

Research Article

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Alsidium oliveiranum sp. nov. (Rhodomelaceae, Rhodophyta), an overlooked species from the southwestern Atlantic based on morphology and DNA sequence data

Silvia M. P. B. Guimarães¹, Luanda Pereira Soares¹, Mutue Toyota Fujii¹ and Pilar Díaz-Tapia^{2,*}

¹Institute of Botany, Nucleus for Research in Phycology, Av. Miguel Estéfano 3687, 04301-012 São Paulo, Brazil

²Coastal Biology Research Group, Faculty of Sciences and Centre for Advanced Scientific Research (CICA), University of A Coruña, A Coruña 15071, Spain

The exploration of seaweed diversity in poorly studied habitats has often led to the discovery of new species. Sand-covered rocks are an example, as they received less attention than sand-free rocky intertidal habitats during seaweed diversity surveys in Brazil. In sand-covered rocks from Espírito Santo and Rio de Janeiro we found an alga whose morphology was unique among rhodomelacean species previously reported in Brazil. With the aim to clarify the taxonomic identity of this species we studied its morphology, as well as its phylogenetic relationships. Molecular analyses resolved this species in the genus *Alsidium* (tribe Alsidieae) and differed from sequenced congeners with divergences ≥ 2.5 and 4.2% in the *rbcL* and *cox1* genes, respectively. Morphological characters were in agreement with the genus *Alsidium*, and differed from other species currently recognized in the genus. The species consisted of a basal crust and scarcely branched erect axes with seven pericentral cells covered by a continuous layer of cortical cells. Reproductive structures were formed on clusters of short determinate branches. Therefore, the new species *A. oliveiranum* is proposed based on morphological and molecular evidence. Our findings contribute to better understand the diversity of the tribe Alsidieae, which is particularly diverse in the Americas.

Key Words: Alsidieae; Brazil; Ceramiales; *cox1*; new species; *rbcL*; red algae; sand-covered rocks

INTRODUCTION

The family Rhodomelaceae includes 1,080 species classified in 158 genera (Guiry and Guiry 2019), and exhibit an extraordinary morphological diversity (Womersley 2003, Díaz-Tapia et al. 2017). Usually, members of this family are easily recognized by their thalli consisting on an axial filament whose cells are surrounded by pericentral cells and by the sympodial branching pattern (Womersley 2003). Moreover, most species in the Rhodomela-

ceae have trichoblasts, apical monosiphonous branches that are usually unpigmented (Maggs and Hommersand 1993, Womersley 2003).

The Rhodomelaceae is the most diverse family of the red algae, and the number of recognized species is continuously growing. The discovery of new species is often related to the finding of cryptic diversity, both when comparing molecular data of specimens from dis-



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*Corresponding Author

E-mail: pdiaz@udc.es

Tel: +34-653535767, Fax: +34-981167065

tant locations or from the same region (e.g., Zuccarello et al. 2015, Muangmai et al. 2016, Savoie and Saunders 2016, Díaz-Tapia et al. 2018, Schneider et al. 2018). More surprisingly, some species with conspicuous morphological differences regarding other members of the family remained unnoticed. One of the causes is that they grow in poorly explored habitats, such as the deep subtidal (Bárbara et al. 2013, Kim and Kim 2014). Intertidal sand-covered rocks are other common, more accessible, but often poorly explored habitat. The presence of sediments in intertidal rocks negatively affects many benthic organisms, and sand-covered rocks host a particular algal assemblage (Airoidi 2003, Díaz-Tapia et al. 2013a) in which several new red algal species have been recently described (Díaz-Tapia and Bárbara 2013, Díaz-Tapia et al. 2013b, D'Archino et al. 2015). During our surveys of marine red algae in this habitat along the coasts of Espírito Santo and Rio de Janeiro (southeastern Brazil), we found abundant populations of a rhodomelacean species consisting of large plants (up to 25 cm in length) with seven pericentral cells and a dense cortication from close to the apices. The morphology of this conspicuous species does not match with the species previously recorded in the region. Even its generic assignment was uncertain, as these characters resemble to some genera of the tribes Chondrieae, Alsidieae or Pterosiphoniae. The objective of this work is to clarify the taxonomic identity and phylogenetic relationships of the Brazilian species using molecular (*rbcL* and *cox1* genes) and morphological data.

MATERIALS AND METHODS

Plants of the targeted species were collected between 1986 and 2017 in Espírito Santo (21°03' S, 40°52' W to 19°52' S, 40°03' W) and Rio de Janeiro (Praia Rasa; 22°44' S, 41°57' W) from the intertidal zone or in the drift (see material examined and Supplementary Table S1). Samples preserved in silica gel desiccant, collected in 2006 and 2014, were used for DNA extraction. Plants for morphological studies were fixed in 4% formalin in seawater. Sections for anatomical studies were made by hand using a razor blade and stained in 1% aqueous aniline blue acidified with 1 N HCl. Sections were photographed on Ilford 50 ASA film (Harman Technology Ltd., Cheshire, UK) with an Olympus BH-2 photomicroscope (Olympus Corporation, Tokyo, Japan). Representative material was deposited in the SP (Institute of Botany) herbarium, São Paulo, Brazil.

DNA was extracted from silica gel-dried material following Saunders and McDevit (2012). Polymerase chain reaction (PCR) amplification was carried out for *rbcL* using the primers F2/R1452, F7/RrbcStart, F7/R893 or F57/rbcLrevNEW (Freshwater and Rueness 1994, Mamoodeh and Freshwater 2011, Saunders and Moore 2013, Díaz-Tapia et al. 2018) and for *cox1* using the primers GwsFn/Cox1R1 (Saunders 2008, Le Gall and Saunders 2010) or GazF1/GazR1 (Saunders 2005). Reactions were performed in a total volume of 25 µL, consisting of 5 µL 5× MyTaq reaction buffer, 0.7 µL 10 µM of forward and reverse primers, 0.125 µL 1 U/µL My Taq DNA Polymerase (Bioline, London, UK), 17.475 µL MilliQ water and 1 µL template DNA. The PCR profile consisted of initial denaturation (93°C for 3 min), 35 cycles of denaturation (94°C for 30 s), primer annealing (45°C for 30 s), and extension (74°C for 90 s) and final extension (74°C for 5 min). The PCR products were purified and sequenced commercially by Macrogen (Seoul, Korea).

Two sequences were generated in this study for the targeted species for each gene (*rbcL* and *cox1*), and blast search in GenBank for the *rbcL* sequences of the Brazilian unidentified species indicated *Alsidium seaforthii* (Turner) J. Agardh, in the tribe Alsidieae, was that the most similar species (similarity 97.23%). Therefore, we downloaded from GenBank all the available *rbcL* and *cox1* sequences for species of the tribe Alsidieae. We also determined five and ten new *rbcL* and *cox1* sequences for species of the genus *Alsidium* C. Agardh from Brazil, the Canary Islands and the Mediterranean Sea (Supplementary Table S1). We selected 17 sequences, one per haplotype, for the *rbcL* and *cox1* phylogenetic analysis. When several sequences were available for a haplotype, we selected the largest. Sequences and their corresponding GenBank accession numbers are listed in Supplementary Table S1. Sequences were aligned using Muscle in Geneious 6.1.8 (Kearse et al. 2012). Phylogenetic trees for *rbcL* and *cox1* were estimated with maximum likelihood (ML) using RAxML 8.1.X (Stamatakis 2014). GTR-Gamma was used as the nucleotide model; branch support was estimated with 100 bootstrap replicates. The genera *Chondria* C. Agardh and *Acanthophora* J. V. Lamouroux (tribe Chondrieae) were selected as outgroup for the *rbcL* and *cox1* trees, respectively. Our outgroup selection was based on the phylogenomic analyses of the major lineages of the Rhodomelaceae that resolved a clade formed by the Chondrieae and Laurencieae as sister to the Alsidieae (Díaz-Tapia et al. 2017).

RESULTS

Phylogeny

The RAxML phylogenetic analysis of *rbcL* sequences (Fig. 1) placed a unique Brazilian species of *Alsidium* in a fully supported clade that included other species of the genus. Within this clade the Brazilian species was placed as sister to *A. seaforthii*, but this relationship was unsupported. The *Alsidium* clade was sister to the fully supported *Digenea* C. Agardh clade. The *cox1* tree (Fig. 2) had a similar topology than the *rbcL* tree. In the *cox1* tree, the Brazilian species was placed as sister to the generitype *Alsidium corallinum* C. Agardh, but again, this relationship was unsupported.

Two *rbcL* and *cox1* sequences for the new Brazilian *Alsidium* were identical. Seven *rbcL* sequences for *A. corallinum* from the Mediterranean Sea (three) and the Canary Islands (four) were identical. Likewise, four *cox1* sequences of *A. triquetrum* (S. G. Gmelin) Trevisan, from Mexico and Brazil, were identical. *A. seaforthii* from Brazil was genetically more diverse in the *cox1* gene, and four haplotypes that diverged by 0.1-2% (1-14 bp) were discovered for the species. Sequence divergence between the Brazilian species and other species of the genus *Alsidium*, was ≥ 2.5 and 4.2% in the *rbcL* and *cox1* genes, respectively. These findings allow for the description of a new species of *Alsidium* from Brazil.

Morphological observations

Alsidium oliveiranum S. M. Guimarães & M. T. Fujii sp. nov. (Figs 3-5)

Diagnosis. Thallus consisting of a basal crust, from which many cylindrical, erect branches are formed. Erect branches up to 1 mm in diameter, with seven pericentral cells and heavily corticated from close to the apices. Vegetative trichoblasts scarcely developed if present. Reproductive structures formed on clustered determinate lateral endogenous branches. Spermatangial branches formed on modified trichoblasts and replacing them, consisting of thin, flat discs, lacking marginal sterile cells. Cystocarps globose. Tetrasporangia spirally arranged on fertile branches, one per segment.

Holotype. SP470454, Sep 10, 2014, M. T. Fujii & P. Díaz-Tapia, sand-covered rocks in the low intertidal.

Type locality. Praia da Cruz; Marataízes, Espírito Santo, Brazil.

Etymology. Named in honor of Dr. Eurico Cabral de Oliveira F°, from the University of São Paulo, for his con-

tributions to our understanding of the Ceramiales from Espírito Santo, Brazil.

Molecular vouchers. MN165085 *rbcL*, MN165073 *cox1*.

Other specimens examined. Espírito Santo: Aracruz County, Praia dos Padres, Oct 22, 1996, S. M. P. B. Guimarães & M. T. Fujii, SP470448. Serra County, Praia da Baleia, Sep 17, 1986, S. M. P. B. Guimarães, SP470447. Anchieta County, Praia de Parati, Jul 20, 1997, S. M. P. B. Guimarães & M. T. Fujii, SP470449; Oct 4, 2006, M. T. Fujii, SP470451; Sep 8, 2014, M. T. Fujii, SP470452. Anchieta County, Ponta dos Castelhanos, Sep 9, 2014, M. T. Fujii, SP470453. Itapemirim County, Praia de Itaoca, Oct 5, 2017, L. P. Soares, SP470455. Marataízes County, Praia da Cruz, Sep 10, 2014, M. T. Fujii, SP470454; Praia de Marataízes, Sep 15, 2001, S. M. P. B. Guimarães & M. T. Fujii, SP470450. Rio de Janeiro: Armação dos Búzios Country, Praia Rasa, Oct 24, 2011, M. T. Fujii, SP470467.

Habitat and distribution. Plants grow up in tufts forming dense intertidal populations in Espírito Santo and Rio de Janeiro, southeastern Brazil (ca. 20° S), growing on sandstone beach reefs or on rocky outcrops often buried by sand and subjected to moderate water movement. Plants were also collected in the drift.

Vegetative morphology. Thalli consisted of a basal crust 2-3 cm in diameter bearing tufted erect axes up to 25 cm high and 1 mm in diameter (Fig. 3A & B). Erect axes terete, scarcely branched alternately to irregular, and bearing clusters of short determinate endogenous lateral branches. Thallus not adhering to herbarium paper when dried; rigid and red to reddish-brown or wine-red in color. Erect axes with polysiphonous structures and heavily corticated. Apical cell of erect axes dome-shaped, 17-25 μm in diameter, dividing transversely forming axial cells which divided longitudinally producing the pericentral cells (Fig. 3C). Branches of vegetative axes formed endogenously close to the apices (Fig. 3G). Cortication developed from close to the apices, initially formed by divisions at the margins of the pericentral cells. Later, the cortical cells formed a continuous layer, so that the pericentral cells were only visible in surface view at the apices. In surface view, cortical cells in middle portions of thalli were rounded to polygonal, 15-55 \times 17-98 μm (Fig. 3D). In cross-section (Fig. 3E), thallus consisted of an axial cell, seven rounded to polygonal pericentral cells, 123-250 μm in diameter, a layer of medullary unpigmented rounded to polygonal cells, 115-150 μm in diameter, and an outer layer of pigmented and rounded to rectangular cortical cells, 27-100 μm in diameter. Secondary pit connections formed between adjacent outer cortical cells. In longitudinal section, pericentral cells

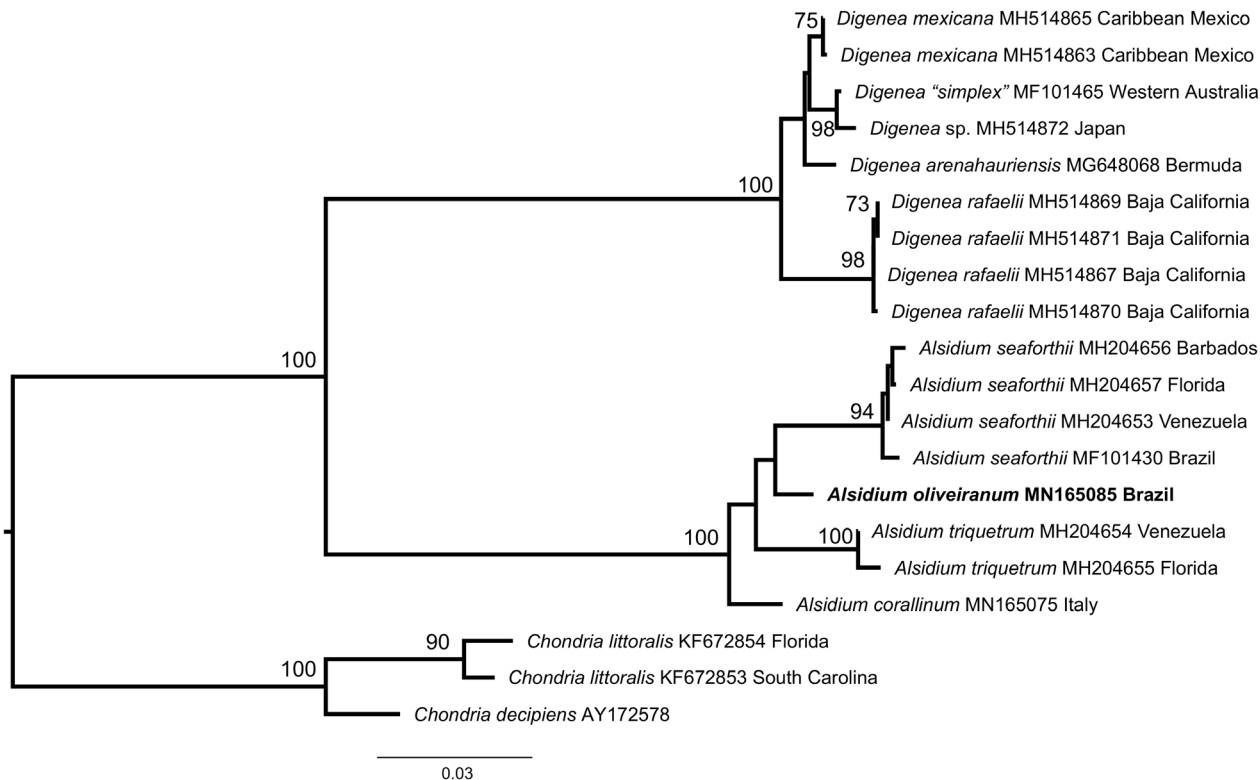


Fig. 1. Phylogenetic tree estimated with maximum likelihood analysis of *rbcl* sequences. Values at nodes indicate bootstrap support (only shown if >70).

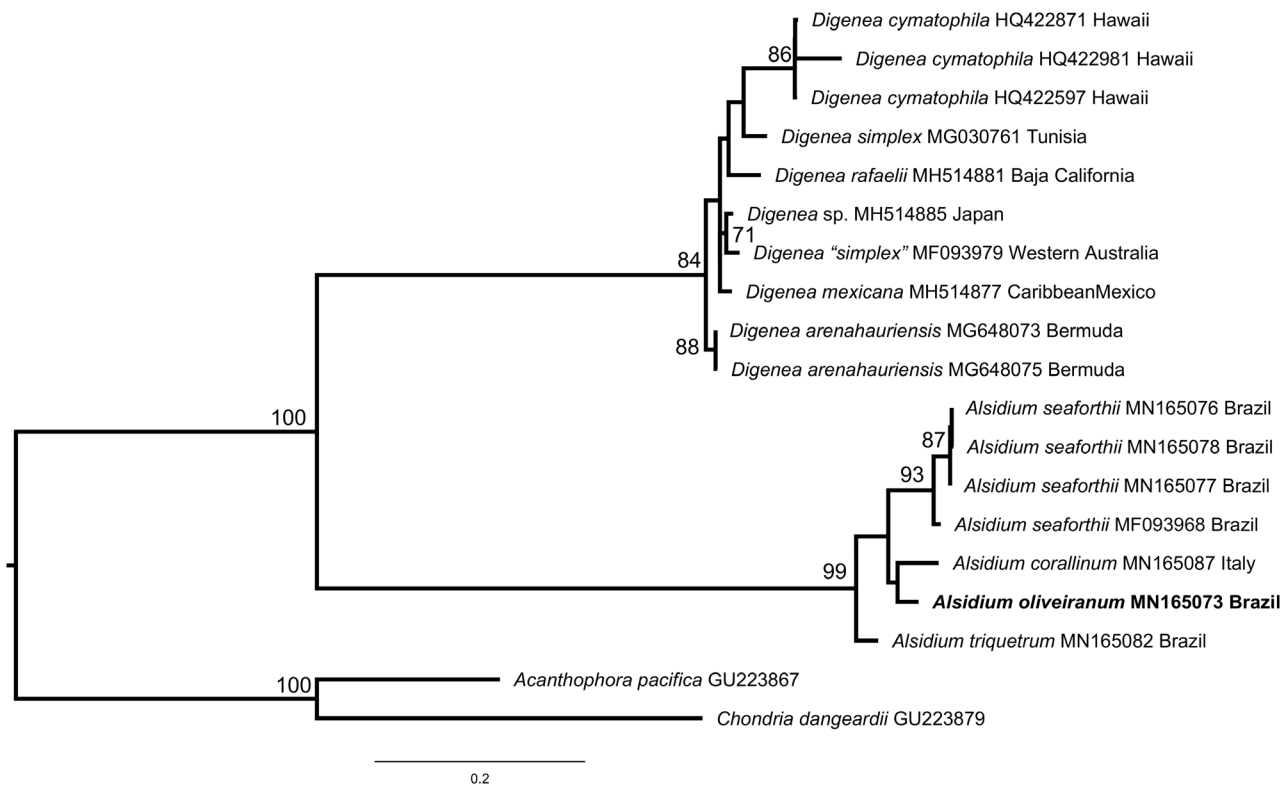


Fig. 2. Phylogenetic tree estimated with maximum likelihood analysis of *cox1* sequences. Values at nodes indicate bootstrap support (only shown if >70).

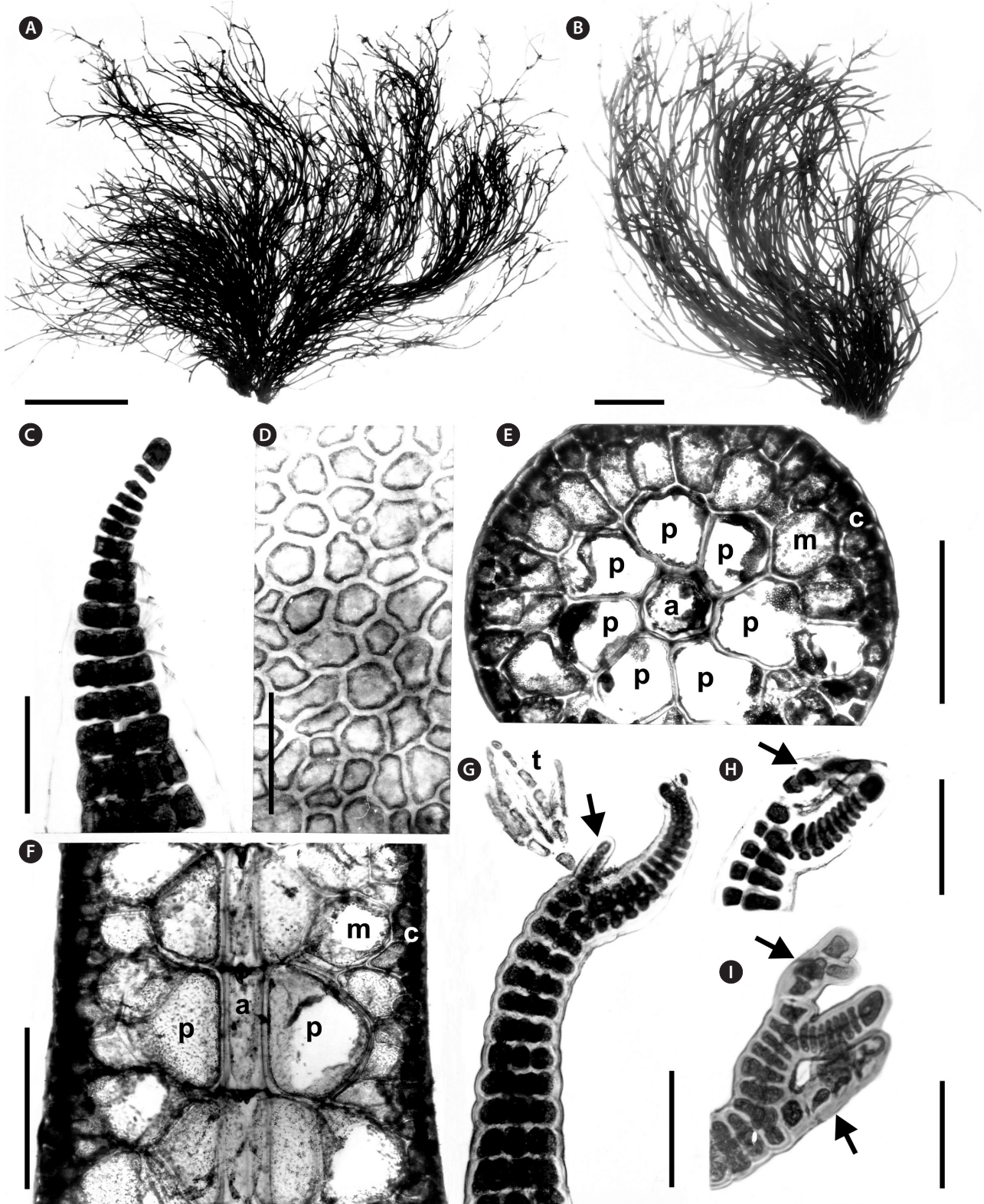


Fig. 3. *Alsidium oliveiranum* sp. nov. (A & B) Habit of tetrasporangial plants. (C) Apex of a branch with a domed-shaped apical cell. (D) Surface view of cortical cells. (E & F) Cross (E) and longitudinal (F) section of an axis showing the central-axial cells (a), pericentral cells (p), medulla (m), and cortex (c). (G) Apex of a vegetative axis with a trichoblast (t) and an endogenous lateral branch (arrow). (H & I) Upper portions of vegetative axis with short trichoblasts (arrows). Scale bars represent: A, 4 cm; B, 2 cm; C, H & I, 50 µm; D & G, 100 µm; E & F, 300 µm.

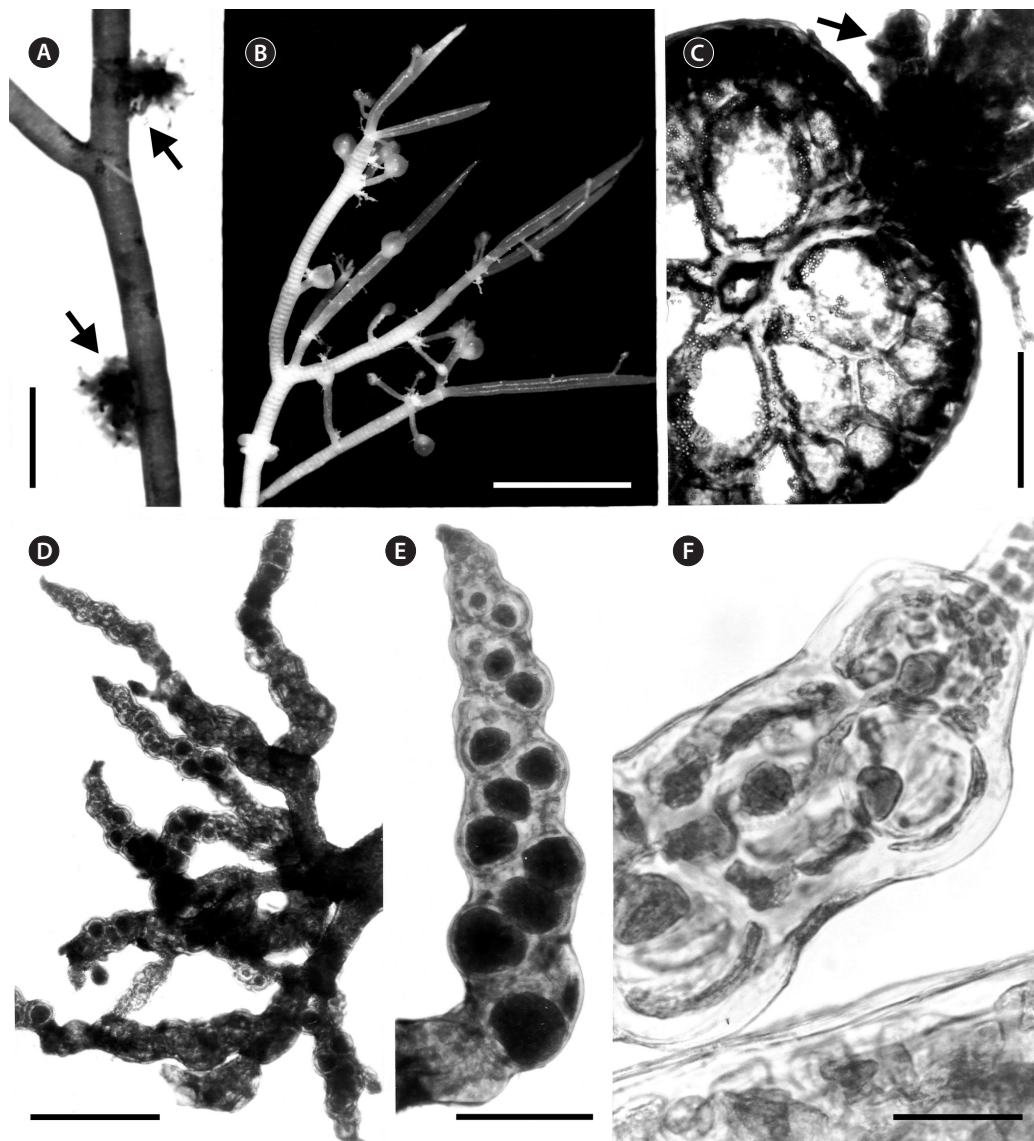


Fig. 4. *Alsidium oliveiranum* sp. nov. (A) Axis of a tetrasporophyte with clusters of tetrasporangial branches (arrows). (B) Female gametophyte with mature cystocarps. (C) Cross-section of a young axis bearing endogenous tetrasporangial branches (arrow). (D) Cluster of tetrasporangial branches. (E) Tetrasporangial branch bearing spirally arranged tetrasporangia. (F) Young tetrasporangia. Scale bars represent: A, 2 mm; B, 5 mm; C & E, 200 μ m; D, 500 μ m; F, 50 μ m.

were rounded to polygonal, with the same length as axial cells (Fig. 3F). Lateral endogenous determinate branches scarce, forming clusters. Trichoblasts rare, formed at the apices of young vegetative branches when present; short, to 140 μ m long, and dichotomously branched 1-2 times (Fig. 3G-I).

Reproductive morphology. Reproductive structures produced on lateral endogenous branches (Fig. 4A-D). Spermatangial branches consisted of thin, flat discs, lacking sterile marginal cells and replacing trichoblasts. In fertile female plants, procarps formed on modified

trichoblasts on the adaxial side of short determinate laterals in a spiral pattern (Fig. 5A & B). Few short vegetative and reproductive trichoblasts present on young female branchlets (Fig. 5D & E). The entire pericarp seems to develop from the third and fourth pericentral cells of the fertile segment (Fig. 5C). Mature cystocarps globose, c. 1 mm in diameter, corticated, slightly flat at the top and with an apical ostiole (Figs 4B, 5F & G). Owing to its growth, mature cystocarps become distal on a short lateral branchlets. Apical portions of branchlets can be observed as ligulate appendices (Fig. 5F). Tetrasporangial

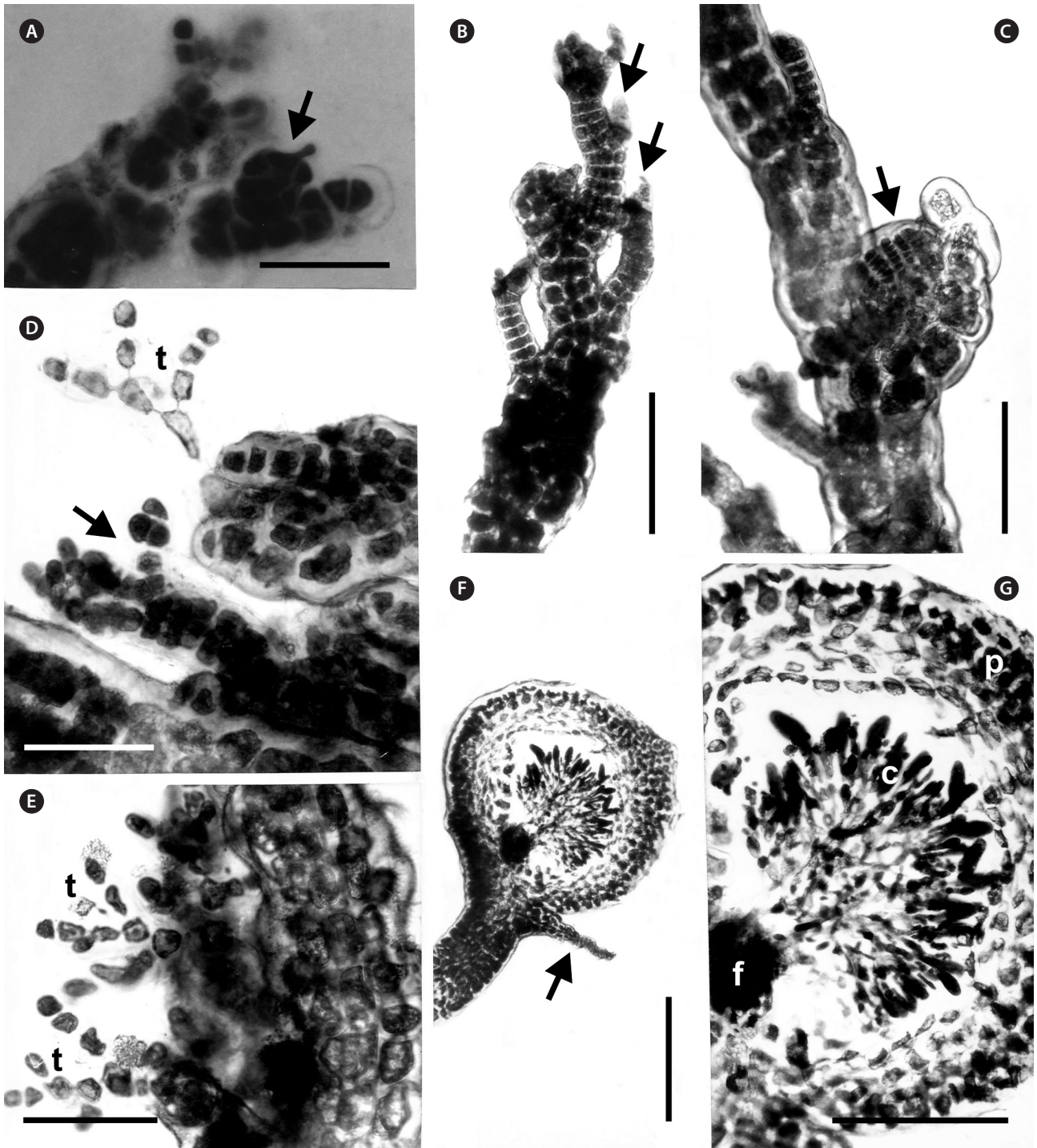


Fig. 5. *Alsidium oliveiranum* sp. nov. (A) Procarp with a four-celled carpogonial branch (arrow). (B) Apex of a female branch with procarps (arrows). (C) Apex of a female plant with a young cystocarp (arrow). (D) Apex of a female branch showing vegetative trichoblasts (t) and a brachyblast (arrow). (E) Apical portion of a female branch with trichoblasts (t). (F & G) Longitudinal section of a cystocarp with a ligulate basal appendix (arrow), 5- to 6-cell layered pericarp (p), carposporangia (c), and a fusion cell (f). Scale bars represent: A, D & E, 50 µm; B & C, 100 µm; F, 500 µm; G, 300 µm.

branches originated endogenously from axial cells and densely clustered (Fig. 4A, C & D), corticated, cylindrical with tapered tips, 1.0-2.0 mm in length and 100-200 µm in diameter (Fig. 4E), distributed sparsely along the thallus. Tetrasporangia 90-100 µm in diameter formed adaxially from pericentral cells. One tetrasporangium occurred in each segment in a spiral sequence, causing swelling on the side where the spores are borne (Fig. 4E & F). Mature tetrasporangia covered by two presporangial cover cells and elongated cortical cells.

DISCUSSION

The Brazilian species in our phylogeny nested in a clade with species of the genus *Alsidium*. The most distinctive morphological characters at the generic level observed in this species were as follows: (1) the thallus consists of a basal crust that produces erect axes; (2) the erect axes are cartilaginous with seven pericentral cells and are corticated beginning close to the apices; (3) spermatangial branches replace the trichoblasts and are plate-like; (4) one tetrasporangia per segment is formed on endogenous determinate branches. All of these characters are in agreement with the current delineation of the genus *Alsidium* (García-Soto and Lopez-Bautista 2018). Moreover, the new species is characterized by a development

of the cortex that differs from *Digenea*, the other genus in the tribe Alsidieae. In *A. oliveiranum*, the formation of the cortex commences close to the apices and the first cortical cells are divided from the margins of the pericentral cells. Later, the cortex continues its development forming a continuous layer that covers the pericentral cells. Cortical development in *A. oliveiranum* is similar to that observed in other corticated members of the genus (*A. seaforthii* and *A. corallinum*) (PD personal observation). The development of the cortex in *Digenea*, by contrast, is unusual compared with *Alsidium* and other Rhodomelaceae. The pericentral cells initially divide into discrete packets of cortical cells that cover each of the pericentral cells (Falkenberg 1901, Norris 1994, Boo et al. 2018, Schneider et al. 2018). Such pattern is conspicuous in the apical parts of *Digenea cymatophila* (R. E. Norris) Díaz-Tapia & Maggs as well as in the determinate branches of other species of *Digenea* (Falkenberg 1901, Norris 1994, Boo et al. 2018, Schneider et al. 2018). Later, the cortex of *Digenea* further develops obscuring this pattern in older parts of the thallus. Therefore, the cortical development is a useful character for delineating *Alsidium* from *Digenea*. Most species in *Digenea* share a common and distinctive habit, as the main axes are densely clothed with short determinate branches. However, the habit of *D. cymatophila*, with scarce determinate branches, differs from other congeners and resembles *Alsidium*. In fact, *D.*

Table 1. Comparison of key morphological characters for distinguishing *Alsidium oliveiranum* and congeneric species (except *A. vagum* whose morphology is largely unknown as its brief protologue provides the only available information)

	Thallus length (cm)	Axes outline	No. of pericentral cells	Determinate branches	Branches bearing reproductive structures	Attachment system	Reference
<i>A. oliveiranum</i> sp. nov.	25	Terete	7	Scarce, forming clusters irregularly arranged	Clustered	Basal crust	This work
<i>A. corallinum</i>	20	Terete	5-8	Abundant, spirally arranged at regular intervals	Non-clustered	Basal crust	Kützting (1865), Einav (2007), Rodríguez-Prieto et al. (2013)
<i>A. helminthochorton</i>	5	Terete	7-9	Scarce, spirally arranged at irregular intervals	Non-clustered	Prostrate axes	Kützting (1865), Rodríguez-Prieto et al. (2013)
<i>A. pacificum</i>	1.5	Terete	7	Scarce	Unknown	Prostrate axes	Dawson (1959)
<i>A. pusillum</i>	1	Terete	5	Scarce	Unknown	Prostrate axes	Dawson (1963)
<i>A. seaforthii</i>	20	Complanate	8-9	Abundant, alternately arranged	Non-clustered or sometimes clustered	Rhizomatous holdfast	García-Soto and Lopez-Bautista (2018)
<i>A. triquetrum</i>	25	Triangular or complanate	7-9	Abundant, spirally arranged forming three rows	Non-clustered or sometimes clustered	Basal disc	Littler and Littler (2000), García-Soto and Lopez-Bautista (2018)

cymatophila was originally assigned to *Alsidium* (Norris 1994) and later transferred to *Digenea* based on its placement in phylogenetic analyses (Díaz-Tapia et al. 2017, Fig. 2 in this work). *A. oliveiranum* is particularly similar in outline morphology to *D. cymatophila*, as both species consist of a basal crust from which scarcely branched erect axes arose, and determinate branches are clustered and mainly formed in relation to reproductive structures. The cortex development is particularly useful for the generic assignment of this pair of species.

Alsidium oliveiranum clearly differs from the other three species of this genus that have been molecularly characterized (*A. triquetrum*, *A. seaforthii*, and *A. corallinum*) by sequence divergence ≥ 2.5 and 4.2% in the *rbcL* and *cox1* genes, respectively. Moreover, they can be distinguished by their morphological characters (Table 1). *A. triquetrum* and *A. seaforthii*, formerly included in the genus *Bryothamnion* Kützing, have compressed or triangular thallus differing from the terete axes in *A. oliveiranum* (Littler and Littler 2000, García-Soto and Lopez-Bautista 2018). Likewise, *A. corallinum* and *A. oliveiranum* differ morphologically in several characters: (1) axes have 5-8 vs. 7 pericentral cells; (2) the thallus being clothed with abundant determinate branches vs. determinate branches being scarce and mainly produced in relation to reproductive structures; and (3) tetrasporangia forming in non-clustered vs. clustered lateral determinate branches (Kützing 1865, Rodríguez-Prieto et al. 2013).

Other four species are currently recognized in the genus *Alsidium* (Guiry and Guiry 2019), but molecular data are unavailable at present. *Alsidium helminthochorton* (Schwendimann) Kützing was originally described and is endemic to the Mediterranean Sea (Kützing 1865, Rodríguez-Prieto et al. 2013). Relevant differences between *A. helminthochorton* and *A. oliveiranum* include: (1) axes with 7-9 vs. 7 pericentral cells; and (2) the attachment by prostrate axes vs. a basal crust (Kützing 1865, Rodríguez-Prieto et al. 2013). Two very small species that differ in the number of pericentral cells, *Alsidium pacificum* E. Y. Dawson and *Alsidium pusillum* E. Y. Dawson, were described in the Palmyra Atoll and the Galapagos Islands, respectively (Dawson 1959, 1963), and were considered conspecific by Norris (1994). Their short erect thalli (up to 15 mm in length) contrast with the long thalli in *A. oliveiranum* (up to 25 cm). Moreover, they have a system of prostrate axes that differs from the basal crust in *A. oliveiranum* (Dawson 1959, 1963). Finally, *Alsidium vagum* (Zanardini) Zanardini was described from the Mediterranean Sea and was not recorded after its original description (Guiry and Guiry 2019). Such description did

not provide morphological details enough to ascertain its identity and further studies are required to clarify its taxonomy (Zanardini 1851, Bompard 1867). Therefore, our molecular and morphological studies clearly distinguish the Brazilian species from previously described species in the genus *Alsidium* and consequently we erected the new species *A. oliveiranum* here.

In Brazil, two species of *Alsidium* were traditionally reported based on morphological studies: *A. seaforthii* and *A. triquetrum*. Both are distributed from the Atlantic North America to Brazil (Guiry and Guiry 2019). *A. oliveiranum* has a much narrower distribution and is endemic to the states of Espírito Santo and Rio de Janeiro; despite our sampling efforts, it was not found elsewhere. In Brazil, *A. seaforthii* has the widest range, extending southward to the coast of São Paulo, while *A. triquetrum* is restricted to the northeastern coast (up to Bahia). *A. oliveiranum* and *A. seaforthii* occur sympatrically on the Espírito Santo and Rio de Janeiro (Guimarães 2006). This result is unsurprising, as this region hosts a particularly high algal species diversity, including rare and endemic species (De Oliveira Filho 1969, Guimarães and Fujii 1998, Chen et al. 2019). Moreover, its seaweed diversity is continuously growing as the use of molecular assisted identification is contributing to the discovery of new species (Nauer et al. 2015, Iha et al. 2016, Ximenes et al. 2017, Brunelli et al. 2019a, 2019b). The high diversity in this peculiar coast has been attributed to the substrate heterogeneity and availability, as well as the particular seawater temperature conditions because this region is a transition zone between tropical and subtropical regions and it is affected by upwelling events (Guimarães 2003).

Our finding contributes to better understand the diversity of the genus *Alsidium* and the tribe Alsidieae. This tribe, with 15 recognized species, has a reduced species diversity compared with other tribes in the Rhodomelaceae and most species have restricted distributions in tropical and subtropical coasts: the Mediterranean and Macaronesian Islands (*A. corallinum* and *A. helminthochorton*), Pacific Islands (*A. pusillum*, *A. pacificum*, and *Digenea cymatophyla*), the Pacific and Atlantic coasts of the Americas (*A. seaforthii*, *A. triquetrum*, "*Bryothamnion*" *pacificum* W. R. Taylor) and South Africa (*D. subarticulata* Simons) (Guiry and Guiry 2019). *Digenea simplex* (Wulfen) C. Agardh is the only species of the tribe that has been widely reported in tropical and subtropical coasts worldwide (Guiry and Guiry 2019). However, the sequencing of material from the type locality, the Mediterranean Sea, led to the discovery of cryptic diversity within what had been referred to as *D. simplex* in the

Americas where three new species have been segregated recently from *D. simplex* (Boo et al. 2018, Schneider et al. 2018). Interestingly, the Atlantic and Pacific coasts of America, with six currently recognized species, host the largest diversity of species of the tribe Alsidieae.

In conclusion, in this paper we described a new species of *Alsidium* endemic to Brazil, a species that was previously overlooked because of the lack of study of its intertidal habitat on sand-covered rocks. Interestingly, this is also the typical habitat for other species of the tribe Alsidieae (e.g., Boo et al. 2018, Schneider et al. 2018). Our work contributes to better understand the species and morphological diversity of the tribe Alsidieae.

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SUPPLEMENTARY MATERIALS

Supplementary Table S1. GenBank accession numbers and collection information of the sequences used in phylogenetic analyses (<https://www.e-algae.org>).

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