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Barcoding the green macroalgae (Ulvophyceae) from the Alagoas coast: new records and an updated checklist

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Abstract

Marine green macroalgae remain poorly characterized along much of the Brazilian coast, particularly in Alagoas, where most records rely exclusively on morphology. This study aimed to assess the diversity of Ulvophyceae along the Alagoas coast using DNA barcoding and to provide a revised state checklist, including new geographic records. Samples collected from eight reef and estuarine sites were examined morphologically and analyzed using *tuf*A, *rbc*L, and LSU rDNA sequences and phylogenetic analysis. We generated 38 new sequences, and our phylogenetic analysis confirmed 20 species, comprising six Ulvales, five Bryopsidales, and nine Cladophorales. Molecular data revealed several discrepancies with morphology, especially in *Ulva* and Cladophorales, and exposed cryptic diversity within the *Cladophora vagabunda* and *Boodlea composita–Phyllodictyon anastomosans* complexes. *Ulva tentaculosa* is recorded for the first time in the Atlantic Ocean, *U. meridionalis* in the South Atlantic, *U. chaugulei* and *U. tepida* in northeastern Brazil, and *U. ohnoi* in Alagoas. The study adds five species to the known Ulvophyceae flora of Alagoas and revises the state total to 79 reported species, nearly twice the number listed in the national database. These findings demonstrate that morphology alone underestimates regional diversity and emphasize DNA barcoding as an essential tool for accurate identification, biodiversity monitoring, conservation, and improvement of public reference databases.

1 Introduction

Ulvophyceae (Chlorophyta) is a diverse clade that primarily includes species occurring in marine, estuarine, and continental waters, as well as in terrestrial habitats (Leliaert et al. 2012). These algae predominate in marine ecosystems where they play important roles as primary producers and ecosystem engineers (Del Cortona et al. 2020). Morphologically, the representatives of the Ulvophyceae are structurally diverse, ranging from unicellular and colonial forms, multicellular forms with coupled mitosis, multicellular bodies composed of multinucleate cells with well-defined cytoplasmatic domains known as siphonocladous, to plants consisting of a single giant tubular cell known as siphonous thallus (Cocquyt et al. 2010; Leliaert et al. 2012).

Around 230 species of green seaweeds are currently reported along the Brazilian coast (Flora e Funga do Brasil 2026), but most have scattered records. This is due to significant knowledge gaps across several regions, indicating that green seaweeds remain poorly characterized along the Brazilian coast (Santos et al. 2026). This applies to the Alagoas littoral, a 220 km stretch in the warm tropical province, characterized by oligotrophic waters and an abundance of sandstone reefs parallel to the coastline, with predominantly seaweed cover (Horta et al. 2001; Rudorff and Gherardi 2008). This region also includes 96 km of the Costa dos Corais Environmental Protection Area, the largest Brazilian marine protected area, which covers several coastal ecosystems including shallow reefs, mangroves, seagrass beds, rhodolith/sponge beds and mesophotic reefs (Ferreira et al. 2001, 2006; Pereira et al. 2021).

Currently, 44 species of Ulvophyceae have been reported for the Alagoas coast, with most of these reports based solely on morphological data from lists and herbarium specimens (Muniz 1993; Carneiro et al. 2018, 2019; Lemos et al. 2017). Over the last two decades, there has been an increasing data on green

seaweeds revealing new cryptic genera and species, which can only be reliably identified based on DNA sequences data (Verbruggen et al. 2005a; Sauvage et al. 2013; Zhao et al. 2018; Ximenes et al. 2017, 2019; Sherwood et al. 2019; Oliveira et al. 2021; Carneiro et al. 2023, 2026; Brito et al. 2024, 2026a, b). In this sense, applying molecular tools, such as DNA barcoding, to assess current diversity can be crucial for a better understanding of species composition.

Given the area's relevance and the knowledge gap regarding seaweeds, our aim is to conduct a floristic assessment of green seaweeds (Ulvophyceae) along the Alagoas coast using DNA *barcoding*. Additionally, we provide a revised list of marine Ulvophyceae for the state, including new records for the country.

2 Material and Methods

Sampling

Exploratory sampling expeditions were performed at eight sites, including six marine reefs and two estuaries (Table S1), between May and August 2019. The samples were collected during low tides on intertidal reefs and by snorkeling in shallow pool areas. In estuaries, samples were collected from sediments and pneumatophores. The samples were collected using spatulas, cleaned of sediment and epiphytic material using tweezers under a stereoscopic microscope, then separated for morphological and molecular studies. For morphological analysis, samples were frozen (-20 ºC) or preserved in 70% ethanol, while at least 5 mm² of each sample were preserved in CTAB saline solution or dried on silica gel for molecular analysis. Morphological analyses were performed using a stereoscopic microscope, Zeiss Axioplan (Oberkotchen, Germany), and an optical microscope, Zeiss, with a Canon digital camera SD950IS (Tokyo, Japan) coupled to them for photographic documentation. Morphological identifications of the specimens were carried out using the main bibliography on seaweeds: Taylor (1960), Joly (1964), Kanagawa (1984), Littler and Littler (1991, 2000), Gestinari (2004), Barata (2004, 2008), Bandeira-Pedrosa et al. (2004), Coto (2007), Oliveira-Carvalho (2008), Alves et al. (2009, 2010, 2011, 2012), Almeida et al. (2012), Almeida (2013), and Rocha (2016). Studied samples were dried and deposited in the Professor Vasconcelos Sobrinho Herbarium (PEUFR) (Index Herbariorum, updated continuously).

Molecular Study

Total DNA from Cladophorales and Bryopsidales samples was extracted following the Doyle and Doyle (1987) 2×CTAB protocol, excluding RNAse incubation. Total DNA from Ulvales and Ulotrichales samples was extracted using the NucleoSpin Plant II Kit (Macherey-Nagel). For molecular identification, we amplified tufA and *rbc*L from Ulvales and Bryopsidales representatives, and LSU (28S) rDNA from Cladophorales, using the specific primers and cycles listed in Tables S2-S3. The polymerase chain reaction was performed in a 25 µl final volume: 6.75 µl of H_2O (Milli-Q), 5 µl of PCR Buffer 5×, 3 µl of $MgCl_2$ 25µM, 5 µl of betaine 5M, 4 µl of dNTP 10µM, 1 µl of primer at 15µM, 0.25 µl of Taq DNA polymerase (5U/µL) and 1 µl of total DNA. The products were purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Piscataway, New Jersey, USA) and then bidirectionally sequenced using the

same primers from the PCR and BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) in an ABI PRISM Genetic Analyzer.

Consensus sequences were assembled using BioEdit v7.0.4.1 (Hall 1999) and compared with GenBank sequences using BLAST. The generated sequences and those from GenBank (Table S4) were aligned using ClustalW (Larkin et al. 2007). For each marker, an alignment dataset was built, comprising 90 sequences for *tuf*A (957 bp), 44 for *rbc*L (1348 bp), and 121 for LSU (658 bp).

The ultrafast Maximum Likelihood (ufML) analyses were performed in IQ-TREE v2.4.0 (Minh et al. 2020), with 1,000× bootstrap replicates for both datasets. The best-fit evolutionary model was determined for each dataset using ModelFinder (Kalyaanamoorthy et al. 2017). For *tuf*A, the best-fit model was TIM3 + F+G4; for *rbc*L, it was TIM2 + F+R2; and TN + I+G4 for LSU. Genetic p-distances within and between species from gene matrices were calculated using MEGA X.

3 Results

We successfully generated 22 new *tuf*A sequences, 6 new *rbc*L sequences, and 10 new LSU rDNA sequences. These results confirmed the presence of 20 species on the Alagoas coast based on DNA sequences, i.e., six Ulvales, five Bryopsidales, and ten Cladophorales (Table 1). The *tuf*A sequences, the main barcode marker for Ulvophyceae, confirmed the presence of ten species on the Alagoas coast, *Caulerpa lentillifera* J. Agardh, *C. racemosa* (Forsskål) J.Agardh var. *macrophysa* (Sonder ex Kützing) W.R.Taylor, *Halimeda incrassata* (J.Ellis) J.V.Lamouroux, *H. opuntia* (Linnaeus) J.V.Lamouroux, *Ulva chaugulei* M.G.Kavale & Kazi, *U. lactuca* Linnaeus, *U. meridionalis* Horimoto & S.Shimada, *U. ohnoi* Hiraoka & S.Shimada, *U. tentaculosa* Lagourgue & Payri, and *U. tepida* Makiyo & S.Shimada. Among these species, *U. tentaculosa* is reported for the first time for the Atlantic Ocean and *U. meridionalis* for the South Atlantic Ocean; *U. chaugulei* and *U. tepida* are reported for the first time for the northeastern coast of Brazil, while *U. ohnoi* is first reported for Alagoas State.

Table 1
Identification and information of samples included in this work.

Taxon	Morphological ID	Locality	Collection date	Voucher	*tuf*A	*rbc*L	LSU
Anadyomene stellata	*Anadyomene* cf. *rhizoidifera*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55554	-	-	X
Boodlea struveoides	*Boodlea struveoides*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55545	-	-	X
Caulerpa lentillifera	*Caulerpa microphysa*	Sonho Verde beach, Paripueira	02.VIII.2019	PEUFR55674	X	-	-
Caulerpa racemosa var. *macrophysa*	*Caulerpa racemosa* var. *macrophysa*	Pontal do Coruripe beach, Coruripe	29.IV.2017	MAC59442	X	-	-
Cladophora vagabunda	*Cladophora vagabunda*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55541	-	-	X
Cladophora vagabunda	*Cladophora vagabunda*	Pontal do Coruripe beach, Coruripe	17.V.2019	PEUFR55519	-	-	X
Cladophora vagabunda	*Cladophora vagabunda*	Pontal do Coruripe beach, Coruripe	17.V.2019	PEUFR55520	-	-	X
Cladophora vagabunda	*Cladophora vagabunda*	Sonho Verde beach, Paripueira	02.VIII.2019	PEUFR55526	-	-	X
Cladophora vagabunda	*Cladophora vagabunda*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55544	-	-	X
Halimeda opuntia	*Halimeda opuntia*	São Miguel dos Milagres beach, São	04.VIII.2019	PEUFR55726	X	-	-

Taxon	Morphological ID	Locality	Collection date	Voucher	*tuf*A	*rbc*L	LSU
		Miguel dos Milagres					
Halimeda incrassata	*Halimeda incrassata*	São Miguel dos Milagres beach, São Miguel dos Milagres	04.VIII.2019	PEUFR55725	X	-	-
Phyllodictyon anastomosans	*Phyllodictyon anastomosans*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55547	-	-	X
Phyllodictyon anastomosans	*Phyllodictyon anastomosans*	São Miguel dos Milagres beach, São Miguel dos Milagres	04.VIII.2019	PEUFR55668	-	-	X
Phyllodictyon sp.	*Phyllodictyon* sp.	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55558	-	-	X
Udotea dotyi	*Udotea dotyi*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55552	-	X	-
Ulva chaugulei	*Ulva flexuosa*	Sonho Verde beach, Paripueira	02.VIII.2019	PEUFR55530	X	-	-
Ulva lactuca		Pontal do Coruripe beach, Coruripe	-	-	X	-	-
Ulva lactuca		-	-	-	X	-	-
Ulva lactuca		-	-	-	X	-	-
Ulva meridionalis		Pontal do Coruripe beach, Coruripe	-	-	X	-	-
Ulva meridionalis		São Miguel dos Milagres	22.VIII.2022	-	X	-	-

Taxon	Morphological ID	Locality	Collection date	Voucher	*tuf*A	*rbc*L	LSU
		beach, São Miguel dos Milagres					
Ulva ohnoi	*Ulva rigida*	Sonho Verde beach, Paripueira	02.VIII.2019	PEUFR55528	X	-	-
Ulva ohnoi	*Ulva rigida*	Sonho Verde beach, Paripueira	02.VIII.2019	PEUFR55529	X	-	-
Ulva ohnoi	*Ulva rigida*	São Miguel dos Milagres beach, São Miguel dos Milagres	-	PEUFR55534	X	-	-
Ulva ohnoi	*Ulva lactuca*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55592	X	X	-
Ulva tentaculosa		Mirante da Sereia beach, Maceió	11.VI.2021	-	X	X	-
Ulva tepida	*Ulva flexuosa*	Mirante da Sereia beach, Maceió	18.IV.2019	PEUFR55514	X	X	-
Ulva tepida	*Ulva flexuosa*	Sonho Verde beach, Paripueira	02.VIII.2019	PEUFR55531	X	X	-
Ulva tepida	*Ulva flexuosa*	Barreiras do Boqueirão beach, Japaratinga	30.VIII.2019	PEUFR55591	X	X	-
Ulva tepida		Mundau-Manguaba Estuarine Complex, Maceió	-	-	X	-	-
Ulva tepida		Mundau-Manguaba Estuarine	-	-	X	-	-

Taxon	Morphological ID	Locality	Collection date	Voucher	*tuf*A	*rbc*L	LSU
		Complex, Maceió					
Ulva tepida		Mundau-Manguaba Estuarine Complex, Maceió	-	-	X	-	-
Ulva tepida		Sonho Verde beach, Paripueira	-	-	X	-	-

The *tuf*A sequences of the samples morphologically identified as *H. opuntia*, *H. incrassata*, and *C. racemosa* var. *macrophysa* matched available sequences in GenBank. *Halimeda incrassata* and *H. opuntia* *tuf*A sequences grouped with their respective authentic clades (Fig. 1), showing low genetic distance, with 0 and 0–0.15%, respectively. *Caulerpa racemosa* var. *macrophysa* from Alagoas was positioned within the *C. racemosa* clade, diverging by 0.1–0.3% from USA and India sequences, which confirms its identity. Conversely, samples morphologically identified as "*C. microphysa*" grouped with *C. lentillifera* and showed higher variation (0.5–0.8% divergence) from *tuf*A sequences from the Indian Ocean.

The *tuf*A sequences of the samples morphologically identified as "*U. rigida*" and one identified as "*U. lactuca*" were identical to sequences of *U. ohnoi* from the type locality and were recovered with high support (ufBS = 99). The samples morphologically identified as "*U. flexuosa*" were recovered within two distinct clades. The sample from Sonho Verde beach was recovered with high support (ufBS = 99) as *U. chaugulei*, with low divergence (0–0.1%) from sequences from Kuwait and Israel. The samples identified as "*U. flexuosa*" from other localities were recovered with full support with *U. tepida* from Israel and Australia, with 0–0.5% genetic divergence. For *U. tentaculosa*, our sequences were identical to sequences available, including a sequence from the type locality of the species (MZ870666). The sequences of *U. meridionalis* show low divergence, 0.1–0.5%, with sequences from the USA and Singapore; although there is no *tuf*A sequence of the holotype, this represents the typical clade.

The *rbc*L analysis was congruent with *tuf*A positioning "*U. flexuosa*" from Mirante da Sereia and Sonho Verde within the *U. tepida* clade, with intraspecific variation ranging from 0–0.6% from sequences from Israel and Mexico. Similarly, sequences of "*U. rigida*" and "*U. lactuca*" were recovered with sequences of *U. ohnoi*, with no divergence from available sequences. The *rbc*L sequence of *U. tentaculosa* was also recovered with high support, along with sequences from the species' type locality. The partial *rbc*L sequence of the sample morphologically identified as *Ud. dotyi* matched sequences from GenBank with low divergence (0.4%), including sequences from Pernambuco.

Together with previously published sequences, our LSU rDNA analysis recovered at least nine species of Cladophorales. Our analysis confirmed the occurrence of *Chaetomorpha antennina* (Bory) Kützing, *Chamaedoris peniculum* (J. Ellis & Solander) Kuntze, *Chaetomorpha costacoralla* J.O.F. Brito, Cassano &

W.A. Gama, *Dictyosphaeria versluysii* Weber Bosse, *Mysticladia nigra* J.O.F. Brito, V. Carneiro, Cassano, W.A. Gama & C.W.N. Moura, *Leliaertia repens* J.O.F. Brito, A.M. Alves, Gestinari & C.W.N. Moura and *Pseudorhizoclonium mangroviorum* Boedeker, Leliaert & A.R. Sherwood. Additionally, *Cladophora vagabunda* (Linnaeus) C. Hoek sequences were recovered as polyphyletic, comprising two distinct clades, indicating the presence of more than one species along the Alagoas coast. The "*C. vagabunda*" 1 clade was 1.25% distinct from "*C. vagabunda*" 2, while sequences of "*C. vagabunda*" 2 showed 0–0.2% intraspecific divergence, and all "*C. vagabunda*" 1 sequences were identical. Both *C. vagabunda* clades from Alagoas might represent a distinct species from the true *C. vagabunda* from Europe.

Our sequences morphologically identified as *Boodlea struveoides*, *Phyllodictyon anastomosans*, and *Phyllodictyon* sp. were recovered in the *Boodlea composita- Phyllodictyon anastomosans* complex clade as a single species, together with a sequence of *Cladophoropsis membranacea* from Cape Verde with 0% divergence. Our sequence, morphologically identified as *Anadyomene* cf. *rhizoidifera*, matched sequences of *A. stellata*, indicating they represent the same species; however, the species was recovered in a polytomy with other *Anadyomene* species.

4 Discussion

Our DNA barcode analysis showed that morphological data reliably delineated some species but failed to delineate all of them. For *Caulerpa lentillifera*, *Caulerpa racemosa* var. *macrophysa*, *Halimeda incrassata*, *H. opuntia* and *Udotea dotyi*, morphological and molecular identification were congruent and feasible. Although our samples of *C. lentillifera* were morphologically identified as *C. microphysa*, this does not constitute a morphological incongruence, as *C. microphysa* was recently synonymized with *C. lentillifera* by Draisma et al. (2025). The separation of these two species has been debated by several authors, including Draisma et al. (2014) and Draisma and Sauvage (2024). However, currently available data on *C. lentillifera* and *C. microphysa* indicate that they represent a cryptic species complex that still needs resolution, although they are currently treated as *C. lentillifera* (Draisma et al. 2025). This species is included in the section Sedoideae, characterized by the presence of pyrenoids associated with large chloroplasts and vesiculated ramuli with constricted pedicels, as supported by molecular phylogenies (Famà et al. 2002; Draisma et al. 2014, 2025; Draisma and Sauvage 2024). The specimens identified as *H. opuntia* were grouped within the same named clade previously established by Verbruggen et al. (2005a), Dijoux et al. (2012), Pongparadon et al. (2015) and Ximenes et al. (2017), although there is no sequence from the type locality of the species. On the other hand, our sequences of *H. incrassata* are grouped with sequences from the type locality.

The morphological identification of *Ud. dotyi* was difficult due to its similarity to the blade siphons of *Ud. dixonii* D.S. Littler & D.S. Littler; both species present abundant siphons with appendages on both sides. However, according to Littler and Littler (1990), *Ud. dixonii* presents branched lateral appendages with rounded tips, while *Ud. dotyi* presents compact lateral appendages with truncate tips. *Udotea dotyi* was not reported on the Brazilian coast until the recent work by Rocha et al. (2023), which confirmed its presence based on samples from Pernambuco, Ceará, and Alagoas.

The most divergent results were found on *Ulva* specimens. *Ulva ohnoi* samples presented denticulations on margins, perforations on the blade, basal cells in cross section taller than wide, and cells of the median portion of the blade quadratic, traditionally attributed to *U. rigida* on the Brazilian coast (Taylor 1960; Barata 2004; Coto 2009; Coto and Pupo 2011). Thus, the sample identified as "*U. lactuca*" exhibited an expanded blade with no perforations and smooth margins, as previously reported for *U. lactuca* on the Brazilian coast (Barata 2004; Coto 2007; Coto and Pupo 2011). This is the first report of *U. ohnoi* for Alagoas. The species was initially described for Japan as a bloom forming seaweed by Hiraoka et al. (2004) and later reported for several parts of the globe as an introduced cryptic species (Flagella et al. 2010; Kirkendale et al. 2013; Melton et al. 2016; Pirian et al. 2016; Krupnik et al. 2018; Miladi et al. 2018; Chávez-Sánchez et al. 2019). Along the Brazilian coast, Batista (2018) first reported this species from Fernando de Noronha and Carneiro et al. (2023) later reported it from Espírito Santo, São Paulo, and Rio de Janeiro. Our results are aligned with the findings of Carneiro et al. (2023), who discussed the overlapping features of *U. ohnoi*, *U. lactuca*, and *U. rigida* on the Brazilian coast, and stated that "*U. rigida*" is a misapplied name for Brazil.

Similarly, our "*U. flexuosa*" corresponded to *U. chaugulei* and *U. tepida*. These misidentifications are not unusual for this group of species, as previously reported by Masakiyo and Shimada (2014), Bast et al. (2014), Rybak (2018), Chávez-Sánchez et al. (2019), and Carneiro et al. (2023), with individuals being misidentified as *U. compressa*, *U. flexuosa*, *U. intestinalis*, and *U. paschima*. It is important to state that *U. tepida* is also reported as an invasive species that can form blooms, also known as green tides, that was initially described for Japan and then reported for Australia (Carl et al. 2014), India (Bast et al. 2014; Bast and Rani 2019), Israel (Krupnik et al. 2018), Mexico (Chávez-Sánchez et al. 2019), USA (Melton and Lopez-Bautista 2021) and Egypt (El-Tablawy et al. 2025). For the Brazilian coast, Carneiro et al. (2023) confirmed this species for the first time and identified at least three candidate species within the *U. tepida* clade, suggesting that it may represent a species complex requiring resolution. Our samples from Alagoas morphologically matched *U. tepida* morphotype 1 described by Carneiro et al. (2023) and were also recovered in the same clade.

The same is reported for *U. chaugulei*, with individuals being misidentified as *U. linza*, *U. flexuosa*, and other tubular *Ulva* species (Kazi et al. 2016; Pirian et al. 2016). Its first report for southeastern Brazil was made by Carneiro et al. (2023) when it was considered a cryptogenic species. *Ulva chaugulei* is also reported as introduced in the Mediterranean Sea (Krupnik et al. 2018).

The identification of *U. meridionalis* and *Ulva tentaculosa* in Brazil was only possible based on DNA sequences. These results highlight the plasticity and the difficulty of morphological delineation in *Ulva* species, indicating that only DNA-based identification can provide a reliable name for *Ulva* representatives.

For Cladophorales members, the LSU sequences generated in this work and those previously published revealed the wide presence of cryptic taxa and the need for DNA sequences for better identification. Previous studies by Brito et al. (2024) and Brito et al. (2026b) confirmed the occurrence of *Dictyosphaeria versluysii* and *Chaetomorpha antennina*, and more recently, Carneiro et al. (2026) also confirmed the identity of *Chamaedoris peniculum* on the Alagoas coast by combining morphological and molecular data.

Additionally, regarding cryptic taxa, Brito et al. (2026a, b) confirmed the occurrence of *Mysticladia nigra* and *Pseudorhizoclonium mangroviorum* on the Alagoas coast. Lastly, special mention goes to *Chaetomorpha costacoralla* and *Leliaertia repens*, which were described from material collected in Japaratinga and the Tatuamunha Estuary, respectively (Brito et al. 2023, 2026a, b).

The data generated in the present work confirmed the presence of *Anadyomene stellata* in Alagoas based on samples that exhibited diffuse rhizoids, similar to *Anadyomene rhizoidifera* (Littler and Littler 1991; Joly and Pereira 1973; Alves et al. 2011). This result reinforces the observations made by Littler and Littler (1991), who discussed a possible overlap in the morphological delineation of these species and pointed out how sections made along the veins could generate artifacts liable to be interpreted as multiple layers. Our results indicate that *A. stellata* might be a plastic species along the Brazilian coast, exhibiting more than one cell layer. Furthermore, the species was reconstructed within a polytomy, indicating that LSU rDNA alone may not provide sufficient phylogenetic resolution. Still within cladophoracean algae, two species complexes were recovered in our analysis: the *Cladophora vagabunda* complex and the *Boodlea composita- Phyllodictyon anastomsans* complex. Our newly generated *C. vagabunda* sequences were recovered in two clades, apart from the European clade of *C. vagabunda*. Gestinari et al. (2009) generated the first SSU rDNA sequences for *C. vagabunda* from Brazil and indicated the need to revise the complex. The most recent phylogenetic study of Cladophoraceae performed by Boedeker et al. (2016) revealed that *C. vagabunda* is a species complex composed of four lineages. Our analysis recovered the Brazilian sequences in two of these lineages, which appear to be widely distributed cryptic tropical species, occurring in the Atlantic and Indian Oceans, that still need taxonomic resolution.

The samples morphologically identified as *Phyllodictyon anastomosans*, *Boodlea struveoides*, and *Phyllodictyon* sp. recovered from the *Boodlea* complex presented a high level of morphological plasticity. Our sequences matched a *Cladophoropsis membranacea* sequence from Cape Verde (AM503484), which, according to Leliaert et al. (2009), represents *Boodlea* sp. 1; this species exhibit morphologies assigned to *C. membranacea*, *C. vaucheriiformis*, *Boodlea siamensis*, and *P. anastomosans*. Although the *Boodlea struveoides* morphology is first recovered in this clade in this work, this is not necessarily new for this group, which represents a complex comprising 13 cryptic species that exhibit a mix of morphologies, preventing reliable typification (Leliaert et al. 2009).

Although genetic distances vary depending on taxonomic groups or molecular markers involved, and some taxa still require further investigation, DNA sequences provide accurate and reliable identification, particularly for genera with few available characters or overlapping morphologies. DNA-based identification often faces the challenge of underrepresented databases, or even a lack of sequences from type localities; nevertheless, phylogenetic analyses can aid in understanding local biodiversity while enhancing genetic databases and providing data to support species monitoring and conservation (Bartolo et al. 2020; Fort et al. 2022).

The revisited flora of Alagoas

The present study adds five species to the known flora of Ulvophyceae from Alagoas, based on DNA sequences, and represents the first barcode assessment of marine green macroalgae from the state. Our results, together with previous and specific genetic data from specimens in Alagoas (Table 2), reinforce the finding that morphological data are not always sufficient to delineate species in green seaweeds, although this remains the most accessible tool for biodiversity surveys. This is highlighted by the findings on cryptic *Ulva* diversity, as well as on Cladophorales diversity, reported here and in previous work on the Brazilian coast. Despite our addition of DNA sequence data from Alagoas, the current knowledge regarding the molecular identification of marine green macroalgae remains very limited, as discussed by Santos et al. (2026). Furthermore, our results indicate that available data in public repositories based on morphology, such as Flora e Funga do Brasil (2026), are not up to date. The revision of the taxa reported for the coast based on published papers, dissertations, and theses revealed the citation of 79 species (Table S5), almost twice the number (44 species) listed in Flora e Funga do Brasil (2026).

Table 2
Taxon reported for Alagoas with molecular data available.

Species	Morfological ID	status	Marker	Reference
Bryopsidales				
Caulerpa lentillifera	*Caulerpa microphysa*	Accepted species	*tuf*A	This work
Caulerpa racemosa var. *macrophysa*	*Caulerpa racemosa* var. *macrophysa*	Accepted species	*tuf*A	This work
Halimeda incrassata	*Halimeda incrassata*	Accepted species	*tuf*A	Ximenes et al. (2017); This work
Halimeda opuntia	*Halimeda opuntia*	Accepted species	*tuf*A	Ximenes et al. (2017); This work
Halimeda aff. *tuna*	*Halimeda tuna*	Cryptic species	*tuf*A	Ximenes (2015)
Udotea dotyi		Accepted species	*rbc*L	Rocha et al. (2023); This work
Cladophorales				
Anadyomene stellata	*Anadyomene* cf. *rhizoidifera*	Accepted species	LSU	This work
Boodlea struveoides		Species complex	LSU	This work
Chaetomorpha antennina		Accepted species	LSU; SSU	Brito et al. (2026)
Chaetomorpha costacoralla	*Chaetomorpha gracilis*	Cryptic species	LSU; SSU	Brito et al. (2026)
Chamaedoris peniculum		Accepted species	LSU; SSU	Carneiro et al. (2026)
Cladophora vagabunda		Species complex	LSU; SSU; ITS1	This work
Dictyosphaeria versluysii		Accepted species	LSU; SSU	Brito et al. (2024)
Leliaertia repens	*Cladophora coelothrix*	Cryptic species	LSU; SSU	Brito et al. (2024)
Mysticladia nigra	*Cladophora prolifera*	Cryptic species	LSU; SSU	Brito et al. (2026)
Phyllodictyon anastomosans		Species complex	LSU	This work
Phyllodictyon sp.		Species complex	LSU	This work

Species	Morfological ID	status	Marker	Reference
Bryopsidales				
Pseudorhizoclonium mangroviorum	*Rhizoclonium riparium*	Cryptic species	LSU; SSU	Brito et al. (2026)
Ulotrichales				
Gayralia brasiliensis		Accepted species	ITS1-5.8S-ITS2	da Silva et al. (2022)
Ulvales				
Ulva chaugulei	*Ulva flexuosa*	Cryptic species	*tuf*A	This work
Ulva lactuca	*Ulva lactuca*	Accepted species	*tuf*A	This work
Ulva meridionalis			*tuf*A	This work
Ulva ohnoi	*Ulva lactuca* and *U. rigida*	Cryptic species	*tuf*A; *rbc*L	This work
Ulva tentaculosa			*tuf*A; *rbc*L	This work
Ulva tepida	*Ulva flexuosa*	Cryptic species	*tuf*A; *rbc*L	This work

Together with the newly reported species from the Brazilian coast and its northeastern portion, we highlight that Brazilian diversity of Ulvophyceae may be underestimated, and further studies using DNA barcoding and/or molecular phylogenies might reveal new taxa or previously unreported species.

Declarations

Conflict of interests

Valéria Cassano is Managing Editor of the Brazilian Journal of Botany, and we assure this article was entirely handled by an Associate Editor.

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Author contribution

JOFB, investigation, formal analysis, writing - original draft preparation; VARC, investigation, formal analysis, writing - original draft preparation; VC, conceptualization, investigation, formal analysis, writing - original draft preparation, project administration, funding acquisition; WAG conceptualization, investigation, formal analysis, writing - original draft preparation.

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Figures

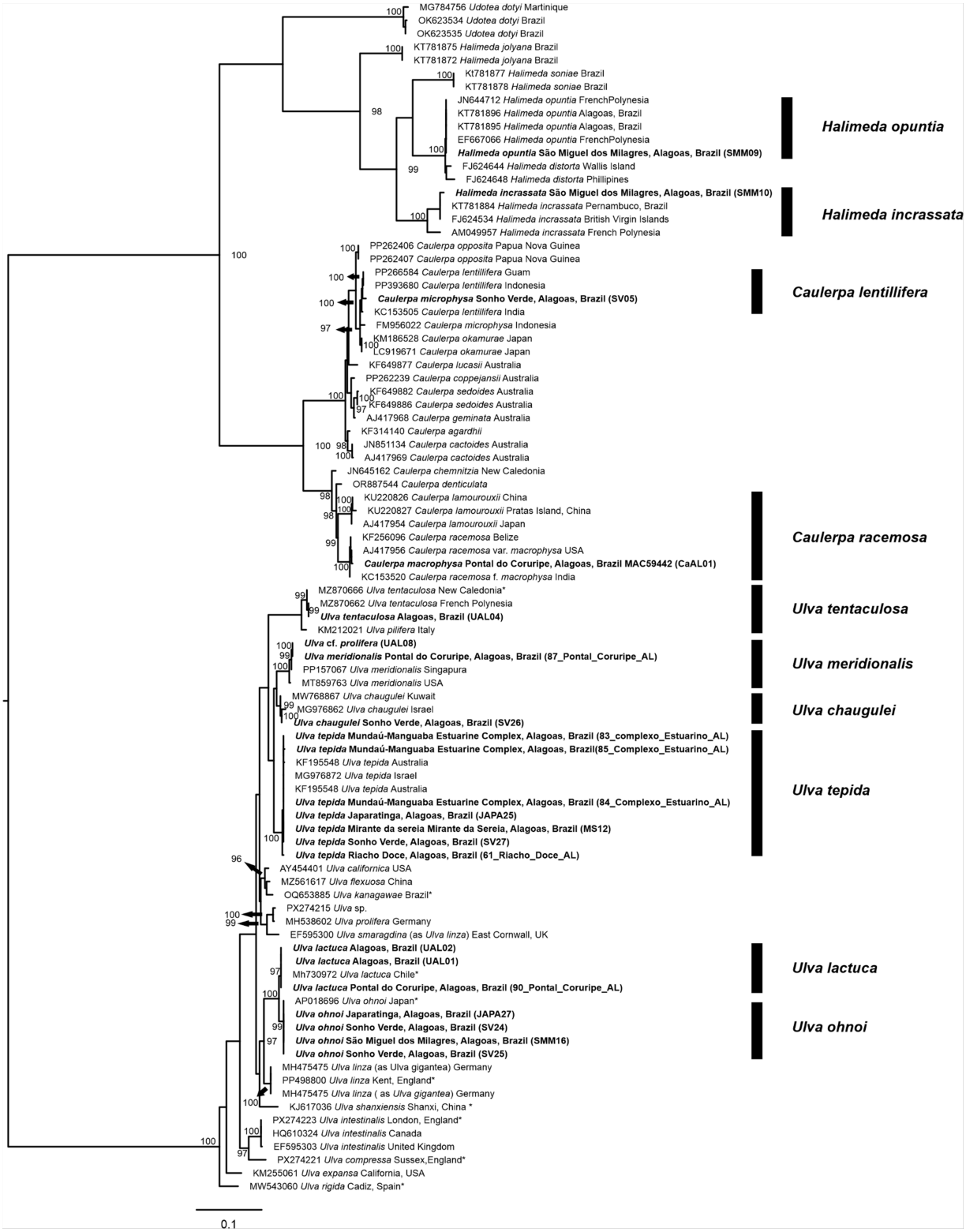


Figure 1

Ultrafast Maximum likelihood (ufML) consensus tree based on *tuf*A sequences. (values above 95 for ufML on branches) * Indicates sequence from the type locality or from a nearby locality. Bold names indicate newly generated sequences

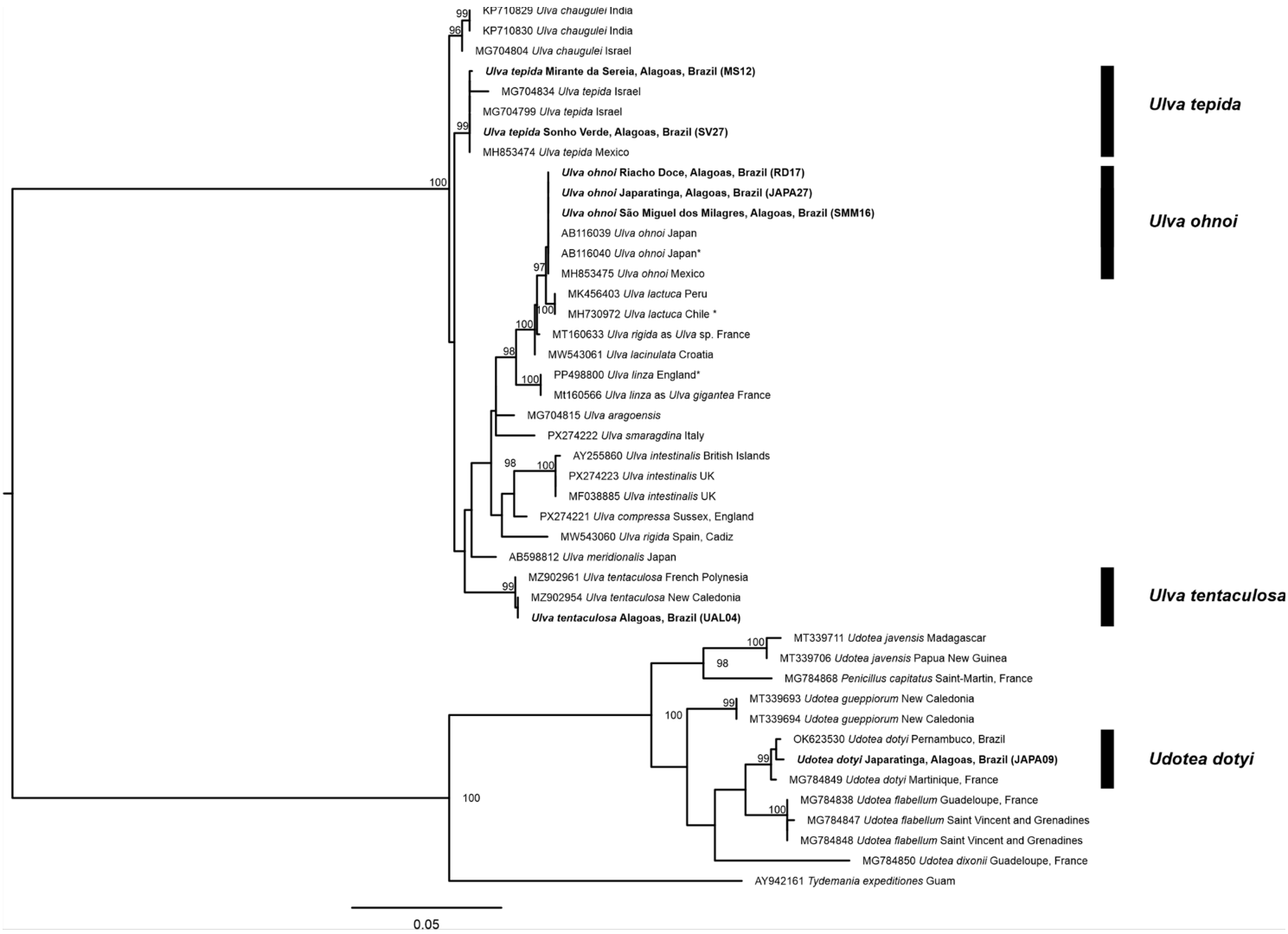


Figure 2

Ultrafast Maximum likelihood (ufML) consensus tree based on *rbc*L sequences. (values above 95 for ufML on branches) * Indicates sequence from the type locality or from a nearby locality. Bold names indicate newly generated sequences

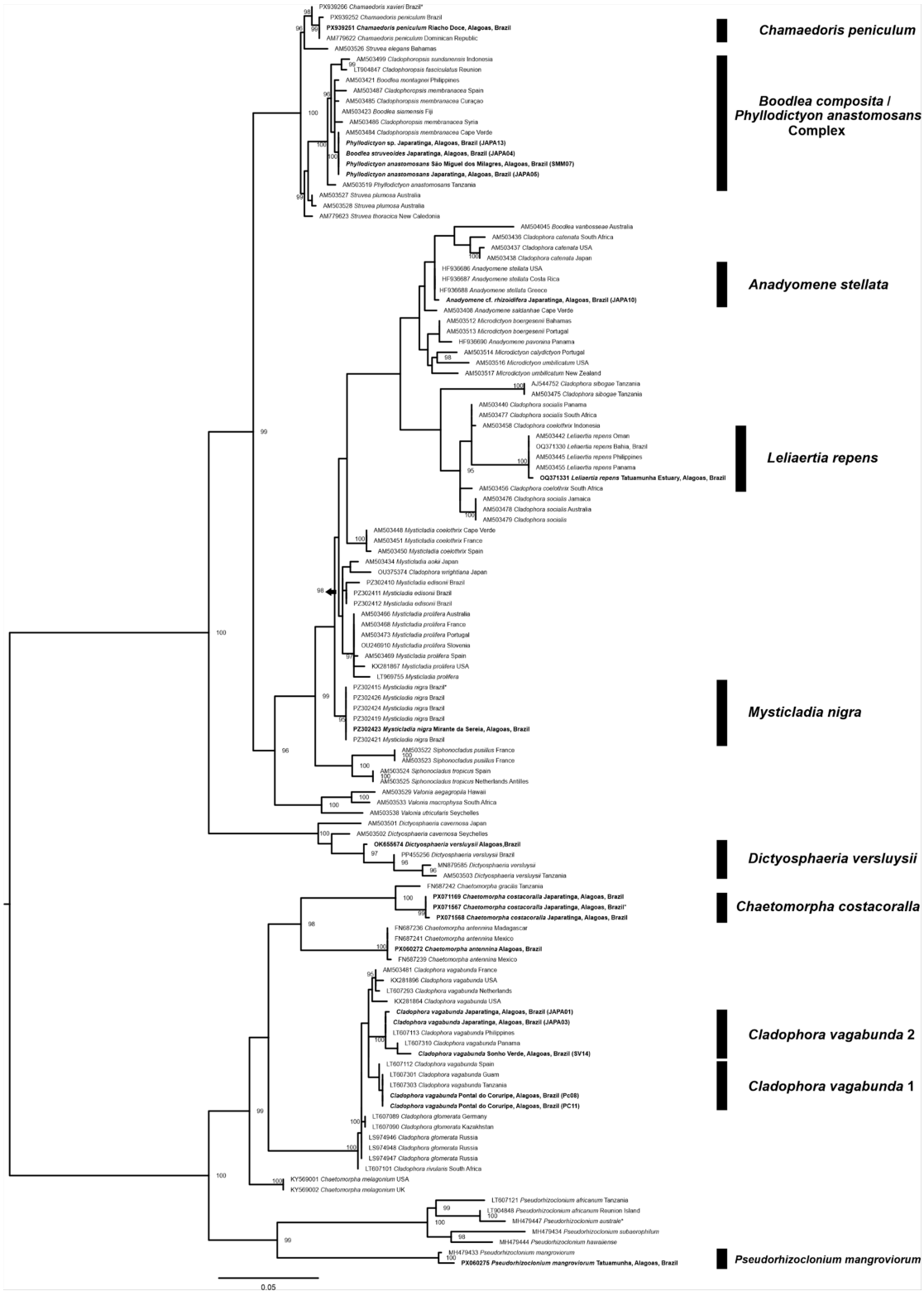


Figure 3

Ultrafast Maximum likelihood (ufML) consensus tree based on LSU rDNA sequences. (values above 95 for ufML on branches) * Indicates sequence from the type locality or from a nearby locality. Bold names indicate newly generated sequences

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Suplementarydata25SETJBVC.docx