

Taxonomic assessment of blade-forming *Ulva* species (Ulvales, Chlorophyta) in the Galápagos Archipelago, Ecuador using DNA sequencing

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Abstract

DNA sequences were obtained from 32 blade-forming *Ulva* specimens collected opportunistically in 2018 and 2019 from four islands in the Galápagos Archipelago, Fernandina, Floreana, Isabela and San Cristóbal. The loci sequenced were nuclear encoded ITS and plastid encoded *rbcL* and *tufA*, all recognized as barcode markers for green algae. Four species were found, *Ulva adhaerens*, *U. lactuca*, *U. ohnoi* and *U. tanneri*, all of which have had their type specimens sequenced, ensuring the correct application of these names. Only one of these, *U. lactuca*, was reported historically from the archipelago. *Ulva adhaerens* was the species most commonly collected and widely distributed, occurring on all four islands. Previously known only from Japan and Korea, this is the first report of *U. adhaerens* from the southeast Pacific Ocean. *Ulva ohnoi* was collected on three islands, Isabela, Floreana, and San Cristóbal, and *U. lactuca* only on the last two. *Ulva tanneri* is a diminutive, 1-2 cm tall, high intertidal species that is easily overlooked, but likely far more common than the one specimen that was collected. This study of blade-forming *Ulva* species confirms that a concerted effort, using DNA sequencing, is needed to document the seaweed flora of the Galápagos Archipelago.

Key Words DNA barcoding ITS, *rbcL*, *tufA*, *Ulva adhaerens*, *U. lactuca*, *U. ohnoi*, *U. tanneri*

Word count:

1 Introduction

Our knowledge of the benthic marine algal flora of the Galápagos Archipelago is based primarily on one late 19th Century collection and three early to mid-20th Century collections, all published in the 20th Century (Farlow 1902, Lemoine 1929, Taylor 1945, Dawson 1963). These published reports were compiled by Silva (1966) in a checklist of 311 species. Subsequent to Silva (1966) the main sources of information on the benthic marine algae, including the updating of name changes, have been the checklists, now online, assembled by the Charles Darwin Foundation, viz. Cyanobacteria (Chiriboga et al. 2014, last updated), Chlorophyta (Ruiz and Ziemmeck 2014, last updated), Phaeophyceae (Ruiz and Ziemmeck 2016a, last updated) and Rhodophyta (Ruiz and Ziemmeck 2016b, last updated). Only one genus of benthic marine algae from the Galapagos Archipelago has been treated in the 21st Century, the siphonous green alga, *Codium* (Chacana et al. 2016). All of these studies were based on the morpho-anatomical examination of the algae using classical light microscopy.

Only two studies (Boo et al. 2016, Anslan et al. 2021) used DNA sequencing to identify algae from the Galápagos. Boo et al. (2016) sequenced the mitogenomes of two herbarium specimens of the red alga genus *Gelidium* (Gelidiales), the holotypes of *G. isabellae* W.R.Taylor and *G. galapagense* W.R.Taylor, both collected by Taylor (1945) and housed in UC (herbarium acronyms follow Thiers 2024). Anslan et al. (2021) performed a metabarcode analysis of algal DNA in fecal samples from two subspecies of the endemic Galapagos marine iguana, *Amblyrhynchus cristatus* subsp. *mertensi* and *A. cristatus* subsp. *godzilla* on Isla San Cristobal. They also sequenced a portion of the *rbcL* gene from field-collected marine macroalgal specimens on Isla San Cristobal from the same sites sampled for *A. cristatus* feces. The algal species were identified mainly by comparing BLASTn analyses of publicly available sequences in NCBI and BOLD.

Many species of marine macroalgae lack diagnostic morpho-anatomical characters (Gabrielson et al. 2018, Hind et al. 2015, Vieira et al. 2014) and/or are morphologically plastic (Gao et al. 2016, Hind et al. 2014, Lewis et al. 1987), so that accurate species identifications are fraught. Since the mid-2000s DNA barcoding has become the preferred method worldwide to identify marine macroalgae (Bartolo et al. 2020, McDevit and Saunders 2009, Saunders and Kucera 2010, Torrano-Silva et al. 2018, Vieira et al. 2021). DNA based species identifications have illuminated just how incorrect many of our identifications have been in the past and revealed a huge amount of cryptic diversity in all benthic marine algae, greens, browns and reds alike, from the tropics to the polar regions. The green macroalgal genus *Ulva* is an exemplar of these problems.

A major advance in the systematics of *Ulva* in recent decades has been the application of DNA sequencing to recent field collected specimens, first by Leskinen and Pamilo (1997, as *Enteromorpha*) and subsequently by many others (e.g., Coat et al. 1998, France (Atlantic); Hayden and Waaland 2004, Northeast Pacific; Heesch et al. 2009, New Zealand; Kraft et al. 2010, Kirkendale et al. 2013, eastern Australia; O'Kelly et al. 2010, Hawai'i; Krupnik et al. 2018, eastern Mediterranean; Chávez-Sánchez et al. 2019, Mexico (Gulf of California); Kang et al. 2019, Korea; Steinhagen et al. 2019 North and Baltic Seas; Xie et al. 2020, China; Dartois et al. 2021, Atlantic, France; Melton et al. 2021, Northeast Atlantic/Gulf of Mexico); Lagourgue et al. 2022, New Caledonia, Tran et al. 2023, Vietnam;. While this approach has been very important to explore the diversity of *Ulva* species, it has not necessarily resulted in the correct application of names for these species. The reason for this is that specimens are placed into species based on DNA sequences, but the historical names applied to these species are based on morpho-anatomical characters. We have known for decades (Papenfuss 1960) that these morpho-

anatomical characters are difficult to apply to *Ulva* species, as the characters are few and the species themselves are morphologically highly variable (Gao et al. 2016).

The second major advance in *Ulva* systematics has been to obtain DNA sequences from *Ulva* type specimens, either by Sanger sequencing a portion of the plastid encoded *rbcL* gene or by high-throughput sequencing to obtain mitochondrial and or plastid genomes (Hanyuda and Kawai 2018, Hughey et al. 2018, 2019, 2021, 2022, 2024). This enables DNA sequences from field-collected material to be directly compared to type sequences, thus ensuring the correct application of names. Indeed, when Fort et al. (2020) compared type specimen sequences to contemporary *Ulva* DNA sequences in GenBank whose identities were determined using morpho-anatomy, many of the over 1,000 named sequences of *Ulva* species in GenBank from localities around the world had been assigned incorrect names. The authors estimated that 21% of named *Ulva* sequences in GenBank were misidentified, and this ranged up to 65% for *U. lactuca*, the generitype species of *Ulva*. These misidentifications have profound consequences for our understanding of the physiology, ecology, biogeography and even commercial utilization of *Ulva* species.

To correctly identify the blade-forming species of *Ulva* in the Galápagos Archipelago, we used targeted PCR methods to characterize field-collected specimens. Four blade-forming *Ulva* species were found, *U. adhaerens* Kaoru.Matsumoto et S. Shimada, *U. lactuca*, *U. ohnoi* M.Hiraoka et S.Shimada, and *U. tanneri* H.S.Hayden et Waaland. These species were also found by Anslan et al. (2021), although *U. adhaerens* was called *Ulva* sp.

2 Materials And methods

Samples of *Ulva* were collected opportunistically from four islands in the Galápagos Archipelago. These are San Cristóbal: four sites, La Lobería, Playa Baquerizo, Punta Carola and

Tijeretas; Fernandina: two sites, Punta Espinosa and Punta Mangle; Floreana: two sites Sur Tres Cuevitas and La Botella; and Isabela: three sites, Concha y Perla, Cuatro Hermanos and Tintorerías (Fig. 1). Specimens were collected by hand via snorkel or SCUBA. Voucher specimens were pressed in the field or at the Galápagos Science Center on Isla San Cristóbal. Fragments for DNA analysis were removed, patted dry and placed into silica gel desiccant. Duplicate voucher specimens were deposited in NCU and QUSF. See Table S1 for collection data and GenBank accession numbers.

Total genomic DNA was extracted and amplified from Galápagos field-collected specimens at the University of North Carolina using the method in Hughey *et al.* (2001). Plastid encoded *rbcL* from field-collected specimens was amplified in two amplicons using the primer pairs *rbcL* start/R750 and F650/*rbcL*end from Shimada *et al.* (2003), with the final sequences trimmed to 1,355 bp to remove primer sequences; plastid encoded *tufA* was amplified using the primer pair *tufG4/tufAR* (Saunders & Kucera 2010), with the final sequence trimmed to 774 bp; nuclear encoded partial SSU, complete ITS1, complete 5.8S, complete ITS2, and partial LSU was sequenced using the primers ITS1 and ITS4 (Shimada *et al.* 2003), with sequences trimmed to 674 bp. Amplification protocols for all PCR reactions followed Hughey *et al.* (2001). PCR products were cleaned with the Qiagen PCR Purification Kit, cycle sequenced, and sent to the DNA Analysis Core Facility at the Marine Sciences Center, University of North Carolina, Wilmington for Sanger sequencing. Sequences were manually aligned and compiled using Sequencher 5.4.6 (Gene Codes Corp., Ann Arbor, Michigan, USA).

Three datasets were prepared, one for each of the sequenced loci, and all were compiled and aligned using MUSCLE (Edgar 2004) as implemented in Geneious Prime (2020.2.4, Biomatters, Auckland, New Zealand) and checked manually. Named sequences of *Ulva* were

downloaded from GenBank to compare to sequences from Galápagos specimens, 50 of ITS ranging in length from 272 bp (ITS2 only was available for *U. 'sublitoralis'*) to 807 bp (depending what proportion of flanking SSU and LSU sequences were included); 50 of *rbcL* ranging in length from 626-1,355 bp; 47 of *tufA* ranging in length from 680-774 bp. Not all of the same loci have been sequenced for all of the *Ulva* species used in the phylogenetic analyses-- for some species only one of the markers is present in GenBank; for others only two are present (see Table S1). Because the datasets did not contain the same taxa, each was analyzed separately. For all datasets three taxa from the family Ulvaceae were used as outgroups, *Percursaria 'percursa'* (C. Agardh) Rosenvinge, *Ulvaria' obscura* var. *blyttii'* (Areschoug) Bliding and *Umbraulva 'japonica'* Bae & I.K. Lee. Throughout this paper, the specific epithets of *Ulva* species whose type specimen(s) have not been sequenced are indicated by ' '. The application of these names to sequences in GenBank may or may not be correct.

Phylogenetic reconstructions with maximum likelihood (ML) and Bayesian inference (BI) for both data sets were carried out using the RAxML (Stamatakis *et al.* 2005) and MrBayes (Huelsenbeck & Ronquist 2001) Geneious Prime plugins, respectively. The RAxML analyses were performed using the GTR + CAT + I model and rapid hill-climbing algorithm for 20 random trees to determine the best starting tree for determining node confidence. Node confidence was then assessed by 1,000 bootstrap replicates and search for best-scoring ML tree. Bayesian analyses were performed using the GTR + gamma + invariable sites model with 4 heated Monte-Carlo Markov Chains for 1,000,000 generations, sampling every 750 generations and with a burn-in period of 250,000 generations.

3 Results

For each of the three loci, the ML and BI phylograms were mostly congruent, and only the BI phylograms (Figs. 2-4) are presented with ML bootstrap values shown at the nodes when $\geq 75\%$ and posterior probabilities when ≥ 0.8 . The Galápagos species occurred in the same major clades within *Ulva* for all three loci (Figs. 2-4). *Ulva adhaerens* consistently occurred in a clade with *U. rigida*, and in *rbcL* was sister to *U. piritoka* for which only an *rbcL* sequence was generated (Fig. 2). *Ulva lactuca* and *U. ohnoi* consistently occurred in a clade with *U. conglobata*, *U. dactylifera*, *U. lacinulata* and *U. taeniata*, with support ranging from moderate (ITS RAxML, Fig. 3) to strong (*rbcL* RAxML, Fig. 2) to full (*rbcL* BI, Fig. 2 and *tufA* RAxML and BI, Fig. 4). Sister taxon relationships among these species varied depending on the locus that was sequenced. *Ulva tanneri* occurred in a clade that was more variable with various combinations of *U. californica*, *U. clathratioides*, *U. 'aragoënsis'* and *U. 'torta'* that lacked support from RAxML and with good to strong support from BI (Figs. 2-4). Note also that the correct application of the names *U. 'torta'* and *U. 'aragoënsis'* is uncertain. Despite its equatorial location, none of the *Ulva* species found in the Galápagos occurs in the large and predominantly tropical to warm water clade (Figs. 2-4) of 14 species (*U. arbuscula*, *U. batuffulosa*, *U. finissima*, *U. iliohaha*, *U. kraftiorum*, *U. meridionalis*, *U. pennata*, *U. planiramosa*, *U. pluriramosa*, *U. scolopendra*, *U. siganiphylla*, *U. spumosa*, *U. tentaculosa*, *U. tepida*, and *U. vietnamensis*).

The DNA sequencing results for all of the markers identified unequivocally the Galápagos *Ulva* specimens to be one of four species, *U. adhaerens*, *U. lactuca*, *U. ohnoi* or *U. tanneri* (Figs. 2-4). Twenty specimen of *U. adhaerens* (Supplemental Figures S1, S2) were collected from sites on all four islands, and 17 were sequenced for *rbcL* with 13 identical to the holotype sequence from Japan and four differing by 1-2 bp; 20 were sequenced for *tufA*, 13 were identical and seven differed by 1-2 bp; 10 were sequenced for ITS, and varied by 2-5 bp from the

holotype sequences from Japan. For *U. lactuca* six specimens (Supplemental Figure S3) were collected from three sites on San Cristóbal and one site on Floreana. All six *rbcL* sequences were identical to each other and to a sequence from the Mediterranean Sea that is linked to the lectotype sequence of *U. lactuca*, and likewise for *tufA*, except for 2 ambiguous bp in one Galápagos sequence. One ITS sequence was obtained and was identical to a *U. lactuca* sequence from Chile. Five specimens of *U. ohnoi* (Supplemental Figure S4) were collected from three sites on San Cristóbal and one each from Isabela and Floreana, and the *rbcL* sequences from these were identical to the *rbcL* holotype sequence from Japan. Likewise, the five *tufA* sequences were identical to a *U. ohnoi tufA* sequence from a specimen from Queensland Australia that can be linked to the *U. ohnoi* holotype specimen. The one ITS sequence from a Galápagos specimen was identical to the ITS sequence from the holotype specimen. Only one specimen of *U. tanneri* was collected from Playa Baquerizo, San Cristóbal (Supplementary Figure S5), and its *rbcL*, *tufA* and ITS sequences were identical to these same sequences from the holotype specimen of *Chloropelta caespitosa* (= *U. tanneri*).

4 Discussion

As a preface to the discussion, what is evident is that all blade-forming *Ulva* species worldwide are cryptic due to their simple construction as a two cell layered blade and their morphological plasticity. It is very difficult to confidently identify *Ulva* species based on morpho-anatomy, either macroscopically (e.g., size and shape of blades, presence/absence of marginal teeth on blade) or microscopically (e.g., cell size, cell shape, number of pyrenoids/cell). All of these characters have been used historically to try to identify *Ulva* specimens to species. An example of the difficulty of identifying *Ulva* specimens using morpho-anatomy are the

studies by Chávez-Sánchez et al. (2017, 2019) in the Gulf of California. In the first paper (Chávez-Sánchez et al. 2017), eight *Ulva* species were identified by morpho-anatomy: *U. acanthophora*, *U. clathrata*, *U. intestinalis*, *U. flexuosa*, *U. lactuca*, *U. lobata*, *U. nematoidea* and *U. rigida*. Subsequently these same specimens were sequenced using the barcoding markers *rbcL*, *tufA* and ITS2 (Chávez-Sánchez et al. 2019). Specimens previously identified by morpho-anatomy as *U. lactuca* and *U. rigida* were *U. ohnoi* by DNA sequencing; *U. intestinalis* was *U. tepida*; *U. clathrata* and *U. flexuosa* were *U. torta*; *U. acanthophora* matched no sequenced *Ulva* specimens in publicly available databases; *U. lobata* and *U. nematoidea* specimens failed to amplify. Thus, none of the specimens that could be sequenced had been correctly identified by morpho-anatomical characters.

Named *Ulva* specimens in herbaria that have not been sequenced may or may not be correctly identified. Sequences in GenBank of species whose type specimens have not been sequenced to correctly apply names also may not be correctly identified. If specimens in a local flora have been sequenced, such that one understands the habitat and seasonality of each *Ulva* species present, then it may be possible to identify these species based on morpho-anatomical characters. But, to our knowledge this comprehensive sequencing has not been done for any local *Ulva* flora anywhere in the world. Moreover, if a species of *Ulva* subsequently is introduced to a flora, it may not be recognized.

For the Galápagos Archipelago, the green algal checklist compiled by Ruiz & Ziemmeck (2014) listed the following *Ulva* species that had been identified historically using morpho-anatomy: *U. fasciata* Delile (type locality: Alexandria, Egypt) that was first reported by Farlow (1902), and *U. lactuca* Linnaeus (type locality: unknown, but possibly the Indo-Pacific), *U. lobata* (Kützinger) Harvey (type locality: Chile) and *U. taeniata* (Setchell) Setchell and

N.L.Garder (type locality: Monterey, California, USA), all reported by Taylor (1945), the last as *U. dactylifera*. However, based on DNA sequencing of type specimens, Hughey et al. (2020) showed that the names *U. fasciata* and *U. lobata* are junior, heterotypic synonyms of *U. lactuca*, thereby reducing the *Ulva* species recognized by morpho-anatomy in the Galápagos Archipelago to two species, *U. lactuca* and *U. dactylifera*.

In contrast, DNA sequences from field-collected, blade-forming, *Ulva* specimens in the Galápagos Archipelago are identical to or highly similar (>99.5%) to sequences from the type specimens of *U. adhaerens*, *U. lactuca*, *U. ohnoi* and *U. tanneri*, conclusively documenting their presence. It is indeed fortunate that type specimens of each of these species have been sequenced, particularly for *U. lactuca* and *U. tanneri* that were described before the advent of DNA sequencing to identify seaweed species. Linnaeus (1753) described *U. lactuca*, the generitype of *Ulva*, and Tanner (1980) described *U. tanneri* (as *Chloropelta caespitosa*). The sequence of the lectotype of *U. lactuca* was made available in a public database (GenBank) and reported in Hughey et al. (2019), and the holotype of *C. caespitosa* was just published (Hughey et al. 2024). Anslan et al. (2021) found these same species, although they called *U. adhaerens*, *Ulva* sp.

Ulva adhaerens was originally described in 2015 from Tenjin-jima, Kanagawa Prefecture, Japan (Matsumoto and Shimada (2015). The study was undertaken to examine small sized (2-4 cm tall) *Ulva* specimens that, when sequenced for ITS and *rbcL*, comprised four different species, *Ulva* sp. 1, later shown by Hughey et al. (2021) to be *U. conglobata*, *U. pertusa* (= *U. australis*), *U. tanneri*, and *Ulva* sp. 2 that was named *U. adhaerens* due to the presence of rhizoids linking adjacent blades. *Ulva adhaerens* was only known from its type locality until some sequences were recently deposited in GenBank from Munseom Island, Korea (*rbcL*

(MT978111-MT978113) and *tufA* (MT978120-MT978122). Thus, it was surprising, not only to find *U. adhaerens* in the Galápagos, but it was the *Ulva* species that we found most commonly (20 samples) and was the only species that occurred on all four islands where we collected, Fernandina, Floreana, Isabela, and San Cristóbal (Table S1). All of the *U. adhaerens* specimens that were collected were small in size ranging from 1-4 cm tall (Supplemental Figures S1, S2), although this size range was typical for nearly all of *Ulva* species collected.

The two *rbcL* sequences from the holotype collection of *U. adhaerens* both have a large (2,521 bp) Group II intron within the gene. Interestingly, this intron was not found in any of the 17 *rbcL* sequences that we obtained from Galápagos specimens. We used the *rbcL* primer pairs designed by Shimada et al. (2003) to sequence this gene from all *Ulva* specimens that we collected. If this intron had been present, we would not have been able to sequence the *rbcL* gene due to the size of the intron. Thirteen of our *U. adhaerens* specimens had *rbcL* sequences identical to *U. adhaerens* from Japan when the intron is removed. Two of four specimens from one site, La Botella, offshore Isla Floreana, differed by the same SNP (single nucleotide polymorphism), and the specimen from Cuatro Hermanos, Isabela differed by two other SNPs, one shared with one of the specimens from Tijeretas, San Cristobal. All *rbcL* sequences from *U. adhaerens* in the Galapagos differed from each other at most by 2 bp.

Ulva piritoka from New Zealand was proposed by Heesch et al. (2021) based only on an *rbcL* sequence, and is sister to *U. adhaerens*. It differs over its 1355 bp *rbcL* sequence by 5 bp from *U. adhaerens*, and none of these base pairs differences is found in any of the various haplotypes of *U. adhaerens*. Given that *U. lactuca* and *U. ohnoi* typically differ by 3 bp over the same length of *rbcL*, *U. piritoka* is recognized as a distinct species from *U. adhaerens*.

Ulva lactuca, first reported for Galápagos Archipelago by Taylor (1945), is present, based on DNA sequencing, on two of the four islands, San Cristóbal and Floreana (Supplemental Figure S3), where blade-forming *Ulva* species were collected. Whether historical specimens in the MICH or CDS herbaria are indeed *U. lactuca*, needs to be confirmed by DNA sequencing. However, this may not be possible with Taylor's specimens as they were preserved in formaldehyde before being pressed. DNA from specimens preserved in this manner has been very difficult to amplify. *Ulva lactuca* occurs in Chile and Peru based on DNA sequenced specimens, so its presence in the archipelago is not surprising.

Another species that was found by both us and Anslan et al. (2021) was *U. ohnoi*. *Ulva ohnoi* (type locality: Tosa Bay, Tosa, Kochi Prefecture, Japan) was described by Hiraoka et al. (2003). Previously, Ohno (1988), while studying a "green tide" (a bloom of one or more *Ulva* species that are free-floating) of *U. australis* (as *U. pertusa*) in southeastern Japan, proposed that another species, "*Ulva sp.*," was present based on temporal and physiological differences. Hiraoka et al. (2003) then performed crossing experiments between the two species and observed that the gametes did not cross, supporting Ohno's hypothesis. They then described *Ulva sp.* as a new species, *U. ohnoi*, based on ITS and *rbcL* sequences from field-collected and cultured specimens that were attached at Tosa Bay and unattached at Naminoue Beach (Okinawa Prefecture, Japan). Subsequently, *U. ohnoi* has been reported from the Northwest Pacific Ocean (Japan and South Korea; attached and unattached), North Central Pacific Ocean (Hawai'i, USA; attached), Northeast Pacific Ocean (Gulf of California, Mexico; attached), Southwest Pacific Ocean (Lord Howe Island, Australia; habitat not reported), Northwest Indian Ocean (Gujarat and Maharastra States, India; attached), Persian Gulf (Iran; attached), Northwest Atlantic Ocean (Florida, USA; attached and unattached), Gulf of Mexico (Alabama and Texas, USA; attached

and unattached), Caribbean Sea (Venezuela; attached), Mediterranean Sea (Italy, Tunisia, Israel; attached). See Table 1 for markers sequenced and references for these reports.

The Galápagos Archipelago *U. ohnoi* specimens were collected from the islands of Floreana, Isabela and San Cristobal, all of the islands from which samples were obtained except for Fernandina. All specimens were epilithic and found from the low intertidal to the subtidal (5 m maximum depth). These specimens were also small in size ranging up to 2-3 cm tall (Supplemental Figure S4). Based on the *tufA* marker, Melton et al. (2016) identified three haplotypes in *U. ohnoi*. All of the Galápagos specimens belong to haplotype 2, which also contains the holotype specimen from Tosa Bay, Japan whose plastid genome (Suzuki et al. 2018) is publicly available in GenBank (AP018696) and from which we extracted the *tufA* sequence. This haplotype is also found in the Northwest Atlantic (Biscayne Bay, Florida, USA), Gulf of Mexico (Florida, Texas, USA; Yucatan, Mexico) (Melton et al. 2016) and the Southwest Pacific (New South Wales, Australia; GenBank JN029329) (Kirkendale et al. 2013). Haplotype 1 is also found in the Gulf of Mexico and in Australia, whereas haplotype 3 is only found in Australia (Melton et al. 2016).

Melton et al. (2016) hypothesized that *U. ohnoi* was non-native to Atlantic Florida and the Gulf of Mexico due to the low genetic diversity found in the *tufA* and ITS1 markers compared to the global genetic diversity. We observed a similar low genetic diversity for the Galápagos specimens of *U. ohnoi*, but collecting was limited to four islands, and a sample size of five is far too low to support any hypothesis about the origins of this species in the archipelago. What is needed, and would be helpful in understanding how long this species has been in the Galápagos in recent historical time, is to sequence *Ulva* herbarium specimens collected in the

20th Century to see if any of those specimens is *U. ohnoi*, as well as a greater sampling effort throughout the archipelago.

All specimens of *U. ohnoi* in the Galápagos were attached. This species has been reported to cause green tides (blooms of unattached specimens) in bays in Japan, South Korea and the USA, but in all other countries the species grows attached (Table 1). No green tides of *U. ohnoi*, nor of any other species of *Ulva*, have been reported from the Galápagos Archipelago.

Ulva tanneri was originally described as *Chloropelta caespitosa* Tanner (1980) from epilithic specimens in the upper littoral zone from San Pedro, Los Angeles County, California, USA. It was established as a monotypic genus based on a different developmental pattern from other *Ulva* species to form the characteristic distromatic blade. Tanner (1980) characterized *C. caespitosa* as forming dense tufts with orbicular, peltate or split thalli ranging in size from a few mm to 60 mm. He also cited specimens from Los Angeles, Laguna, La Jolla and Pacific Beach, California. Stewart (1991) included *C. caespitosa* in her survey of the marine algae and seagrasses of San Diego County, California based on morpho-anatomy. Joska and Bolton (1992) examined and cultured thalli from Dalebrook, South Africa, and concluded they represented *C. caespitosa*. Using morphology and culture studies, Lima and Fukusumi (1996) reported *C. caespitosa* from Japan.

Based on phylogenetic analyses using ITS sequences and *rbcL* gene sequences of *C. caespitosa* from Kobe, Japan, Hayden *et al.* (2003) concluded that *Chloropelta* should not be recognized as a separate genus. They proposed the new combination, *Ulva tanneri* H.S.Hayden & Waaland, the currently accepted name. Additional molecular studies have supported the transfer, including the analysis of specimens from Monterey, California, USA (Hayden & Waaland 2004), Brisbane, Australia (Kraft *et al.* 2010) and North Island, New Zealand (Nelson

et al. unpublished). Based on morpho-anatomy, Wysor (2004) included *U. tanneri* (as *C. caespitosa*) in his annotated list of marine Chlorophyta from Panama, and Huisman et al. (2007) also recorded *U. tanneri* from Hawai'i, USA. Fernández-García et al. (2011) also reported *U. tanneri* from Costa Rica.

DNA sequences generated from the holotype specimen of *U. tanneri* (Hughey et al. 2024) are the same as those named *U. tanneri* in GenBank, showing that they were correctly identified. The phylogenetic analyses published by Hayden *et al.* (2003) using ITS and *rbcL* DNA sequences placed *U. tanneri* sister in position to *U. californica* and *Enteromorpha* sp. 1. A following study (Hayden & Waaland 2004), using the same two genetic markers, resolved *U. tanneri* in a clade sister to *U. californica* and *U. prolifera* O.F.Müller. Kraft *et al.* (2010), in an investigation of *Ulva* from southern Australia also using ITS and *rbcL* sequences, found that their new species *U. clathratioides* L.G.Kraft, Kraft & R.F.Waller and *U. tanneri* were closely related, and sister to *U. californica*. More recent analyses using *rbcL* sequences (Hughey *et al.* 2019, 2020, 2021), are consistent with these previous results as well as the findings reported herein. They show that *U. tanneri* occupies a strong (BI) to unsupported (RAxML) sister relationship to *U. clathratioides* in the *rbcL* and *tufA* phylograms, also unsupported in ITS.

In addition to previously published DNA sequences of *U. tanneri* from California, USA, Japan, and eastern Australia and New Zealand, the data herein confirm that *U. tanneri* is present in the Galápagos Archipelago. The specimens are small (< 1 cm tall, Supplemental Figure S5), but this is typical for species found in high intertidal habitats. This and Anslan's et al. (2021) finding of *U. tanneri* on Isla San Cristóbal should not be used to infer that *U. tanneri* is a recent introduction to the Galápagos Archipelago. Rather, it is likely that this species has been present historically, but has been misidentified in collections by using morpho-anatomy or, more likely,

is not represented in historical collections. High intertidal macroalgae frequently are overlooked entirely or not collected in surveys as they are assumed to be depauperate specimens of species found lower in the intertidal. Still, historical voucher specimens of *Ulva* species in herbaria should be sequenced to determine their correct identities and to test hypotheses about how long *U. adhaerens*, *U. ohnoi* and *U. tanneri* have been present in the Galápagos Archipelago.

Based on Anslan et al. (2021) the Galápagos marine iguanas (*Amblyrhynchus cristatus*) are consuming all four species of *Ulva* that both studies have found using DNA barcoding, at least on San Cristóbal, and we think this likely occurs on the other islands in the archipelago. We also have observed the green sea turtle, *Chelonia mydas*, consuming *Ulva* species on the islands where specimens were collected for this study.

Despite its historical biological importance and the uniqueness of the terrestrial flora and fauna of the Galápagos Archipelago, the seaweed flora is very poorly known. Only one of the blade-forming *Ulva* species, *U. lactuca*, previously reported for the archipelago based on morpho-anatomical characters, is confirmed with DNA sequencing. This study and Anslan et al. (2021) have demonstrated for the first time the presence of *U. adhaerens*, *U. ohnoi* and *U. tanneri* not only from the Galápagos Archipelago, but from the entire southeast Pacific Ocean. A concerted effort is needed to document, with DNA sequencing, the seaweed flora of this treasured and unique archipelago.

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Author Contributions

Paul W. Gabrielson: Concept, field collections, DNA sequencing, phylogenetic analyses, writing; Anna Claire Smith: Field collections, DNA sequencing, writing; John F. Bruno: Concept, field collections, writing; Todd J. Vision: Concept, writing; Margarita Smith: Concept, field-collections, writing.

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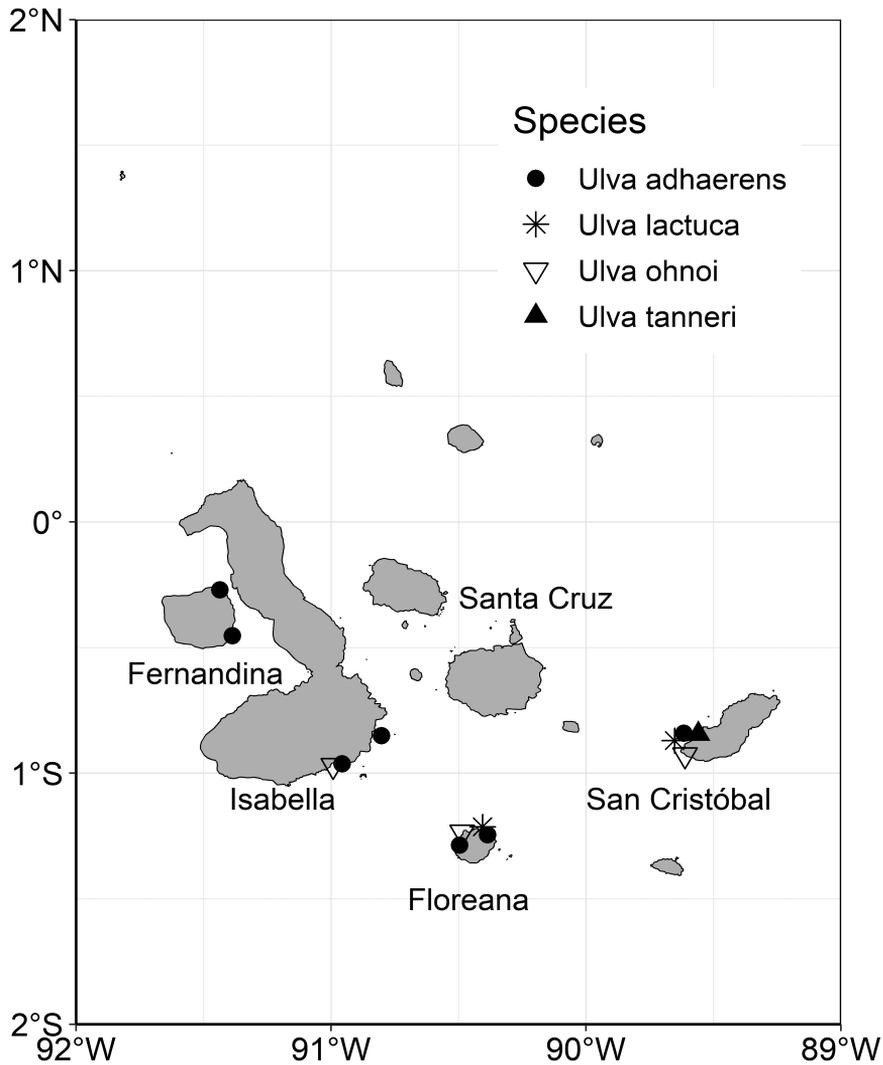
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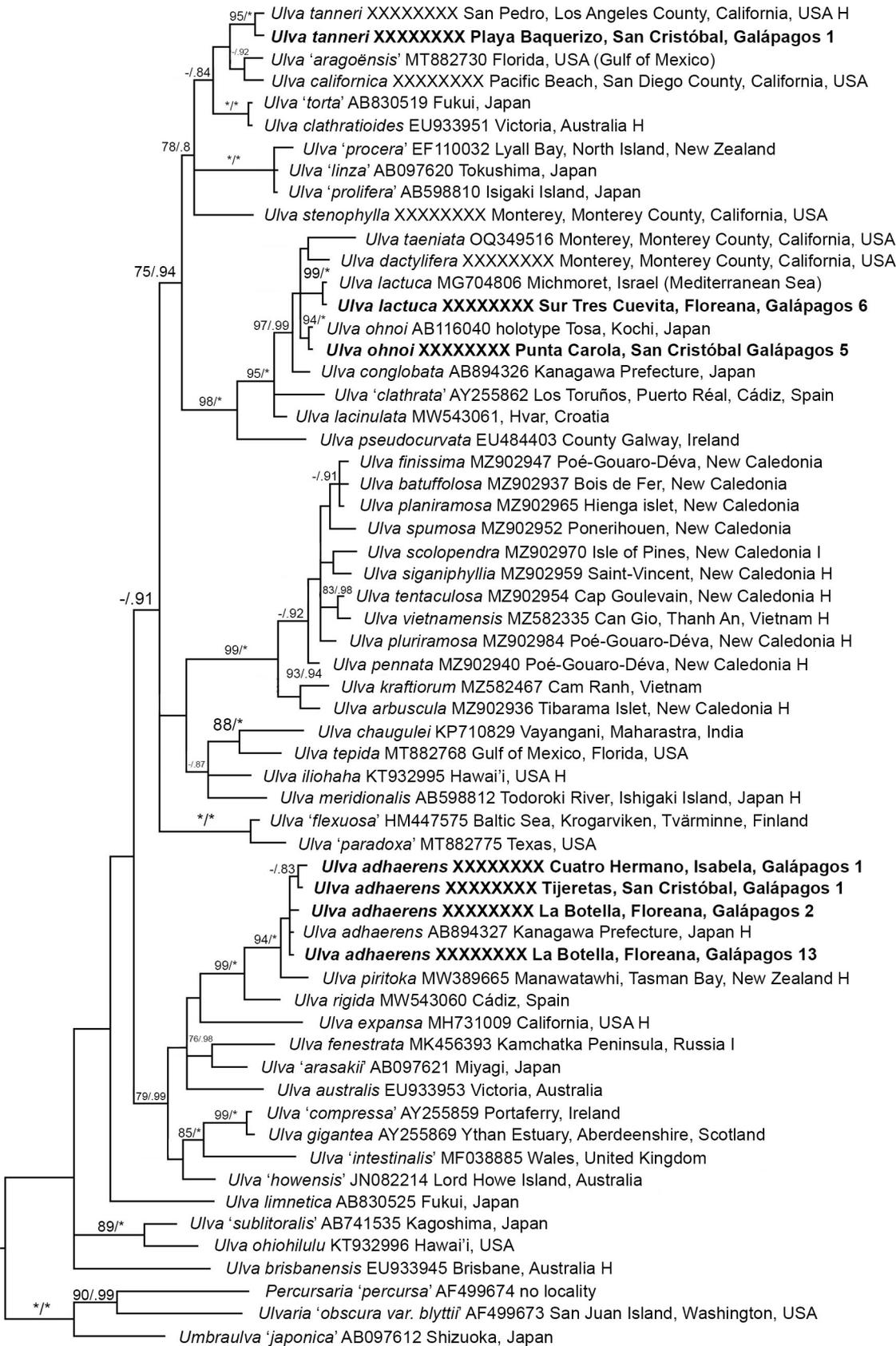
Figure 1. Map of the Galápagos Archipelago with major islands named and where *Ulva* specimens were collected.

Figure 2. Phylogram of *Ulva* species based on *rbcL* sequences. RAxML and Bayesian analyses yielded the same topology. Branch lengths are from ML analysis. The topology is rooted with outgroups *Percursaria 'percursa'*, *Ulvaria' obscura* var. *blyttii'* and *Umbraulva 'japonica'* (family Ulvaceae). Galápagos sequences in bold; species names in '' have not had their type specimens sequenced. Support values at each node are shown as bootstrap percentage/Bayesian posterior probability. Bootstrap percentages (nreps=1,000) are shown when $\geq 75\%$; Bayesian posterior probabilities are shown when ≥ 0.8 , and * indicates full support. The single letters at ends of some localities indicate: H=holotype; I=isotype. Numbers at end of Galápagos sequences indicate total number of identical sequences.

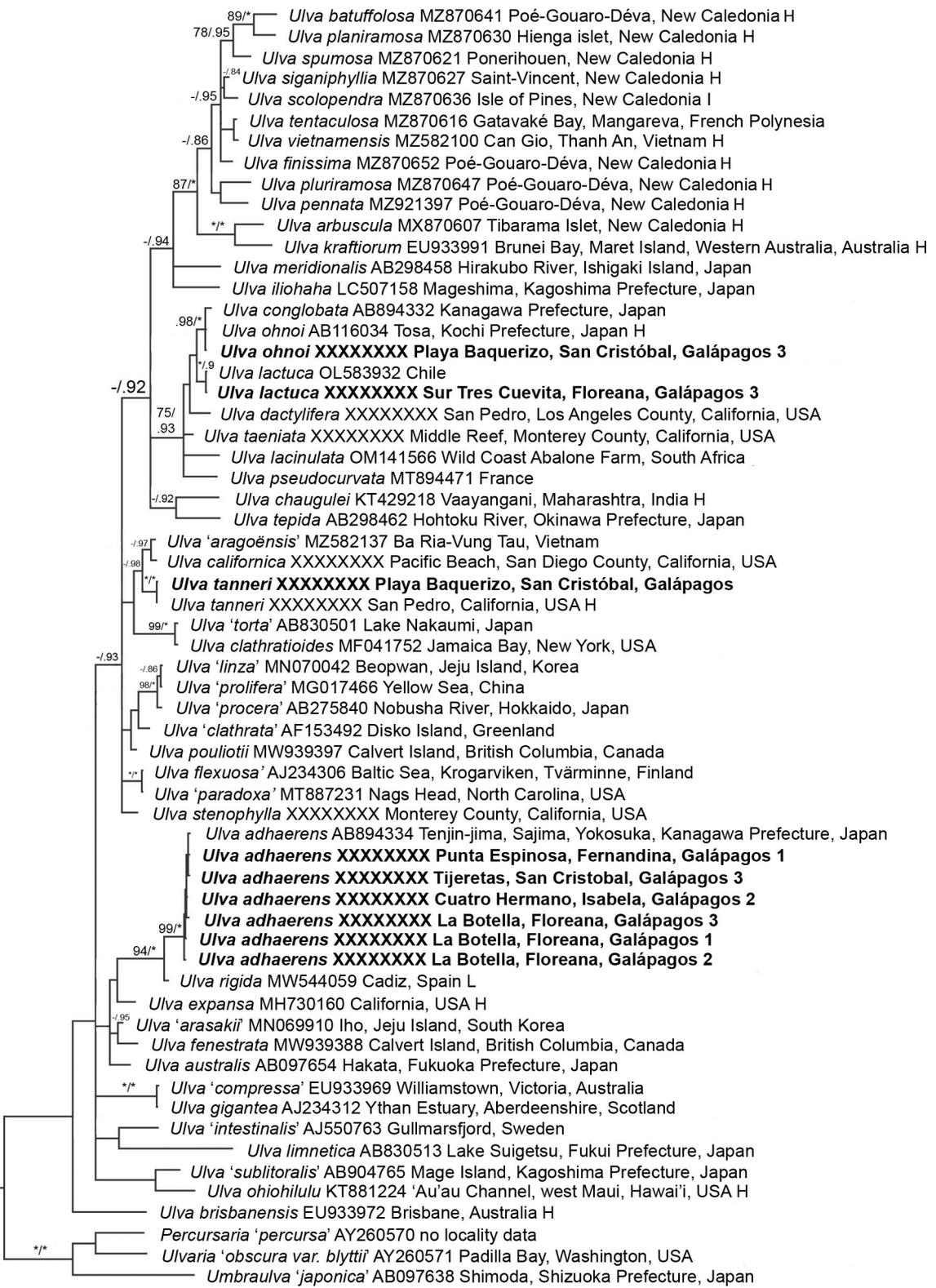
Figure 3. Phylogram of *Ulva* species based on ITS sequences. RAxML and Bayesian analyses yielded the same topology. Branch lengths are from ML analysis. The topology is rooted with outgroups *Percursaria 'percursa'*, *Ulvaria' obscura* var. *blyttii'* and *Umbraulva 'japonica'* (family Ulvaceae). Galápagos sequences in bold; species names in '' have not had their type specimens sequenced. Support values at each node are shown as bootstrap percentage/Bayesian posterior probability. Bootstrap percentages (nreps=1,000) are shown when $\geq 75\%$; Bayesian posterior probabilities are shown when ≥ 0.8 , and * indicates full support. The single letters at ends of some localities indicate: H=holotype; I=isotype. Numbers at end of Galápagos sequences indicate total number of identical sequences.

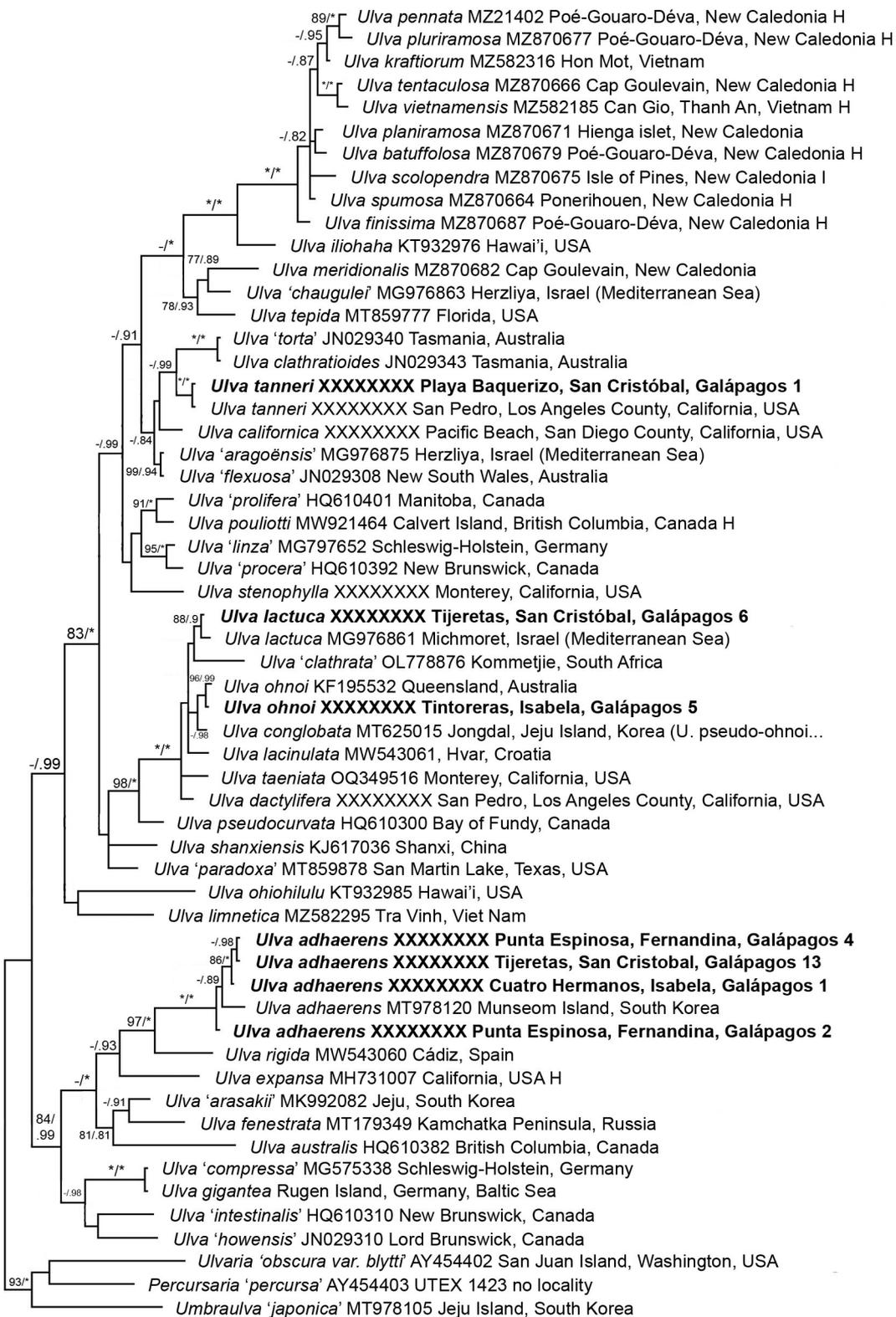
Figure 4. Phylogram of *Ulva* species based on *tufA* sequences. RAxML and Bayesian analyses yielded the same topology. Branch lengths are from ML analysis. The topology is rooted with outgroups *Percursaria 'percursa'*, *Ulvaria' obscura* var. *blyttii'* and *Umbraulva 'japonica'* (family Ulvaceae). Galápagos sequences in bold; species names in '' have not had their type specimens sequenced. Support values at each node are shown as bootstrap percentage/Bayesian posterior probability. Bootstrap percentages (nreps=1,000) are shown when $\geq 75\%$; Bayesian posterior probabilities are shown when ≥ 0.8 , and * indicates full support. The single letters at ends of some localities indicate: H=holotype; I=isotype. Numbers at end of Galápagos sequences indicate total number of identical sequences.





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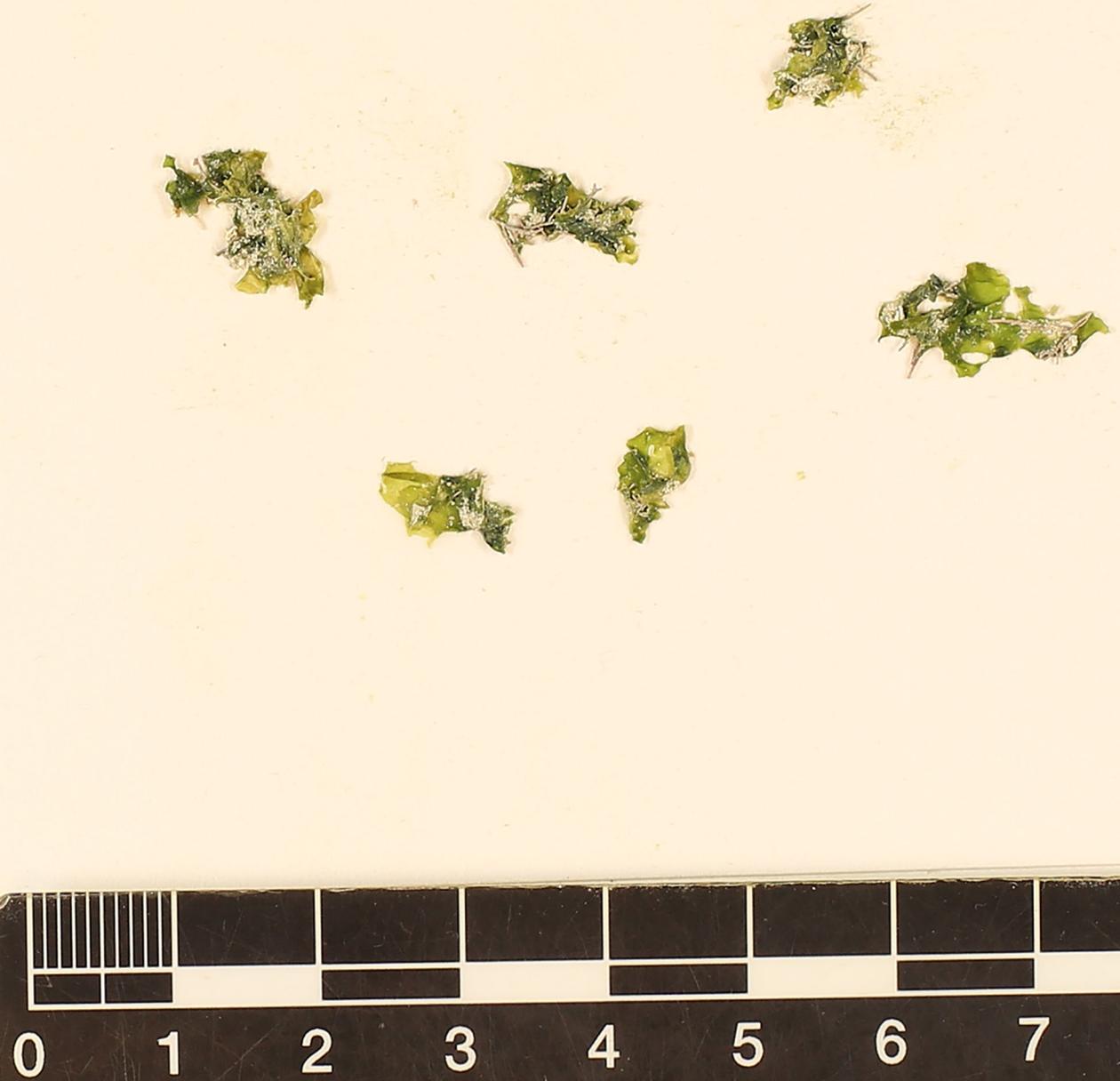
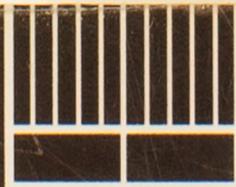


Figure S1. *Ulva adhaerens* (NCU 673046) epilithic, 2 m depth, Concha y Perla, Isabela, Galápagos Archipelago, Ecuador.



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Figure S2. *Ulva adhaerens* (NCU 673105), epilithic 1.5 m depth, Tijeretas, San Cristóbal, Galápagos Archipelago, Ecuador.



Figure S3. *Ulva lactuca* (NCU 673126) epilithic, 12 m depth, Sur Tres Cuevitas, Floreana, Galápagos Archipelago, Ecuador.



Figure S4. *Ulva ohnoi* (NCU 673106), epilithic 5 m depth, Tintoreras, Isabela, Galápagos Archipelago, Ecuador.



Figure S5. *Ulva tanneri* (NCU 675092), epilithic, high intertidal, Playa Baquerizo, San Cristóbal, Galápagos Archipelago, Ecuador.

Table S1. List of specimens sequenced or used in phylogenetic analyses, including herbarium number, collection data and GenBank Accession number. Taxa are listed in alphabetical order. Species in single quotes ' ' have not had their type specimens sequenced, and cited specimens may not be that species. Cited specimens preceded by a # are collection numbers, not herbarium accession numbers; - indicates no sequence. Sequences with * were used in concatenated analyses. *Boreolithothamnion* species with more than one sample are listed north to south; *Lithothamnion* species east to west.

Species	Herbarium Accession or Collection #	Collection Data	GenBank Accession #		
			ITS	<i>rbcL</i>	<i>tufA</i>
<i>Percursaria</i> 'purcursa'	#UWCC MA230	no collection data	AY260570	AF499764	AY454403
<i>Ulvaria</i> 'obscura' var. <i>blyttii</i>	WTU 344838	Padilla Bay (San Juan Island), Washington, USA, 25.iv.1997, no habitat data, leg. H. S. Hayden	AY260571	AF499673	AY454403
<i>Umbraulva</i> 'japonica'	SAP 095050	Shimoda, Shizuoka Prefecture, Japan, 13.iii.2000, no habitat data, unknown collector	AB097638	AB097612	-
<i>Umbraulva</i> 'japonica'	#MSK SYWT98	Shinyang, Jeju, South Korea, 2.i.2018, 8–15m in depth in subtidal areas, unknown collector	-	-	MT978105
<i>Ulva adhaerens</i>	TNS AL 183435	Kanagawa, Sajima, Tenjin-jima Kanagawa Prefecture, Japan, 24.iv.2012, intertidal, unknown collector	AB894334	AB894327	-
<i>Ulva adhaerens</i>	#MSK GA00073	Munseom Island, South Korea, no	-	-	MT978120

		date, no habitat data, unknown collector			
<i>Ulva adhaerens</i>	NCU 673046 G232	Concha y Perla, Isabela, Galápagos, 25.vii.2019, epilithic 2m depth, P. Gabrielson & A. Smith		XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673146 G10	Punta Espinosa, Fernandina, Galápagos, 28.iii.2018, epilithic 12m depth, J. Bruno & M. Brandt	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673143 G5	Punta Espinosa, Fernandina, Galápagos, 28.iii.2018, epilithic 12m depth, J. Bruno & M. Brandt	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673123 G1	Punta Espinosa, Fernandina, Galápagos, 20.iii.2018, epilithic 10m depth, J. Bruno & M. Brandt	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673125 G29	Punta Espinosa, Fernandina, Galápagos, 13.viii.2018, epilithic 12m depth, J. Bruno, I. Silva & D. Fernandez	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673127 G3	Punta Mangle, Fernandina, Galápagos, 26.iii.2018, epilithic 7.9m depth, J. Bruno & M. Brandt	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673110 G11	Punta Mangle, Fernandina, Galápagos, 28.iii.2018, epilithic 7.9m depth, J. Bruno & M. Brandt	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673112 G8	La Botella, Floreana, Galápagos, 26.iii.2018, epilithic 4.6m depth, J. Bruno & M. Brandt		XXXXXXXXXX	XXXXXXXXXX

<i>Ulva adhaerens</i>	NCU 673116 G7	La Botella, Floreana, Galápagos, 27.iii.2018, epilithic 7.3m depth, J. Bruno & M. Brandt	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673115 G23	La Botella, Floreana, Galápagos, 31.iii.2018, epilithic 6.3m depth, J. Bruno & M. Brandt		XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673113 G4	Punta Espinosa, Floreana, 20.iii.2018, epilithic 12m depth, J. Bruno & M. Brandt		XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673105 G92	Tijeretas, San Cristóbal, Galápagos, 15.vii.2019, epilithic 1.5m depth, P. Gabrielson & A. Smith	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673120 G91	Tijeretas, San Cristóbal, Galápagos, 15.vii.2019, epilithic 2m depth, P. Gabrielson & A. Smith	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU 673124 G113	Playa Baquerizo, San Cristóbal, Galápagos, 18.vii.2019, epilithic 1-2m depth, P. Gabrielson		XXXXXXXXXX	XXXXXXXXXX
<i>Ulva adhaerens</i>	NCU673159 G240	Cuatro Hermanos, Isabela, Galápagos, 26.vii.2019, epilithic 16m depth, A. Smith, I. Silva & M. Brandt	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva 'aragoënsis'</i>	#LA VT 06 (no voucher)	Long Son, Vũng Tàu, Bà Rịa– Vũng Tàu, Vietnam, 26.xi.2017, attached on a boat in the estuary, unknown collector	MZ582137	-	-
<i>Ulva 'aragoënsis'</i>	UNA 00072193	Florida, USA (Gulf of Mexico), 24.iii.2014, intertidal to shallow	-	MT882730	-

		subtidal, <i>leg.</i> J. T. Melton III			
<i>Ulva 'aragoënsis'</i>	#HER 2 TC	Herzliya, Israel (Mediterranean Sea), xii.2015-i.2016, intertidal rock abrasion platforms at low tide, <i>leg.</i> N. Krupnik	-	-	MG976875
<i>Ulva 'arasaki'</i>	#U 100	Iho, Jeju Island, South Korea, iv.2015, no habitat data, unknown collector	MN069910	-	-
<i>Ulva 'arasaki'</i>	SAP 095062	Shizugawa, Miyagi Prefecture, Japan, 11.v.1999, no habitat data, unknown collector	-	AB097621	-
<i>Ulva 'arasaki'</i>	#U 099	Iho, Jeju Island, South Korea, iv.2015, no habitat data, unknown collector	-	-	MK992082
<i>Ulva arbuscula</i>	NOU 218800	Tibarama Islet, New Caledonia, 1.vii.2020, high intertidal zone on beach rock mostly coral islet, <i>leg.</i> C. Peignon	MZ870607	MZ902936	-
<i>Ulva australis</i>	SAP 095066	Hakata, Fukuoka Prefecture, Japan, 28.ix.2009, free-floating, unknown collector	AB097654	-	-
<i>Ulva australis</i>	MELU LK 029	Flinders Pier, Flinders, Victoria, Australia, 1.x.2006, no habitat data, unknown collector	-	EU933953	-
<i>Ulva australis</i>	UNB GWS005800	British Columbia, Canada, 28.x.2006, no habitat data, <i>leg.</i> H. Kucera & D. Riddell	-	-	HQ610382
<i>Ulva batuffolosa</i>	NOU 218754	Poé-Gouaro-Déva, New Caledonia, 1.vii.2019, intertidal and subtidal substratum attached	MZ870641	-	MZ870679

		on shallow rocks and corals or epiphytes, <i>leg. S. Gobin</i>			
<i>Ulva batuffolosa</i>	NOU 218810	Bois de Fer, New Caledonia, 1.i.2019, intertidal and subtidal substratum attached on shallow rocks and corals or epiphytes, <i>leg. S. Gobin</i>	-	MZ902937	-
<i>Ulva 'beytensis'</i>	no identifier	Hanumandandi, Beyt Island, Gujarat, India, no date, no habitat data, unknown collector	-	HM142168	-
<i>Ulva brisbanensis</i>	MELU LK 014	Brisbane, Queensland, Australia, 10.viii.2006, 5 km upstream from mouth of Brisbane River, on floating walkway just above waterline, <i>leg. G. Kraft & R. Herrington</i>	EU933972	EU933945	-
<i>Ulva californica</i>	NY 01089139	Pacific Beach south of La Jolla, San Diego County, California, USA, no date, on rocks near high water mark, <i>leg. Mrs. E. Snyder</i>	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva chaugulei</i>	#CAL/ALG./029	Vayangani, Maharashtra, India, 6.ix.2014, no habitat data, unknown collector	KT429218	-	-
<i>Ulva chaugulei</i>	#ARC U 303A	Vayangani, India, 6.ix.2014, no habitat data, unknown collector	-	KP710829	-
<i>Ulva chaugulei</i>	#HER 1 TD	Herzliya, Israel (Mediterranean Sea), xii.2015-i.2016, intertidal rock abrasion platforms at low tide, <i>leg. N. Krupnik</i>	-	-	MG976863
<i>Ulva 'clathrata'</i>	no identifier	Disko Island, Greenland, no date,	AF153492	-	-

		no habitat data, unknown collector			
<i>Ulva 'clathrata'</i>	#623	Los Toruños, Puerto Réal, Cádiz, Spain, no date, no habitat data, unknown collector	-	AY255862	-
<i>Ulva 'clathrata'</i>	#D2991 GT	Kommetjie, South Africa, 18.ii.2019, attached, <i>leg.</i> J. J. Bolton	-	-	OL778876
<i>Ulva clathratoides</i>	#BBC 20150529MP #ULVA 06	Jamaica Bay, New York, USA, no date, no habitat data, unknown collector	MF041752	-	-
<i>Ulva clathratoides</i>	MELU LK 027	Victoria, Australia, 20.iv.2006, no habitat data, unknown collector	-	EU933951	-
<i>Ulva clathratoides</i>	UNB GWS015137	Tasmania, Australia, 20.i.2010, G.W. Saunders, no habitat data, <i>leg.</i> L. Kraft & K. Dixon	-	-	JN029343
<i>Ulva 'compressa'</i>	MELU LK 011	Williamstown, Victoria, Australia, 30.v.2006, no habitat data, unknown collector	EU933969	-	-
<i>Ulva 'compressa'</i>	#544	Portaferry, Ireland, no date, no habitat data, unknown collector	-	AY255859	-
<i>Ulva 'compressa'</i>	#S 1003	Schleswig-Holstein, Germany, 16.iii.2016, shallow lagoons and estuaries, <i>leg.</i> S. Steinhagen	-	-	MG575338
<i>Ulva conglobata</i>	TNS AL 183434	Kanagawa Prefecture, Japan, 23.vi.2013, intertidal, unknown collector	AB894332	AB894326	-
<i>Ulva conglobata</i>	#MSK U41 JD SM D 3	Jongdal harbor, Jeju Island, South Korea (<i>U. pseudo-ohnoi</i>),	-	-	MT625015

		21.viii.2017, intertidal to subtidal and floating, unknown collector			
<i>Ulva dactylifera</i>	UC 205622	San Pedro, Los Angeles County, California, USA, ix.1908, exposed rocks, uppermost subtidal, <i>leg.</i> N.L. Gardner	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva expansa</i>	UC 98481	California, USA, 4.vi.1901, Floating and apparently attached to sandy rocks, <i>leg.</i> W. A. Setchell	MH730160	MH731009	MH731007
<i>Ulva fenestrata</i>	UBC A90778	Calvert Island, British Columbia, Canada, 16.vi.2014, subtidal, <i>leg.</i> S. Lindstrom	MW939388	-	-
<i>Ulva fenestrata</i>	UBC A57002	Kamchatka Peninsula, Russia, no date, no habitat data, unknown collector	-	MK456393	MT179349
<i>Ulva finissima</i>	NOU 218760	Poé-Gouaro-Déva, New Caledonia, 1.i.2019, shallow water on rocks or epiphytes on seagrass, <i>leg.</i> S. Gobin	MZ870652	-	MZ870687
<i>Ulva finissima</i>	NOU 218792	Poé-Gouaro-Déva, New Caledonia, 1.i.2019, shallow water on rocks or epiphytes on seagrass, <i>leg.</i> S. Gobin	-	MZ902947	-
<i>Ulva flexuosa</i>	#E 45	Baltic Sea, Krogarviken, Tvärminne, Finland, no date, no habitat data, unknown collector	AJ234306	HM447575	-
<i>Ulva flexuosa</i>	#LAK 10	New South Wales, Australia, 29.vi.2010, Manyana Beach rock platform, <i>leg.</i> L. Kirkendale,	-	-	JN029308

<i>Ulva gigantea</i>	#U44	France, xi.2017-ix.2019, no habitat data, unknown collector	MT894471	-	-
<i>Ulva gigantea</i>	UNB GWS005692	Bay of Fundy, Canada, 11.i.2007, no habitat data, <i>leg.</i> H. Kucera	-	-	HQ610300
<i>Ulva 'howensis'</i>	UNB GWS023390	Lord Howe Island, Australia, 23.ix.2010, Far Rocks, Signal Point, Lord Howe, <i>leg.</i> G. T. Kraft	-	JN082214	JN029310
<i>Ulva iliohaha</i>	#personal:Hiroshi Kawai KU d21725	Mageshima, Kagoshima Prefecture, Japan	LC507158	-	-
<i>Ulva iliohaha</i>	#ARS 08535	Hawai'i, USA, 2004-2013, 40 to 125 m depths, unknown collector	-	KT932995	KT932976
<i>Ulva 'intestinalis'</i>	no identifier	Gullmarsfjord, Sweden, 1993-1999, upper littoral, unknown collector	AJ550763	-	-
<i>Ulva 'intestinalis'</i>	#UI LM F1	Wales, United Kingdom, iii.2015-iv.2016, no habitat data, unknown collector	-	MF038885	-
<i>Ulva 'intestinalis'</i>	UNB GWS003820	New Brunswick, Canada, 28.v.2006, SE of Beaver Harbor on rocky cliffs, Bay of Fundy, <i>leg.</i> H. Kucera	-	-	HQ610310
<i>Ulva kraftiorum</i>	MELU LK 052	Brunei Bay, Maret Island, Western Australia, Australia, 17.iv.2007, unknown collector	EU933991	-	-
<i>Ulva kraftiorum</i>	VNM 00028437	Khanh Hoa, Cam Ranh, Vietnam, 4.iv.2019, no habitat data, unknown collector	-	MZ582467	-
<i>Ulva kraftiorum</i>	BR	Kien Giang, Hòn Một, Vietnam,	-	-	MZ582316

	5010175914731V	28.i.2018, no habitat data, unknown collector			
<i>Ulva lacinulata</i>	#HG 2	Wild Coast Abalone Farm, South Africa, 11.vi.2018, nearby seashores (Kommetjie, Sea Point, Eersterivier and Knysna), <i>leg.</i> J. J. Bolton & B. M. Macey	OM141566	-	-
<i>Ulva lacinulata</i>	L 0054997	Hvar, Croatia, no date, no habitat data, <i>leg.</i> M. Botteri	-	MW543061	MW543061
<i>Ulva lactuca</i>	PC 0042814	Chile, no date, no habitat data, unknown collector	OL583932	-	-
<i>Ulva lactuca</i>	#MIC 5 TF	Michmoret, Israel (Mediterranean Sea), xii.2015-i.2016, intertidal rock abrasion platforms at low tide, <i>leg.</i> N. Krupnik	-	MG704806	MG976861
<i>Ulva lactuca</i>	NCU 673109 G65	Punta Carola, San Cristóbal, Galápagos, 10.vi.2019, epilithic 1m depth, A. Smith		XXXXXXXXXX	XXXXXXXXXX
<i>Ulva lactuca</i>	NCU 673122 G75	Playa Baquerizo, San Cristóbal, Galápagos, 9.vii.2019, epilithic, intertidal, A. Smith		XXXXXXXXXX	XXXXXXXXXX
<i>Ulva lactuca</i>	NCU 673108 G123	Playa Baquerizo, San Cristóbal, Galápagos, 18.vii.2019, epilithic, mid-intertidal, <i>leg.</i> P. Gabrielson & A. Smith			
<i>Ulva lactuca</i>	NCU 673121 G158	Sur Tres Cuevitas, Floreana, Galápagos, 24.vii.2019, epilithic 12m depth, A. Smith, I. Silva, M. Brandt & P. Gabrielson	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva lactuca</i>	NCU 673126	Sur Tres Cuevitas, Floreana,		XXXXXXXXXX	XXXXXXXXXX

	G160	Galápagos, 24.vii.2019, epilithic 12m depth, A. Smith, I. Silva, M. Brandt & P. Gabrielson			
<i>Ulva lactuca</i>	NCU 673111 G25	Tijeretas, San Cristóbal, Galápagos, 31.iii.2018, epilithic 2m depth, J. Bruno & M. Brandt	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva limnetica</i>	0 31	Lake Suigetsu, Fukui Prefecture, Japan, 10.ix.2010, unknown collector	AB830513	AB830525	-
<i>Ulva limnetica</i>	VNM 00028363	Trà Vinh, Vietnam, xii.2017, no habitat data, unknown collector	-	-	MZ582295
<i>Ulva 'linza'</i>	#U 447	Beopwan, Jeju Island, Korea, unknown date, no habitat data, unknown collector	MN070042	-	-
<i>Ulva 'linza'</i>	SAP 095061	Tokushima, Japan, 12.iii.2000, no habitat data, unknown collector	-	AB097620	-
<i>Ulva 'linza'</i>	S 851	Schleswig-Holstein, Germany, v.2016, no habitat data, leg. S. Steinhagen	-	-	MG797652
<i>Ulva meridionalis</i>	SAP 102989	Hirakubo River, Ishigaki Island, Japan, 30.iv.2004, riverine and littoral habitats, unknown collector	AB298458	-	-
<i>Ulva meridionalis</i>	RH 010	Todoroki River, Ishigaki Island, Japan, 16.ii.2009, no habitat data, unknown collector	-	AB598812	-
<i>Ulva meridionalis</i>	NOU 218832	Cap Goulevain, New Caledonia, 1.i.2020, no habitat data, leg. C. Payri,	-	-	MZ870682
<i>Ulva ohiohilulu</i>	#ARS 07528	'Au'au Channel, west Maui,	KT881224	-	-

		Hawai'i, USA, 2004-2013, 40 to 125 m depths, unknown collector			
<i>Ulva ohiohilulu</i>	#ARS 08539	Hawai'i, USA, 2004-2013, 40 to 125 m depths, unknown collector	-	KT932996	-
<i>Ulva ohiohilulu</i>	#ARS 08546	Hawai'i, USA, 2004-2013, 40 to 125 m depths, unknown collector	-	-	KT932985
<i>Ulva ohnoi</i>	SAP 095155	Tosa, Kochi Prefecture, Japan H, 12.ii.2000, rocks in the intertidal zone in Tosa Bay, unknown collector	AB116034	AB116040	-
<i>Ulva ohnoi</i>	#GC1	Queensland, Australia, no date, intertidal environment, unknown collector	-	-	KF195532
<i>Ulva ohnoi</i>	NCU 673119 G133	Playa Baquerizo, San Cristóbal, Galápagos, 18.vii.2019, epilithic, low intertidal, <i>leg.</i> P. Gabrielson	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva ohnoi</i>	NCU XXXXXX G325	Punta Carola, San Cristóbal, Galápagos, 1.viii.2019, epilithic, 2 m depth, <i>leg.</i> P. Gabrielson & A. Smith	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva ohnoi</i>	NCU XXXXXX G79	La Loberia, San Cristóbal, Galápagos, 18.vii.2019, epilithic, low intertidal, P. Gabrielson		XXXXXXXXXX	XXXXXXXXXX
<i>Ulva ohnoi</i>	NCU 673114 G12	La Botella, Floreana, Galápagos, 26.iii.2018, epilithic 4.6m depth, J. Bruno & M. Brandt	?	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva ohnoi</i>	NCU 673106 G205	Tintorerías, Isabela, Galápagos, 25.vii.2019, epilithic 5m depth, I. Silva & M. Brandt	-	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva 'paradoxa'</i>	UNA 00072559	Nags Head, North Carolina, USA,	MT887231	-	-

		22.iv.2015, intertidal to shallow subtidal, unknown collector			
<i>Ulva 'paradoxa'</i>	UNA 00072122	San Martin Lake, Texas, USA, 28.iii.2013, intertidal to shallow subtidal, <i>leg.</i> J. T. Melton III	-	MT882775	MT859878
<i>Ulva pennata</i>	NOU 218731	Poé-Gouaro-Déva, New Caledonia, 5.ii.2019, on subtidal sandy terraces or attached to high-to-mid intertidal coral rubble, <i>leg.</i> S. Gobin	MZ921397	-	-
<i>Ulva pennata</i>	NOU 218795	Poé-Gouaro-Déva, New Caledonia, 1.i.2020, intertidal to subtidal zones, <i>leg.</i> S. Van.Wynsberge & M. Brisset	-	MZ902940	MZ921402
<i>Ulva piritoka</i>	AK 379911	Manawatawhi, Tasman Bay, New Zealand, 19.iv.2013, subtidally at depths of 14-24 m, <i>leg.</i> R. D'Archino	-	MW389665	-
<i>Ulva planiramosa</i>	NOU 218807	Hienga islet, New Caledonia, 28.vi.2020, high intertidal zone on beach rock mostly on coral islets, <i>leg.</i> C. Peignon	MZ870630	MZ902965	MZ870671
<i>Ulva pluiramosa</i>	NOU 218730	Poé-Gouaro-Déva, New Caledonia, 5.ii.2019, on eroded corals in shallow water, <i>leg.</i> S. Gobin	MZ870647	MZ902984	-
<i>Ulva pluiramosa</i>	NOU218738	Poé-Gouaro-Déva, New Caledonia, 5.ii.2019, on eroded corals in shallow water, <i>leg.</i> S. Gobin	-	-	MZ870677

<i>Ulva pouliotii</i>	#SCL 15161	Calvert Island, British Columbia, Canada, 1.iv.2012, subtidal, <i>leg.</i> S. Lindstrom	MW939397	-	MW921464
<i>Ulva 'procera'</i>	SAP 100130	Nobusha River, Hokkaido, Japan, 19.iv.2004, no habitat data, <i>leg.</i> Naoko Yokoyama	AB275840	-	
<i>Ulva 'procera'</i>	WELT A027528	Lyall Bay, North Island, New Zealand, 8.iii.2005, no habitat data, unknown collector	-	EF110032	-
<i>Ulva 'procera'</i>	UNB GWS 006271	New Brunswick, Canada, 28.viii.2007, no habitat data, <i>leg.</i> K. Roy	-	-	HQ610392
<i>Ulva 'prolifera'</i>	#16	Yellow Sea, China, 16.vii.2017, no habitat data, unknown collector	MG017466	-	-
<i>Ulva 'prolifera'</i>	#RH 008	Isigaki Island, Japan, 16.ii.2009, no habitat data, unknown collector	-	AB598810	-
<i>Ulva 'prolifera'</i>	UNB GWS 005322	Manitoba, Canada, 21.viii.2006, no habitat data, <i>leg.</i> G.W. Saunders, B. Clarkston & D. McDevit	-	-	HQ610401
<i>Ulva pseudocurvata</i>	#SY0501	Ythan Estuary, Aberdeenshire, Scotland, no date, no habitat data, unknown collector	AJ234312	-	-
<i>Ulva pseudocurvata</i>	#SY0501	Ythan Estuary, Aberdeenshire, Scotland, no date, no habitat data, unknown collector	-	AY255869	-
<i>Ulva rigida</i>	LD 14294	Cadiz, Spain, no date, no habitat data, <i>leg.</i> L. A. Cabrera	MW544059	MW543060	MW543060

<i>Ulva scolopendra</i>	NOU 218813	Isle of Pines, New Caledonia, 18.i.2020, forming diffuse mat on sandy bottom of barrier reef, <i>leg.</i> R. Legendre	MZ870636	MZ902970	MZ870675
<i>Ulva shanxiensis</i>	SAS 06035	Shanxi, China, 7.iv.2006, no habitat data, <i>leg.</i> Bo Li	-	-	KJ617036
<i>Ulva siganiphyllia</i>	NOU 218822	Saint-Vincent, New Caledonia, 7.vii.2020, forming large masses on sandy 10m depth terraces of barrier reefs, <i>leg.</i> M. Dumas	MZ870627	MZ902959	-
<i>Ulva spumosa</i>	NOU 218856	Ponerihouen, New Caledonia, 16.ix.2020, shallow water on rocky substrata, <i>leg.</i> C. Payri	MZ870621	MZ902952	MZ870664
<i>Ulva stenophylla</i>	NCU 679143	Monterey County, California, 17.v.2021, partially buried in sandy pools at the low tide mark on the north side of Middle Reef, Spanish Bay, Pebble Beach, <i>leg.</i> J. Hughey	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva stenophylloides</i>	MELU LK 024	Lighthouse Reef, Pt. Lonsdale, Victoria, Australia, no date, no habitat data, unknown collector	XXXXXXXXXX	EU933950	XXXXXXXXXX
<i>Ulva 'sublitoralis'</i>	#zk1-3	Kagoshima, Japan, 7.iii.2007, Mage island offshore subtidal (33.4 m), <i>leg.</i> R. Terada	AB904765	-	-
<i>Ulva 'sublitoralis'</i>	SAP 106385	Mage Island, Kagoshima Prefecture, Japan, 15.v.2008, no habitat data, unknown collector	-	AB741535	-
<i>Ulva taeniata</i>	NCU 679144	Monterey County, California,	XXXXXXXXXX	-	-

		17.v.2021, partially buried in sandy pools at the low tide mark on the north side of Middle Reef, Spanish Bay, Pebble Beach, <i>leg.</i> J. Hughey			
<i>Ulva tanneri</i>	NCU 675092 G122	Playa Baquerizo, San Cristóbal, Galápagos, 18.vii.2019, epilithic high intertidal, P. Gabrielson & A. Smith	XXXXXXXXXX	-	XXXXXXXXXX
<i>Ulva tanneri</i>	UBC A57963	Point Fermin, San Pedro, Los Angeles County, California, USA, 26.v.1976, upper intertidal zone, <i>leg.</i> C. Tanner	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
<i>Ulva tentaculosa</i>	NOU 218840	Gatavaké Bay, Mangareva, French Polynesia, 1.i.2020, <i>leg.</i> S. Andrefouet	MZ870616	-	-
<i>Ulva tentaculosa</i>	NOU 218829	Cap Goulevain, New Caledonia, 12.vii.2020, high intertidal zone on coral debris, <i>leg.</i> C. Payri	-	MZ902954	MZ870666
<i>Ulva tepida</i>	SAP 102993	Hohtoku River, Okinawa Prefecture, Japan, 09.v.2005, riverine and littoral habitat, unknown collector	AB298462	-	-
<i>Ulva tepida</i>	UNA 00072155	Gulf of Mexico, Florida, USA, 11.viii.2012, intertidal to shallow subtidal, <i>leg.</i> J. T. Melton III	-	MT882768	-
<i>Ulva tepida</i>	UNA 00072321	Florida, USA, 27.iii.2014, intertidal to shallow subtidal, <i>leg.</i> J. T. Melton III	-	-	MT859777
<i>Ulva 'torta'</i>	#17 10	Lake Nakaumi, Japan, 23.v.2009,	AB830501	-	-

		no habitat data, unknown collector			
<i>Ulva 'torta'</i>	#1 29	Fukui, Japan, 15.vii.2010, no habitat data, unknown collector	-	AB830519	-
<i>Ulva 'torta'</i>	#LAK 43	Port Lincoln, South Australia, Australia, 22.x.2010, intertidal, on boulder wall, leg. Pia Winberg	-	-	JN029340
<i>Ulva vietnamensis</i>	BR 5010175912676V	Cần Giờ, Thanh An, Vietnam, 5.xii.2017, mangrove forest, unknown collector	MZ582100	MZ582335	MZ582185