Merging the cryptic genera *Radicilingua* and *Calonitophyllum* (Delesseriaceae, Rhodophyta): molecular phylogeny and taxonomic revision

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In memory of phycologist Annette (Netty) Elizabeth Little.

Radicilingua Papenfuss and *Calonitophyllum* Aregood are two small genera of the family Delesseriaceae that consist of only three and one taxonomically accepted species, respectively. The type species of these genera, *Radicilingua thysanorhizans* from England and *Calonitophyllum medium* from the Americas, are morphologically very similar, with the only recognized differences being vein size and procarp development. To date, only other two species were recognized inside the genus *Radicilingua*: *R. adriatica* and *R. reptans*. In this study, we analysed specimens of *Radicilingua* collected in the Adriatic and Ionian Sea (Mediterranean), including a syntype locality of *R. adriatica* (Trieste, northern Adriatic Sea), alongside material from near the type locality of *R. thysanorhizans* (Torpoint, Cornwall, UK). The sequences of the *rbc*L-5P gene fragment here produced represent the first molecular data available for the genus *Radicilingua*. Phylogenetic reconstruction showed that the specimens from the Adriatic and Ionian Seas were genetically distinct from the Atlantic *R. thysanorhizans*, even if morphologically overlapping with this species. A detailed morphological description of the Mediterranean specimens, together with an accurate literature search, suggested that they were distinct also from *R. adriatica* and *R. reptans*. For these reasons, a new species was here described to encompass the Mediterranean specimens investigated in this study: *R. mediterranea* Wolf, Sciuto & Sfriso. Moreover, in the *rbc*L-5P tree, sequences of the genera *Radicilingua* and *Calonitophyllum* grouped in a well-supported clade, distinct from the other genera of the subfamily Nitophylloideae, leading us to propose that *Calonitophyllum medium* should be transferred to *Radicilingua*.

Key Words: *Calonitophyllum*; Delesseriaceae; *Radicilingua media* comb. nov.; *Radicilingua mediterranea* sp. nov.; *rbc*L; systematics

INTRODUCTION

Radicilingua Papenfuss (1956) is a small genus in the family Delesseriaceae with three currently accepted species: the type species *Radicilingua thysanorhizans* (Holmes) Papenfuss, *R. adriatica* (Kylin) Papenfuss and *R. reptans* (Kylin) Papenfuss (Guiry and Guiry 2020). *Radicilingua* has been reported along the Mediterranean and northeastern Atlantic coasts (Guiry and Guiry 2020). *Calonitophyllum* Aregood (1975) is a monotypic genus in the same family, based on *Calonitophyllum medium* (Hoyt) Aregood from North and South America, which has never been found in the Mediterranean Sea.

Detailed morphological analyses were previously conducted on the type species of *Radicilingua*. Kylin (1924) carefully analysed the growth of *R. thysanorhizans* (as *Rhizoglossum thysanorhizans* [Holmes] Kylin) and described the differentiation of the characteristic microscopic blade veins; Huvè and Riouall (1970) illustrated young plants, marginal proliferations and rhizoid formation. Kylin (1956) observed the reproductive structures and reported scattered cystocarps, gonimoblasts bearing abundant carpospores and tetrasporangia in sori near the thallus tips. A detailed description of English specimens was also reported by Maggs and Hommersand (1993). Conversely, the other two species of *Radicilingua*, *R. adriatica* and *R. reptans*, reported from the Mediterranean Sea in several checklists (e.g., Gómez Garreta et al. 2001, Sfriso and Curiel 2007, Bottalico et al. 2016, Gallardo et al. 2016, Petrocelli et al. 2019), are inadequately known. Another hindrance to correct identification is the absence of DNA sequences for *Radicilingua* in public databases.

Morphological and reproductive characters of *C. medium* were reported by Aregood (1975). *C. medium* resembles *R. thysanorhizans* in its vegetative development, but it is distinguishable by very prominent macroscopic veins and by its reproductive structures. Aregood (1975) reported that the procarp of *C. medium* is characterized by a single carpogonial filament and a single large persistent sterile cell, which is absent in *R. thysanorhizans*. This character is not easy to observe, especially in thalli that are not fully mature. Moreover, the formation of macroscopic veins is restricted to extreme basal regions and does not occur in males or young thalli (Aregood 1975). For these reasons, the distinction between species belonging to these genera is very difficult.

The goals of this study were to produce the first molecular data available for the genus *Radicilingua* and to compare Atlantic and Mediterranean specimens. More-over, the phylogenetic relationship between the morphologically overlapping genera *Radicilingua* and *Calonitophyllum* was investigated.

MATERIALS AND METHODS

Sampling

Specimens morphologically identified as *Radicilingua* sp. were collected from December 2019 to April 2020 in the Adriatic and Ionian Seas: three sampling sites in the Venice lagoon (station 1: 45°25′35.2″ N, 12°24′49.0″ E; station 2: 45°19′38.9″ N, 12°19′46.1″ E; station 3: 45°16′16.5″ N, 12°15′0.32″ E), one in Trieste (station 4: 45°38′10.5″ N, 13°45′27.6″ E) and one in the Mar Piccolo of Taranto (station 5: 40°29′39″ N, 17°19′22″ E). In March 2021, cystocarpic plants were also found in the Mar Piccolo of Taranto (station 5).

Two specimens of the type species of *Radicilingua*, *R. thysanorhizans*, were supplied from the personal herbarium of phycologist Annette (Netty) Elizabeth Little, née Twitchin (born Nov 4, 1948, died Jul 15, 2002), which her husband David Little donated, after her death, to the British Phycological Society. The two analyzed specimens were collected in July 1986 from Duke Rock, Plymouth, England, near the type locality of *R. thysanorhizans*, and in August 1984 in Pembrokeshire, Wales (Fig. 1A & B), respectively.

Molecular analyses

Genomic DNA was extracted using the Genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA). Amplification of the *rbc*L-5P gene fragment (about 700 bp) was carried out with the primer pairs F57-R753 (Freshwater and Rueness 1994), following Wolf et al. (2018). The obtained PCR products were cleaned using the HT ExoSAP-IT (Thermo Fisher Scientific) and sequencing was carried out at the Eurofins Genomics Sequencing Service (Eurofins Genomics GmbH, Ebersberg, Germany), with the same primers employed in the amplification reactions. The GeneStudio sequence analysis software (http://genestudio.com/) was used to assemble the final consensus sequences. The new sequences (one for each sampling site) were deposited in the International Nucleotide Sequence Database Collaboration (INSDC) repositories, through the European Nucleotide Archive (ENA) platform, with the following accession numbers: LR991380-LR991386.
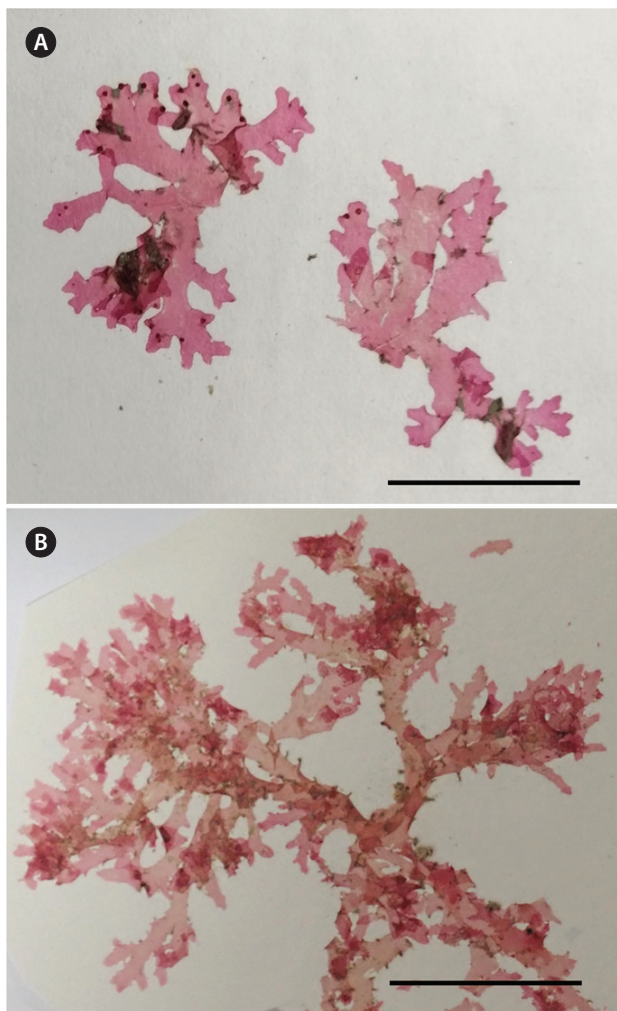
Fig. 1. *Radicilingua thysanorhizans* (Holmes) Papenfuss. (A) Habit of specimens collected in Pembrokeshire (United Kingdom) in August 1984, leg: A. E. Little, det: C. A. Maggs. (B) Habit of the specimen collected in Plymouth (United Kingdom) in July 1986, leg: A. E. Little, det: C. A. Maggs. Scale bars represent: A & B, 25 mm.

Using the BLAST program, available at the USA National Center for Biotechnology Information (NCBI) web server (http://www.ncbi.nlm.nih.gov), the obtained sequences were compared with those present in the INSDC archives.

A dataset of *rbc*L-5P sequences was created including the newly obtained sequences and other suitable sequences available in the INSDC repositories, following the most recent classifications for the focal taxa (Lin et al. 2001, 2004*a*, 2004*b*, Lin and Fredericq 2003, Díaz-Tapia et al. 2019). Representative sequences of the family Delesseriaceae were included, and in particular sequences of the subfamilies Nitophylloideae, Sarcomenioideae, Delesserioideae, and Phycodryoideae. Two representative

sequences of the Rhodomelaceae (*Odonthalia dentata*, [Linnaeus] Lyngbye, INSDC accession: KU564463, and *Rhodomela confervoides* [Hudson] P. C. Silva, INSDC accession: MH277301) were used as the outgroup to orient the tree. Supplementary Table S1 lists the Delesseriaceae specimens with *rbc*L-5P sequences included in the phylogenetic analyses.

A multiple sequence alignment was generated with MUSCLE (Edgar 2004) and used for phylogenetic analyses; it included 58 sequences for a total of 630 aligned positions. Phylogenetic analyses based on the neighbour joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods were performed with MEGA X (Kumar et al. 2018). For ML, the model that best fit the data was GTR + G + I, as suggested by the "Find best DNA Models" tool implemented in MEGA under the Bayesian information criterion (Schwarz 1978). A non-parametric bootstrap re-sampling (Felsenstein 1985) of 1,000 replicates was performed to test the robustness of the tree topologies. Bayesian inference (BI) analyses were carried out with MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). The analyses included two independent Markov chain Monte Carlo (MCMC) runs, each composed of four chains (three heated and one cold); each MCMC ran for 2×10^6 generations, sampling trees every 100 generations. The sampling of the posterior distribution was considered to be adequate if the average standard deviation of split frequencies was ≤0.01. The first 5,000 trees were discarded as burn-in, as determined by stationarity of log likelihood assessed using Tracer version 1.5 (Rambaut and Drummond 2007). The consensus topology and posterior probability values were then calculated from the remaining trees. The final tree figure was created with Inkscape v. 0.92. An alignment of the *rbc*L-5P sequences of only the subfamily Nitophylloideae was also obtained with MUSCLE to calculate the percent identities within this group (Supplementary Table S2).

Morphological analyses

Specimens were preserved in 4% formaldehyde/seawater solution and were observed using a light microscope Optika B-510PH (Optika Srl, Ponteranica, BG, Italy) equipped with a digital image acquisition system. Final pictures, suitable for publication, were created with GIMP v. 2.8.22 (https://www.gimp.org) and Inkscape v. 0.92 (https://www.inkscape.org).

RESULTS

Molecular analyses

The phylogenetic reconstruction based on the *rbc*L-5P gene fragment (Fig. 2) highlighted different subfamilies and corresponding tribes of the family Delesseriaceae. Of these, the subfamily Nitophylloideae was not monophyletic, except for the topologies obtained with MP and BI methods (data not shown). The sequences of the Mediterranean *Radicilingua* specimens formed a highly supported clade (100 / 100 / 100 / 1.00), sister to another highly supported group (100 / 100 / 100 / 1.00) consisting of the two *R. thysanorhizans* specimens from England. The group formed by the Mediterranean and the English *Radicilingua* clades was sister taxon with strong statistical support (100 / 95 / 100 / 1.00) to a highly supported cluster (99 / 99 / 100 / 1.00) formed by two *Calonitophyllum medium* sequences from the United States. The two *C. medium rbc*L sequences were deposited in GenBank by Lin et al. (2001) from specimens collected in North Carolina (type locality of the species) and Texas. The similarities among the *rbc*L-5P sequences of the specimens belonging to the *Radicilingua* and *Calonitophyllum* clades were 96.35–96.67% (i.e., the nucleotide divergence was 3.33–3.65%). The *rbc*L-5P similarities among the sequences of the remaining Nitophylloideae clades, identified at the genus level and including different species of the corresponding genera, were: 92.86% for *Nitophyllum* Greville, 95.87–96.51% for *Augophyllum* S. M. Lin, Fredericq & Hommersand, 96.03% for *Valeriemaya* A. J. K. Millar & Wynne and 87.78–98.41% for *Martensia* K. Hering (Supplementary Table S2). Except for the two American specimens of *C. medium*, which were 99.84% similar (one nucleotide difference in 630 aligned positions), all the other Nitophylloideae specimens attributed to the same species showed 100% *rbc*L-5P sequence identity.

Based on the obtained molecular and phylogenetic data, the following taxonomic change is proposed.

Radicilingua media (Hoyt) Wolf, Sciuto & Sfriso, comb. nov.

Basionym. *Nitophyllum medium* Hoyt 1920, Bull. U. S. Bur. Fish. 36: 494, pl. CV; pl. CXIV: figs 4–5; text-fig. 35.

Synonyms. *Calonitophyllum medium* (Hoyt) Aregood, 1975; *Hymenena media* (Hoyt) W. R. Taylor 1960.

Holotype. United States National Museum, Jul 12, 1907, No. 2, leg: W. D. Hoyt.

Type locality. Bogue Beach, Beaufort, North Carolina.

Distribution. North America (North Carolina, South Carolina, Florida, Texas) and South America (Venezuela).

Description. Aregood (1975): "Plants large (to 20 cm high), rosy-red blades comprised of narrow (0-5-1.5 cm wide) branches; holdfast a prominent branched and somewhat calcified, perennial structure, creamy-pink in colour; tetrasporophytes with conspicuous veins, large and proliferous, spores borne in sori scattered over entire surface of plant; cystocarps also widely scattered, abundant; procarps formed on one or both sides of fertile segment, each comprised of one carpogonial branch and a single very large sterile cell; fertile segment also bears a sterile pericentral cell in addition to the supporting cell; gametophytes somewhat dimorphic; males lack macroscopic veins, are smaller and nearly dichotomous."

Based on the obtained molecular and phylogenetic data and on the morphological evidence reported below, to encompass the Adriatic and Ionian specimens the following new species is here described.

Radicilingua mediterranea Wolf, Sciuto & Sfriso sp. nov. (Figs 3–5)

Diagnosis. Plants 2–4 cm high, pink to red in colour, composed of membranous single-layered blades. Blades erect at the apices, composed of axes with rounded or pointed margins. Branching usually irregular, pseudodichotomously to alternately. Blades traversed by microscopic veins, 1–2 parallel to the central vein. At the apices veins connected by other transverse veins, forming an irregular rectangular pattern. Margin of blades with a large apical cell broad and flat to domelike and narrow in form. Blades anchored to the substratum by a discoid holdfast. Tetrasporangia in irregular sori, scattered over the upper half fertile blade. Cystocarps spherical with a non-protruding ostiole and a two-layered pericarp.

Holotype. Voucher A000840 at PAD Herbarium, Botanical Garden Padova (Italy).

Type locality. Venice Lagoon, Venice, North Adriatic, Italy.

Etymology. The specific epithet (fem. adj.) refers to the area (i.e., the Mediterranean Sea) where it was found up to now.

Molecular voucher. LR991382 (*rbc*L-5P).

Vegetative morphology. Thalli were 2–4 cm high, pink to red in colour (Fig. 3A), often forming aggregates of 10–15 cm, composed of overlapping membranous single-layered blades, 40–50 µm in thickness, tangled and often adherent to each other through marginal unicellular rhizoids (haptera) grouped in bundles that can reach
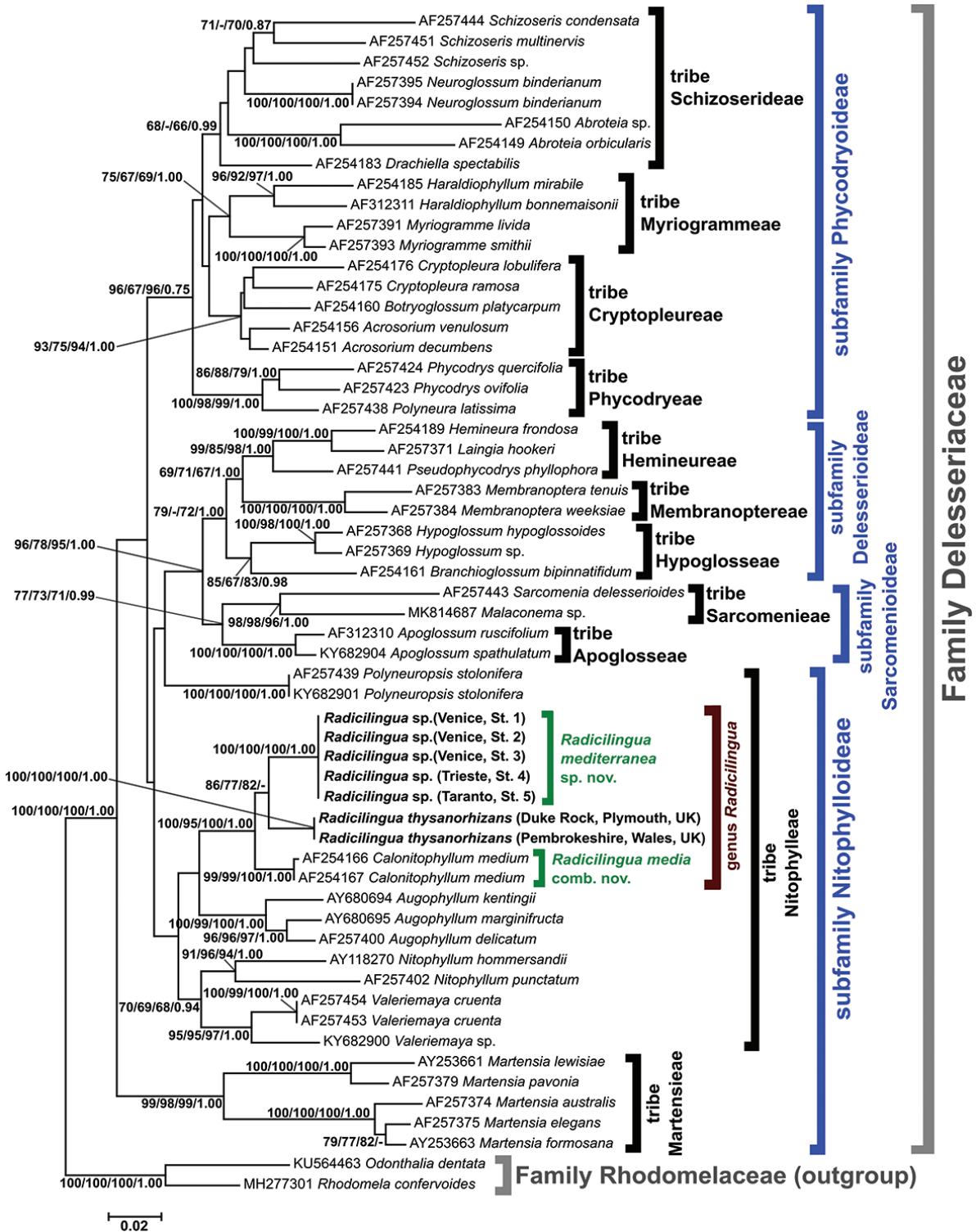
Fig. 2. Phylogenetic reconstruction of the family Delesseriaceae based on the partial *rbc*L gene. The topology obtained with neighbour joining (NJ) method is depicted and the support values from NJ bootstrap, maximum parsimony bootstrap, maximum likelihood bootstrap and Bayesian inference posterior probabilities are reported, respectively. Only bootstrap supports ≥50% and posterior probabilities ≥0.70 are shown. Values for nodes that obtained support in only two of the phylogenetic analyses were omitted. For each of the downloaded sequences, the International Nucleotide Sequence Database Collaboration (INSDC) accession number is reported followed by the species name. The sequences obtained in this work are in boldface font. Square brackets indicate families, subfamilies and tribes. Scale bar represents: expected number of nucleotide substitutions per site.
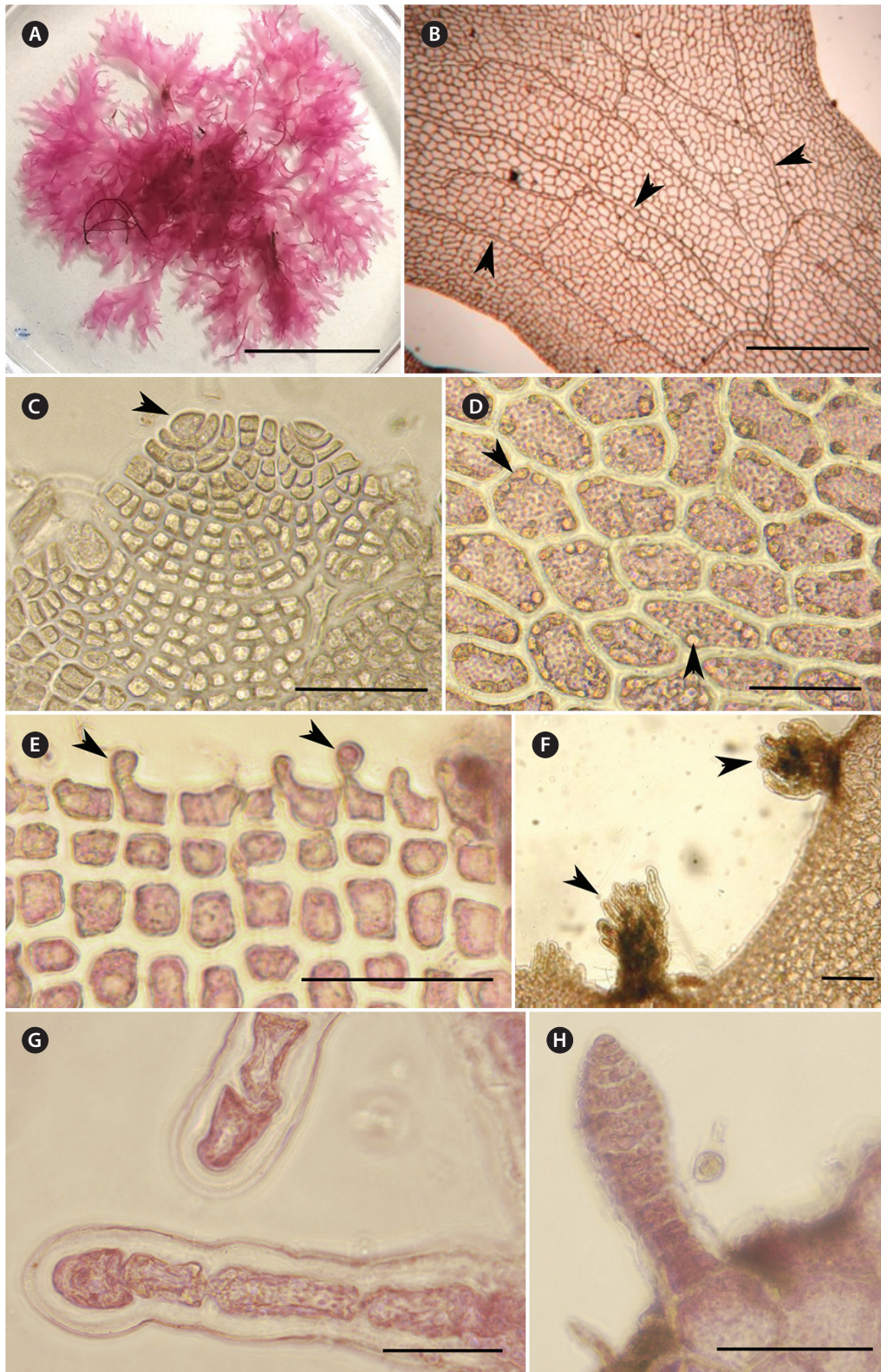
Fig. 3. *Radicilingua mediterranea* sp. nov., vegetative morphology. (A) Habit of the thallus. (B) Irregular microscopic veins (arrowheads). (C) Margin of a young blade showing the apical cell (arrowhead). (D) Surface view of parietal discoid plastids (arrowheads). (E) Detail of the margin of a blade showing irregular surface cells (arrowheads). (F) Marginal rhizoidal filaments (arrowheads). (G) Detail of a single rhizoidal filament. (H) Young new blade arising from surface cells of the holdfasts. Scale bars represent: A, 25 mm; B, 200 μm; C–F, 50 μm; G & H, 100 μm.

300–500 µm in length. Blades became erect at the apices, which were composed of axes with rounded or pointed margins. Branching was usually irregular, pseudodichotomously to alternately, resulting in deeply lobed thalli (Fig. 3A). Blades were traversed by microscopic veins, usually 3 cells thick in cross-section, which, in surface view, were composed by a single, rarely double, row of elongate cells, 15–90 µm long × 10–30 µm wide (Fig. 3B), connected by conspicuous pit connections. Veins were mostly parallel, 1–2 lateral veins parallel to a central vein. At the apices of the blade lateral veins were often connected to the central one by other transverse veins, forming an irregular rectangular pattern (Fig. 3B). At the margin of blades, a large apical cell was present (10 µm in diameter) (Fig. 3C), varying in form from broad and flat to domelike and narrow. This initial gave rise to a primary cell row composed of cells arranged in semi-circular arrays around the apical cell (Fig. 3C). Inner surface cells were irregular, rectangular to polygonal, 30–100 µm long × 20–60 µm wide, with conspicuous secondary pit connections, and were characterized by numerous parietal discoid plastids (Fig. 3D). At the margin of the blade the cells were smaller with elongate-elliptical projections (Fig. 3E). Blades were anchored directly to the substratum by a discoid holdfast and secondarily attached by uniseriate, multinucleate rhizoidal filaments extending from marginal surface cells (Fig. 3F & G). New young blades arose from the surface cells of the holdfasts (Fig. 3H).

Reproductive morphology. Tetrasporangia were borne in sori formed over the upper half of the entire thallus on both sides of the blade. Tetrasporangial sori were irregular in shape, scattered over the upper half fertile blade and arranged on both sides of the apical veins (Fig. 4A & B). Mature tetrasporangia were spherical-ellipsoid, tetrahedrally divided, 40–50 µm in diameter (Fig. 4C). Cystocarps were scattered on both sides of the thallus (Fig. 5A). When mature they were spherical, 335–575 µm in diameter with a non-protruding ostiole and a two-layered pericarp (Fig 5B). Carposporangia were ovoid to spherical, 24–39 × 36–78 µm (Fig. 5C).

DISCUSSION

In this study we report the first molecular data for the genus *Radicilingua* Papenfuss and analyse the phylogenetic relationship between this genus and the morphologically overlapping *Calonitophyllum* Aregood.
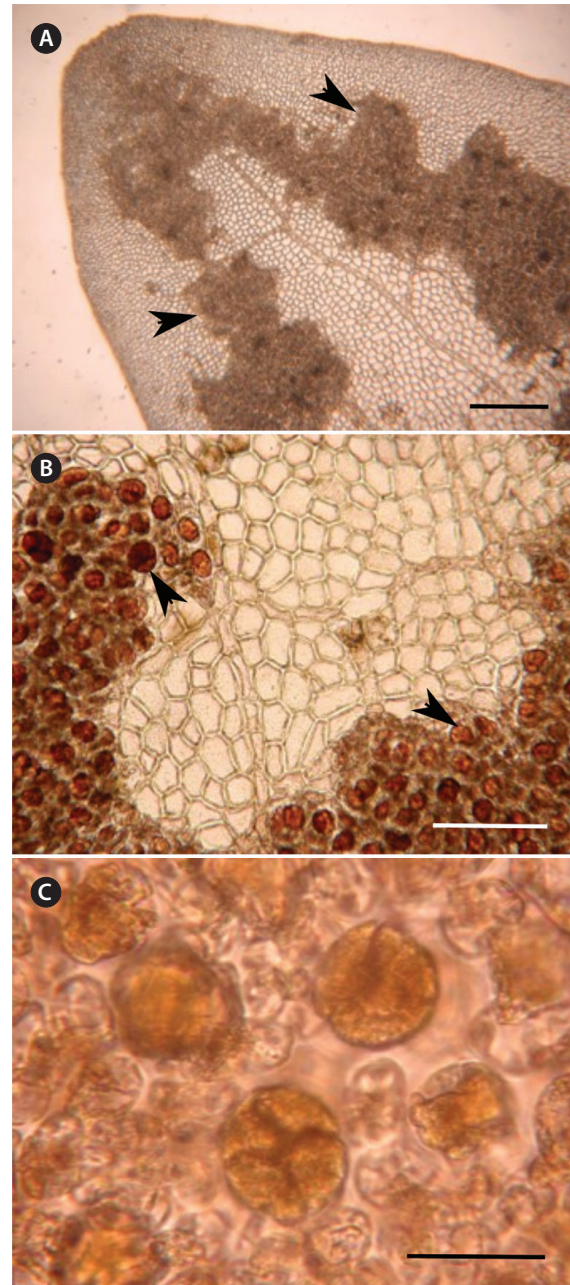
The taxonomic treatment of the two genera has un-

Fig. 4. *Radicilingua mediterranea* sp. nov., tetrasporangial morphology. (A) Surface view of mature tetrasporangial sori scattered over a fertile blade (arrowheads). (B) Detail of the tetrasporangial sori showing numerous tetrasporangia (arrowheads). (C) Detail of mature tetrasporangia. Scale bars represent: A, 100 µm; B, 200 µm; C, 50 µm.

dergone several changes in the past. Holmes (1873) described the type species of the genus *Radicilingua* as *Nitophyllum thysanorhizans* Holmes, after analysing northeastern Atlantic Nitophylleae Willkomm from Cornwall, England. In 1924, Kylin moved the species to the genus *Rhizoglossum* (as *Rhizoglossum thysanorhi-*

Fig. 5. *Radicilingua mediterranea* sp. nov., female thalli. (A) Habit of female blade with numerous cystocarps. (B) Surface view with cystocarps at different levels of maturity. (C) Transverse section of a mature cystocarp. Scale bars represent: A, 5 mm; B, 200 μm; C, 100 μm.

zans [Holmes] Kylin), but Kylin's *Rhizoglossum* is an illegitimate name because later homonym of Presl's (1845) *Rhizoglossum*. Therefore, later Papenfuss (1956) erected the new genus *Radicilingua nomen novum* to include the northeastern Atlantic *Radicilingua thysanorhizans* (Holmes) Papenfuss and two Mediterranean species: *Radicilingua adriatica* (Kylin) Papenfuss and *Radicilingua reptans* (Kylin) Papenfuss.

During the same period, on the other side of the Atlantic Ocean (western Atlantic), Hoyt (1920) described *Nitophyllum medium* from material collected on offshore reefs and in the drift along the coast of North Carolina (USA). Subsequently, Taylor (1960) proposed the combination *Hymenena media* (Hoyt) W. R. Taylor, based on the superficial resemblance of this veined plant to representatives of the genus *Hymenena* Greville from South Africa, Pacific North America, and Australia. But, at around the same time, Wagner (1954) reported that the morphology and development of the procarp and carposporophyte of plants in North Carolina revealed no relationship to *Hymenena* (typified by *H. venosa* [L.] C. Krauss). Indeed, procarp development of the North Carolina plants was similar to that of *Nitophyllum bonnemaisonii* (C. Agardh) Greville, a European species, while early apical growth of the blades and subsequent vein formation were similar to that in *Radicilingua* (= *Rhizoglossum*) *thysanorhizans* (Holmes) Papenfuss (Holmes 1873, Kylin 1924, Papenfuss 1956, Huvè and Riouall 1970). To solve this taxonomic issue, Aregood (1975) erected a new monotypic genus for the North Carolina populations: *Calonitophyllum* Aregood. She reported that *C. medium* resembled *R. thysanorhizans* in vegetative developmental pattern, but was distinguishable by very prominent macroscopic veins and by a procarp characterized by a single carpogonial filament and a single large persistent sterile cell.

These characters are not easy to observe, especially in immature and vegetative thalli (Aregood 1975). Moreover, macroscopic veins are not present in males and in young thalli and the number of cell layers is not specified (Aregood 1975). On the contrary, Hoyt (1920) described the veins of *N. medium* as one cell wide, rarely two cells wide. Thus, further morphological analyses of American *C. medium* are needed to clarify the vein size.

In the light of all this, currently the taxonomic status of these taxa is controversial and distinguishing the two genera is very difficult with the only morphological observations.

In the present study, the *rbc*L-5P phylogenetic reconstruction shows that the *Radicilingua* and *Calonitophyllum* specimens group together with strong statistical support and the *rbc*L-5P sequence percent identities calculated among the members of this clade are comparable to intrageneric *rbc*L-5P values for other genera of the subfamily Nitophylloideae (Supplementary Table S2). This suggests that the genera *Radicilingua* and *Calonitophyllum* are congeneric, and the name *Radicilingua* Papenfuss (Papenfuss 1956) has priority. Therefore, we here propose to transfer the only species described so far for the genus *Calonitophyllum*, *C. medium* (Aregood 1975), to the genus *Radicilingua* as *Radicilingua media* (Hoyt) Wolf, Sciuto and Sfriso comb. nov.

The phylogenetic reconstruction shows that the Mediterranean *Radicilingua* specimens are genetically distinct from the northeastern Atlantic *R. thysanorhizans* sampled from the United Kingdom. The *rbc*L-5P sequence divergences found between the Mediterranean and northeastern Atlantic *Radicilingua* lineages are comparable to the *rbc*L-5P interspecific divergences calculated for other genera of the subfamily Nitophylloideae (Supplementary Table S2).

Despite their phylogenetic divergence, our Adriatic and Ionian samples are morphologically very similar to *R. thysanorhizans*, even if they present the following two differences: (1) a smaller number of lateral parallel veins (1–2 on both sides of the principal vein) in our samples, compared to the myriad of veins forming a trellis-like pattern in *R. thysanorhizans*; (2) the form of the cystocarp ostiole: non-protruding in our samples, protruding in *R. thysanorhizans*. Our samples differ also from the other two species of the genus. In fact, *R. adriatica*, originally described as *Rhizoglossum adriaticum* by Kylin (1924), can be distinguished from our samples by the smaller size of the thallus (1 cm in *R. adriatica*, 2–4 cm in the Adriatic and Ionian specimens) and by the presence of a single parallel vein on both sides of the central vein. Moreover, Kylin (1924) did not observe a rectangular pattern of veins at the apices of the blades as present in our samples. *R. reptans*, in contrast to the other species, is easily recognizable because parallel veins are completely absent (Kylin 1924). It is a very rare species (Sfriso and Curiel 2007) that was reported in the Venice lagoon for the first time only in the 1990s (Curiel et al. 1996).

For these reasons, we attribute the Mediterranean samples to the new species *R. mediterranea* Wolf, Sciuto & Sfriso. Our results show that *R. mediterranea* and *R. thysanorhizans* are not easily distinguishable by a morphological point of view, since they can exhibit overlapping morphological characters, but they are clearly distinct at the species level based on molecular analyses (Fig. 2).

During our sampling surveys, we did not find any specimens of the other *Radicilingua* species reported for the Mediterranean Sea. Concerning records of *R. thysanorhizans*, it is possible that, due to the overlapping morphology with our specimens, this Atlantic species has never been present in the Mediterranean Sea and that the previously collected specimens attributed to this taxon were misidentified. Similar misidentifications are not new for red algal specimens collected in the Mediterranean Sea, as reported by previous studies. For example, Wolf et al. (2011) demonstrated that Adriatic samples of *Ceramium* Roth with characters falling inside the morphological range of one or more Atlantic species of this genus, and previously identified as those species, were indeed phylogenetically distinct from their Atlantic counterparts (Wolf et al. 2011). Recently, based on molecular analyses and an important diacritical morphological character, Serio et al. (2020) recognized and described the new species *Melanothamnus testudinis* Serio et al., generally overlooked in the Mediterranean records and previously confused with the morphologically very similar Pacific species *Polysiphonia carettia* Hollenberg.

Concluding, with this study we provide the first molecular data for *R. thysanorhizans* and we describe the new species *R. mediterranea*. Moreover, our molecular results demonstrate that the morphologically overlapping genera *Radicilingua* and *Calonitophyllum* are congeneric, *Radicilingua* having priority; so the type species of *Calonitophyllum* is here transferred to *Radicilingua*.

ACKNOWLEDGEMENTS

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Table S1. List of the organisms belonging to the family Delesseriaceae whose sequences are included in the phylogenetic analyses based on the *rbc*L gene (https://www.e-algae.org).

Supplementary Table S2. Percent identity matrix for the 5P-*rbc*L gene fragment (630 aligned positions) of the subfamily Nitophylloideae (https://www.e-algae.org).

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