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Phylogenetic analysis of the *Cladophora coelothrix* complex, including the description of the new genus *Leliaertia* (Cladophorales, Ulvophyceae)

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ABSTRACT
Molecular phylogenetic studies have revealed the polyphyletic nature of *Cladophora* as traditionally circumscribed, which is separated into four main clades: Cladophoraceae, *Siphonocladus*, Pithophoraceae and Pseudocladophoraceae. Several taxonomic proposals have recently been made to resolve its polyphyletic nature. The present work focuses on the cryptic diversity of *Cladophora coelothrix*, within the *Siphonocladus* clade. Based on new collections from northeastern Brazil and phylogenetic analyses of partial SSU and LSU rDNA sequence alignments, we propose the new genus and species *Leliaertia repens* to accommodate one of the cryptic species hitherto included in *C. coelothrix*, occurring in the tropical Atlantic, Indian and Pacific Oceans. *Leliaertia repens* was recovered in the *Siphonocladus* clade and is phylogenetic distinct from the genuine *Cladophora* species in the Cladophoraceae. The new species occurs in marine and estuarine environments and forms conspicuous mats of semi-prostrate filaments. It represents a cryptic species and genus since morphological features, including the diameter and length: diameter ratio of the apical cell, show high levels of overlap with other species, including *C. socialis*. Our phylogenetic data indicate that *L. repens*, *C. coelothrix* and *C. socialis* represent a complex of cryptic species with at least four lineages, some of which co-occur in tropical seas.

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Chlorophyta; Cryptic species; *Leliaertia repens sp. nov.*; Molecular phylogeny; Nucleus-encoded LSU rDNA

INTRODUCTION

The green algal genus *Cladophora* Kützing is characterized by branched, uniseriate filaments composed of multinucleate cells. The simple morphology makes species identification notoriously difficult (Bakker *et al.* 1995; Gestinari *et al.* 2009; Boedeker *et al.* 2016). Phylogenetic analyses based on nuclear ribosomal DNA sequence data revealed that the genus, as initially circumscribed, is polyphyletic and that many species present cryptic diversity (Bakker *et al.* 1994; Hanyuda *et al.* 2002; Leliaert *et al.* 2007). *Cladophora* species are distributed in the four main clades of the Cladophorales: the Cladophoraceae, which include the type of *Cladophora*, *C. oligoclona* (Kützing) Kützing; the *Siphonocladus* clade *sensu* Boedeker *et al.* (2012); the Pithophoraceae; and the Pseudocladophoraceae (Leliaert *et al.* 2007; Boedeker *et al.* 2012, 2016; Taylor *et al.* 2017). A number of taxonomic proposals have already been made to resolve this polyphyly, e.g. the erection of genera such as *Pseudocladophora* C. Boedeker & Leliaert (Boedeker *et al.* 2012) or the reinstatement of *Lychaete* J. Agardh (Wynne 2017), but some taxonomic entities still need revision.

Cladophora coelothrix Kützing was originally described based on material from the Gulf of Genova, Italy, in the Mediterranean Sea (Kützing 1843; van den Hoek 1963). Its original description has no more details than: a marine filamentous species, with a flaccid, dichotomous thallus, and cells 6–8 times longer than wide (Kützing 1843). The species differs from other *Cladophora* species by a combination of characters, including semi-prostrate cushions with rhizoids formed from stolon-like branches, and long, cylindrical apical cells that are 50–210 μm wide (Kraft 2000; Leliaert & Coppejans 2003; Alves *et al.* 2012b; Almeida *et al.* 2012). *Cladophora coelothrix* is currently regarded as a widely distributed species in the tropical to subtropical Atlantic, Indian and Pacific Oceans, occurring from marine to estuarine environments (Taylor 1960; van den Hoek 1963; Womersley 1984; van den Hoek & Chihara 2000; Leliaert & Coppejans 2003; Wysor & Kooistra 2003; Coppejans *et al.* 2004; Gestinari *et al.* 2010;

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Cormaci *et al.* 2014; Pellizzari *et al.* 2017; Guiry & Guiry 2023). In van den Hoek's sectional classification, *C. coelothrix* is the type species of section *Repentes*, which groups species with cushion-like thalli, branches composed of long cells laterally inserted and rhizoids at basal cell poles (van den Hoek 1963). This section also includes *C. sibogae* Reinbold, *C. socialis* Kützing and *C. subsimplex* Kützing.

Despite its morphological affinities with *Cladophora* species, *C. coelothrix* has been positioned within the *Siphonocladus* clade in molecular phylogenetic studies, and separate from the *Cladophora* clade containing the type in the Cladophoraceae (Bakker *et al.* 1994; Leliaert *et al.* 2003, 2007; Taylor *et al.* 2017). However, molecular studies regarding the South Atlantic species are scarce and the most comprehensive Cladophorales phylogeny to date indicates the presence of cryptic diversity in *C. coelothrix*, as currently conceived (Leliaert *et al.* 2007). Furthermore, due to the lack of morphological descriptions concerning most of the sequences available in GenBank, and the low number of sequences from tropical specimens, a reliable taxonomic treatment for *C. coelothrix* has not yet been achieved. Based on newly produced molecular sequences (SSU and LSU rDNA) and geographical distribution data we propose here a new genus and species, *Leliaertia repens*, to accommodate a taxon hitherto included in the *C. coelothrix* complex within the *Siphonocladus* clade.

MATERIAL AND METHODS

Samples were collected in four localities of the Brazilian Northeast coast (Table 1). Samples were frozen or preserved in 70% ethanol for morphological observations, while fragments of these specimens were preserved in ethanol 70%, CTAB gel, or were silica-dried for molecular analysis. External morphology and cell observations were performed in a Zeiss Axioplan stereoscopic microscope (Zeiss, Oberkochen, Germany) and photographed using a Digital Camera SD950IS (Canon, Tokyo, Japan). The chemical nature of crystalline inclusions was observed according to Yasue (1969), Leliaert & Coppejans (2004) and Alves *et al.* (2012a). Birefringence was observed using phase contrast interference (Nomarski) microscopy. The samples studied were dried and deposited in the herbaria of the Universidade Estadual de Feira de Santana (HUEFS) and Professor Vasconcelos Sobrinho of the Universidade Federal Rural de Pernambuco (PEUFR) (Table 1). Distribution data were obtained from Taylor (1960), van den Hoek (1963), Womersley (1984), van den Hoek & Chihara (2000), Wysor & Kooistra (2003), Leliaert & Coppejans (2003), Coppejans *et al.* (2004), Leliaert *et al.* (2007), Gestinari *et al.* (2010), Cormaci *et al.* (2014), Pellizzari *et al.* (2017), Flora e Funga do Brasil (2022) and Guiry & Guiry (2023).

Total DNA was extracted using the Doyle & Doyle (1987) 2×CTAB protocol excluding RNAse incubation. We amplified the SSU (18S) and LSU (28S) rDNA regions using the primers SR1-SS11H and SSU897-18SC2 for SSU rDNA, and C1FL-D2FL for LSU rDNA (Leliaert *et al.* 2003, 2007). The polymerase chain reaction was performed in 25 µl final volume: 6.75 µl of H_2O miliQ, 5 µl of PCR Buffer 5×, 3 µM $MgCl_2$, 1 M betaine, 1.6 µM dNTP, 0.6 µM of each primer, 1.25 units of Taq DNA polymerase and 1 µl of total DNA. The PCR program for SSU rDNA started with an initial denaturation for 3 min in 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min 30s at 72°C, and final extension of 1 min at 72°C. The LSU rDNA program started with an initial denaturation for 3 min in 94°C, followed by 35 cycles of 30s at 94°C, 30s at 53°C, 30s at 72°C, and final extension of 3 min at 72°C. PCR products were checked in agarose gels, EDTA and UniSafe Dye (Invitrogen, Waltham, Massachusetts, USA), with bands compared with a 1kb DNA Ladder (Invitrogen). The products were purified using Illustra GFX PCR DNA and Gel Purification Kit (GE Healthcare Life Sciences, Piscataway, New Jersey, USA), and then bidirectionally sequenced using the same primers from PCR and Big Dye Terminator Cycle Sequencing Ready to Reaction Kit (Applied Biosystems, Foster City, California, USA) in an ABI PRISM Genetic Analyzer.

Consensus sequences were built using BioEdit v7.0.4.1 (Hall 1999) and compared to sequences in GenBank using the BLAST tool. The generated sequences and sequences acquired from GenBank (Table S1) were aligned using ClustalW (Larkin *et al.* 2007) and the final alignment was manually refined. Three datasets were generated for phylogenetic analysis: 1) partial SSU alignment with 40 sequences from GenBank (1,480 bp) and one newly generated in this work; 2) LSU alignment including 68 sequences from GenBank (519 bp) and four newly generated in this work; and 3) concatenated SSU+LSU sequences of the same individuals (2,225 bp), including 56 sequences (28 were from GenBank). The SSU+LSU sequences were manually concatenated based on the alignment of each marker individually to make sure to not overlap different regions. All datasets included representatives of the Cladophoraceae and *Siphonocladus* clades. For a better view of these clades, the focus of this work, sequences of Pithophoraceae and Pseudocladophoraceae were not included in LSU dataset, and *Aegagropila linnaei* Kützing (Pithophoraceae) was

Table 1. Material examined in the present study.

Taxon	Herbarium voucher	Collection site	Collection date	SSU rDNA	LSU rDNA
Leliaertia repens gen. & sp. nov.	PEUFR55536	Tatuamunha Estuary, Porto de Pedras, Alagoas, Brazil	4 Aug. 2019	OQ359159	OQ371331
Leliaertia repens gen. & sp. nov.	HUEFS217732	Mirante da Sereia beach, Maceió, Alagoas, Brazil	27 Apr. 2013		OQ371328
Leliaertia repens gen. & sp. nov.	HUEFS217733	Enseada do Pedrão beach, Vera Cruz, Itaparica Island, Bahia, Brazil	20 Mar. 2011		OQ371330
Cladophora coelothrix	HUEFS217731	Pedra do Xaréu beach, Cabo de Santo Agostinho, Pernambuco, Brazil	25 Apr. 2013		OQ371329

taken as outgroup in the concatenated data set. The best evolutionary model was calculated and selected using jModelTest using AIC criterion (Darriba *et al.* 2012), with GTR+I+G being the best suited model for all data sets. Maximum Likelihood (ML) analyses were performed using MEGAX (Kumar *et al.* 2018) with 1,000 bootstrap replicates. Bayesian Inference analyses were performed in MrBayes v3.2.2 (Ronquist *et al.* 2012), with two runs and four Markov chains Monte Carlo, with 5,000,000 generations and sampling one tree every 1,000 generations, with burn-in verified with the software Tracer v1.7 (Rambaut *et al.* 2018). The consensus ML tree was used as used as base for the phylogenetic trees in Figs 1, 2, and bootstrap (BS) and posterior probability (PP) values above 50% and 0.5, respectively, are indicated at the tree nodes.

RESULTS

Phylogenetic analyses

The generated partial SSU and LSU rDNA sequences were 783 bp and 541–568 bp long, respectively (Table 1). In all three data sets (Figs 1, 2, S1), the sequenced specimens were grouped within the *Siphonocladus* clade (*sensu* Boedeker *et al.* 2012) and were distantly related to *Cladophora* species in the Cladophoraceae clade, which includes the type species of *Cladophora, C. rivularis* (Linnaeus) Kuntze (regarded as synonymous with *C. oligoclona*).

Accessions morphologically identified as *C. coelothrix* were recovered as non-monophyletic. Our phylogenetic trees based on the LSU dataset (Fig. 1) and the concatenated dataset of SSU +LSU (Fig. 2) indicate that *C. coelothrix* and *C. socialis*, as hitherto circumscribed, represent a species complex. Sequences of *C. sibogae* grouped together as sister clade to the *C. socialis* plus *L. repens* clade (Fig. 1) and, although this was supported only by posterior probability (PP = 0.96), it is probably closely related to these clades. The sequences assigned to *C. coelothrix* are positioned in three different clades (Fig. 1). Clade 1 groups with moderate to high support (BS = 92%; PP = 0.96) sequences of *C. coelothrix* from the North Atlantic Islands (Canary Islands, Spain, and Cape Verde), Europe (Roscoff, France) and the Mediterranean Sea, including a sequence from Corsica, France, which is close to the type locality, the Gulf of Genova, Italy. Based on geographical distribution, we consider this clade as the authentic *C. coelothrix* clade. The samples of '*Cladophora coelothrix*' from the Brazilian coast represent a species complex composed of two clades (clades 2 and 4). Clade 2 (BS = 100%; PP = 1) groups three sequences from Brazil (OQ371328, OQ371331, OQ371330) and sequences from the Atlantic, Pacific and Indian Oceans identified as *C. coelothrix* (herein described as a new genus and species, *Leliaertia repens*). Clade 4 included a single sequence identified as *C. coelothrix* from Brazil (Pernambuco state; OQ371329) in a moderately supported (BS = 86%; PP = 0.95) sister relationship to specimens from Australia (AM503478; named as *Cladophora socialis*), Jamaica (AM503476), and from an unknown locality (AM503479). The clade 3 grouped sequences from Panama (AM503440) and South Africa (QM503477) with moderate support (BS = 94%; PP = 0.88).

The genetic distances of LSU sequences within the *L. repens* clade vary from 0.0% to 0.6%, whereas the interspecific distances between *L. repens* and *C. coelothrix* (clade 1) ranged from 4.4% to 5.0%, reinforcing the idea that they represent distinct species. The genetic distance between the *L. repens* clade and clades 2 and 4 was lower, varying from 1.6% to 2.2%.

Overall, the phylogeny inferred from the concatenated dataset of SSU+LSU (Fig. 2) was congruent with that of the LSU dataset. Clade 1 grouped sequences of *C. coelothrix* from France, including a sequence from Corsica (AM503451), with high support (BS = 96%; PP = 1), forming a sister clade to *C. prolifera* (Roth) Kützing and *C. aokii* Yamada. The type sequence of *Leliaertia repens* (PEUFR55536; OQ359159 and OQ371331 – previously identified as '*Cladophora coelothrix*') is grouped with a sequence from the Philippines (F135) with maximal support (BS = 100%; PP = 1), forming the clade 2, which is positioned relatively distant from the authentic *C. coelothrix* (clade 1). Clade 2 forms a bigger clade together with clades 3 and 4 (both containing sequences named as *C. socialis*) with high support (BS = 99%; PP = 1). The combined dataset recovers the available sequences in these three clades, grouping sequences named as '*C. socialis*' (clades 3 and 4) and sequences now named *L. repens* (clade 2), although the relationships between them are weakly to moderately supported (Fig. 2). The combined data set displays a better resolution for the phylogenetic position of clades 2, 3 and 4 (Fig. 2), with their representatives forming a sister clade to Anadyomenaceae with moderate support from ML only (BS = 92%).

The SSU data set shows low phylogenetic resolution (Fig. S1), with '*C. coelothrix*' from Brazil (OQ359159) grouping with sequences from tropical to subtropical regions (named as *C. coelothrix* and *C. socialis*), with high support (BS = 98%; PP = 1). Sequences from Brazil (OQ359159) and Thailand (AM498749) are identical, while the genetic distance in this clade varies from 0.0% to 0.5% among all sequences (OQ359159/Brazil, AM498749/Philippines – named as *C. coelothrix*; and AM498751/Australia, AM498752/Panama, AB971263/Thailand – named as *C. socialis*). Considering the current identification of these sequences, *C. coelothrix* and *C. socialis* appear to be polyphyletic, positioned in two different clades in the SSU phylogenetic tree (Fig. S1).

Morphological analysis

Specimens from Alagoas and Bahia showed greater thallus dimensions (up to 15 cm), forming intricate mats, whereas specimens from Pernambuco showed smaller mats (0.5–5.0 cm), with irregular and sparse branching, showing no distinction between an erect and prostrate axis, with stolon bearing intercalary rhizoids and short erect branches. However, diameter and length of apical cells overlap between specimens from Bahia (47.5–97.5 µm in diameter × 110–1,113 µm in length), Alagoas (55–163.5 µm in diameter × 110–1,214 µm in length) and Pernambuco (55–170 µm in diameter × 220–2,000 µm in length).

Considering the current knowledge, and combining the generated and available molecular data with morphological characterization, we propose a taxonomic adjustment, describing a new genus and species to accommodate '*Cladophora coelothrix*' of clade 2 (Table 2).

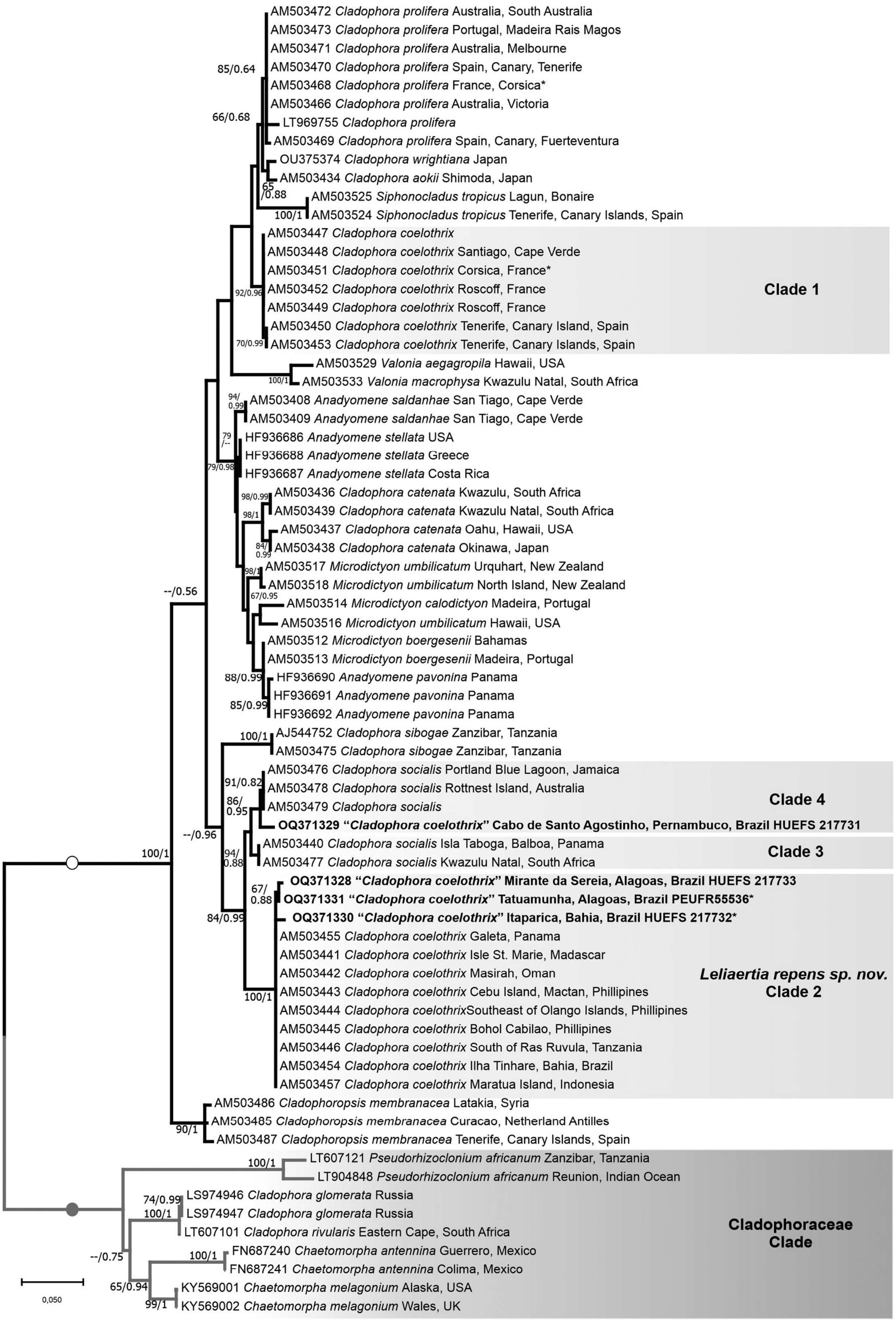


Fig. 1. Maximum likelihood (ML) consensus tree based on LSU rDNA (values above 50/0.5 for BS/PP are shown in branches). *indicates sequence from the type locality or from a nearby locality. The white circle indicates the *Siphonocladus* clade. The gray circle indicates the Cladophoraceae clade.

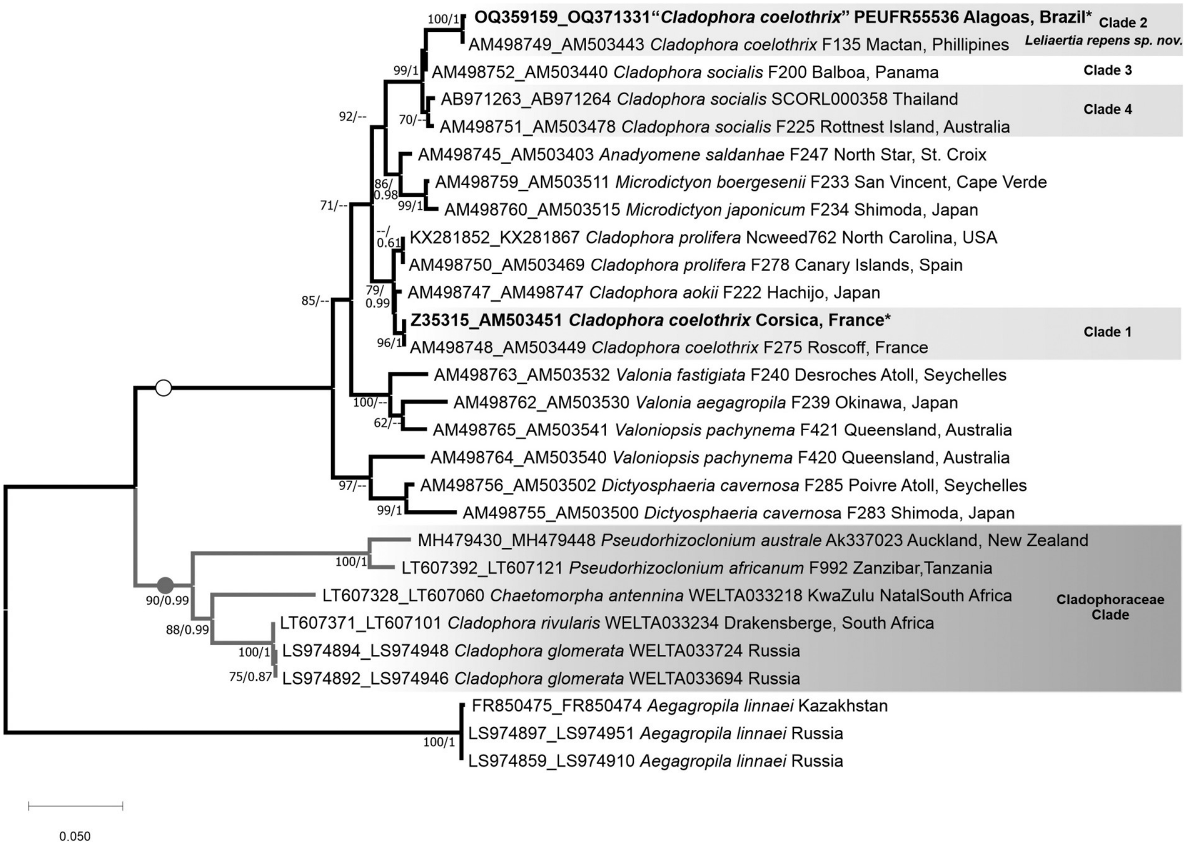


Fig. 2. Maximum likelihood (ML) consensus tree based on concatenated SSU+LSU rDNA (values above 50/0.5 for BS/PP are shown in branches). *indicates sequence from type locality or from a nearby locality. Accession number given as SSU_LSU. The white circle indicates the *Siphonocladus* clade. The gray circle indicates the Cladophoraceae clade.

Leliaertia J.O.F. Brito, A.M. Alves, Gestinari & C.W.N. Moura *gen. nov.*

DESCRIPTION: Thallus composed of uniseriate, branched filaments forming irregular cushions, attached to substrate by unicellular or multicellular rhizoids arising from basal pole of the cells of prostrate or erect branches. Thallus densely and irregularly branched, cells long and cylindrical, lateral branches arising from apical poles often opposite. Presence of hapteroidal cells attaching filaments to each other.

TYPE SPECIES: *Leliaertia repens* J.O.F. Brito, A.M. Alves, Gestinari & C. W.N. Moura *sp. nov.*

ETYMOLOGY: The generic name honors to Dr. Frederik Leliaert for his remarkable contributions to the knowledge of cladophoralean algae.

Leliaertia repens J.O.F. Brito, A.M. Alves, Gestinari & C.W. N. Moura *sp. nov.*

Figs 3–17

DIAGNOSIS: Morphologically indistinguishable from *Cladophora coelothrix* (cryptic genus and species) but differing in LSU and SSU rDNA sequences.

DESCRIPTION: Thallus uniseriate filamentous, dark green to brownish, semi-prostrate, forming cushion-like mats, firm, up to 15 cm in diameter and 1.1 cm high, attached to the substratum by unicellular or multicellular rhizoids arising from the distal pole of prostrate cells. Intercalary cell division in any portion of the thallus. Prostrate axes with cylindrical cells, 80.6–138.5 µm wide, 285–1,619 µm long, densely and irregularly branched, unilateral to pseudodichotomously, with up to two orders of branches, sometimes adhering to one another by hapteroidal cells. Erect axes unilaterally to pseudodichotomously branched with wall formation delayed, with low distinction from prostrate axes and long, cylindrical cells. Apical cells cylindrical, straight to slightly curved, 47.5–163.5 µm wide, 97.5–1,635 µm long, length:width ratio 4–17. Cells with birefringent star-shaped clusters of needle-shaped crystalline inclusions.

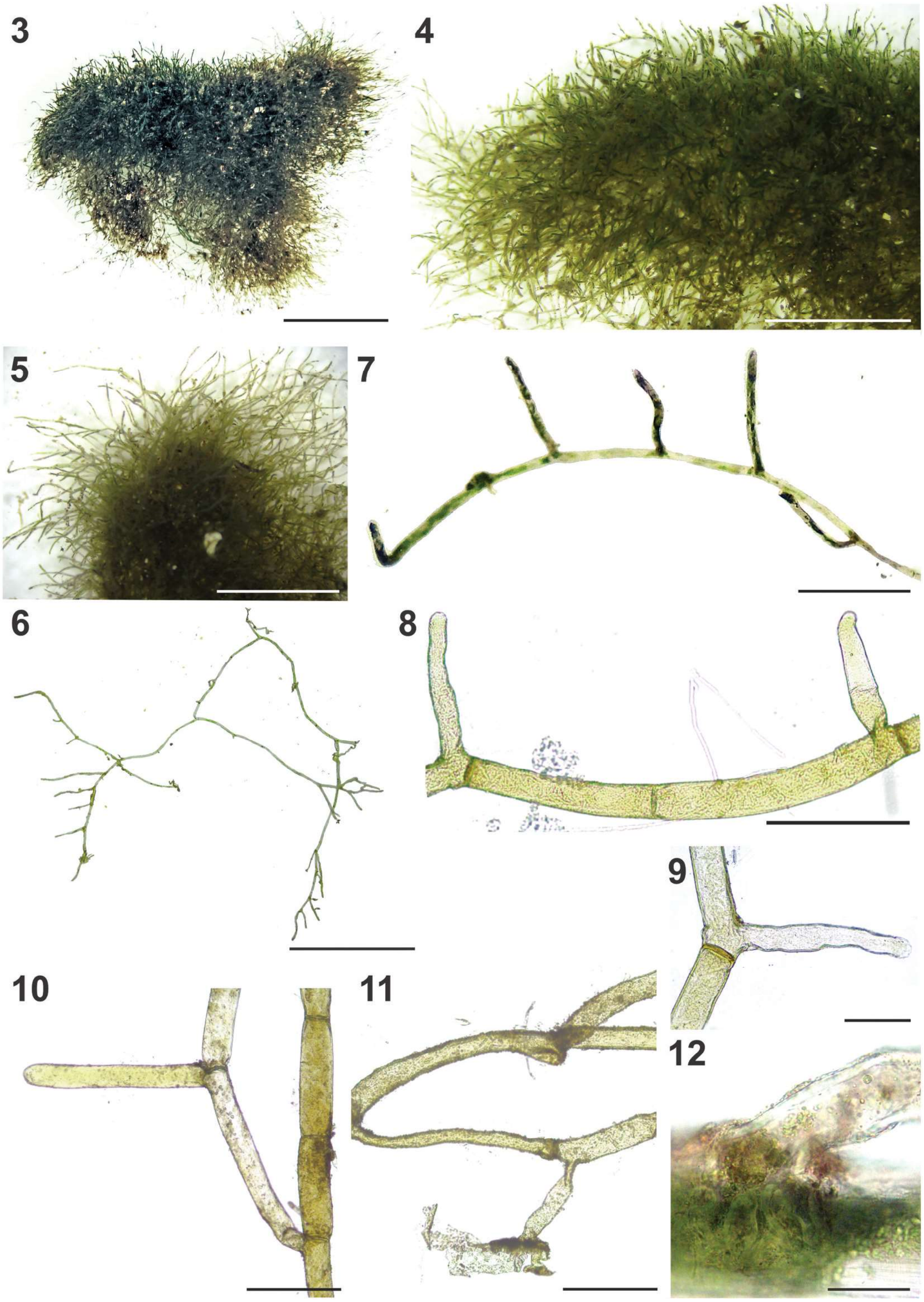
HABITAT: Specimens growing in marine environments in the intertidal zones of rocky reefs, in estuarine areas of mangroves on pneumatophores, and on sandy substrata.

HOLOTYPE: PEUFR55536, deposited in the Professor Vasconcelos Sobrinho Herbarium (PEUFR), Universidade Federal Rural de Pernambuco, Recife, Brazil, collected 4 August 2019 by J.O.F. Brito. GenBank accessions: LSU rDNA, OQ371331; SSU rDNA, OQ359159.

TYPE LOCALITY: Brazil, Alagoas, Porto de Pedras, Tatuamunha estuary, 9°13.737′S, 35°20.285′W, growing in mangrove on sediment and on pneumatophores.

Table 2. Morphological comparison of *Leliaertia gen.* & *sp. nov*. and other genera of Cladophorales.

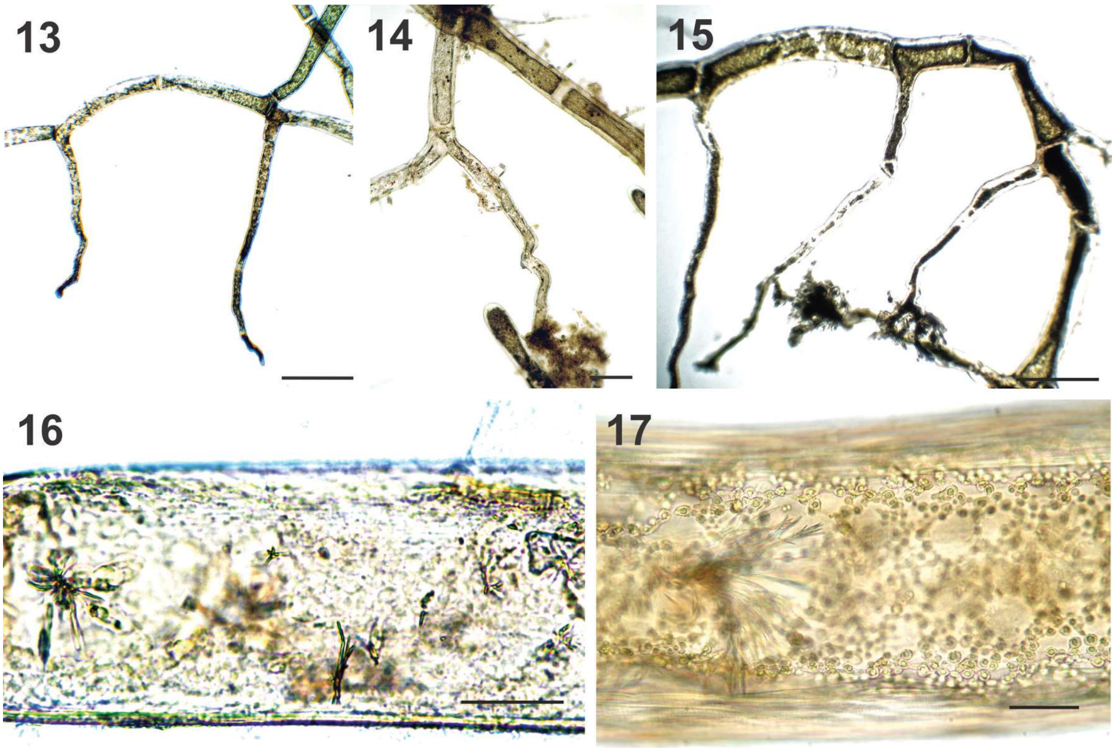
Taxa	*Leliaertia repens*	*Anadyomene* J.V. Lamouroux	*Cladophora* Kützing	*Dictyosphaeria* Decaisne	*Microdictyon* Decaisne	*Siphonocladus* F. Schmitz	*Valonia* C. Agardh
Habit	Cushion-like mats, composed of irregularly branched filaments, attached by rhizoids or hapteroidal cells	Blade-like thalli, simple to imbricate, composed of large, elongated adhering cells, attached by rhizoids	Erect, delicate to robust, attached by single or multiple basal cells	Vesicular globose, composed of inflated cells adhering by tenacular cells, attached by hapteroidal cells	Reticulate blades, composed of filaments adhering to each other by anastomoses, attached by rhizoids	Erect to prostrate, forming tufts, branched, attached by multicellular rhizoids	Vesicular globose, composed of inflated cells, attached by hapteroidal cells
Stipe	Absent	Present in some species	Absent	Absent	Absent	Absent	Absent
Thallus reinforcement	Present, by entangling of filaments and hapteroidal cells	Absent	Absent	Absent	Absent	Present, by tenacular cells	Absent
Mode of cell division	Centripetal wall ingrowths	Centripetal wall ingrowths	Centripetal wall ingrowths	Segregative cell division	Centripetal wall ingrowths	Segregative cell division	Lenticular cell divisions
Crystalline cell inclusions	Star-shaped groups of needle-like, birefringent	-	Cubical cell inclusions, single or fused	Elongated elliptical to irregular rod shaped, single or clustered in cruciate to star-shaped aggregates	Star-shaped or irregular groups of needle-like, birefringent	Star-shaped or irregular groups of needle-like, birefringent	Star-shaped or irregular groups of needle-like, birefringent
Habitat	Marine and estuarine, intertidal	Marine, intertidal and subtidal	Marine and estuarine, intertidal to subtidal	Marine, intertidal	Marine, subtidal	Marine, subtidal	Marine, intertidal to subtidal
Distribution	Global, tropical	Global, tropical to temperate	Global, tropical to temperate	Global, tropical to subtropical	Global, tropical to temperate	Global, tropical to temperate	Global, tropical to subtropical
Taxonomic Notes		Non-monophyletic genus	Non-monophyletic genus		Non-monophyletic genus		Non-monophyletic genus
References		Littler & Littler (1991); Leliaert & Coppejans (2004); Leliaert *et al.* (2007); Alves et al. (2011a)	Leliaert & Coppejans (2004); Boedeker *et al.* (2016)	Leliaert & Coppejans (2004); Leliaert *et al.* (2007); Alves *et al.* (2012a)	Leliaert & Coppejans (2004); Leliaert *et al.* (2007); Alves et al. (2011a)	Leliaert & Coppejans (2004); Leliaert *et al.* (2007); Alves *et al.* (2012a)	Leliaert & Coppejans (2004); Leliaert *et al.* (2007); Alves *et al.* (2010)



Figs 3–12. Habit and morphology of *Leliaertia repens gen. & sp. nov.*
Fig. 3. General aspect of the thallus. Scale bar = 1 cm.
Figs 4, **5**. Details of semiprostrate apical branches. Scale bars = 2.5 mm.
Figs 6, **7**. Details of filaments with unilateral to irregular branching. Scale bars = 2.5 mm.
Figs 8–10. Cylindrical apical cells with delayed cell wall formation. Scale bars = 200 µm.
Figs 11, **12**. Hapteroidal cells on the prostrate branches. Scale bars = 200 µm.

PARATYPES: Brazil, Bahia, Vera Cruz, Enseada do Pedrão beach, 13° 3.488′S, 38°42.275′W, growing under rocky reefs, in pools of the intertidal region, collected 20 March 2011 by C.W.N. Moura *et al.* (HUEFS217733; GenBank accession for LSU rDNA, OQ371330); Brazil, Alagoas, Maceió, Praia da Sereia, growing under rocky reefs, in pools of the intertidal region, collected 27 April 2013 by A.M. Alves & G.B.C. Mascarenhas (HUEFS 217732; GenBank accession for LSU rDNA, OQ371328).

ETYMOLOGY: Specific epithet from Latin *repens*, referring the prostrate or creeping mode of thallus growth.



Figs 13–17. Habit and morphology of *Leliaertia repens gen. & sp. nov.*
Figs 13–15. Unicellular and multicellular rhizoids from prostrate axis. Scale bars = 200 µm.
Figs 16, **17**. Star-shaped group of needle-like crystalline cell inclusions. Scale bars = 50 µm .

DISTRIBUTION: Available DNA sequences confirm the presence of this species in Brazil, Indonesia, Philippines, Madagascar, Oman, Panama and Tanzania.

DISCUSSION

On the generic proposal of *Leliaertia*

In current taxonomy, the delineation of macroalgal genera and higher taxonomic ranks relies on different criteria, leading to occasional arbitrariness due to variations in emphasis by different authors (Entwisle & Weston 2005; Zuccarello *et al.* 2022). In recent years, the use of molecular phylogenetic data has resulted in the description or re-circumscription of several algal genera, with clade distinctiveness and support serving as a significant criterion. This has led to an increase in the number of newly described genera and, in some cases, disagreements regarding whether to split existing genera or retain them in a wider sense (De Clerck *et al.* 2013; Gurgel *et al.* 2018; Lyra *et al.* 2021; Zuccarello *et al.* 2022). We agree that that proposals for generic classification based on phylogenetic data should ideally rely on well-supported clades obtained from different methods and multiple genes, resulting in stable phylogenetic trees (Zuccarello *et al.* 2022). This approach is particularly crucial in taxonomically complex groups where the delimitation of taxa lacks morphological diagnosis and primarily depends on molecular data.

Several phylogenetic studies have delimited genera and families within the Cladophorales based on clade support observed in phylogenies inferred from SSU and partial LSU rDNA sequences, occasionally supplemented with ITS data (Leliaert *et al.* 2007, 2022; Boedeker *et al.* 2012, 2016; Zhao *et al.* 2018; Zhu *et al.* 2020). This approach seems to be suitable given the taxonomic complexity of the Cladophorales, characterized by extensive cryptic diversity and morphological variation that crosses traditional generic boundaries (Leliaert *et al.* 2009, 2011; Boedeker *et al.* 2016, 2017; Sherwood *et al.* 2019; Zhao *et al.* 2021). A notable example is the *Cladophora*-complex, with species exhibiting a simple morphology, often lacking distinct morphological features, and displaying considerable genetic divergence. Currently, *Cladophora*-like representatives are found in several genetically distinct clades: *Aegagropila* Kützing, *Cladophora s.s., Lychaete, Pseudocladophora, Willeella* Børgesen, and several clades in the *Siphonocladus* clade.

Although the genus *Cladophora* has undergone several taxonomic changes as cited above, this genus remains polyphyletic with its current circumscription, and needs further revision. As previously shown by Bakker *et al.* (1994), Hanyuda *et al.* (2002), Leliaert *et al.* (2003), Taylor *et al.* (2017), and now by our phylogenetic data, '*Cladophora coelothrix*' is placed in the *Siphonocladus* clade, genetically removed not only from *Cladophora s.s.* but also from other members of the Cladophoraceae.

Understanding the complex nature of *Cladophora* species, and the genetic distinction and phylogenetic position of several lineages, namely *Cladophora aokii, C. catenata* Kützing, '*C. coelothrix*', *C. prolifera*, '*C. socialis*', *C. sibogae* and

C. wrightiana Harvey, are required for establishing new circumscriptions that may lead to the proposition of potential new genera, aiming at establishing a monophyletic circumscription of *Cladophora*. However, our phylogenetic data could only provide sufficient molecular evidence for a species of the '*Cladophora coelothrix*' complex (the one in clade 2), for which we propose *Leliaertia gen. nov.*, fully supported by the analysis of the LSU rDNA and concatenated SSU+LSU data sets. This clade had been previously recovered with full support in BI analyses based on LSU sequences (Leliaert *et al.* 2007), but it could not be reliably reconstructed in concatenated analyses at that time due to availability of only one sample included in their analyses (F135 accession: AM498749 and AM503443).

We cannot disregard the possibility that the lineages referred in clades 3 and 4 (Figs 1, 2) belong to this new genus, given the high support for a sister relationship in the concatenated tree and the moderate to high support in the LSU tree, which was also verified previously by Leliaert *et al.* (2007) for LSU and with higher support. The same is observed for the sequences named as *C. sibogae* from which only LSU sequences were available, and which were recovered with support only in one analysis (PP = 0.96). For this case, new sequences, especially from SSU rDNA, may perhaps provide a better resolution and ascertain the position of *C. sibogae*. The sequences in clades 3 and 4 were named as *C. socialis* and *C. coelothrix* (one only from Brazil), but none of them belong to the type specimens or came from the type localities, nor even from locations close to them. Therefore, proposals for new combinations by transferring these two species to the genus *Leliaertia* would be arbitrary and could lead to unnecessary nomenclatural instability, since the phylogenetic position of authentic *C. socialis* and *C. coelothrix* cannot be inferred from the data currently available. With the proposal of *Leliaertia, Cladophora* still remains polyphyletic and in need of taxonomic revision, which will require overcoming the methodological difficulties in molecular studies and the absence of type-materials, and obtaining sequences from topotypes. At this point, our proposal may represent a starting point for taxonomic adjustments for several taxa currently included in *Cladophora* that are recovered outside the genus clade, and are therefore in need of revision, as previously cited.

Resolving part of *Cladophora coelothrix* species complex

Based on available sequence data, clade 1 (Fig. 18), appears to have a restricted distribution, occurring along the European Atlantic coast, the Mediterranean Sea and Atlantic Islands (Canary Islands and Cape Verde). Due to proximity of the sequenced specimen in this clade to the Gulf of Genova, the type locality of *C. coelothrix* (Kützing 1843), this clade is assumed to represent the *bona fide C. coelothrix*. Womersley (1984) and South & Skelton (2003) refer to Livorno, Italy, as the type locality of *C. coelothrix*, two areas located very close to each other. On the other hand, *Leliaertia repens* (clade 2) seems to be more widely distributed at lower latitudes, occurring along the Western Tropical Atlantic, Western Indo-Pacific and Central Indo-Pacific marine regions (Fig. 18). Along the Brazilian coast, the distribution of this new species

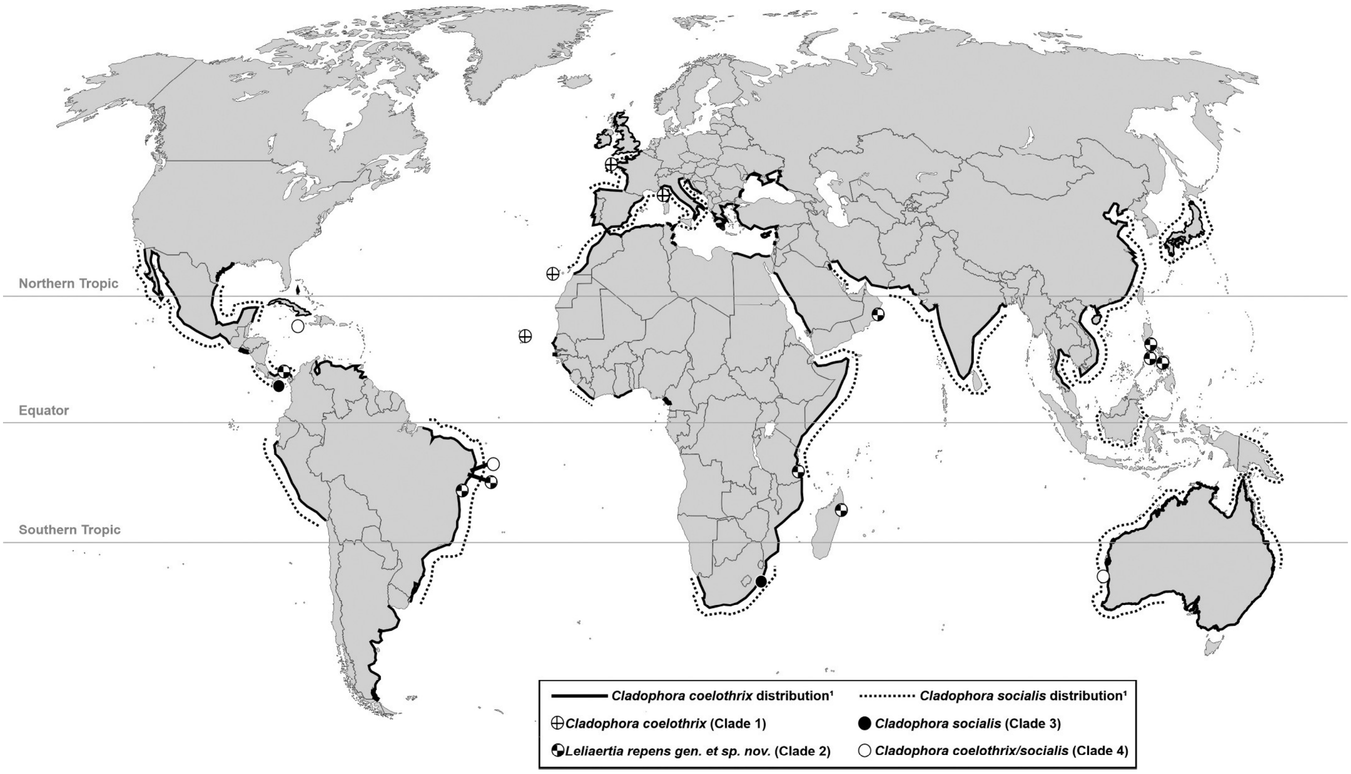


Fig. 18. World distribution of *Cladophora coelothrix s.s.*, *Leliaertia repens sp. nov.*, *Cladophora socialis* and '*Cladophora coelothrix*' *s.l.* lineages. [1]According to Guiry & Guiry (2023) and Flora e Funga do Brasil (2022).

is restricted to warm tropical waters on the northeast coast, being found in marine to estuarine areas, showing high tolerance to saline fluctuations.

Based on the phylogenetic placement, the full support in the LSU and concatenated LSU+SSU analysis, and on different distribution patterns, it is reasonable to propose a new taxon, *Leliaertia repens* to accommodate '*Cladophora coelothrix*' clade 2 as a distinct lineage in relation to the authentic *C. coelothrix* (clade 1). The low resolution obtained with SSU sequences (Fig. S1) is perhaps related to a higher level of conservation of this gene within some representatives of Cladophorales or the low number of available sequences.

The cell dimensions used to distinguish *C. coelothrix*-like species from *C. socialis*, which was previously referred to as a stable and dependable trait, appear to be unreliable, as they exhibit a continuum of overlapping dimensions (Kraft 2000; Leliaert & Coppejans 2003, 2006; Coppejans *et al.* 2004; Gestinari *et al.* 2010; Alves *et al.* 2012b). The Brazilian specimens are in accordance with the previous descriptions of *Cladophora coelothrix* worldwide (Table 3). In this sense, although morphological data indicate slight differences in mat size and apical cell length between Brazilian individuals of *Leliaertia repens* and '*Cladophora coelothrix*' (HUEFS217731), it would be unwise establish these traits as diacritic for both lineages based on a single sample. The overlapping morphology between species in clades 1, 2, 4 and with *Cladophoropsis fasciculata* (Kjellman) Wille of the *Boodlea* complex (Leliaert & Coppejans 2003, 2006), which includes cushion-like growth, presence of hapteroidal rhizoids, delayed cross-wall formation and overlapping apical cell dimensions, indicates that this simple habit and morphology evolved several times in the *Siphonocladus* clade, and does not represent a monophyletic and morphologically cohesive group as suggested by Leliaert *et al.* (2007).

The observation of star-shaped groups of needle-like crystalline cell inclusions in *Leliaertia repens* is in accordance with the results described by Leliaert & Coppejans (2004) for '*Cladophora coelothrix*' from South Africa. The South African material may represent *L. repens*, and from our knowledge there is no description of crystalline cell inclusion from European samples of *C. coelothrix*, therefore being unclear whether there is any difference in crystalline cell inclusions between the species.

Several names have been considered synonyms of *Cladophora coelothrix*, and given the lack of clear diagnostic characters, associated with the lack of sequences of type material, we provide a list of possible early synonyms that might be conspecific with lineages here discussed, and for which the phylogenetic position requires confirmation (Table 4). Cribb (1960) first indicated that *Cladophoropsis peruviana* M. Howe and *C. limicola* Setchell were perhaps representatives of *Cladophora s.l.*, based on the lack of segregative cell division in both species, and this was later reinforced by Leliaert & Coppejans (2006) in their revision of *Cladophoropsis* taxa. Both species are referred as mud-inhabiting, occurring in marine to estuarine areas in the Tropics (Cribb 1960; Leliaert & Coppejans 2006). In addition, *Cladophora patentiramea* (Montagne) Kützing needs to be revised using phylogenetic data, since it represents another tropical species that falls within the morphological range of the *C. coelothrix* complex, sometimes overlapping with *C. socialis* in apical cells dimension (van den Hoek & Chihara 2000; Huisman 2015).

Table 3. Morphological comparison between *Cladophora coelothrix* and '*Cladophora coelothrix*' lineages worldwide.

Taxa	*Leliaertia repens* *gen. & sp. nov.*	*C. coelothrix* *sensu* van den Hoek (1963)	'*C. coelothrix*' *sensu* Womersley (1984)	'*C. coelothrix*' *sensu* Coppejans *et al.* (2002)	'*C. coelothrix*' *sensu* Coppejans *et al.* (2004)	'*C. coelothrix*' *sensu* Leliaert & Coppejans (2003)	'*C. coelothrix*' *sensu* Gestinari *et al.* (2010)	'*C. coelothrix*' *sensu* Alves *et al.* (2012b)	*C. coelothrix* *sensu* Cormaci *et al.* (2014)
Habit	Cushion-like mats	Cushions	Tufts to cushions	Cushions	-	Cushion-like mats	Tufts to cushions	Dense tufts like cushion	Cushion-like mats
Rhizoid	Unicellular or multicellular from basal poles of prostrate to erect axis	At basal poles	From basal pole of prostrate cells	From basal poles of the cells	-	Uni-multicellular, from basal poles of prostrate cells	Unicellular from basal cells, from basal poles of main axis cells	From any part along the thallus	Uni-multicellular from basal pole of axial cells
Growth form	Apical cell and intercalary divisions	Apical cell and intercalary divisions	-	-	-	Apical cell and intercalary divisions	-	Apical cell and intercalary divisions	Apical cell and intercalary divisions
Branching pattern	Unilateral to pseudodichotomous	Irregular	Irregular	Irregular	-	Subterminal	Pseudodichotomous or unilateral	Irregular, unilateral, pseudodichotomous and polytomous	Irregular
Apical cell diameter	47.5–163.5	55–215	60–140	65–95	55–140	57–75	(81–)98(–118)	(50–)77(–110)	(55–)80(–160)
Apical cell length: width ratio	4–17	4–22	3–16	5–12	-	5–15	(6–)8(–11)	(3–)7(–13)	5–20
Habitat	Marine and estuarine	-	Marine	Marine	Marine	Marine	Marine	Marine and estuarine	Marine
Locality	Brazil	Europe	Australia	Mozambique	Rodrigues, Mauritius	South Africa	Brazil	Brazil	Mediterranean

Table 4. Species which need to be evaluated as potential synonyms of *Leliaertia repens sp. nov.*, and taxa currently regarded as synonyms of *Cladophora coelothrix* and *C. socialis*.

	Type	Distribution	Notes
Possible conspecific taxa of *Leliaertia repens*			
Cladophoropsis peruviana M. Howe (1914, p. 30)	Holotype: La Palisada, Peru; leg. Coker 370 (NY) (Leliaert & Coppejans 2006, p. 673)	Tropical	This mud-inhabiting species falls within the morphological range of *Cladophora coelothrix* (Leliaert & Coppejans 2006, p. 673)
Cladophoropsis limicola Setchell (1924, p. 176)	Holotype: Tutuila Island, Samoa; leg. Setchell 1167 (NY) (Leliaert & Coppejans 2006, p. 672)	Tropical	This mud-inhabiting, estuarine species falls within the morphological range of *Cladophora coelothrix* (Leliaert & Coppejans 2006, p. 672)
Cladophora patentiramea (Montagne) Kützing (1849, p. 416). Basyonym: *Conferva patentiramea* Montagne (1842, p. 15)	Holotype: Society Is. - Windward, Tahiti, French Polynesia (BM001045491) (Silva *et al.* 1996, p. 778)	Tropical	Falls within the morphological range of *Cladophora coelothrix* (van den Hoek & Chihara 2000; Huisman 2015, p. 54). Currently accepted taxonomically (*fide* Guiry & Guiry 2023)
Considered as synonyms of *C. coelothrix*			
Cladophora repens Harvey (1849, pl. CCXXXVI), new name for *Conferva repens* J. Agardh (1842, p. 13), *nom. illeg.* (*non* Dillwyn 1802)	Marseille, France; Lenormand (TCD) (van den Hoek 1963, p. 41)	Temperate	Synonym of *Cladophora coelothrix* (*fide* John *et al.* 2004, p. 13). Restricted to the Mediterranean (Harvey 1849, pl. CCXXXVI)
Conferva spongiosa Zanardini (1847, p. 250), *nom. illeg.* (later homonym of *Conferva spongiosa* Hudson 1762)	Isotype: Venice, Italy; probably in (L, 937.278.387) (van den Hoek 1963, p. 41)	Temperate	—
Cladophora bryopsoides Zanardini *ex* Frauenfeld (1855, p. 12)	Isotype: Makarska, Dalmatian coast, Croatia; probably (L, 937.217.387) (van den Hoek 1963, p. 41)	Temperate	—
Siphonocladus modonensis (Kützing) Bornet in De Toni (1889, p. 359). Basionym: *Cladophora* (*Aegagropila*) *modonensis* Kützing (1849, p. 416)	Holotype: Modon, Morea Peninsula, Peloponnesos, Greece; leg. Bory St. Vincent. Specimen dedit amic. Lenormand No. 61 (Leliaert & Coppejans 2006, p. 672)	Temperate	*Cladophora modonensis* is probably referable to *C . coelothrix* Kützing; other Mediterranean specimens identified as *C. modonensis* are often referable to *Cladophoropsis membranacea* (Leliaert & Coppejans 2006, pp. 672, 673)
Cladophoropsis concrescens (Reinbold) Wille in Engler & Prantl (1910, p. 116). Basionym: *Siphonocladus concrescens* Reinbold (1898, p. 88)	Lectotype: Rhodos Island, Greece; leg. J. Nemetz 46 (M) (Leliaert & Coppejans 2006, p. 672)	Temperate	This species is referable to *C. coelothrix* (Leliaert & Coppejans 2006, p. 672)
Cladophoropsis fallax Schiffner (1933, p. 304)	Holotype: Lacroma Island, near Ragusa (Dubrovnic), South Dalmatia, Croatia; leg. F. Berger, Schiffner Algae Marinae no. 964, (NY); isotype (BM) (Leliaert & Coppejans 2006, p. 672)	Temperate	Reduced to a synonym of *Cladophora coelothrix* Kützing by Leliaert & Coppejans (2006, p. 672)
Considered as synonyms of *C. socialis*			
Cladophora patentiramea f. *longiarticulata* Reinbold in Weber-van Bosse (1913, p. 84)	Syntype localities: Indonesia: Kangean Island and Waru Bayu, Seram (Silva *et al.* 1996, p. 781)	Tropical	This species is currently a synonym of *Cladophora socialis* Kützing (Huisman 2015, p. 56)
Cladophoropsis infestans Setchell (1924, p. 177)	Holotype: Tutuila Island, Samoa; leg. Setchell 1134, (NY); Isotype (BM) (Leliaert & Coppejans 2006, p. 672)	Tropical	Reduced to a synonym of *Cladophora socialis* Kützing. The type of *Cladophoropsis infestans* is morphologically indistinguishable from *C. socialis* (Leliaert & Coppejans 2006, p. 672)

To confirm the phylogenetic value of these and other diagnostic criteria, the use of a pragmatic approach, which includes proposing new genera/species only after analysing molecular data from type material or type locality seems to be more suitable, and is commonly followed in studies of algae (Montecinos *et al.* 2017; Sherwood *et al.* 2019; Hoshino *et al.* 2021; Coutinho *et al.* 2021). The proposal of the new genus *Leliaertia* based mainly on molecular data, along with habitat and distributional data, reinforces the notion that the morphological delineation of *Cladophora* as filamentous, uniseriate branched thalli seems to be impracticable, since this morphology is widely found in different clades (Leliaert *et al.* 2007; Boedeker *et al.* 2012). Moreover, new taxonomic revisions are needed for *C. coelothrix* (clade 1 and 4), *C. prolifera*, '*C. socialis*' (clade 3 and 4) and '*C. sibogae*', preferably based on a combination of morphological and genetic data. In addition to our taxonomic proposals and understanding that clade 1 represents the true *C. coelothrix*, further studies are needed especially for tropical lineages such as HUEFS217731. The familial placement of clades 2 (*Leliaertia repens*), 3 and 4 cannot be resolved since our concatenated data placed them as sister to Anadyomenaceae with moderate support only for ML. Possibly, a family may be recognized, but further molecular data are needed to revise the family-level classification in the clade.

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