genera of the Pterosiphoniae were resolved in two main lineages, one group including *Echinothamnion*, *Gredgaria*, *Aphanocladia*, *Lophurella*, and *Periphykon*, characterized by having four pericentral cells and the other group including the remaining nine genera, characterized by having 5–16 pericentral cells. The two Australian species studied in this work, a priori assigned to *Aphanocladia* because they resemble *A. stichidiosa*, were closely related to one another and were placed in the phylogeny in a fully

supported clade with species of the genus *Lophurella* (Figure 1). One of the studied species was placed as sister to other *Lophurella* species, and *A. stichidiosa* was placed as sister to all of them; both placements were with full support. The whole *Lophurella* clade was sister to *Echinothamnion*, including a genome of topotype material of the generitype *E. hystrix*. The generitype of *Aphanocladia* (*A. delicatula*) was resolved in a clade with the closely related generitype *Gredgaria maugeana*, which was sister to species morphologically assigned to “*Echinothamnion*” from New Zealand. Therefore, *Aphanocladia* is currently non-monophyletic, and the placement of *A. stichidiosa* requires revision. We propose to transfer this species to the genus *Lophurella*, to which we also assign the new species discovered in Australia.

The tree inferred using the genome-scale phylogeny as a backbone and adding 31 additional *rbcL* sequences (Figure 2) was congruent with the genome-scale tree. Lineages of the tribe Pterosiphoniae generally have clear biogeographical affinities (Figure 2). Most genera are distributed in specific regions, and only five genera are naturally distributed in more than one region. The clade including *Lophurella*, *Aphanocladia*, *Echinothamnion*, *Pollexfenia*, and *Periphykon* is restricted to the southern hemisphere, specifically to Australia, New Zealand, and South America. The only exception is *L. stichidiosa*, which occurs in Australia and Europe.

Morphological observations

Lophurella pseudocorticata Díaz-Tapia sp. nov. (Figure 3)

Etymology: “*pseudocorticata*” refers to the incomplete cortication of the thalli.

Holotype designated here: Male gametophytes and non-reproductive plants collected from a unique scraping of a turf from sand-covered low intertidal rocks on 13 November 2016, leg. Pilar Díaz-Tapia. MEL2457785.

Type locality: Shipwreck Creek, Mallacoota, Victoria, Australia.

Paratypes: Two vouchers of material collected in the type locality from two different scrapings of turfs from sand-covered low intertidal rocks on 13 November 2016, leg. Pilar Díaz-Tapia. MEL2457790, tetrasporophytes and female gametophytes; MEL2457764, non-reproductive plants.

Description

Thalli up to 2 cm in height, form turfs of densely entangled axes (Figure 3a). Thalli are dorsiventral, composed of extensive prostrate axes bearing rhizoids

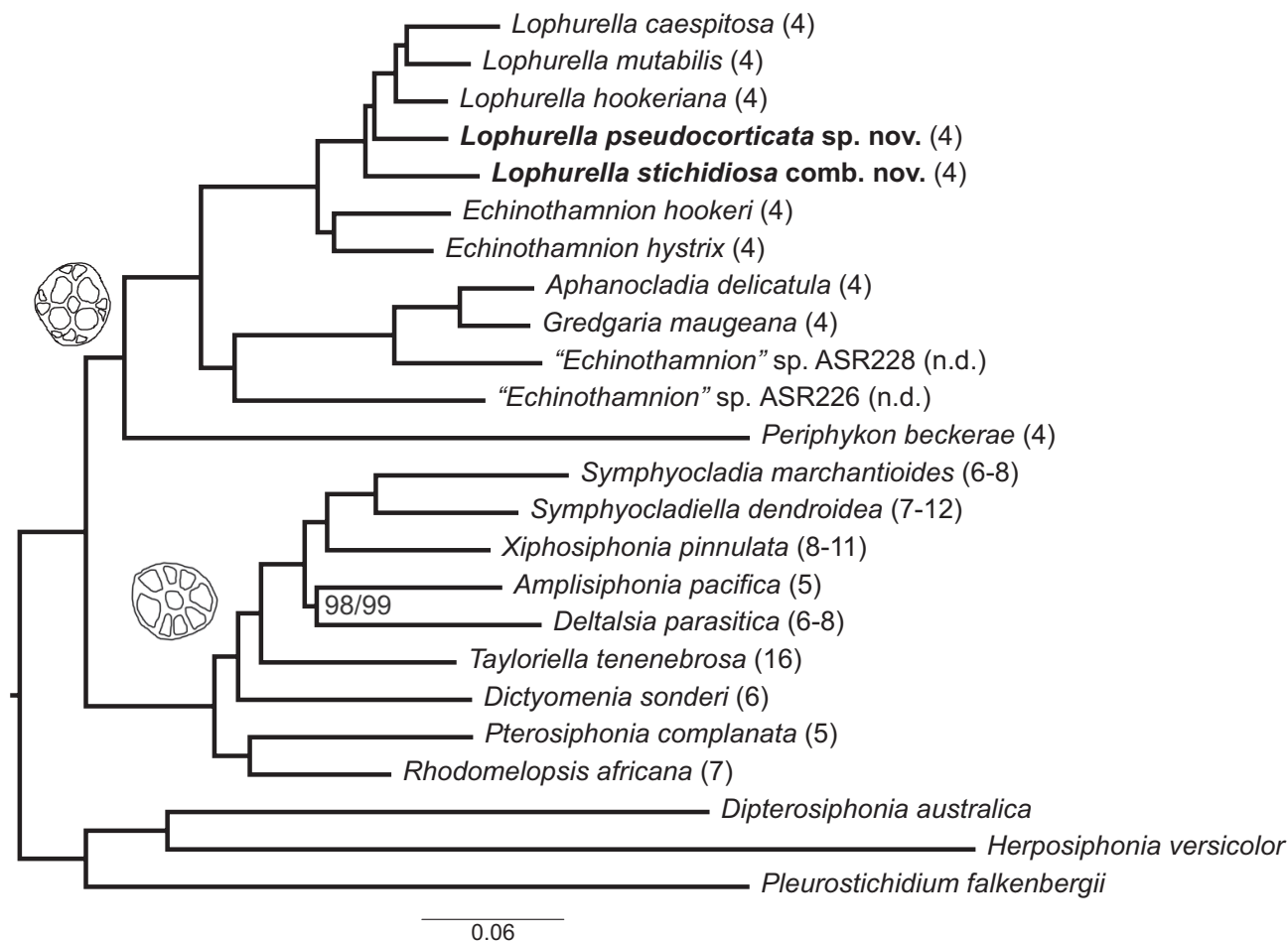


FIGURE 1 Maximum likelihood phylogeny of the tribe Pterosiphonieae based on nucleotide alignment of the 197 concatenated genes from the plastid genomes. All branches have full bootstrap support except the one for which bootstrap values are indicated on branches (as non-parametric/ultrafast bootstrap). The number of pericentral cells is indicated between brackets. Names of the two focus species of this work are printed in bold.

ventrally and erect axes dorsally which are unbranched or scarcely and irregularly branched up to three orders (Figure 3b). Thalli are dark red to black in color, texture rigid.

Axes consisting of an axial cell surrounded by four pericentral cells (Figure 3c,d). Cortication developing a few segments below the apices in both prostrate and erect axes, growing between pericentral cells (Figure 3e–h). Cortication remains incomplete throughout the thallus so that all pericentral cells are at least partially uncovered (Figure 3f–h), but parts of the thallus between segments can be completely surrounded by a layer of cortical cells (Figure 3d,g). Cortication is denser at mid parts of erect axes and sparse in the prostrate axes.

Prostrate axes growing from obtuse apical cells, 20–25 μm in diameter, increasing in diameter to 150–220 μm , composed of segments with a 1–2 length diameter ratio (L/D; Figure 3i). Rhizoids arising in the distal ends of the pericentral cells, one per segment, rarely two, cut off from the pericentral cells, consisting of a filament 30–60 μm in diameter and a terminal digitate multicellular hapteron composed of

dichotomously branched filaments up to three cells in length (Figure 3j). Branch initials formed exogenously, before the division of the pericentral cells, on every segment in a $\frac{1}{4}$ spiral. Some initials producing branches at irregular intervals, which become erect axes dorsally oriented and further prostrate axes laterally oriented.

Erect axes growing from obtuse apical cells, 22–30 μm in diameter, increasing in diameter to 170–280 μm in mid and basal parts, composed of segments 0.8–1.6 L/D. Branch initials formed exogenously, before the division of the pericentral cells, on every segment in a $\frac{1}{4}$ spiral (Figure 3k). All branches adventitious, formed far from the apices from some branch initials, irregularly arranged (Figure 3l,m). Trichoblasts absent.

Gametophytes dioecious. Spermatangial branches formed on densely branched lateral branches growing at mid parts of the axes (Figure 3n), growing one per segment in a $\frac{1}{4}$ spiral. Spermatangial axes are cylindrical, incurved, 200–325 μm in length, and 70–100 μm in diameter, with 1–2 apical sterile cells at maturity (Figure 3o). Mature cystocarps located at the mid parts

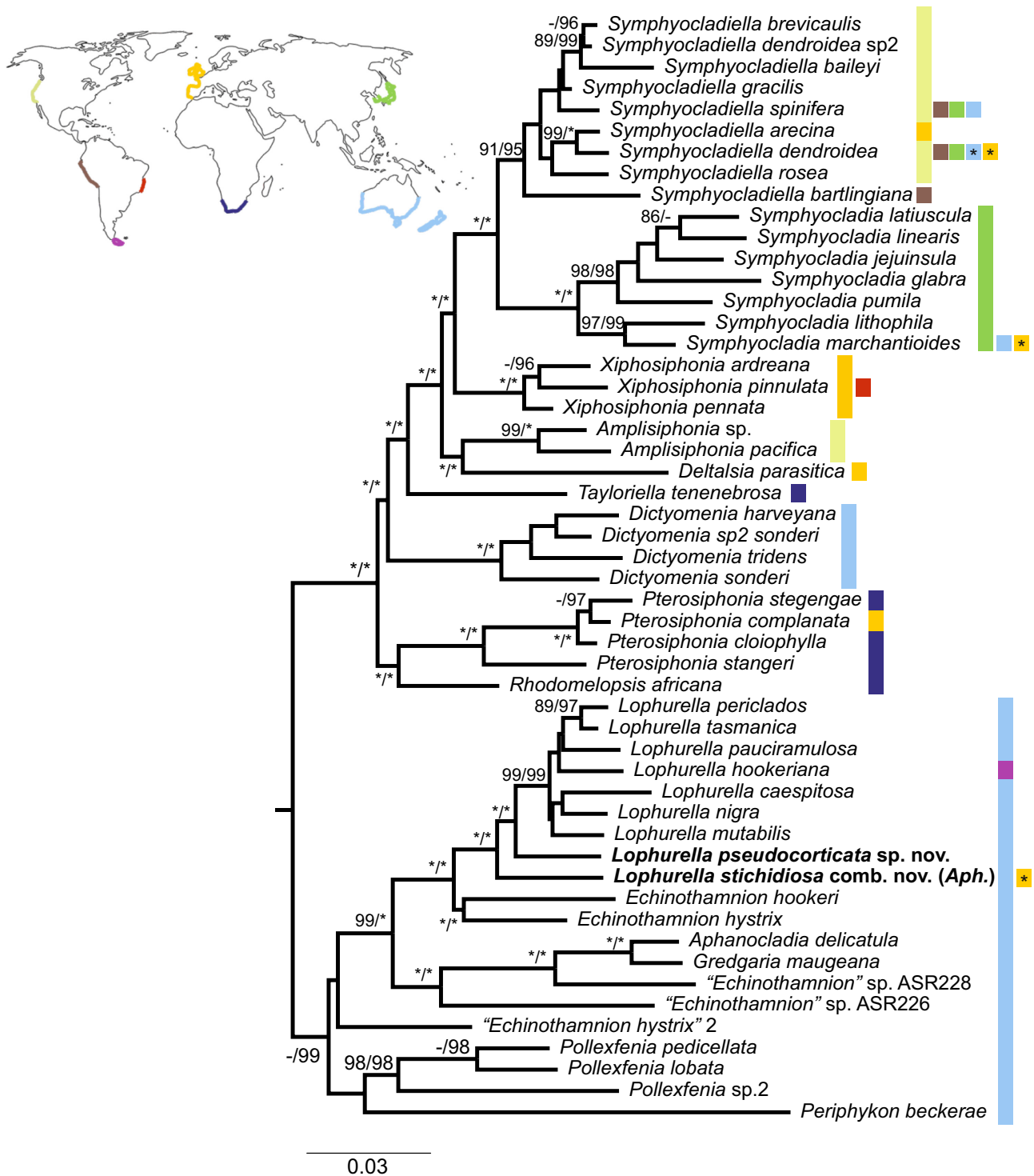


FIGURE 2 Maximum likelihood tree of the tribe Pterosiphonieae using the genome-scale plastid phylogeny based on nucleotides as a constraint and incorporating *rbcl* sequences for additional 31 species. The two focus-species of this work are printed in bold. Branch support values are indicated on branches as non-parametric/ultrafast bootstrap when $\geq 85\%$ and $\geq 95\%$, respectively; asterisks represent full support. Outgroup species were removed from the tree for clarity. The color of the bars to the right of the phylogeny indicates the distribution of lineages according to the map; asterisks indicate regions where the species are considered introduced. The distribution map is based on molecularly confirmed species records. [Color figure can be viewed at wileyonlinelibrary.com]

of erect axes, globose, $500\ \mu\text{m}$ in height and $550\ \mu\text{m}$ in diameter (Figure 3p). Carpospores $15\text{--}20 \times 40\text{--}55\ \mu\text{m}$.

Tetrasporangia formed on densely branched lateral branches growing at mid parts of the axes (Figure 3q),

forming spiral series (Figure 3r), subspherical, $60\text{--}85\ \mu\text{m}$ in diameter, tetrahedrally divided, with two presporangial and one postsporangial cover cells (Figure 3s).

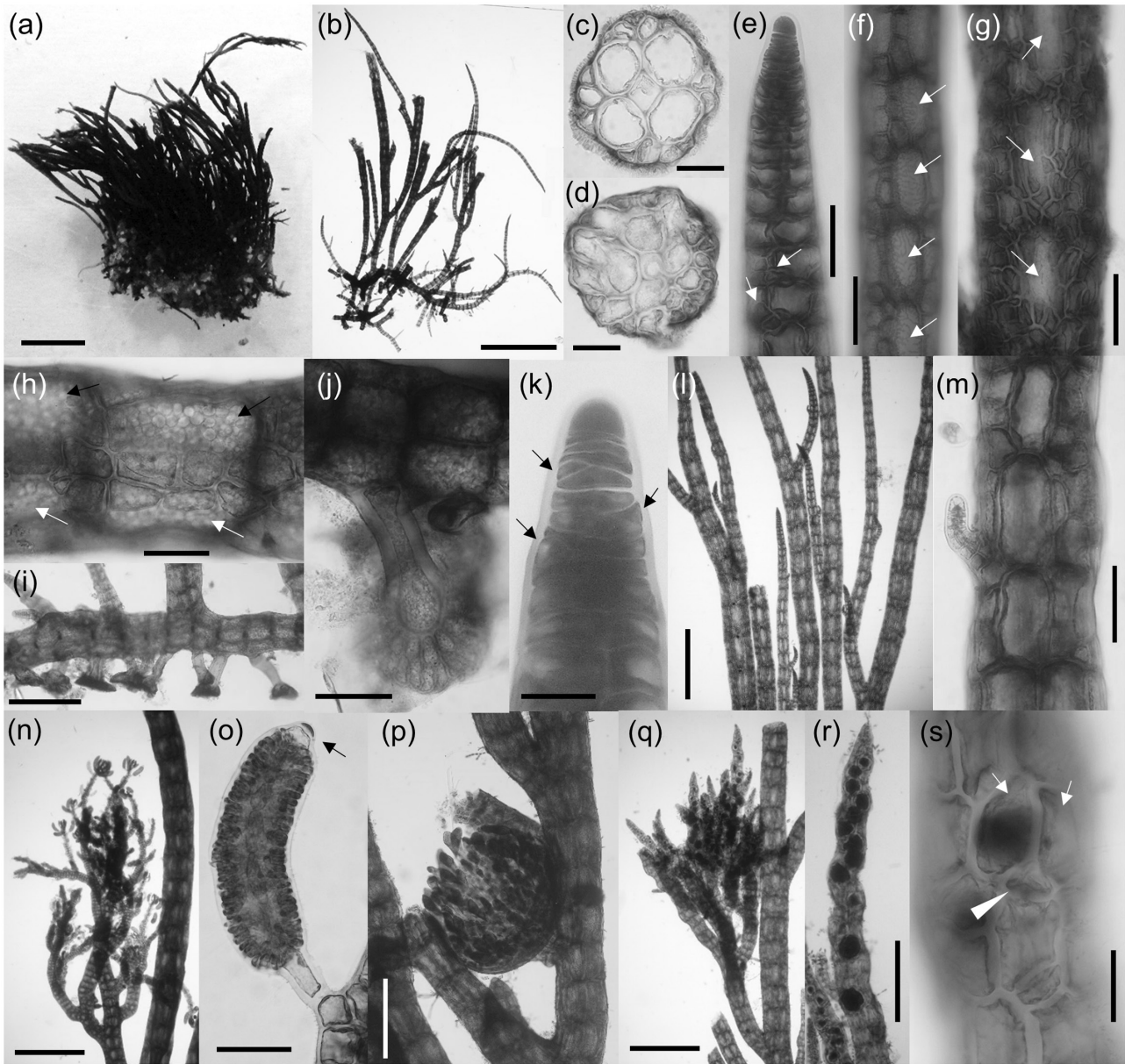


FIGURE 3 *Lophurella pseudocorticata* sp. nov. (a) Turf. (b) Habit. (c and d) Cross section of erect axes with four pericentral cells surrounded by an incomplete (c) or complete layer of cortical cells (d). (e) Apex of an erect axis with cortical cells (arrows) formed a few segments below the apical cell. (f and g) Surface view of a young (f) and old (g) part of an axis incompletely covered by cortical cells, so that the pericentral cells (arrows) are visible. (h) Prostrate axis with slight cortication formed between the pericentral cells (arrows). (i) Prostrate axes with up to one rhizoid per segment. (j) Rhizoid cut off from a pericentral cell, terminating in a multicellular discoid pad. (k) Apex of an erect axis with branch initials (arrows) formed on every segment in a $\frac{1}{4}$ spiral. (l) Erect axes irregularly branched. (m) Erect axis with an adventitious branch. (n) Spermatangial branches formed on densely branched lateral branches. (o) Spermatangial branch with two sterile apical cells. (p) Cystocarp. (q) Lateral branch profusely branched bearing tetrasporangia. (r) Tetrasporangia forming spiral series. (s) Tetrasporangia with two presporangial (arrows) and one postsporangial (arrowhead) cover cells. Figs (b, e, g–i, k–m) MEL2457764; Figs (c, d, n–o) MEL2457785; Figs (a, f, j, p–s) MEL2457790. Scale bars: (a) 5 mm; (b) 2 mm; (c–e) 70 μ m; (f–g) 130 μ m; (h) 70 μ m; (i) 300 μ m; (j) 70 μ m; (k) 25 μ m; (l) 650 μ m; (m) 70 μ m; (n) 650 μ m; (o) 100 μ m; (p) 300 μ m; (q) 650 μ m; (r) 300 μ m; (s) 35 μ m.

Habitat and distribution

Lophurella pseudocorticata was collected forming nearly monospecific algal turfs growing on sand-covered rocks from the low intertidal. It has been only observed in a single wave-exposed site in eastern Victoria, Australia.

Lophurella stichidiosa (Funk) Díaz-Tapia comb. nov. (Figure 4)

Basionym: *Polysiphonia stichidiosa* Funk, 1955: 138. Beiträge zur Kenntnis der Meeresalgen von Neapel: Zugleich mikrophotographischer Atlas. *Pubblicazioni della Stazione Zoologica di Napoli*, 25(Suppl.), 1–178.

Homotypic synonym: *Aphanocladia stichidiosa* (Funk) Ardré, 1969: 1751. Remarques et précisions sur le genre *Aphanocladia* Falk. (Rhodomélacées, Cérámiales). *Compte Rendu Hebdomadaire des Séances de l'Académie des Sciences. Paris. Série D*, 269, 1751–1754.

Description of Australian specimens

Thalli up to 2 cm in length, forming turfs of densely entangled axes. Thalli composed of prostrate and erect axes, but without a clear dorsiventral structure, all axes can eventually develop rhizoids at basal, mid, and distal parts. As a result, the extent of prostrate axes is variable, and thalli can be predominantly erect (Figure 4a) or prostrate (Figure 4b). Axes irregularly branched, up to three orders. Thalli pink to dark red in color, texture flaccid.

Axes consisting of an axial cell surrounded by four pericentral cells (Figure 4c,d). Cortication developing a few segments below the apices, growing between pericentral cells (Figure 4e,f). Cortication remains incomplete throughout the thallus so that all pericentral cells are at least partially uncovered, but parts of the thallus between segments can be completely surrounded by a layer of cortical cells (Figure 4d,f).

Axes growing from obtuse apical cells (Figure 4g), 17.5 µm in diameter, increasing in diameter to 80–200 µm, composed of segments 0.4–1.3 L/D. Branch initials formed exogenously, before the division of the pericentral cells, on every segment in a ¼ spiral (Figure 4g). Rhizoids arising in the distal ends of the pericentral cells, one per segment, cut off from the pericentral cells, consisting of a filament 20–50 µm in diameter and a terminal digitate multicellular hapteron composed of dichotomously branched filaments up to 5 cells in length (Figure 4h,j). Axes producing adventitious branches at irregular intervals (Figure 4i).

Sexual reproductive structures not observed. Tetrasporangia formed on erect branches (Figure 4k), forming marked spiral series (Figure 4l), subspherical, 65–90 µm in diameter, tetrahedrally divided, with two presporangial and one postsporangial cover cells.

Habitat and distribution

Lophurella stichidiosa grows as part of algal turfs intermixed with other species. It has been observed from the low intertidal to the upper subtidal (up to 5 m depth) on sites ranging from wave-exposed to sheltered. Our results show that this species is present in Victoria, Australia, in addition to its known distribution

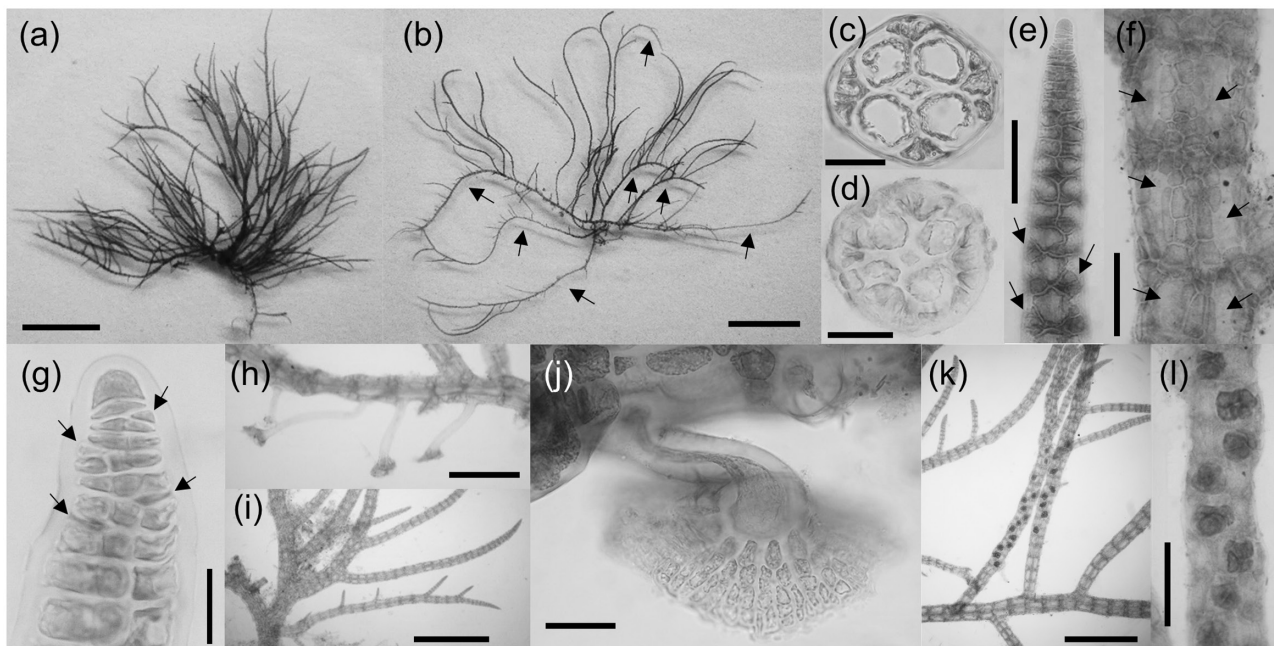


FIGURE 4 *Lophurella stichidiosa* comb. nov. from Australia. (a, b) Habit (arrows indicate the presence of rhizoids). (c, d) Cross sections of axes with four pericentral cells and an incomplete (c) or complete (d) outer layer of cortical cells. (e) Apex of an axis with cortication (arrows) formed some segments below the apical cell. (f) Surface view of an axis with incomplete cortication around the pericentral cells (arrows). (g) Detail of the apex of an axis with branch initials (arrows) formed on every segment in a ¼ spiral. (h) Prostrate part of an axis with rhizoids. (i) Axes. (j) Rhizoid with a multicellular discoid pad. (k) Branches bearing tetrasporangia. (l) Tetrasporangia forming spiral series. Figs (a, e, f, h, k–l) PD721; Figs (b, c) PD2778; Figs. (d, g) PD2718; Fig. (i) PD2827; Fig. (j) PD2773. Scale bars: (a) 3.5 mm; (b) 4 mm; (c, d) 50 µm; (e) 70 µm; (f) 140 µm; (g) 20 µm; (h) 300 µm; (i) 800 µm; (j) 50 µm; (k) 800 µm; (l) 180 µm.

along southern European coastlines including the Mediterranean Sea and the Atlantic Iberian Peninsula, as well as the Canary Islands and the Azores.

DISCUSSION

Systematics of *Lophurella pseudocorticata* sp. nov. and *L. stichidiosa* comb. nov

Our molecular analyses and morphological observations resolved the taxonomic identity of the two studied Australian entities as separate species, one corresponding to *Lophurella stichidiosa* with the other proposed as a new species, *L. pseudocorticata* sp. nov. The sequence divergence between them is 3.7%–4.1% in the *rbcL* marker, and they can be morphologically distinguished. *Lophurella pseudocorticata* is thicker (170–280 µm) with a rigid texture; it is dark red to black in color, with a clear distinction of prostrate and erect axes and with tetrasporangia formed on densely branched laterals. By contrast, *L. stichidiosa* is thinner (80–200 µm) with a flaccid texture; it is pink to dark red in color with unclear differentiation between prostrate and erect axes (all axes can eventually develop rhizoids), and the tetrasporangia are formed on lateral branches that are not profusely divided. Australian *rbcL* sequences of *L. stichidiosa* were identical to sequences from Europe, confirming its identification. Moreover, morphological characters observed in *L. stichidiosa* from Australia agree with previous descriptions of this species from Europe (Ardré, 1970; Coppejans, 1983; Cremades & Bárbara, 1990; Funk, 1955; Rojas-González & Afonso-Carrillo, 2003), so we can be confident that the European and Australian populations belong to the same species.

Both *L. stichidiosa* and *L. pseudocorticata* were placed in *Lophurella* as they were unequivocally resolved as sister lineages to a clade of known *Lophurella* species. However, their main morphological characters resemble *Aphanocladia*, *Gredgaria*, and/or *Lophurella*. These characters include the cortication formed near to the apical cells but remaining incomplete throughout the axes, the exogenous formation of branch initials on every segment of which only some develop into branches, the irregular branching pattern, and the individually formed, non-clustered, rhizoids.

The cortication pattern of the two studied species is unusual, as cortication in most Rhodomelaceae is typically lighter in younger parts of axes and becomes denser towards the base (Maggs & Hommersand, 1993; Womersley, 2003). One of the most relevant distinguishing characters of *Lophurella* is the heavy cortication formed close to the apices, increasing in thickness basally, resulting in several layers of cortical cells that completely surround the pericentral cells (Díaz-Tapia, Maggs, et al., 2019; Schmitz & Falkenberg, 1897;

Womersley, 2003), but *L. stichidiosa* and *L. pseudocorticata* differ from other *Lophurella* in this trait. The only rhodomelacean genera with a similar cortication pattern to that seen in the two studied species are *Gredgaria* and *Aphanocladia*. In fact, *L. stichidiosa* was originally described as a member of *Polysiphonia* and later transferred to *Aphanocladia* based on a detailed comparative study of the ontogenetic development of branches of the generitype *A. delicatula* and European specimens of *L. stichidiosa* (Ardré, 1969, 1970, as *A. stichidiosa*). Both species, as well as *L. pseudocorticata*, form branch initials on every segment before the division of the pericentral cells, but only some of them further develop into branches (Ardré, 1970; this work). Some other *Lophurella* spp. also have this character (Díaz-Tapia, Maggs, et al., 2019), so it does not appear to be exclusive to *Aphanocladia*.

In addition to our phylogenetic results, what other features might suggest that *Lophurella stichidiosa* is more closely aligned with *Lophurella* than with *Aphanocladia*? An important difference between *Lophurella* and *Aphanocladia* is that rhizoids are individual versus clustered, respectively (Table 1). This character had been previously described in most species assigned to *Aphanocladia* (Table 2) showing that *L. stichidiosa* differed from most other species in the genus, but its potential significance in the delineation of genera has been underestimated before. Rhizoid anatomy has recently emerged as one of the main characters distinguishing higher-level groupings in the Rhodomelaceae (Bustamante et al., 2017; Díaz-Tapia, Maggs et al., 2017). The arrangement of branches appears to be another trait distinguishing *Aphanocladia* and *Lophurella*. Branches are alternately arranged every two segments in *Aphanocladia* (Tables 1 and 2), while they are spirally or irregularly arranged in *Lophurella*. These characters further support the placement of the two studied species in *Lophurella*.

In summary, even if *Lophurella pseudocorticata* and *L. stichidiosa* resemble *Aphanocladia* because of the similarities in their cortication pattern, we present ample evidence that both species are more closely related to *Lophurella*. Accordingly, we transfer *L. stichidiosa* to this genus from *Aphanocladia*. *Lophurella pseudocorticata* was erected as a new species, as it clearly differs morphologically (Tables 1 and 2) and genetically from other similar or related species. Our results clearly confirm that cortication levels vary in *Lophurella*, the definition of which needs to be amended to include *L. stichidiosa* and *L. pseudocorticata*. *Lophurella* can be distinguished morphologically from other genera in the Pterosiphonieae by a combination of characters that include having four pericentral cells, individually formed rhizoids, and terete main axes with similar cortication degrees to branches (Table 1).

Beyond the systematics of the studied species, our work provides further evidence of the utility of

TABLE 1 Comparison of selected morphological characters among genera of the Pterisophoniae with four pericentral cells.

	<i>Aphanocladia</i>	<i>Lophurella</i>	<i>Echinothamnion</i>	<i>Gredgaria</i>	<i>Heterostroma</i>	<i>Polllexfenia</i>	<i>Periphykon</i>
Distribution	New Zealand ^a (South Africa, South America)	Australasia ^a , South America	Australasia ^a	Australasia ^a	(Australia ^a)	Australia ^a (South America, California)	(Indonesia ^a , Brazil) Australia
Thallus	Complanate	Terete	Terete	Complanate	Blade-like	Blade-like	Blade-like
Cortication	Incomplete	Incomplete to heavy	Heavy (absent on determinate branches)	Incomplete	Absent	Absent	Absent
Branches arrangement	Alternate	Spiral or irregular	Spiral	Alternate	Alternate	Alternate	Alternate
Vegetative trichoblasts	Absent	Present or absent (species specific)	Present	Absent	Present	Present	Absent
Rhizoids	Clustered	Individual	–	Clustered	Clustered	–	Clustered
Spermatangial branches	–	Cylindrical	Cylindrical	–	Cylindrical	Cylindrical	Flattened
References	Adams (1994), Díaz-Tapia, pers. obs.	Díaz-Tapia, Maggs, et al. (2019), Womersley (2003), this work	Womersley (2003)	Wilcox (2018), Womersley (2003)	Kraft & Wynne (1992)	Womersley (2003)	Huisman (2018)

^aIndicates the region of the type species; distribution between brackets indicates records of species molecularly not studied; – indicates characters not described. Note: additional characters distinguish blade-like genera (see discussion in Kraft & Wynne, 1992).

TABLE 2 Comparison of selected morphological characters among *Aphanocladia*, *Gredgaria*, and the two studied species of *Lophurella*.

	<i>Aphanocladia delicatula</i>	<i>Aphanocladia ecorticata</i>	<i>Aphanocladia pacifica</i>	<i>Aphanocladia robusta</i>	<i>Aphanocladia skottsbergii</i>	<i>Gredgaria maugeana</i>	<i>Lophurella stichidiosa</i>	<i>Lophurella pseudocorticata</i>
Distribution	New Zealand	South Africa	Chile	Argentina	Chile	Australia; New Zealand	Australia, Europe	Australia
Axes outline	Complanate	Terete	Complanate	Complanate	Terete	Complanate	Terete	Terete
Rhizoids	Clustered	Clustered	Clustered	Clustered	–	Clustered	Individual	Individual
Axes diameter (μm)	150–250	60	225–345	150–300	175–250	160–300	80–200	170–280
Branching	Alternate every 2 segments	Alternate every 2 segments	Alternate every 2 segments	Alternate every 2 segments	Alternate every 2 segments	Alternate every 2 segments	Irregular	Irregular
Cortication	Incomplete	Absent	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete	Incomplete
References	Adams (1994), P. Díaz-Tapia, pers. obs.	Stegenga et al. (2004)	Joly & Alveal (1968)	Pujals (1967)	Levring (1941)	Wilcox (2018), Womersley (2003)	Ardré (1970); this work	This work

phylogenomic approaches for resolving classification issues at various taxonomic levels (Costa et al., 2016; Díaz-Tapia, Maggs, et al., 2017; Díaz-Tapia, Pasella, et al. 2019; Díaz-Tapia, Rodríguez-Buján, et al., 2022; Lyra et al., 2021). The topology of the *rbcL* and plastid genomes trees (Figure S1 vs. Figure 1) was similar with respect to the two studied species. However, relationships of *Lophurella pseudocorticata* remained unresolved in the single-gene phylogeny, leaving open the possibility that *L. pseudocorticata* and *L. stichidiosa* were actually sister species and a potential separate genus. By contrast, the plastid genome tree fully resolved their relationships, rejecting the possibility of a separate genus and supporting their placement in *Lophurella*.

Although not the main focus of this work, our study also revealed a very close relationship between *Aphanocladia delicatula* from New Zealand and *Gredgaria maugeana*, originally described from Australia and also known in New Zealand (Wilcox, 2018), two genera that are morphologically very similar (Table 1). *Gredgaria* was originally placed in the tribe Herposiphonieae and described as having eight pericentral cells (Womersley, 2003). However, this character has been misinterpreted, and *G. maugeana* has four pericentral cells (Díaz-Tapia, pers. obs.), which probably prevented the comparison of this species at the time of its erection within the earlier described genus *Aphanocladia*. Also, both *A. pacifica* and *A. robusta* are very similar to each other and to *A. delicatula* and *G. maugeana* (Table 2) and molecular data are needed to clarify their relationships. Unresolved taxonomic issues in this clade of the Pterosiphonieae are even more complex, as our phylogeny shows that three species morphologically identified as *Echinothamnion* from New Zealand and Western Australia were resolved as non-sister to topotype material of the generitype (*E. hystrix* from George Town, Tasmania). Further investigations of species and genus delineation in these Pterosiphonieae genera are, therefore, warranted.

***Lophurella stichidiosa*: Native to Australia and an old introduction in Europe**

Despite *Lophurella stichidiosa* being remarkably morphologically distinctive, it remained unrecorded in Australia in previous studies. Surprisingly, it was commonly observed in several sites in Victoria in our study, including Port Phillip Bay, a well-studied area (Womersley, 2003). Likewise, *L. pseudocorticata* is morphologically distinctive, but the absence of previous records is less surprising, as we observed it only in a single site in the easternmost Victoria despite our extensive sampling efforts in other environmentally similar sites in Victoria and Tasmania. Its distribution is probably wider, and additional surveys, possibly

north into New South Wales, might provide new records. Both *L. stichidiosa* and *L. pseudocorticata* were most probably overlooked in previous studies as they are small (2 cm) and inhabit intertidal and shallow subtidal algal turfs. This type of assemblage is common and abundant along the Australian coastline, but its algal species diversity has been poorly explored. Thus, our study contributes to the characterization of the Australian turf-forming species evidencing that further efforts are required to uncover their species diversity (Díaz-Tapia et al., 2018, 2020; Díaz-Tapia, Maggs, et al., 2019).

The occurrence of *Lophurella stichidiosa* in disjunct regions, Europe and Australia, strongly suggests that this species has been introduced in one of them. The finding of identical *rbcL* sequences in both regions further supports this hypothesis, as some genetic differentiation would be expected from distant populations of species widely distributed naturally (Díaz-Tapia et al., 2018; Dijoux et al., 2014). Our phylogenetic analyses showed that most lineages of the Pterosiphoniae have specific biogeographical affinities, and the clade including *Lophurella*, *Echinothamnion*, *Gredgaria*, and *Aphanocladia* is restricted to the southern hemisphere, mainly Australasia. The only exception is the presence of *L. stichidiosa* in Europe, which combined with the Australasian distribution of the wider lineage suggests that this species is an Australasian native introduced to Europe. This region is one of the main sources of introduced seaweeds in southern Europe (van der Loos et al., 2023; Williams & Smith, 2007). Although most seaweeds introduced from this region were recognized as such relatively early on (e.g., *Asparagopsis armata*, *A. taxiformis*, *Caulerpa* spp.;

van der Loos et al., 2023; Verlaque et al., 2007), the small and inconspicuous *L. stichidiosa* was described as a Mediterranean new species in 1955, without any suspicion that it could have been introduced (Funk, 1955). Similarly, *Dictyota cyanoloma* was recently described from the Mediterranean and Macaronesia (Tronholm et al., 2010), but later phylogeographic work showed the species to be introduced from Australia (Steen et al., 2017). In both cases, their recognition as introduced species has been possible thanks to the determination of DNA sequences from distant regions and the introduced and native regions have been identified using phylogenetic approaches. Alternatively, a less plausible hypothesis that explains the current known disjunct distribution of *L. stichidiosa*, with a single *rbcL* haplotype known in both Australia and Europe, could be that it is a widely distributed species that remained overlooked in the coasts between these two regions and that maintains some genetic flow between them.

The first record of *Lophurella stichidiosa* in Europe dates back to 1955 in Naples (Funk, 1955), and the potential introduction vector is uncertain. The original collection of the species was at about 10 m depth, near the Naples harbor and aquarium (Funk, 1955), both built before the description of *L. stichidiosa*, so shipping or an accidental aquarium escape are plausible introduction vectors, which is in line with what is presumed for many other introduced seaweeds (Verlaque et al., 2007). Of course, it remains unknown whether Naples was the point of introduction or if its presence in this city was the result of spread from another point of introduction. We could not confirm whether the Naples aquarium had species imported from Australia in the 1950s.

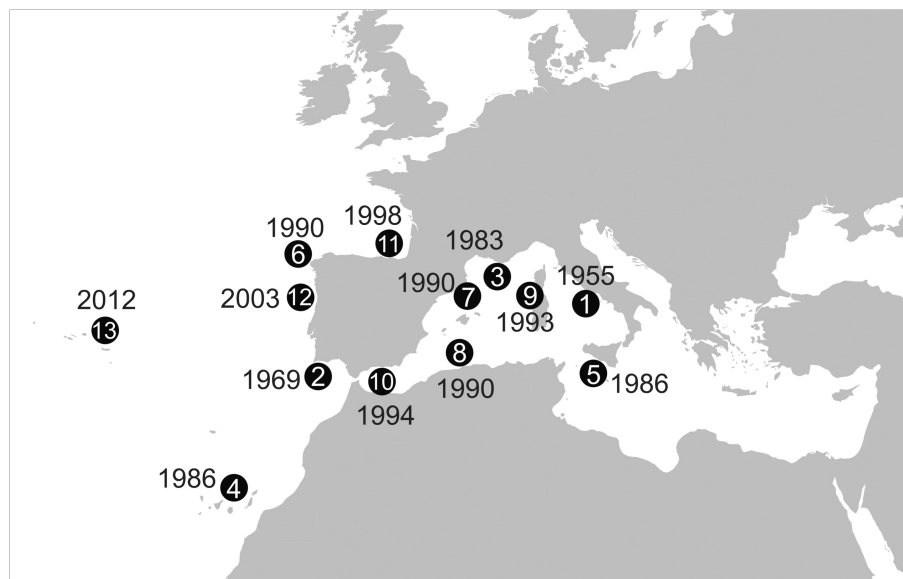


FIGURE 5 Distribution of *L. stichidiosa* in Europe indicating the date of first regional records. (1) Funk (1955), (2) Ardré (1969), (3) Coppejans (1983), (4) Price et al. (1986), (5) Giaccone et al. (1986), (6) Cremades & Bárbara (1990), (7) Ballesteros (1990), (8) Seridi (1990), (9) Cossu et al. (1993), (10) González García & Conde Poyales (1994), (11) Santolaria et al. (1998), (11) Araújo et al. (2009), (12) León-Cisneros et al. (2012).

After its first record in the Mediterranean, *Lophurella stichidiosa* was detected in other southern European regions, following a pattern that suggests its progressive dispersal (Figure 5). Again, whether the species is spreading naturally or its spread is mediated by human activities remains unknown, and it is probably both. *L. stichidiosa* grows as part of algal turfs in intertidal and shallow subtidal rocky reefs, and it has been observed in sites with a variety of human pressures, from highly modified to almost pristine coasts. Therefore, potential dispersal vectors cannot be easily deduced from the habitat of *L. stichidiosa* in the introduced region. Interestingly, it differs from many other introduced seaweeds, which are often common in ports or near aquaculture facilities (Arenas et al., 2006; Díaz-Tapia, Bárbara, et al., 2017; Petrocelli et al., 2019). However, the most recent record of *L. stichidiosa* in the Atlantic was in the Azores (León-Cisneros et al., 2012), an archipelago placed 1300 km from mainland Europe, suggesting human intervention in the dispersal of the species in Europe.

In conclusion, our characterization of turf-forming species from Australia and Europe and the study of their phylogenetic relationships uncovered a new introduced species in Europe. Record data suggest a relatively rapid expansion of its distribution (Figure 5), so potential new records in adjacent European regions can be expected in the future. *Lophurella stichidiosa* is small in size and easily overlooked, and its potential impacts on native communities are not obvious. However, its abundance is increasing regionally, and it is nowadays a common seaweed in algal turfs from at least the northwestern Iberian Peninsula and the Azores (P. Díaz-Tapia, pers. obs.). This type of community is expanding in temperate regions as kelp forests and canopies of Fucales decline (Filbee-Dexter & Wernberg, 2018; O'Brien & Scheibling, 2018; Provera et al., 2021). Introduced species are often predicted to increase in abundance and distribution as a consequence of global change (Atkinson et al., 2020; Félix-Loaiza et al., 2022; Santin et al., 2022). Considering these trends, the potential role of *L. stichidiosa* in marine community shifts, in combination with other introduced or invasive turf-forming species that often grow together, should be further studied.

AUTHOR CONTRIBUTIONS

Pilar Díaz-Tapia: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (supporting); investigation (lead); methodology (equal); writing - original draft (lead). **Wendy A. Nelson:** Data curation (supporting); funding acquisition (supporting); writing - review and editing (supporting). **Heroen Verbruggen:** Conceptualization (supporting); formal analysis (supporting); funding acquisition (lead); methodology (supporting); project administration (lead); software (lead); writing - review and editing (lead).

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DATA AVAILABILITY STATEMENT

DNA sequences were deposited in NCBI GenBank under accession numbers OQ731394-403 (plastid genomes) and OQ731919-33 (*rbcL* sequences).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1.

Table S1.

Table S2.

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