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Revealing the diversity of the genus *Ulva* (Ulvales, Chlorophyta) in southeastern Brazil, with a description of *Ulva kanagawae sp. nov.*

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ABSTRACT

The green algal genus *Ulva* is one of the most widely distributed macroalgal genera. The taxonomy of *Ulva* is problematic due to its simple morphology. The study of the diversity of this genus has undergone great advances using molecular data, leading to changes in the taxonomic status of species, and the recognition of species complexes and cryptic species. Here we reassessed *Ulva* species from southeastern Brazil using molecular data. We recognized 10 taxa, among which only three previously reported species were confirmed by molecular data: *U. compressa, U. lactuca* and *U. ohnoi*, the latter recently recorded from insular waters in Fernando de Noronha Archipelago, northeastern Brazil. Our phylogenetic analyses and species delimitation methods strongly supported the establishment of *Ulva kanagawae sp. nov*. The species traditionally cited for southeastern Brazil, *U. flexuosa, U. linza, U. rigida* and *U. paradoxa*, proved to be misapplied names of *U. tepida, U. aragoënsis, U. ohnoi/U. lactuca* and *U. torta*, respectively. One taxon, *Ulva* sp., remains unnamed and needs further studies. *Ulva chaugulei* and *U. tanneri*, both considered here as cryptogenic species, are new occurrences for Brazil expanding their geographic distribution to the Atlantic Ocean and western Atlantic Ocean, respectively. In addition, our analysis of sequences from type materials revealed that *U. pseudo-ohnoi* is a heterotypic synonym of *U. conglobata*. This first systematic molecular study of *Ulva* species in Brazil points out that more extensive sampling is needed to reveal the true diversity of the genus in Brazilian waters.

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INTRODUCTION

The green macroalgal genus *Ulva* Linnaeus has a worldwide distribution in marine, estuarine and freshwater environments, and is one of most speciose genera of green macroalgae, with 102 species names accepted taxonomically and 181 synonyms (Guiry & Guiry 2023). The simple morphology of *Ulva* species and few diagnostically valuable characters, combined with significant intra- and inter-specific variation, often associated with environmental conditions (Blomster *et al.* 1998; Hayden *et al.* 2003; Brodie *et al.* 2007; Coto & Pupo 2009), make the reliable assignment of a name to *Ulva* specimens a challenging task. In this context, molecular data have clarified the taxonomic status of many species and revealed new species, as well as species complexes and cryptic diversity (Hofmann *et al.* 2010; Hughey *et al.* 2019; Steinhagen *et al.* 2019a, b; Melton & López-Bautista 2021; Lagourgue *et al.* 2022; Santiañez & De Clerck 2023; Tran *et al.* 2023).

In general, molecular data have not been congruent with species identification based on morpho-anatomical data (Kazi *et al.* 2016; Chávez-Sánchez *et al.* 2019; Lagourgue *et al.* 2022). Furthermore, in the most recent worldwide review of *Ulva*, considering the data available in GenBank for the three most used markers (ITS rDNA, *rbc*L and *tuf*A), Tran *et al.* (2022) showed that about 8% of the unique haplotypes of *Ulva* were attributed to more than one named species, indicating misidentifications or intraspecific morphological variation. Major advances in the understanding of *Ulva* taxonomy have been especially achieved by the successful sequencing of type material. These advances have resolved some longstanding taxonomic problems with species synonymizations and the correction of misapplied names (Mareš *et al.* 2011; Hanyuda & Kawai 2018; Hughey & Gabrielson 2022). Molecular studies are also continuously revealing introduced or invasive *Ulva* species which are the main cause of green tides in the world (Hiraoka *et al.* 2004; Hughey *et al.* 2018; Suzuki *et al.* 2018; Melton & López-Bautista 2021).

The tropical and subtropical Atlantic Ocean is poorly sampled for *Ulva* using molecular methods (Tran *et al.* 2022) and, particularly Brazil, with its *c.* 8,000 km of coastline, represents a huge gap in the western South American Atlantic, having no published DNA sequences. *Ulva* species have a wide distribution along the Brazilian coast, extending from Maranhão state (northeastern Brazil) to Rio Grande do Sul state (southern Brazil) (Flora e Funga do Brasil 2022), including oceanic islands (Atol das Rocas, Fernando de Noronha, São Pedro and São Paulo, Abrolhos, Trindade and Martim

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Vaz; Villaça *et al.* 2006). Most *Ulva* citations for Brazil are based on traditional morpho-anatomical studies and floristic surveys, which culminated in the record of 15 taxa, including 13 species, one subspecies and one form (Ugadim 1973; Flora e Funga do Brasil 2022). The Brazilian southeastern region comprises most of the *Ulva* taxa citations: 13 of the 15 recorded for the country (Table 1). The remaining two species, *Ulva polyclada* Kraft (as '*Enteromorpha multiramosa* Bliding', *nom. inval.*) and *U. ohnoi* Hiraoka & S. Shimada were cited only for Atol das Rocas (Oliveira Filho & Ugadim 1974, 1976; Villaça *et al.* 2010) and Fernando de Noronha Archipelago (Batista 2018), respectively.

Molecular studies for *Ulva* in Brazil are still extremely scarce, limited to laminar species, with restricted sampling and based on a single marker. For instance, Martins (2016) used *tuf*A for the first time to confirm the identification of *U. lactuca* Linnaeus populations (as *U. fasciata* Delile) in Rio de Janeiro state (southeastern Brazil). Afterwards, Batista (2018) generated the first *rbc*L sequences for *U. lactuca* (including the *U. fasciata* morphotype), covering the northeastern (Bahia state and Fernando de Noronha Archipelago, Pernambuco state) and southern (Paraná and Santa Catarina states) Brazilian regions. In addition, the occurrence of *U. ohnoi* was first detected in Fernando de Noronha through *rbc*L sequences and was considered a possible introduced species in Brazil (Batista 2018).

The scarcity of molecular data on *Ulva* along the Brazilian coast, therefore, led us to perform the first systematic study of *Ulva* focusing on an extensive sampling effort from the southeastern Brazilian coastline. We combined morphology, phylogeny and species delimitation methods for selected clades, as a first step to reveal the true diversity and distribution of *Ulva* species on the Brazilian coast.

Table 1. Current knowledge of *Ulva* diversity in southeastern Brazil. In bold currently recognized species, from this study. Not in bold, names either not collected or misapplied.

Taxa	Distribution	Taxonomic status based on this study
***Ulva aragoënsis* (Bliding) Maggs**	RJ, SP	Matched the current molecular species concept. Previously misidentified as *U. linza*
Ulva chaetomorphoides (Børgesen) H.S. Hayden *et al.*[3,4,6,8,11] (as *Enteromorpha ramulosa* (Smith) Carmichael)[7]	ES, RJ, SP	Not found in this study. DNA sequence not available in the databases
***Ulva chaugulei* M.G. Kavale & Kazi**	RJ, SP	First record. Cryptogenic species. Matched the current molecular species concept including the topotype (Kazi *et al.* 2016)
Ulva clathrata (Roth) C. Agardh[2,4,5,6,11,12] (as *E. crinita* Nees)[8]	ES, RJ, SP	Not found in this study
***Ulva compressa* Linnaeus**[1,2,6,13,14]	ES, RJ, SP	Found only in ES Matched the current molecular species concept
Ulva flexuosa Wulfen[1,3.4,5,6,7,8,11,12,13] [as *E. flexuosa* (Wulfen) J. Agardh subsp. *flexuosa*[3]; also as *E. lingulata* J. Agardh)[3,5]	ES, RJ, SP	Misapplied name in southeastern Brazil to *Ulva tepida*
Ulva flexuosa f. *submarina* (Collins & Hervey) M.J. Wynne (as *E. flexuosa* f. *submarina* Collins & Hervey)[7]	SP	Not found in this study
Ulva hookeriana (Kützing) H.S. Hayden *et al.* [as *E. bulbosa* (Suhr) Montagne][14]	RJ	Doubtful record (Oliveira Filho 1977)
Ulva intestinalis Linnaeus[2,4]	RJ, SP	Not found in this study
Ulva kanagawae sp. nov.	SP	New species, *Ulva linza* morphotype
***Ulva lactuca* Linnaeus**[1–14]	ES, RJ, SP	Matched the current molecular species concept including the holotype (Hughey *et al.* 2019)
Ulva linza Linnaeus[2,3,5,6,8,9,11,12,13]	ES, RJ, SP	Misapplied name in southeastern Brazil to *Ulva aragoënsis*. *U. linza* morphotype split into three different genetic species
***U. ohnoi* Hiraoka & S. Shimada**	ES, RJ, SP	First record for southeastern Brazil. Cited for northeastern Brazil by Batista (2018). Matched the current molecular species concept including the holotype (Hiraoka *et al.* 2004)
U. paradoxa C. Agardh[12] [as *E. paradoxa* (C. Agardh) Kützing[8]; as *U. flexuosa* subsp. *paradoxa* (C. Agardh) M.J. Wynne][14]	ES, RJ, SP	Found only in RJ Misapplied name in southeastern Brazil to *Ulva torta*
U. prolifera O.F. Müller[2,4,8,11,13]	ES, RJ, SP	Not found in this study
U. ralfsii (Harvey) Le Jolis[10,14]	RJ	Not found in this study. Doubtful record. Similar to *E. paraxoda sensu* Kanagawa (now *U. torta*)
U. rigida C. Agardh[6,11,12,13]	ES, RJ, SP	Previously misidentified as *U. ohnoi* or *U. lactuca*
***Ulva tanneri* H.S. Hayden & Waaland**	RJ	First record. Cryptogenic species. Matched the current molecular species concept including sequence from region close to the type locality (Saunders 2014)
***Ulva tepida* Masakiyo & S. Shimada**	ES, RJ, SP	Matched the current molecular species concept. Previously misidentified as *U. flexuosa*
***Ulva torta* (Mertens) Trevisan**	RJ, SP	Matched the current molecular species concept including sequence from region close to the type locality (Steinhagen *et al.* 2019a). Previously misidentified as *U. paradoxa sensu* Kanagawa
***Ulva* sp.**	ES	No genetic correspondence with any sequenced species. *Ulva linza* morphotype

Taxa identified in this study are highlighted in bold. [1]Taylor (1930), [2]Taylor (1931), [3]Joly (1957), [4]Taylor (1960), [5]Joly (1965), [6]Yoneshigue-Braga (1970), [7]Ugadim (1973), [8]Kanagawa (1983), [9]Yoneshigue (1985), [10]Horta (2000), [11]Barata (2004), [12]Coto & Pupo (2009), [13]De-Paula *et al.* (2020a), [14]Flora e Funga do Brasil (2022). ES: Espírito Santo, RJ: Rio de Janeiro, SP: São Paulo.

MATERIAL AND METHODS

Sampling and morphological analyses

Ulva samples were collected at 48 geo-referenced sites in southeastern Brazil comprising the states of Espírito Santo, Rio de Janeiro and São Paulo, between the coordinates 18° 19.8973′S and 25°18.6298′S. The samples were collected in the intertidal zone on rocks, sandstone reefs, mangrove, in estuaries, during low tide in 2013, 2014, 2015 and 2019.

For morphological study, a fragment of each thallus was fixed in 4% formalin-seawater or absolute ethanol and then pressed as herbarium sheets. Another fragment of the same thallus was stored in silica gel for molecular analyses. The samples were analysed showing the most common habit and its morphological variations. Transverse hand sections were obtained with a razor blade and stained with 0.5% aqueous acidified aniline blue. Pyrenoids were stained with acidic Lugol solution (Berlyn & Miksche 1976). Details of the thalli and microscopic features were recorded with a Sony W570 digital camera (Tokyo, Japan) coupled to a Stemi 305 EDU stereomicroscope (Zeiss, Göttingen, Germany) and a Primo Star optical microscope (Zeiss). For each measurable vegetative and reproductive characteristic, a set of 10 measurements were made, whenever possible, with randomly chosen specimens from different collection sites. For such measurements, minimum and maximum values were given as length × diameter. The complete set of material examined is shown in Table S1. Vouchers were deposited in the herbaria of University of São Paulo (SPF), Federal University of Rio de Janeiro (RFA), and Institute of Environmental Research (SP), Brazil. Herbaria abbreviations follow the Index Herbariorum (Thiers 2023).

Molecular analysis

Total DNA was extracted after pulverizing the silica-dried material in liquid nitrogen using the NucleoSpin© Plant II-Macherey-Nagel (Bethlehem, Pennsylvania, USA) following the manufacturer's instructions. For PCR, the *tuf*A marker was amplified using the primer pair *tuf*AF and *tuf*AR following Famà *et al.* (2002). For *rbc*L, we used two overlapping pairs of primers: F623-603 (Curtis *et al.* 2008) and R1396-1372 (Lam & Zechman 2006); and F22-41(Curtis *et al.* 2008) and R689-667 (Hanyuda *et al.* 2000) following the PCR cycles described by Loughnane *et al.* (2008). PCR was performed for each marker in a final volume of 25 µL: 1× PCR *buffer*, 1.6 mM of dNTP, 1.2 mM of betaine, 3.0 of mM $MgCl_2$, 0.4 mM of each primer, 0.31U of *Taq* DNA polymerase (Promega Corp., Madison, Wisconsin, USA) and 1 µL of DNA. All PCR products were purified using the GFX™ PCR DNA or Gel Band Purification kit (GE Healthcare, Buckinganshire, UK), following the manufacturer's instructions. Purified amplicons were sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, California, USA), with the PCR primers on an ABI PRISM 3130DNA Genetic Analyzer (Applied Biosystems). Consensus sequences and multiple alignments for both *tuf*A and *rbc*L sequences were constructed using ClustalW implemented in BioEdit v7.0.4.1 (Hall 1999); each consensus sequence was manually corrected afterwards by visual inspection of the original electropherograms. For each marker, an alignment was built with the sequences generated in this study plus those available in GenBank (Tables S1, S2).

Phylogenetic analyses

For selection of sequences available in GenBank, we prioritized those generated from type specimens, topotypes and sequences from publications. The most appropriated model of sequence evolution for maximum likelihood (ML) and Bayesian inference (BI), TIM3+F+I+G4 for both markers, was selected under the Akaike information criterion (AIC) implemented in IQ-TREE webserver (Trifinopoulos *et al.* 2016). ML analysis was performed with 2,000 non-parametric bootstrap (BS) replicates (Felsenstein 1985). BI analysis was performed using MrBayes v3.2.2 (Ronquist *et al.* 2012), with the following parameters: four chains of the Markov chain Monte Carlo (one hot and three cold) for two independent runs, sampling one tree every 1,000 generations for 5,000,000 generations, starting with a random tree. We discarded the first 50,000 generations in both runs as the burn-in to build the consensus tree and computing the posterior probabilities (PP). The best tree was visualized using FigTree v1.4.4 (Rambaut 2018). *Umbraulva* E.H. Bae & I.K. Lee, *Ryuguphycus* H. Kawai, Hanyuda & Kitayama and *Gemina* V.J. Chapman were used as outgroups. For *tuf*A and *rbc*L matrices, genetic distances were calculated using uncorrected '*p*' distances in PAUP v4.0 beta 10 (Swofford 2002).

Species delimitation methods

We applied three primary species delimitation methods (SDMs) for selected *tuf*A clades, namely: Assemble Species by Automatic Partitioning (ASAP; Puillandre *et al.* 2021), the Poisson Tree Processes (PTP; Zhang *et al.* 2013), and the General Mixed Yule Coalescent Model (GMYC; Pons *et al.* 2006).

The ASAP analyses were performed with the online implementation (https://bioinfo.mnhn.fr/abi/public/asap/#) using a GTR distance matrix as input, previously built in PAUP. ASAP analysis was run splitting groups below 0.01 probability and only the best ASAP-score partition was considered. For PTP analysis we used a maximum-likelihood (ML) tree constructed as described above but excluding outgroup sequences. PTP was performed on the online website (http://species.h-its.org) under the following parameters: 100,000 MCMC generations, thinning = 100 and burn-in = 0.1. Only the PTP ML approach was considered. For GMYC analyses, one Bayesian ultrametric tree was estimated employing the birth and death speciation model (BD) available in BEAST v2.6.0 (Bouckaert *et al.* 2019). The GMYC ultrametric tree was constructed using the GTR evolutionary model. The parameters were as described above for Bayesian analyses. GMYC single and multiple threshold approaches were executed using the package '*splits*' in R v1.0 (Fujisawa & Barraclough 2013).

The secondary species hypotheses (SSHs) were established based on the congruence between the different SDM approaches combined with genetic distance, phylogeny and morphological data.

RESULTS

Molecular analyses

A total of 308 samples of *Ulva* were collected in southeastern Brazil from which we generated 154 new sequences, 116 for *tuf*A and 38 for *rbc*L (Table S1). For the *tuf*A marker we built a dataset with 120 sequences including those obtained from GenBank (Table S2) with a final alignment of 853 bp. Identical sequences were removed from the *tuf*A alignment. For the *rbc*L marker, a dataset with 144 sequences was built including those obtained from GenBank (Table S2) with a final alignment of 1,354 bp.

Our *tuf*A (Fig. 1) and *rbc*L (Fig. S1) phylogenies showed similar topologies with nine clades formed by the species sequenced in this study, namely: *Ulva aragoënsis* (Bliding) Maggs, *U. chaugulei* M.G. Kavale & Kazi ('*chaugulii*'), *U. compressa* Linnaeus, *U. lactuca, U. ohnoi, U. torta* (Mertens) Trevisan, *U. tanneri* H.S. Hayden & Waaland, *U. tepida* Masakiyo & S. Shimada, and a distinct clade, designated '*Ulva sp. nov.*'. A single sample (OQ653874, *tuf*A) that did not group with any other sequences was maintained as *Ulva* sp. Most clades were moderately to robustly supported for both markers (>80% BP; >0.97 PP), except for *U. aragoënsis*, in the *tuf*A phylogeny (Fig. 1), and *U. torta*, in the *rbc*L phylogeny (Fig. S1), both supported only for BI.

Based on the *tuf*A marker, the three different SDMs (ASAP, PTP and GMYC) were applied to selected clades of Brazilian sequences (*Ulva aragoënsis, U. chaugulei, U. torta, U. tanneri, U. tepida, Ulva sp. nov*. and *Ulva* sp.; Fig. 1). The applied SDMs were mostly consistent showing a similar number of primary species hypotheses (PSH) resulting in 11 consensus PSH; PTP was less conservative generating 12 PSH, whereas GMYC single-threshold was more conservative with only seven PSH generated. Nine secondary species hypotheses (SSH) were determined for the selected Brazilian clades (Fig. 1).

We initially identified our sequences of *Ulva tepida* as '*U. flexuosa*' based on morpho-anatomical characteristics (Kanawaga 1983). Three SDM methods, excluding single-threshold GMYC, identified three PSH, which could be attributed to three distinct morphologies, representing three SSH. The SSH1 lineage corresponded to the typical morphology of the species, whereas SSH2 and SSH3 corresponded to the morphological variants identified in this study as morphotypes 2 and 3. The morphotype 3 also included a sequence from Florida, USA (Melton & López-Bautista 2021).

Considering all Brazilian *U. tepida* sequenced for *tuf*A, this species showed the highest intraspecific divergence, up to 1.4% (Table S3). In the *rbc*L analyses, the Brazilian *U. tepida* sequences differences ranged up to 0.97% (Table S4).

The moderate to well-supported clade of *U. flexuosa* Wulfen was phylogenetically distant from our *U. tepida* sequences (as '*U. flexuosa*') for both markers (Figs 1, S1). Interspecific divergence for *tuf*A between *U. tepida* and *U. flexuosa* ranged from 3.8% to 4.94%, and was slightly lower for *rbc*L (2.55%–3.26%), confirming that none of our sequences corresponded to the European *U. flexuosa*.

Two of the four SDMs resolved *U. chaugulei* as a single entity (SSH4; Fig. 1). Our *tuf*A sequences of *U. chaugulei* differed by only 0.12%–0.31% (Table S3). In the *rbc*L phylogeny, the sister relationship of *U. chaugulei* and *U. tepida* received high support (Fig. S1). Our *rbc*L sequences of *U. chaugulei* were 100% identical and diverged from the topotype sequences (India) only by 0.23% (Fig. S1; Table S4).

Our single *tuf*A sequence of *Ulva* sp. (OQ653874) was initially identified as '*U. linza*' based on its distromatic thallus in its central portion and tubular monostromatic margin. All SDM resolved *Ulva* sp. as an independent entity (SSH5) that requires further collections and sequencing of other molecular markers to define its taxonomic status. Our *Ulva* sp. (OQ653874) is not phylogenetically related to any *U. linza*, including a sequence of *U. linza* (EF595300) from UK, East Cornwall, near the type locality, positioned within the *Ulva linza-procera-prolifera* complex, named the LPP clade (Fig. 1).

The Brazilian sequences of *U. aragoënsis* were morphologically identified as '*U. linza*' for their distromatic central thalli and tubular monostromatic margins. Our *tuf*A sequences clustered with *U. aragoënsis* as defined by Krupnik *et al.* (2018). This clade grouped *U. aragoënsis* (also as *U. mediterranea* Alongi, Cormaci & G. Furnari) from Israel and USA, sequences identified as '*U. prolifera*' from India, and '*U. flexuosa*' from different localities (Fig. 1). All SDM applied to the *U. aragoënsis* clade recovered only one taxonomic entity (SSH6). Our *U. aragoënsis* is not phylogenetically related to *U. linza* from the UK (EF595300).

In the *tuf*A analysis, *U. tanneri* was sister to *U. torta* with high support for BI (Fig. 1). SDMs applied to the *U. tanneri* clade were completely congruent supporting all sequences as a single species (SSH7) and clustered with a sequence from near the type locality (California, USA, KM255002; Fig. 1). The sister relationship between *U. tanneri* and *U. torta* was not well-supported by *rbc*L (Fig. S1).

The Brazilian *tuf*A sequences of *U. torta* grouped with sequences from Germany and Australia (Tasmania, as *U. clathratioides* L.G. Kraft, Kraft & R.F. Waller) plus *Ulva* sp. (USA; Fig. 1). All SDMs applied to the *U. torta* clade recovered only one taxonomic entity (SSH8; Fig. 1). The type locality of *U. torta* is Germany, North Sea, East Frisian Islands, Norderney (Silva *et al.* 1996). Two sequences from Germany (MH538694 and MH475496; Steinhagen *et al.* 2019a) used in our analyses were collected in areas close to the type locality, particularly sequence MH538694, from the North Sea, Nordstrand, Schleswig-Holstein, and are considered here as authentic *U. torta*, which showed a genetic divergence from Brazilian sequences of up to 0.3%. In the *rbc*L analysis, our Brazilian sequence of *Ulva torta* grouped with *U. torta* from Japan, USA and Australia (the latter as *U. clathratioides*; Fig. S1). The Brazilian sequence diverged from other *U. torta* by 0.07%–0.34%.

Ulva sp. nov., initially identified as '*U. linza*', formed an independent and well-supported clade in the *tuf*A analyses

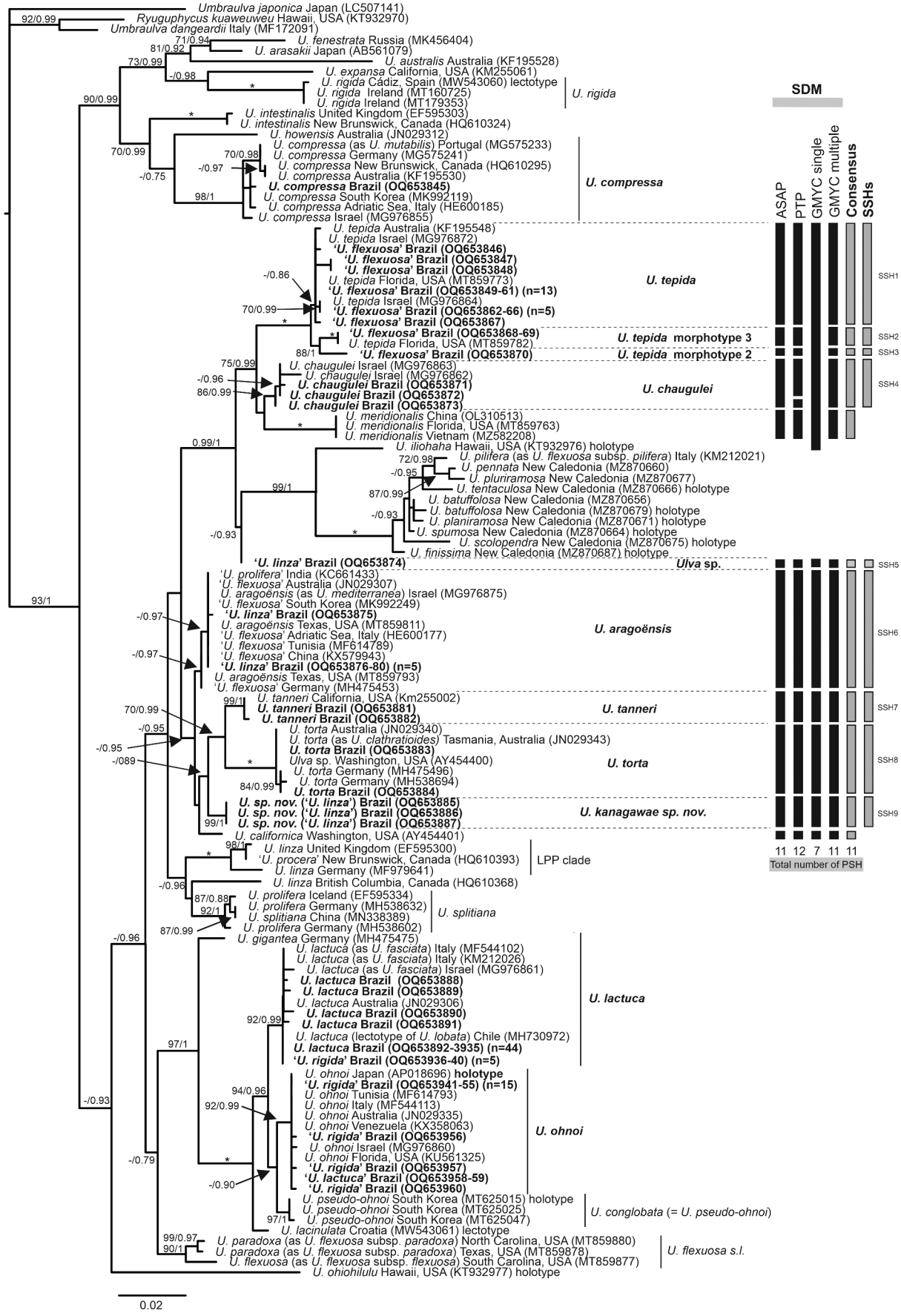


Fig. 1. Maximum-likelihood derived topology of *tuf*A sequences for *Ulva* taxa, and results of species-delimitation methods. Bootstrap percentages and Bayesian posterior probabilities (BP/PP) are indicated on the branches. Samples generated in this study are in bold; '-' indicates lack of bootstrap support/posterior probability or values under 70 /0.7; *, full support; N, number of samples. Black vertical bars represent each of the PSH species delimitation methods applied. Consensus of species delimitation methods and SSH are represented by vertical grey bars.

(Fig. 1). However, its phylogenetic relationship to other *Ulva* species was not well-supported. Morphologically, *Ulva sp. nov*. showed a very distinct habit from other '*U. linza*' morphotypes, forming narrow, very tangled thalli, despite its distromatic central thallus with tubular monostromatic margins. All SDMs applied supported a single taxonomic entity (SSH9) for these samples (Fig. 1). Our *tuf*A sequences of *Ulva sp. nov*. were 100% identical. *Ulva sp. nov*. diverged from *U. linza* (EF595300, UK) by 2.63%.

The Brazilian sequences of *Ulva lactuca* included some samples initially identified as '*U. rigida*', especially due to its laminar thalli with marginal teeth (Figs 1, S1). In the *tuf*A analyses, *U. lactuca* showed close relationship with *U. ohnoi* and *U. pseudo-ohnoi* Hyung W. Lee, Jeong Chan Kang & M.S. Kim with moderate to high support (94% BP; 0.96 PP; Fig. 1). The Brazilian *tuf*A sequences of *U. lactuca* formed a clade with sequences from Australia, Italy and Israel plus the lectotype of *U. lobata* (Kützing) Harvey from Chile, sequenced and synonymized with *U. lactuca* by Hughey *et al.* (2019). The authentic European *U. rigida* clade including its lectotype from Cádiz, Spain, sequenced by Hughey *et al.* (2021b), was distantly related to the samples identified as '*U. rigida*' from Brazil (Fig. 1). In the *rbc*L analyses, the relationships among *U. lactuca, U. ohnoi* and '*U. pseudo-ohnoi*' were not resolved but do not contradict the *tuf*A phylogeny (Fig. S1). The Brazilian *rbc*L sequences of *U. lactuca* clustered with the holotype of *U. lactuca*, the epitype of *U. fasciata* (Egypt) and the lectotype of *U. lobata* (Chile).

Our *Ulva ohnoi* sequences were initially identified as '*U. rigida*' (with marginal teeth), or as '*U. lactuca*' (without marginal teeth). The *tuf*A Brazilian sequences formed a clade with sequences from several localities, and with an authentic culture of *U. ohnoi*, strain KU-MACC: KU-3321 (type locality Japan, Kochi Prefecture, Tosa Bay, AP018696; Fig. 1). Authentic *U. rigida* was distantly related to the Brazilian sequences of *U. ohnoi*. Our *rbc*L sequences showed a similar relationship of our *tuf*A sequences (Fig. S1).

Our *rbc*L phylogeny grouped *U. pseudo-ohnoi* and *U. conglobata* Kjellman in a high supported clade for BI (0.95 PP; Fig. S1). Partial *rbc*L sequences (110 bp) from the type material of *U. conglobata* (*U. conglobata* f. *conglobata* MT815850, lectotype, and *U. conglobata* f. *densa* Kjellman MT815853, holotype; Hughey *et al.* 2021a) were included in our analyses and compared with *U. pseudo-ohnoi* (Fig. S1). Another sequence of *U. conglobata* (as *Ulva* sp., AB894326, from Japan, its type locality) was 1,350 bp long (Matsumoto & Shimada 2015) and grouped in this same clade. The complete sequence of *U. conglobata* from Japan was 100% identical to the holotype and isotype of *U. pseudo-ohnoi*.

A single Brazilian sequence of *U. compressa* (ES91) clustered in a *U. compressa* clade with sequences from different localities in both *tuf*A and *rbc*L analyses (Figs 1, S1). There is no *tuf*A sequence of *U. compressa* from the type locality (probably Bognor, Sussex, England; Hayden *et. al.* 2003) for comparison. Our *rbc*L analyses included a sequence from Ireland, near the type locality, and whose divergence from the Brazilian sequence was low, only 0.15% (Fig. S1).

Morphological analyses

Using new collections of *Ulva* from southeastern Brazil, we recognize 10 taxa of *Ulva*, three of which with distromatic blades: *U. lactuca, U. ohnoi* and *U. tanneri*; three with entirely tubular monostromatic thallus: *U. compressa, U. tepida* and *U. torta*; three with distromatic blades with tubular margins: *U. aragoënsis, Ulva sp. nov*. and *Ulva* sp.; and one with specimens that were either completely tubular or formed distromatic blades with tubular margins: *U. chaugulei*. The molecular and morphological approaches carried out added to a detailed analysis of the Brazilian literature, allowing us to re-assess the names of *Ulva* species identified from southeastern Brazil, and revealing that species names traditionally cited for this region, *U. flexuosa, U. linza, U. paradoxa* and *U. rigida*, are misapplied names. Our SDM and phylogenetic results supported the description of a new species for an entity sister to *U. torta* and *U tanneri* from Brazil (see below and Figs 2–6). *Ulva tepida* constituted a species complex that could be split into three distinct species (Figs 7–16). Detailed morphological data, including descriptions, illustrations, misapplied names and remarks for other species studied are presented in Supplementary material (Morphological descriptions of the studied Brazilian *Ulva* species; Figs S2–S39).

Ulva kanagawae V. Carneiro, N.T. Martins & Cassano *sp. nov.*

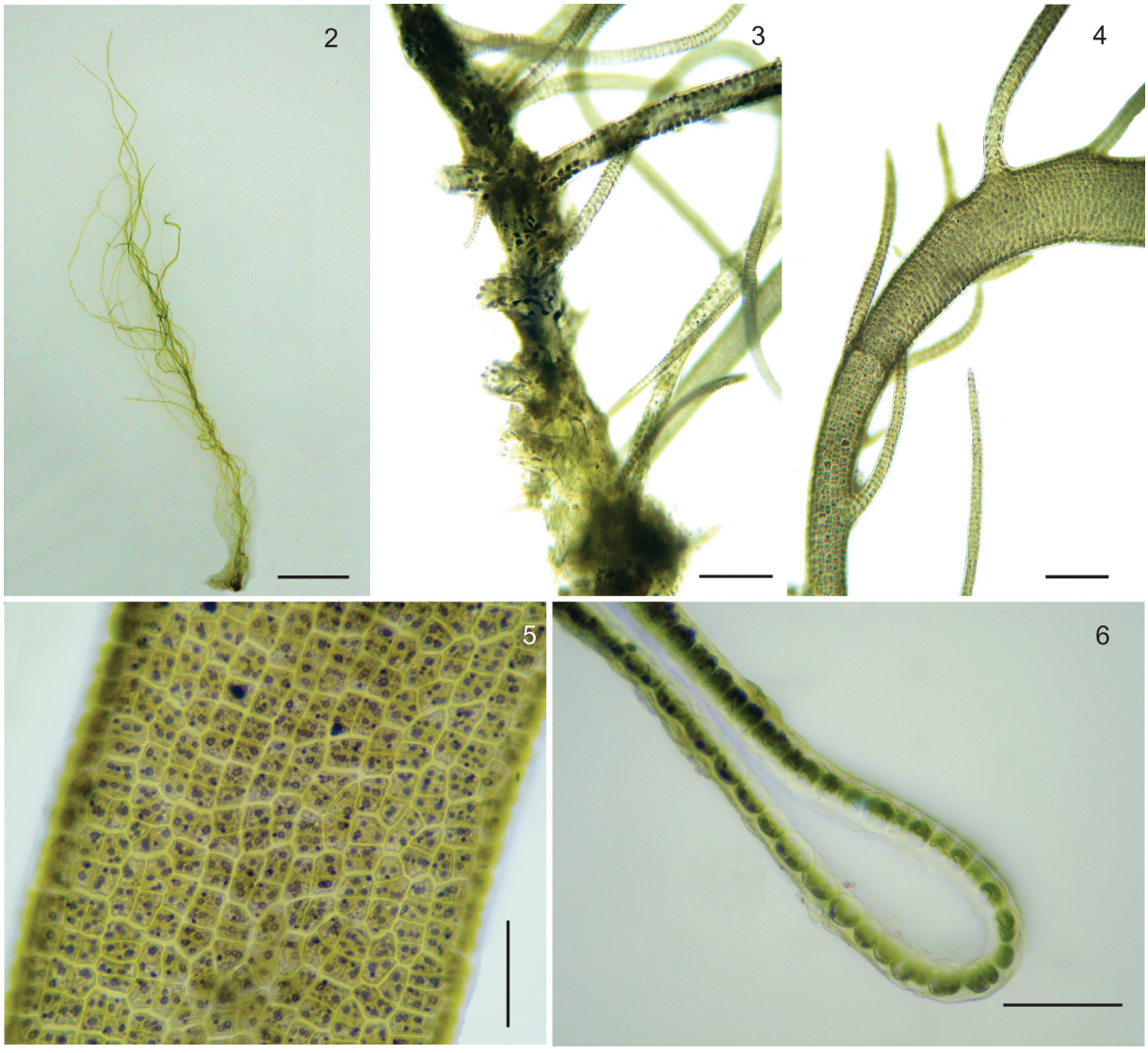
Figs 2–6

DESCRIPTION: Thallus membranous in texture, often gregarious, light green in colour, 8.0–17 cm high (Fig. 2), attached to the substratum by a tiny discoid holdfast, 82.5 µm diameter (Fig. 3). Stipe cylindrical, elongate and simple, 30–55 µm in diameter. Main axes cylindrical in basal portions, simple or branched into up to two orders of branches, often mutually tangled, 30–55 µm in diameter; main axes compressed in the mid- to apical region of the thallus, with narrow ribbon-like frond, tortuous, constricted and spirally twisted in some portions, 97.5–167.5 µm wide. Lateral branches with the same characteristics as the main axes, except for a smaller diameter, 15–45 µm. Proliferations abundant at the base of the thallus, filiform, simple, tortuous, tapered towards the apices, 15–45 µm in diameter. Short spine-like branchlets sparsely disposed on the stipe, 15–30 µm in diameter, uni- or multi-seriate (Fig. 4) ending by a triangular apical cell. In surface view, cells are square to rectangular, sometimes angular, irregularly arranged in the compressed portions, 15–40 × 5.0–25 µm (Fig. 5). In transverse section, thallus distromatic at the central portions and monostromatic at the margins (Fig. 6); tubular base entirely monostromatic, with square to slightly rectangular cells, 12.5–15 × 10–12.5 µm. Thallus thickness 25–32.5 µm in the mid-upper portions. Chloroplasts are parietal, cup-shaped with (1–2)3–4(–5) pyrenoids per cell (Fig. 5). Fertile cells in the apical portions of the thallus, darker in colour.

HOLOTYPE: SPF58660, collected 26 September 2019 by V.A.R. Carneiro & R. Oliveira, deposited in the herbarium of University of São Paulo (SPF), São Paulo, Brazil. GenBank accession for *tuf*A sequence of holotype: OQ653885; for *rbc*L sequence of holotype: OQ687080.

ISOTYPE: SP514236, deposited in the herbarium of the Institute of Environmental Research, São Paulo, Brazil. GenBank accession of *tuf*A sequence: OQ653886.

PARATYPE: SPF58661, Boqueirão beach, channel 3, collected 26 September 2019 by V.A.R.Carneiro & R.Oliveira, deposited in the



Figs 2–6. *Ulva kanawagae sp. nov.*
Fig. 2. Habit of the thallus showing spirally twisted axes. Scale bar = 1 cm.
Fig. 3. Detail of base showing holdfast. Scale bar = 200 µm.
Fig. 4. Detail of branchlets. Scale bar = 100 µm.
Fig. 5. Surface view of the apical portion showing cells with multiple Lugol-stained pyrenoids. Scale bar = 50 µm.
Fig. 6. Transverse section showing the tubular monostromatic margin. Scale bar = 50 µm.

herbarium of the University of São Paulo (SPF), São Paulo, Brazil. GenBank accession of *tuf*A sequence: OQ653887.

TYPE LOCALITY: 23°58.15′S, 46°20.73′W, José Menino Beach, channel 1, Santos, São Paulo, Brazil.

HABITAT: Brackish environment. Epilithic, growing on the edges of artificial rainwater channels 1 and 3 connected to the sea (José Menino and Boqueirão beaches, Santos, São Paulo) forming dense mats, usually associated with sediments; also growing on pieces of wood and other debris in the channels. Associated with *Ulva tepida*.

ETYMOLOGY: The species is named in honour of Dr. Amélia Iaeca Kanagawa, a Brazilian phycologist, for her contributions to our knowledge of the green macroalgae of Brazil.

REMARKS: Although *Ulva kanagawae* has the anatomical characteristic of *U. linza* (Joly 1957; Kanagawa 1983; Barata 2004; Coto & Pupo 2009), it differs from '*U. linza*' (now *U. aragoënsis*) by the smaller width of the laminar portions, the presence of branching and the number of pyrenoids, often higher. Furthermore, *U. kanagawae* differs from all tubular taxa studied in Brazil due to its unique tangled and coiled thalli, which are difficult to dissociate. It was not closely related to the LPP clade that contained sequences of *U. linza*, including one from the region of the type locality (United Kingdom, EF595300) considered here as authentic *U. linza*. All SDMs resolved our samples as a distinct species. Therefore, our results strongly support the proposal of a new species, *Ulva kanagawae sp. nov.*

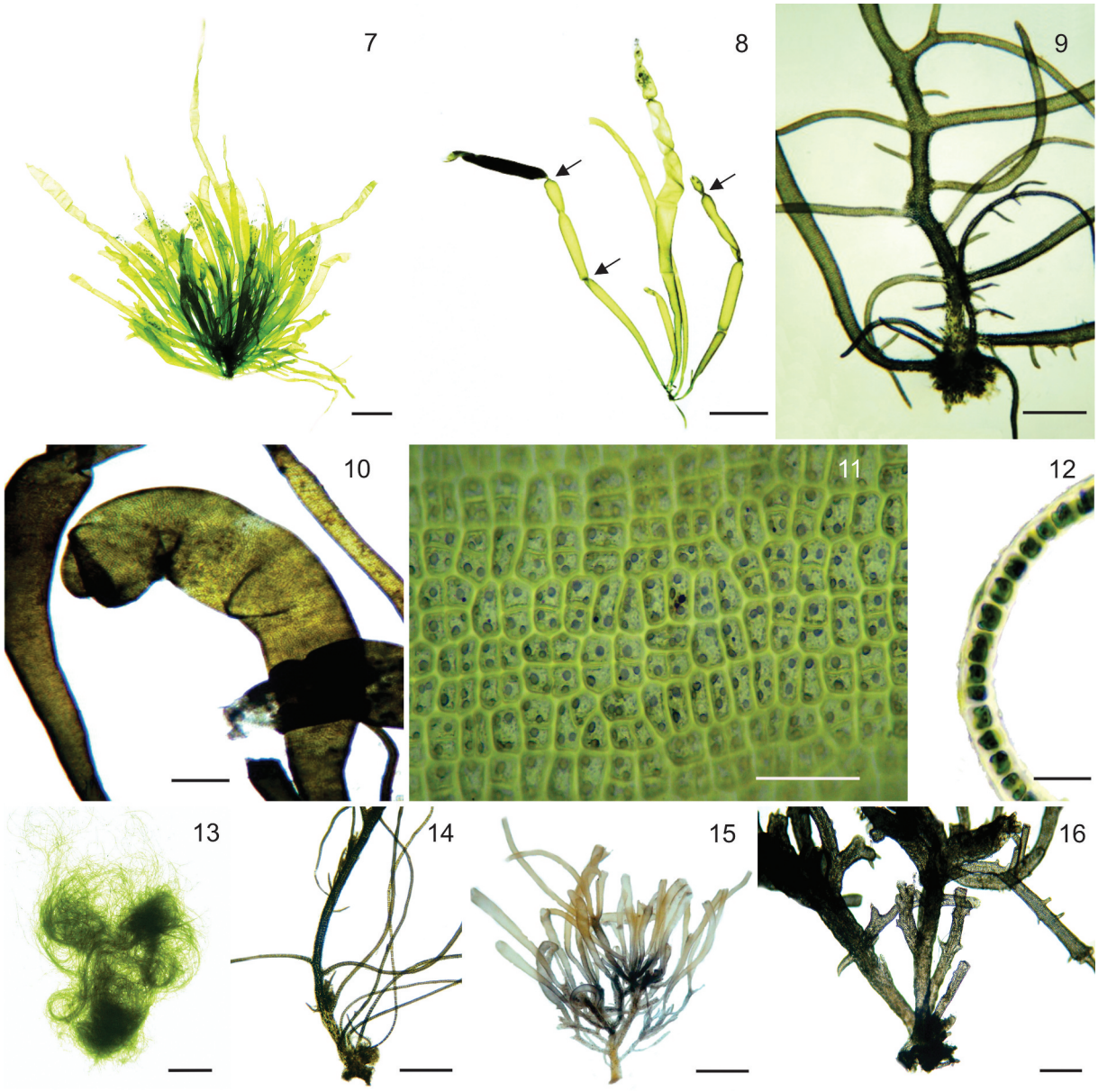
Ulva tepida Masakiyo & S. Shimada (2014, p. 11, figs 4, 16–33)

Figs 7–16

HETEROTYPIC SYNONYMS: *Ulva paschima* Bast in Bast *et al.* (2014, Article e109295, p. 6 of 8, fig. 1); *Ulva sapora* J.A. Phillips, R.J. Lawton & C. Carl in Phillips *et al.* (2016, p. 59, figs 2–9).

TYPE LOCALITY: Enoshima Island, Fujisawa, Kanagawa Prefecture, Japan.

DESCRIPTION: Thallus membranous or rough in texture, gregarious, light to dark green in colour, 1.9–8.7 cm high (Figs 7, 8), attached to the



Figs 7–16. *Ulva tepida*, and its three morphotypes.
Figs 7, **8**. Morphotype 1. Habit of thalli showing tubular and constricted axes (arrows). Scale bar = 5 mm.
Fig. 9. Morphotype 1. Detail of base showing holdfast. Scale bar = 600 µm.
Fig. 10. Morphotype 1. Detail of dilated apical portion of the thallus. Scale bar = 200 µm.
Fig. 11. Morphotype 1. Surface view of the basal portion showing cells with stained pyrenoids. Scale bar = 50 µm.
Fig. 12. Morphotype 1. Transverse section of base showing tubular monostromatic thallus. Scale bar = 25 µm.
Fig. 13. Morphotype 2. Habit of the thallus showing entangled filaments. Scale bar = 1 cm.
Fig. 14. Morphotype 2. Detail of base showing holdfast. Scale bar = 2 cm.
Fig. 15. Morphotype 3. Habit of the thallus showing branched tubular frond. Scale bar = 1 mm.
Fig. 16. Morphotype 3. Detail of base showing discoid holdfast. Scale bar = 500 µm.

substratum by one or more discoid holdfasts, 125–350 µm diameter. Thallus tubular radially or irregularly branched at the base (Fig. 9), compressed, inflated, constricted (Fig. 8) or pleated in the upper portions, 60–3,000 µm wide (Fig. 10). Stipe inconspicuous. Lateral branches cylindrical, sparse or abundant, simple or irregular to unilaterally branched, often becoming wider and compressed towards the apices, 15–1,500 µm wide. Spine-like branchlets disposed at the base of the lateral branches and main axes, uni- or multi-seriate, ending by a triangular apical cell, 12.5–17.5 µm diameter (Fig. 9). In surface view, cells are square to rectangular, regularly arranged in longitudinal rows only at the tubular base (Fig. 11), and irregularly arranged in the compressed mid-apical portions, 5.0–30 × 5.0–25 µm. In transverse section, thallus entirely monostromatic with rectangular to square cells, 12.5–32.5 × 7.5–20 µm (Fig. 12). Thallus thickness 87.5–100 µm in the compressed upper portions. Chloroplasts are parietal, laminar or cup-shaped with (1–)2–4(–5) pyrenoids per cell (Fig. 11). Fertile cells in the apical portions of the thallus, darker in colour.

Two other morphotypes of *U. tepida* (morphotypes 2 and 3) were observed with distinct morphology from the typical one. Morphotype 2 has filamentous thalli, very entangled, flexible, gregarious, 0.5–10.1 cm high (Fig. 13), with main axes narrower than typical *U. tepida*, 60–162.5 µm wide. Thalli branched at the base with lateral branches narrower than main axes, simple, very elongated and tortuous, regular throughout, 30–45 µm wide (Fig. 14). In surface view, cell dimensions are slightly larger than typical *U. tepida*, 7.5–40 × 10–30 µm, and with higher number of pyrenoids per cell (1–)2–4(–7).

Morphotype 3 has thalli rough in texture, gregarious, smaller than two other morphotypes, 0.4–0.7 cm high (Figs 15, 16), with main axes narrower than typical *U. tepida*, 160–170 µm wide. Lateral branches short, often wider than main axes, simple or profusely branched in the upper portions, 50–210 µm wide (Fig. 15). Apices of lateral branches are slightly obtuse, often with irregularly inflated portions. In surface view, cells slightly larger than typical *U. tepida*, 7.5–20 × 5.0–47.5 µm, and with higher number of pyrenoids per cell than the two other morphotypes, (1–)3–4(–8). Both morphotypes are entirely tubular monostromatic with spine-like branchlets present at the base of the main axes and with cells arranged in longitudinal rows throughout the thalli.

HABITAT: Marine and brackish environments. Epilithic or epizoic on the Brown Mussel *Perna perna* Linnaeus in intertidal zone, in protected to wave-exposed rocky shores, generally forming tufts. Also growing on artificial substrates, such as plastic bags, or partially buried in sand. Species common in the studied area, collected in most of the sampling sites, associated with *U. aragoënsis, U. chaugulei* and *U. lactuca*. Morphotypes 2 and 3 growing in brackish or marine environments. Morphotype 2 was rare, collected only in Santos, São Paulo state, growing on the edges of artificial rainwater channel 3, forming dense tufts. Morphotype 3 was rare, collected only in Rio Escuro, Ubatuba, São Paulo state, found in mangrove or epilithic in intertidal zone in exposed wave exposure on rocky shore.

REMARKS: Our specimens of *U. tepida* showed the greatest degree of morphological variation (Figs 7–16) among the tubular species analysed, with the distinct morphologies corroborated by most SDMs applied. Initially we identified them as '*U. flexuosa*' based on morphological characteristics according to Brazilian literature: thallus tubular simple or branched at the base, compressed in the upper portions, variable in width, with cells arranged in longitudinal rows only at the base, and 2–5 pyrenoids per cell (Kanagawa 1983; Barata 2004; Coto & Pupo 2009). However, overall, most the Brazilian specimens (Figs 7–12, typical morphology) agreed with the original description of *U. tepida* by Masakiyo & Shimada (2014). The filamentous morphotype 2, found in brackish waters (Figs 13–14), was morphologically similar to *U. tepida* from Israel (Krupnik *et al.* 2018) by the long and narrow branched tubular thallus, similar to hairs (<1 mm wide), as well as the filiform thalli described for *U. tepida* (as *U. sapora*) from Australia by Phillips *et al.* (2016), but with up to 10 pyrenoids per cell. The morphotype 3 found in brackish (mangrove) and marine waters (Figs 15–16) showed smaller tubular thalli not compressed, rough in texture, and with a higher number of pyrenoids per cell, up to eight. Misapplied names for southeastern Brazil and additional remarks are available in Supplementary material.

DISCUSSION

Our phylogenetic results supported ten distinct taxa for southeastern Brazil. Most of them can be recognized as distinct species, whereas *U. tepida* corresponded to a species complex, corroborating Melton & López-Bautista (2021). The SDMs applied to *tuf*A sequences of selected clades supported our phylogenetic results recognizing nine independent taxa, including an undescribed species, *Ulva* sp., and splitting *U. tepida* into three separate, hypothetical species.

The DNA barcode marker *tuf*A proved to be effective for the application of different species delimitation methods, being largely consistent for our dataset. The effectiveness of this marker was recently confirmed by Tran *et al.* (2022), whose results for different SDM were more congruent across methods than those obtained for *rbc*L and ITS, demonstrating that *tuf*A is a more suitable marker for species delimitation in *Ulva*. Tran *et al.* (2022) verified the greater tendency to oversplit species using ITS and *rbc*L than *tuf*A.

Of the 15 taxa commonly reported for Brazil, only three were confirmed by molecular data: *U. compressa, U. lactuca* and *U. ohnoi*. Species traditionally cited for the southeastern Brazilian marine flora, such as *U. flexuosa, U. paradoxa, U. linza* and *U. rigida* were revealed to be misapplied names of *U. tepida, U. torta, U. aragoënsis* and *U. ohnoi*/*U. lactuca*, respectively.

Ulva ohnoi, U. lactuca and *U. tepida* were the most common species in the studied area, recorded for several localities in southeastern Brazil. Particularly, *U. ohnoi* has a wide and unrecognized distribution along the southeastern coast. In contrast, *U. chaugulei, U. compressa, U. kanagawae, U. tanneri, U. torta* and *Ulva* sp. were uncommon.

Four taxa with several citations for southeastern Brazil, namely *U. chaetomorphoides* (Børgesen) H.S. Hayden *et al.*, *U. clathrata* (Roth) C. Agardh, *U. intestinalis* Linnaeus and *U. prolifera* O.F. Müller (Yoneshigue-Braga 1970; Mitchell *et al.* 1979; Yoneshigue 1985; Reis & Yoneshigue-Valentin 1996; Gestinari *et al.* 1998; Barata 2004; De-Paula *et al.* 2020a) were not found in this study (Table 1). Three of these species have sequences available in GenBank (*U. clathrata, U. intestinalis* and *U. prolifera*), although no sequences are from the type specimens, and only *U. prolifera* has a sequence from the topotype based on the ITS2 region and the 5S ribosomal spacer region (Cui *et al.* 2018). For *U. chaetomorphoides* there is no DNA sequence data. Therefore, based on our *tuf*A and *rbc*L sequences there is no genetic evidence of any sequences from Brazil of *U. clathrata, U. intestinalis* and *U. prolifera*. There is the possibility that these species were not collected due to the difficulty in distinguishing them in the field. In addition, *U. clathrata* is considered common in mangroves in Brazil (Mitchell *et al.* 1979), areas less sampled in this study. Although it is known that changes in floristic composition can occur due to anthropogenic impacts (Oliveira Filho & Berchez 1978; Taouil & Yoneshigue-Valentin 2002; De-Paula *et al.* 2020b), it is possible that these species, in fact, do not occur in Brazil, or that they are perhaps misapplied names, as they were reported based only on morphology.

Ulva chaugulei and *U. tanneri* are new occurrences for Brazilian waters. After its original description, *U. chaugulei* was reported from Iran (Pirian *et al.* 2016), Israel (Krupnik *et al.* 2018) and China (Xie *et al.* 2020), and was considered as a potentially introduced species into the Mediterranean Sea or, perhaps, previously reported as *U. linza* for the area (Krupnik *et al.* 2018). This first record of *U. chaugulei* for Brazil also expands its geographic distribution to the Atlantic Ocean. The two sampling sites of *U. chaugulei* are close to important ports, the São Sebastião Port and the Santos Port Complex, São Paulo, the latter the largest port in Latin America. Similarly, *U. tanneri* was sampled in an area close to the Port of Rio de Janeiro, Guanabara Bay, one of the busiest in the country. This species is cited from Pacific North America, California (Tanner 1980; Hayden & Waaland 2004) and Mexico (Aguilar-Rosas *et al.* 2005), Central American Pacific, Panama (Fernández-García *et al.* 2011), Japan (Lima & Fukusumi 1996; Hayden & Waaland 2004; Matsumoto & Shimada 2015), Hawaiian Islands (Huisman *et al.* 2007), Australia and New Zealand (Kraft

et al. 2010; Nelson *et al.* 2021), and South Africa on the east coast of the Atlantic (Joska & Bolton 1992; Stegenga *et al.* 1997). Thus, this first occurrence of *U. tanneri* on the Brazilian coast also expands its geographic distribution to the western Atlantic Ocean. Despite their occurrence in areas of intense ship traffic, we cannot confirm that both are recent introductions on the Brazilian coast due to the difficulty in identification based on morphology. Thus, we consider them as cryptogenic species.

Our study showed a high morphological plasticity in *Ulva* species, which makes morphological identification extremely difficult, and it is almost impossible to determine characters that have diagnostic value capable of effectively separating species. *Ulva* species have recognized external morphological crypticity (Lagourgue *et al.* 2022) and even the required close morpho-anatomical analyses used to distinguish them, as pointed out by Hughey *et al.* (2019), Steinhagen *et al.* (2019b) and Lagourgue *et al.* (2022), can lead to misidentifications due to overlapping characters previously considered diagnostic, as well as the interpretation of characteristics that can be subjective; for example, *U. tepida* was firstly identified as '*U. flexuosa*' (this study) and as '*U. intestinalis*' by Chávez-Sánchez *et al.* (2019), although the morphological characteristics that led to either identification essentially were the same. Likewise, our *U. torta* is a misapplied name of *U. paradoxa sensu* Kanagawa (1983), whereas the same species corresponded to two different morphotypes, '*U. clathrata*' and '*U. flexuosa*' in Chávez-Sánchez *et al.* (2019). In addition, the presence of lateral branches ending by uniseriate filaments, a characteristic used for the identification of *U. paradoxa sensu* Kanagawa (1983), was not observed in other reports of *U. torta* (Bliding 1963; Boraso de Zaixso 2004, 2013; Cormaci *et al.* 2014; Chávez-Sánchez *et al.* 2019; Steinhagen *et al.* 2019a).

Still for tubular *Enteromorpha*-like thalli, distromatic thalli with tubular margins are described for the first time for *U. aragoënsis* based on the Brazilian material, while our specimens of *U. chaugulei* had either distromatic or entirely monostromatic thalli, a variation described by Xie *et al.* (2020) from China but not found in the original specimens by Kazi *et al.* (2016). The '*U. linza*' morphology (thallus centrally distromatic with tubular margins) was convergent in three genetically distinct species, *U. aragoënsis, U. kanagawae sp. nov.* and *Ulva* sp. None of the three was within the LPP clade which included a sequence of *U. linza* (EF595300, UK, East Cornwall, Greenaway), from a region near the type locality, Sheerness, Kent, England (Hayden *et al.* 2003). Since sequences of the type specimen are lacking, we regarded the sequence EF595300 as authentic *U. linza*. Therefore, our results confirm that '*U. linza*' is a misapplied name for southeastern Brazil, and that the distrosmatic/monostromatic morphology that was previously used for segregating species in the Brazilian literature (Joly 1957) is not of diagnostic value in identifying species with tubular thalli.

Our tubular specimens of *U. tepida* were initially identified as '*U. flexuosa*' based on morphological characteristics, as defined in previous Brazilian works (Kanagawa 1983; Barata 2004; Coto & Pupo 2009). None of our *U. tepida* sequences clustered in the authentic European *U. flexuosa* clade (Mareš *et al.* 2011; Hiraoka *et al.* 2017). Thus, we considered '*U. flexuosa*' as a misapplied name of *U. tepida* for southeastern Brazil. The SDM analyses indicated that *U. tepida* is a species complex, and specimens showed the greatest morphological variation with three morphotypes recognized. Similar result was obtained by Melton & López-Bautista (2021) where high divergence based on plastid sequences led them to consider dividing the *U. tepida* clade into two different species. However, the ITS2 dataset did not support the separation of *U. tepida*, which was maintained by them as a single species. Although most SDMs divided our *U. tepida* into three different hypothetical species, we consider it premature to establish new species for these and suggest that further molecular studies are needed, including a broader sampling and the use of other markers (e.g. ITS).

For laminar species, part of the samples with '*U. lactuca*' morphology (thallus expanded with smooth margins) and '*U. rigida*' morphology (thallus expanded with marginal teeth) corresponded to *U. ohnoi*, as verified also by Chávez-Sánchez *et al.* (2019). Therefore, presence or absence of marginal teeth is not a reliable feature to separate these species. Taking into account that our sequenced samples of *U. ohnoi* and *U. lactuca* grouped with authentic *U. ohnoi* (Hiraoka *et al.* 2004; Suzuki *et al.* 2018) and *U. lactuca* (Hughey *et al.* 2019), and that none of our samples previously identified as '*U. rigida*' clustered in the authentic European clade of this species (Hughey *et al.* 2021b), we argue that '*U. rigida*' is a misapplied name for *U. ohnoi* for the southeastern region of Brazil, constituting its first record for that region and for the continental portion of Brazil.

The genetic divergences observed between species for the two markers used in this study are compatible with those described by others (Saunders & Kucera 2010; Kirkendale *et al.* 2013; Steinhagen *et al.* 2019a, b; Melton & López-Bautista 2021). The major clade formed by *U. lactuca* and *U. ohnoi* are closely related with low interspecific divergence, (similar to Melton & López-Bautista 2021), but reproductively isolated, as shown by cross-breeding tests (Hiraoka *et al.* 2004). *Ulva conglobata* (as '*U. pseudo-ohnoi*') is also closely related to *U. ohnoi* and *U. lactuca*, showing low interspecific minimum values between itself and *U. ohnoi* (0.42% for *tuf*A; 0.3% for *rbc*L) and *U. lactuca* (0.68% *tuf*A; 0.47% for *rbc*L). Cross-breeding tests would be the way to determine whether these species are reproductively isolated or not.

Although most *Ulva* clades can be confidently circumscribed based on current molecular species concepts, it will still be necessary to review the boundaries of these species complexes that involve, for example, taxa currently considered conspecific with *U. tepida* (*U. paschima* and *U. sapora*) and at least five infraspecific taxa of *U. flexuosa*, such as subsp. *paradoxa*, of which the elevation to species level is not widely accepted (Melton & López-Bautista 2021; Guiry & Guiry 2023). In addition, the proposition of new species for *Ulva* is made in spite of several problems, such as the fact that there are many named species, many of them have been synonymized, and the absence of sequences from type specimens for most species. For example, many *Ulva* species with a worldwide distribution and highly cited, such as *U. aragoënsis, U. compressa,*

U. intestinalis, U. linza and *U. torta* do not have their type specimens sequenced, which, if successful, may change the current taxonomic status of *Ulva* species. However, considering the large number of described species, it may be difficult or even impossible to obtain sequences for all type material without which there is no way to ensure whether new collections are indeed undescribed species or could be assigned old names (De Clerck *et al.* 2013; Verbruggen 2014; Leliaert & De Clerck 2017). In this context, it seems to be more appropriate to take a pragmatic approach proposing new species based on molecularly defined types as advocated by Sherwood *et al.* (2019) as not naming potentially new lineages would result in their diversity not being recognized within the Linnaean taxonomic scheme. Thus, we assumed a pragmatic path proposing *U. kanagawae sp. nov*. Although it has been possible to identify it by a combination of morphological characteristics that distinguishes it from the other studied species, these characters are variable within and between species; therefore, its proposition is based on its phylogenetic position and on the SDM applied to *tuf*A.

Our *rbc*L phylogeny and genetic distances supported the recognition of the conspecificity of *Ulva pseudo-ohnoi* and *U. conglobata*. *Ulva pseudo-ohnoi* was previously treated as *Ulva* sp. 1 by Matsumoto & Shimada (2015), who highlighted its morphological similarity to *U. conglobata*. Despite this, Lee *et al.* (2019) decided to describe *Ulva* sp. 1 as the new species *U. pseudo-ohnoi* from South Korea, arguing that Matsumoto & Shimada (2015) did not provide molecular proof of the original material of *U. conglobata*. The comparison of the sequenced type materials of *U. conglobata* (Hughey *et al.* 2021a) and *U. pseudo-ohnoi* (Lee *et al.* 2019) showed that *U. pseudo-ohnoi* should be reduced to a heterotypic synonym of *U. conglobata*. Furthermore, Hughey *et al.* (2021a) synonymized *U. conglobata* f. *densa* with the typical form as a single species, *U. conglobata*.

This study comprised a first comprehensive effort to unveil the diversity of *Ulva* in Brazil, which still needs broader sampling, expanding to the coasts of northeastern and southern Brazil and covering under-sampled environments, such as mangroves. Added to this a detailed morphological analysis, the use of other molecular markers, such as ITS, and phylogenomic analyses that promote a more robust phylogeny are essential to better understand the phylogenetic relationships within the genus and clarify the diversity of *Ulva* on the Brazilian coast.

Taxonomic change

Ulva pseudo-ohnoi and *U. conglobata* are here considered conspecific.

Ulva conglobata Kjellman (1897, p. 10, pl. 2, figs 1–7; pl. 3, figs 9–14). Syntype localities: Yokohama, Goto and Amakusa, Japan (Kjellman 1897, p. 11). Heterotypic synonyms: *Ulva pseudo-ohnoi* Hyung W. Lee, Jeong Chan Kang & M.S. Kim in Lee *et al.* (2019, p. 257, figs 3A, 3B, 3D–3F); *U. conglobata* f. *densa* Kjellman (1897, p. 11, pl. 2, figs 8–11; pl. 3, fig. 15); *U. rigida* f. *densa* (Kjellman) Feldmann (1937, p. 197).

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