Research Article

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A DNA barcode inventory of the genus *Ulva* (Chlorophyta) along two Italian regions: updates and considerations

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Abstract: The genus *Ulva* Linnaeus 1753 is well known for its global distribution and containing many taxonomically debated species. Their morphological plasticity and cryptic nature overrepresent and underrepresent, respectively, the biodiversity of these species. The difficulty in morphologically identifying *Ulva* species has led to the accumulation of many species names that are currently considered synonyms. The correct identification of these species is crucial due to their significant role in marine ecosystems and mariculture. In the last 30 years, several checklists and taxonomic contributions have been made from the Italian coast on *Ulva* species, almost entirely based on morphological studies and only a few papers with molecular data have been published. In this study, samples deposited at the Institute of Marine Sciences (ISMAR) and Phycological Lab (PHL) herbaria have been reviewed by sequencing the chloroplast-encoded elongation factor Tu (*tuf*A) barcode marker. The results obtained provide an update of the DNA barcode inventory of *Ulva* species for the Lagoon of Venice and the Strait of Messina, two ecosystems characterized by a high algal biogeographical diversity and continuous introduction due to anthropogenic activities. Here, ten *Ulva* species were identified based on molecular data. Furthermore, we record the presence of *Ulva chaugulei* on the coast of Tunisia.

Keywords: *Ulva*; Mediterranean Sea; *Ulva chaugulei*; *tuf*A; LTER-Italy

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1 Introduction

The study of the genus *Ulva* (Chlorophyta) is always full of surprises, as Papenfuss (1960) already reported in his works. Due to phenotypic plasticity and prompt morphological adaptation to changes in the natural environment – in marine and some freshwater ecosystems – the identification of species of *Ulva* is particularly challenging, and many taxonomic synonyms have been generated over time.

In the last few years, extensive molecular investigations of *Ulva* species have been performed around the world with nuclear (ITS1-5.8S-ITS2) and chloroplast (*rbc*L and *tuf*A) barcoding markers (Figure 1). This is reflected in numerous records (Chávez-Sánchez et al. 2019; Hofmann et al. 2010; Kirkendale et al. 2013; Manghisi et al. 2011; Miladi et al. 2018; Steinhagen et al. 2019; Woo and Ki 2017; Xie et al. 2020) that have led to many rearrangements and taxonomic revisions of the *Ulva* species (Cui et al. 2018; Hughey et al. 2019, 2021, 2022; Krupnik et al. 2018; Rybak et al. 2014). As recorded by Guiry and Guiry (2023), only 155 of the 622 taxa described at the species and infraspecific levels are currently accepted; additionally, 105 names are currently of uncertain status.

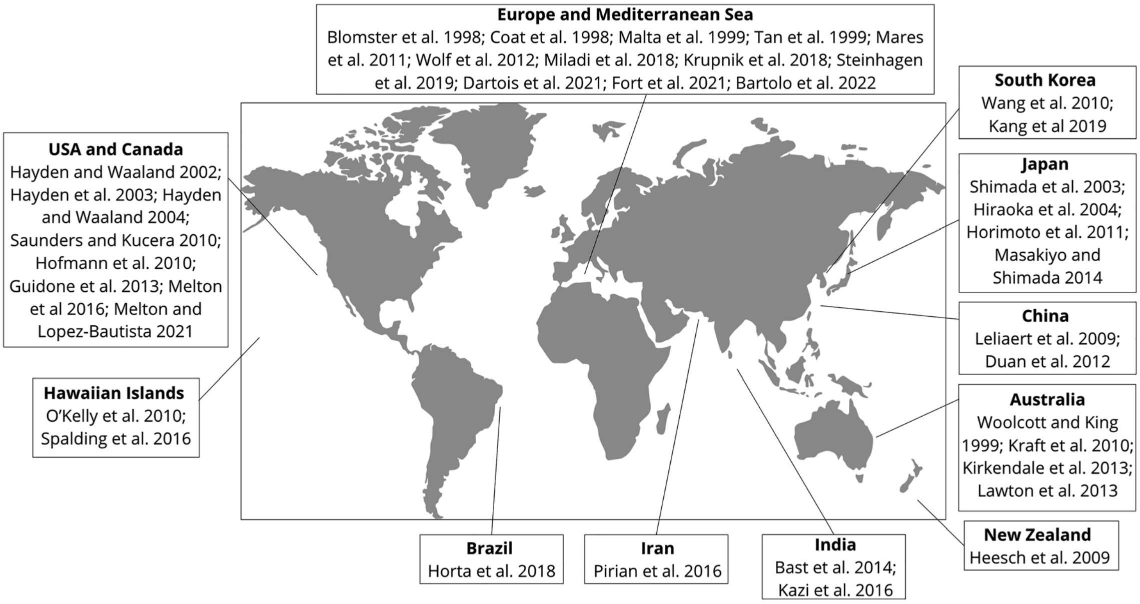

Figure 1: Optimized investigations of *Ulva* species through barcoding markers around the world.

The Italian seaweed flora includes over 800 taxa (Furnari et al. 2010). The biogeographical origin of this biodiversity is characterized by a high incidence of Atlantic (41.87 %), followed by Mediterranean (25.74 %) and cosmopolitan taxa (21.51 %), whereas a lower percentage of species have Indo-Pacific (5.06 %), Circumtropical (4.03 %) and Circumboreal (1.79 %) origins (Furnari et al. 2010). However, according to taxonomic and molecular revisions, seaweed biodiversity data are constantly being updated and revised. Unveiling the biodiversity will allow us to better understand the phenomenon of introduced or Non-Indigenous Species (NIS).

Along the Italian coasts, several checklists and taxonomic contributions have been made to the species of *Ulva* in the last 30 years (Cormaci et al. 2014; Cuomo et al. 1993; Flagella et al. 2010; Manghisi et al. 2011; Miladi et al. 2018; Petrocelli et al. 2019; Serio et al. 2009; Sfriso 2010a, b; Wolf et al. 2012). In 2019, 19 species of this taxon were reported (Cormaci et al. 2014; Miladi et al. 2018). However, the knowledge of both diversity and distribution was almost entirely based on morphological studies and there have only been a few published papers dealing with molecular data (Miladi et al. 2018; Wolf et al. 2012).

The need to explore the diversity of *Ulva* species is further due to the ecological and economic importance of these algae (Melton and Lopez-Bautista 2021), and are often well represented in the Transitional Water Ecosystems (TWE). Represented mainly by estuaries, deltas and lagoons, the TWE are intermediate in time or space between marine and nonmarine environments (Tagliapietra et al. 2009). In this study, the diversity of *Ulva* species was assessed in two Italian TWE, over a period of 10 years.

A molecular approach by sequencing the *tuf*A molecular marker (plastid) was used to compile a DNA barcode inventory and to update the reported species list.

2 Materials and methods

2.1 Study area

The samples revised in this study were collected during different seasons, between 2009 and 2019, from the intertidal to shallow subtidal from the Lagoon of Venice (Veneto Region), the Lake Ganzirri and the Strait of Messina (Sicily Region) (Figure 2; Supplementary Table 1).

The Lagoon of Venice is the largest Italian lagoon and the Mediterranean transitional environment with the highest floristic richness; however, this ecosystem is exposed to intense anthropogenic pressures, including the introduction of alien species. In order to obtain the best representation of *Ulva* species, 24 sampling stations falling within the four classification areas on the basis of the Macrophyte Quality Index (MaQI, Sfriso et al. 2009) were chosen (Figure 2A).

Furthermore, of these research sites, 8 belong to the Long-Term Ecological Research (LTER) network LTER-Italy (Armeli Minicante et al. 2019), an essential component of worldwide efforts to improve our knowledge of the structure

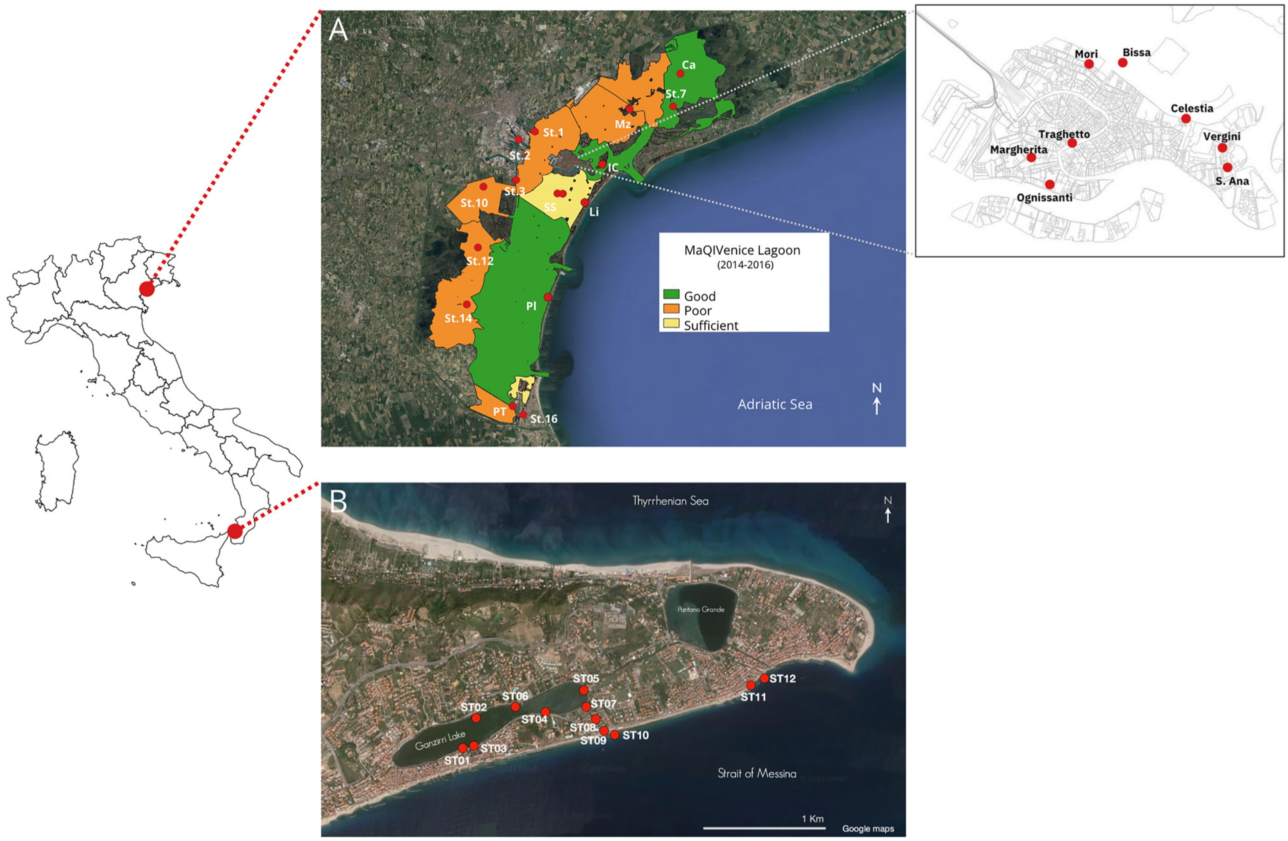

Figure 2: Sampling sites from the Lagoon of Venice (A), Lake Ganzirri and the Strait of Messina (B).

of ecosystems and of their long-term response to environmental, societal and economic drivers (Mirtl et al. 2018).

The Strait of Messina and Lake Ganzirri, in Capo Peloro Lagoon are noteworthy areas in the Mediterranean Sea, with special and unique biological communities, and are included in the "Natura 2000" network and in the Sites of Community Interest, respectively. A floristic list based on literature data was performed by Serio et al. (2009) for Lake Ganzirri, whereas there are few recent studies based on molecular methods for these two environments (Bertuccio et al. 2014). The 12 sampling stations include 7 stations in Lake Ganzirri (ST01-ST07), 4 stations along the Strait of Messina coast (ST09-ST12), and one station (ST08) in the channel which connects Lake Ganzirri to the sea (Figure 2B).

2.2 Algal material processing

All samples collected from the Lagoon of Venice and from Sicilian coasts were pressed in herbarium sheets, vouchered, and deposited in the Herbarium of the Institute of Marine Sciences of Venice (ISMAR, https://www.archiviostudiadriatici.it/index.php/it/) and the Phycological Lab Herbarium (PHL) of the University of Messina, respectively. Abbreviations of herbaria follow the online Index Herbariorum (Thiers 2024).

For each voucher, two subsamples were prepared: one dried in silica gel for molecular analysis, and a second preserved in 4 % formalin in seawater for morphological studies.

2.3 DNA sequencing

For PhL vouchers, the DNA was extracted using a modified CTAB-extraction method (Miladi et al. 2018). The plastid *tuf*A gene was PCR amplified as described in Saunders and Kucera (2010), using tufGF4 and tufAR as primers. Sequencing reactions were performed by an external company (Macrogen Europe, The Netherlands).

For ISMAR vouchers, a QIAGEN DNeasy Plant kit (QIAGEN, Valencia, CA, USA) was used to extract DNA. The *tuf*A molecular marker was amplified by PCR according to Saunders and Kucera (2010). PCR products were run on a gel electrophoresis (1 % agarose gel) and bands were cut from the gel and cleaned with a QIAGEN MinElute Gel Extraction kit (QIAGEN, Valencia, CA, USA). Sanger sequencing of the

PCR products was performed at the University of Kentucky (UK) HealthCare Genomics Centre in Lexington, Kentucky.

2.4 Molecular analyses

Fifty-three *tuf*A sequences from the Mediterranean Sea were trimmed and assembled with Geneious v11.1.5 (Biomatters Ltd., Auckland, New Zealand; available at http://www.geneious.com/). After performing a BLASTn (Altschul et al. 1990) of the sequences, 90 *tuf*A sequences were selected from GenBank. This included sequences from the type material of *Ulva lacinulata* (MW543061), *Ulva ohnoi* (AP018696), *Ulva expansa* (MH731007), *Ulva fenestrata* (MK456404), and *Ulva rigida* (MW543060). *Percursaria percursa* (AY454403), *Umbraulva kaloakulau* (KT932974), and *Ulvaria obscura* (HQ610406) were used as an outgroup. In total, 143 sequences were aligned with MAFFT (Katoh and Standley 2013; Katoh et al. 2002) to form an 802 bp alignment. A maximum likelihood analysis was then performed with RAxML v8 (Stamatakis 2014) using a GTR + I + G model of nucleotide evolution and 1,000 bootstrap replicates. The resulting tree was then edited with FigTree v1.4 (Rambaut 2012). Sequence divergences were calculated with MEGA7 (Kumar et al. 2016). The 'pairwise deletion' option was selected for the gaps and missing data treatment. Taxonomic names were assigned based on molecular species concepts from previous studies. Intraclade divergences from Melton and Lopez-Bautista (2021) were taken into consideration when assigning taxonomic names.

3 Results and discussion

The 52 *tuf*A sequences generated from coastal Italy in this study clustered in 10 distinct clades: *Ulva australis* (six samples), *U. californica* (two samples), *U. compressa* (11 samples), *U. flexuosa* subsp. *flexuosa* (one sample), *U. lacinulata* (15 samples), *U. lactuca* (eight samples), *U. linza/procera/prolifera* (three samples), *U. ohnoi* (two samples), *Ulva* sp. 1 (one sample), and *Ulva* sp. 2 (three samples) (Figures 3–5; Table 1). Nine of the species in this study had an intraclade divergence of 1 % or less, which falls within intraspecific divergences from previous studies (e.g., Kirkendale et al. 2013; Melton and Lopez-Bautista 2021). The intraclade divergence of *U. compressa* (0–1.4 %) was the largest in this study; however, it has been previously noted that this group could potentially represent a cryptic clade instead of a single species (Melton and Lopez-Bautista 2021).

3.1 *Ulva australis* Areschoug

From the analysis of the data obtained from the Lagoon of Venice, we report *U. australis* Areschoug (Figure 5) as established in the area between Celestia and Certosa Island, with a good MaQI index. However, the latest samplings also report it in more inland areas of the lagoon, including Porto S. Leonardo and ST14, and with a poor MaQI index.

According to Sfriso et al. (2023), *U. australis* is now the most widespread NIS in the Lagoon of Venice. It is possible that the species growth is being aided by the decrease in the lagoon's trophic conditions.

This Indo-Pacific species was introduced in the Mediterranean Sea by mollusc farming and recorded, as *U. pertusa* Kjellman, from the Thau Lagoon (France) (Cormaci et al. 2004), the Gulf of Naples (Flagella et al. 2010) and the Lagoon of Venice (Manghisi et al. 2011). ITS1 and *rbc*L data of *U. pertusa* from Spain suggested their synonymy with *U. australis* (Couceiro et al. 2011). Wolf et al. (2012) confirmed the presence of *U. australis* in the Lagoon of Venice by analyses based on *rbc*L and *tuf*A markers.

Womersley (1984) reported *U. laetevirens* among synonyms of *U. australis*, pointing out that the sizes of cells of the lectotype of *U. laetevirens* were superimposable to those of *U. australis*. Subsequently, Phillips (1988) considered *U. australis* to be a synonym of *U. rigida*.

Furthermore, molecular investigations performed by Kraft et al. (2010) have demonstrated that *U. rigida* was distinct from *U. australis.* The recent phylogenetic analysis of *rbc*L gene sequences by Hughey et al. (2021), supports the taxonomic synonymy between *U. laetevirens* and *U. australis* assumed by Womersley (1984).

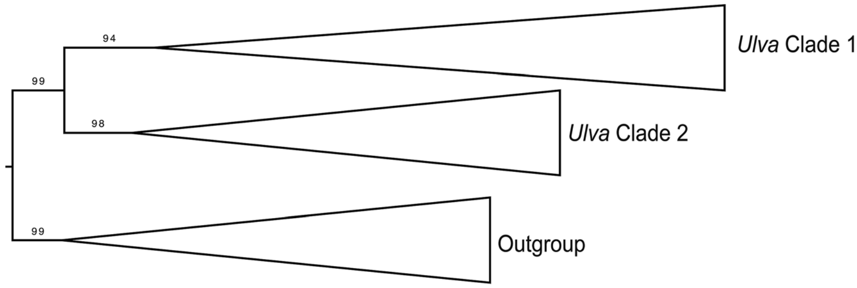

Figure 3: A maximum likelihood (ML) phylogenetic tree of *Ulva* based on *tuf*A (802 bp alignment). The "Clade 1" and "Clade 2" for two major groups of *Ulva* species were collapsed and shown in detail in Figures 4 and 5, respectively. The phylogenetic tree was run with a GTR + I + G model of nucleotide evolution and 1,000 bootstrap replicates.

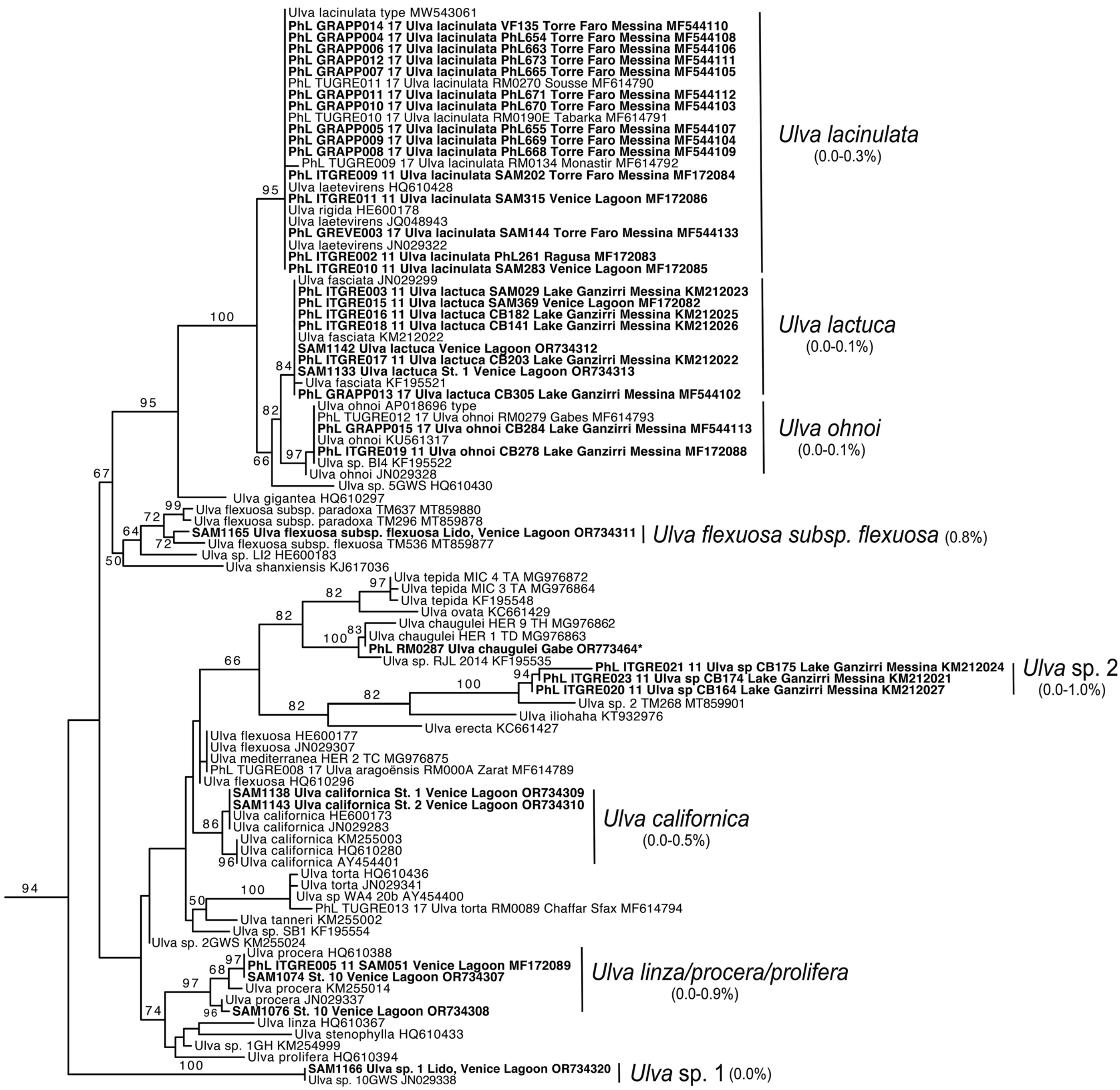

Figure 4: *Ulva* "Clade 1" from the maximum likelihood (ML) phylogenetic tree based on *tuf*A (802 bp alignment). The phylogenetic tree was run with a GTR + I + G model of nucleotide evolution and 1,000 bootstrap replicates. Samples from this study are in bold. Sequences from the type specimen are labelled as 'type'. Pairwise distances were calculated for each species. *Specimen of *U. chaugulei* (RM0287) from Tunisian coast.

In the Lagoon of Venice, Sfriso (2010a), probably following Phillips (1988), listed *U. australis* among synonyms of *U. rigida*. Santarelli (1931) listed *Ulva lactuca* f. *laciniata* (Wulfen) J. Agardh from Trani (Adriatic Sea), indicating the species is synonymous with *U. australis* Kützing; as reported by the author, this species was collected in Adriatic Sea also by Van Den Bosch, Kützing and Hauck.

Whereas Schiffner (Schiffner and Vatova 1938) labelled samples of *U. lactuca* var. *laciniata* (Wulfen) J. Agardh, including also the "f. *australis*" in the "Vatova and Schiffner" collection, hosted at the Natural History Museum of Venice. Observing these herbarium samples in the "Vatova and Schiffner" collection, we do not exclude the possibility that *U. australis* was already present in the Lagoon of Venice in the early 1990s.

3.2 *Ulva californica* Wille

We report the presence of *Ulva californica* Wille (Figure 4) in the most internal sites of the Lagoon of Venice, ST01 and

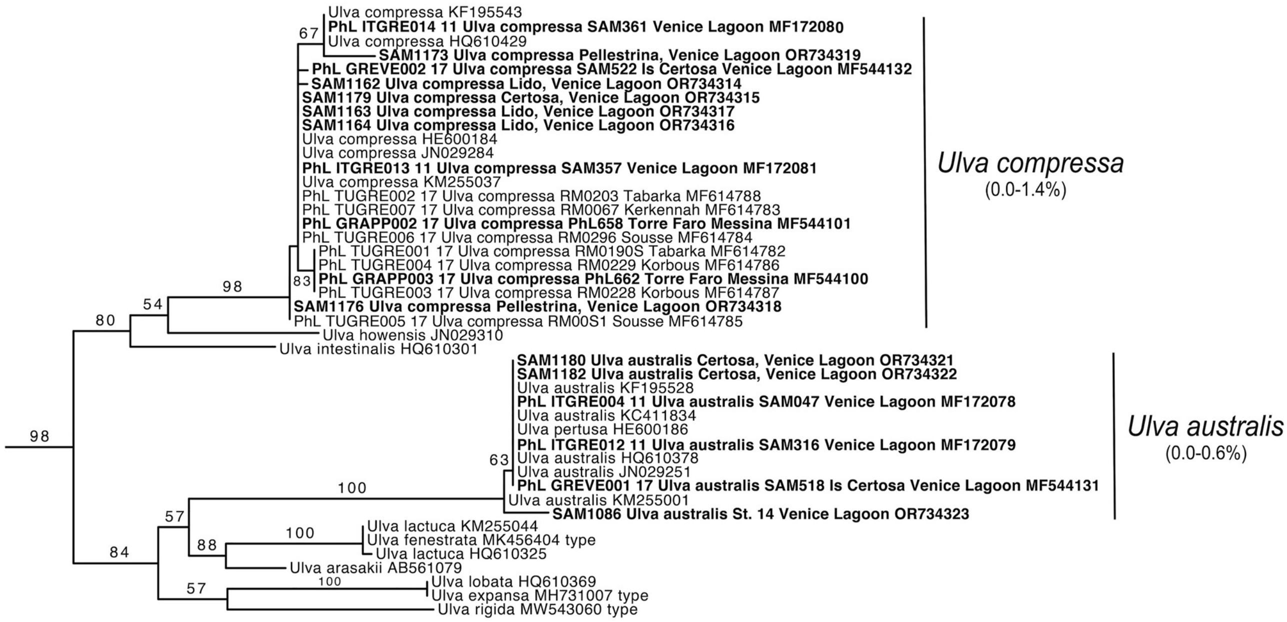

Figure 5: *Ulva* “Clade 2” from the maximum likelihood (ML) phylogenetic tree based on *tuf*A (802 bp alignment). The phylogenetic tree was run with a GTR + I + G model of nucleotide evolution and 1,000 bootstrap replicates.

ST02. This species was first reported in 2012 in the historic centre of Venice by Wolf et al. (2012).

U. californica was first described in 1899 from La Jolla, California (Collins et al. 1899). Opinions about the original range of the species are very different (Wei et al. 2022), from a distribution limited to North America (Scagel 1989; Wolf et al. 2012) or wider, including European temperate coasts (Loughnane et al. 2008). New and numerous reports of this species have been made in different biogeographical areas through molecular data, including Japan (Kawai et al. 2007), New Zealand (Heesch et al. 2009), Italy (Wolf et al. 2012) and Australia (Kirkendale et al. 2013).

3.3 *Ulva compressa* Linnaeus

The species was collected in the marine sites of Torre Faro (ST10 and ST11); in the Lagoon of Venice, *Ulva compressa* Linnaeus (Figure 5) was collected in Ottagono S. Pietro in Volta, Pellestrina, Lido and Porto S. Leonardo. Due to the high intraclade divergence, it is possible that this group represents a species complex (Melton and Lopez-Bautista 2021).

U. compressa is a very common marine green macroalga, distributed on the coasts of Asia, Europe, and America (Guiry and Guiry 2023), with a high intraspecific morphological plasticity, from the narrow, blade-like morphotype, to the foliose free-floating thallus (Liu and Melton 2021). The species could grow rapidly in eutrophic environments, accumulating massive biomass and causing notorious green tides (Blomster et al. 2002).

3.4 *Ulva flexuosa* subsp. *flexuosa* Wulfen

The only sample of *Ulva flexuosa* subsp. *flexuosa* Wulfen (SAM1165; Figure 4) was collected at the Lido station (sufficient MaQI Index) in the spring of 2019.

According to Cormaci et al. (2014), *U. flexuosa* colonizes several habitats, both sheltered and exposed; however, it is more common in brackish lagoon environments and in canals with poor water exchange (Cormaci et al. 2014).

In the Lagoon of Venice, Pignatti (1962), reported *U. flexuosa* (as *Enteromorpha flexuosa*) as a species characteristic of the photophilous community of *Fucetum virsoidis*, established on the north-eastern Adriatic rocky coasts in the presence of large tidal ranges, low average temperatures, low salinity, and eutrophic waters (Pignatti 1962).

Three subspecies of *U. flexuosa* were reported for the Lagoon of Venice by Sfriso et al. (2009): *U. flexuosa* Wulfen subsp. *biflagellata* (Bliding) Sfriso et Curiel, *U. flexuosa* Wulfen subsp. *paradoxa* (C. Agardh) M. J. Wynne and *U. flexuosa* Wulfen subsp. *pilifera* (Kützing) M. J. Wynne.

Table 1: Specimens of *Ulva* analysed by DNA barcoding in this study; previous names have been assigned both by morphological and molecular analyses.

Herbarium code	Specimen ID	Previous name	Name resulting from this study	Collectors	Collection date	Country	Region	Exact site	*tuf*A GenBank/BOLD accession number
PHL	CB164	*Ulva* sp.	*Ulva* sp. 2	Clara Bertuccio	24/06/2009	Italy	Sicily	Lake Ganzirri, ST01	GB# KM212027 BOLD# ITGRE020-11
PHL	CB284	*Ulva ohnoi*	*Ulva ohnoi*	Clara Bertuccio	13/10/2009	Italy	Sicily	Lake Ganzirri, ST01	GB# MF544113 BOLD# GRAPP015-17
PHL	CB174	*Ulva* sp.	*Ulva* sp. 2	Clara Bertuccio	24/06/2009	Italy	Sicily	Lake Ganzirri, ST02	GB# KM212021 BOLD# ITGRE023-11
PHL	CB175	*Ulva* sp.	*Ulva* sp. 2	Clara Bertuccio	24/06/2009	Italy	Sicily	Lake Ganzirri, ST02	GB# KM212024 BOLD# ITGRE021-11
PHL	CB182	*Ulva lactuca*	*Ulva lactuca*	Clara Bertuccio	24/06/2009	Italy	Sicily	Lake Ganzirri, ST03	GB# KM212025 BOLD# ITGRE016-11
PHL	CB141	*Ulva lactuca*	*Ulva lactuca*	Clara Bertuccio	17/06/2009	Italy	Sicily	Lake Ganzirri, ST05	GB# KM212026 BOLD# ITGRE018-11
PHL	CB203	*Ulva lactuca*	*Ulva lactuca*	Clara Bertuccio	14/07/2009	Italy	Sicily	Lake Ganzirri, ST05	GB# KM212022 BOLD# ITGRE017-11
PHL	CB305	*Ulva lactuca*	*Ulva lactuca*	Clara Bertuccio	20/11/2009	Italy	Sicily	Lake Ganzirri, ST05	GB# MF544102 BOLD# GRAPP013-17
PHL	SAM029	*Ulva lactuca*	*Ulva lactuca*	Simona Armeli Minicante	18/03/2010	Italy	Sicily	Lake Ganzirri, ST05	GB# KM212023 BOLD# ITGRE003-11
PHL	CB278	*Ulva ohnoi*	*Ulva ohnoi*	Clara Bertuccio	13/10/2009	Italy	Sicily	Lake Ganzirri, ST06	GB# MF172088 BOLD# ITGRE019-11
PHL	PhL665	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST08	GB# MF544105 BOLD# GRAPP007-17
PHL	PhL668	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST08	GB# MF544109 BOLD# GRAPP008-17
PHL	PhL669	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST08	GB# MF544104 BOLD# GRAPP009-17
PHL	PhL670	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST08	GB# MF544103 BOLD# GRAPP010-17
PHL	PhL671	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST08	GB# MF544112 BOLD# GRAPP011-17
PHL	PhL673	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST08	GB# MF544111 BOLD# GRAPP012-17
PHL	PhL654	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST09	GB# MF544108 BOLD# GRAPP004-17
PHL	PhL655	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST09	GB# MF544107 BOLD# GRAPP005-17

Table 1: (continued)

Herbarium code	Specimen ID	Previous name	Name resulting from this study	Collectors	Collection date	Country	Region	Exact site	*tuf*A GenBank/BOLD accession number
PHL	SAM143	*Umbraulva dangeardii*	*Umbraulva dangeardii*	Simona Armeli Minicante	21/04/2010	Italy	Sicily	Torre Faro, ST09	GB# MF172091 BOLD# ITGRE007-11
PHL	SAM144	*Ulva* sp.	*Ulva lacinulata*	Simona Armeli Minicante	21/04/2010	Italy	Sicily	Torre Faro, ST09	GB# MF544133 BOLD# GREVE003-17
PHL	SAM202	*Ulva* sp.	*Ulva lacinulata*	Simona Armeli Minicante	10/06/2010	Italy	Sicily	Torre Faro, ST09	GB# MF172084 BOLD# ITGRE009-11
PHL	VF135	*Ulva* sp.	*Ulva lacinulata*	Valeria Fiore	05/02/2010	Italy	Sicily	Torre Faro, ST09	GB# MF544110 BOLD# GRAPP014-17
PHL	PhL662	*Ulva compressa*	*Ulva compressa*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST10	GB# MF544100 BOLD# GRAPP003-17
PHL	PhL663	*Ulva* sp.	*Ulva lacinulata*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST10	GB# MF544106 BOLD# GRAPP006-17
PHL	PhL658	*Ulva compressa*	*Ulva compressa*	Antonio Manghisi	09/03/2016	Italy	Sicily	Torre Faro, ST11	GB# MF544101 BOLD# GRAPP002-17
PHL	SAM175	*Umbraulva dangeardii*	*Umbraulva dangeardii*	Simona Armeli Minicante	09/06/2010	Italy	Sicily	Torre Faro, ST12	GB# MF172090 BOLD# ITGRE008-11
PHL	SAM283	*Ulva laetevirens*	*Ulva lacinulata*	Simona Armeli Minicante	28/06/2010	Italy	Veneto	Lagoon of Venice, Bocca di Porto di Lid o	GB# MF172085 BOLD# ITGRE010-11
PHL	SAM047	*Ulva australis*	*Ulva australis*	Simona Armeli Minicante	22/03/2010	Italy	Veneto	Lagoon of Venice, Celestia	GB# MF172078 BOLD# ITGRE004-11
PHL	SAM051	*Ulva linza*	*"Ulva linza/procera/prolifera"*	Simona Armeli Minicante	22/03/2010	Italy	Veneto	Lagoon of Venice, Celestia	GB# MF172089 BOLD# ITGRE005-11
ISMAR	SAM1179	*Ulva* sp.	*Ulva compressa*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Isola della Certosa	GB# OR734315
ISMAR	SAM1180	*Ulva* sp.	*Ulva australis*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Isola della Certosa	GB# OR734321
ISMAR	SAM1182	*Ulva* sp.	*Ulva australis*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Isola della Certosa	GB# OR734322
PHL	SAM518	*Ulva australis*	*Ulva australis*	Simona Armeli Minicante	09/05/2011	Italy	Veneto	Lagoon of Venice, Isola della Certosa	GB# MF544131 BOLD# GREVE001-17
PHL	SAM522	*Ulva compressa*	*Ulva compressa*	Simona Armeli Minicante	09/05/2011	Italy	Veneto	Lagoon of Venice, Isola della Certosa	GB# MF544132 BOLD# GREVE002-17
ISMAR	SAM1162	*Ulva* sp.	*Ulva compressa*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Lido (canale)	GB# OR734314

Table 1: (continued)

Herbarium code	Specimen ID	Previous name	Name resulting from this study	Collectors	Collection date	Country	Region	Exact site	*tuf*A GenBank/BOLD accession number
ISMAR	SAM1163	*Ulva* sp.	*Ulva compressa*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Lido (canale)	GB# OR734317
ISMAR	SAM1164	*Ulva* sp.	*Ulva compressa*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Lido (canale)	GB# OR734316
ISMAR	SAM1165	*Ulva* sp.	*Ulva flexuosa* subsp. *flexuosa*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Ido (canale)	GB# OR734311
ISMAR	SAM1166	*Ulva* sp.	*Ulva* sp. 1	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Lido (canale)	GB# OR734320
PHL	SAM357	*Ulva compressa*	*Ulva compressa*	Simona Armeli Minicante	30/06/2010	Italy	Veneto	Lagoon of Venice, Ottagono S. Pietro in Volta	GB# MF172081 BOLD# ITGRE013-11
ISMAR	SAM1173	*Ulva* sp.	*Ulva compressa*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Pellestrina	GB# OR734319
ISMAR	SAM1176	*Ulva* sp.	*Ulva compressa*	Simona Armeli Minicante	17/05/2019	Italy	Veneto	Lagoon of Venice, Pellestrina	GB# OR734318
ISMAR	SAM1133	*Ulva* sp.	*Ulva lactuca*	Simona Armeli Minicante	18/09/2018	Italy	Veneto	Lagoon of Venice, St. 1	GB# OR734313
ISMAR	SAM1138	*Ulva* sp.	*Ulva californica*	Simona Armeli Minicante	18/09/2018	Italy	Veneto	Lagoon of Venice, St. 1	GB# OR734309
ISMAR	SAM1074	*Ulva* sp.	*"Ulva linza/procera/prolifera"*	Simona Armeli Minicante	24/05/2018	Italy	Veneto	Lagoon of Venice, St. 10	GB# OR734307
ISMAR	SAM1076	*Ulva* sp.	*"Ulva linza/procera/prolifera"*	Simona Armeli Minicante	24/05/2018	Italy	Veneto	Lagoon of Venice, St.10	GB# OR734308
ISMAR	SAM1086	*Ulva* sp.	*Ulva australis*	Simona Armeli Minicante	24/05/2018	Italy	Veneto	Lagoon of Venice, St. 14	GB# OR734323
ISMAR	SAM1142	*Ulva* sp.	*Ulva lactuca*	Simona Armeli Minicante	18/09/2018	Italy	Veneto	Lagoon of Venice, St. 2	GB# OR734312
ISMAR	SAM1143	*Ulva* sp.	*Ulva californica*	Simona Armeli Minicante	18/09/2018	Italy	Veneto	Lagoon of Venice, St. 2	GB# OR734310
PHL	SAM315	*Ulva laetevirens*	*Ulva lacinulata*	Simona Armeli Minicante	30/06/2010	Italy	Veneto	Lagoon of Venice, Porto S. Leonardo	GB# MF172086 BOLD# ITGRE011-11
PHL	SAM316	*Ulva australis*	*Ulva australis*	Simona Armeli Minicante	30/06/2010	Italy	Veneto	Lagoon of Venice, Porto S. Leonardo	GB# MF172079 BOLD# ITGRE012-11
PHL	SAM361	*Ulva compressa*	*Ulva compressa*	Simona Armeli Minicante	30/06/2010	Italy	Veneto	Lagoon of Venice, Porto S. Leonardo	GB# MF172080 BOLD# ITGRE014-11
PHL	SAM369	*Ulva lactuca*	*Ulva lactuca*	Simona Armeli Minicante	30/06/2010	Italy	Veneto	Lagoon of Venice, Porto S. Leonardo	GB# MF172082 BOLD# ITGRE015-11
PHL	RM0089	*Ulva torta*	*Ulva torta*	Ramzi Miladi	28/06/2014	Tunisia		Chaffar, Sfax	GB# MF614794 BOLD# TUGRE013-17
PHL	RM0279	*Ulva ohnoi*	*Ulva ohnoi*	Ramzi Miladi	10/08/2015	Tunisia		Gabes	GB# MF614793 BOLD# TUGRE012-17
PHL	RM0287	*Ulva* sp.	*Ulva chaugulei*	Ramzi Miladi	10/08/2015	Tunisia		Gabes	GB# OR773464
PHL	RM0067	*Ulva compressa*	*Ulva compressa*	Ramzi Miladi	01/06/2014	Tunisia		Kerkennah	GB# MF614783 BOLD# TUGRE007-17

Table 1: (continued)

Herbarium code	Specimen ID	Previous name	Name resulting from this study	Collectors	Collection date	Country	Region	Exact site	*tuf*A GenBank/BOLD accession number
PHL	RM0228	*Ulva compressa*	*Ulva compressa*	Ramzi Miladi	08/04/2015	Tunisia		Korbous	GB# MF614787 BOLD# TUGRE003-17
PHL	RM0229	*Ulva compressa*	*Ulva compressa*	Ramzi Miladi	08/04/2015	Tunisia		Korbous	GB# MF614786 BOLD# TUGRE004-17
PHL	RM0134	*Ulva laetevirens*	*Ulva lacinulata*	Ramzi Miladi	03/08/2014	Tunisia		Monastir	GB# MF614792 BOLD# TUGRE009-17
PHL	RM00S1	*Ulva compressa*	*Ulva compressa*	Ramzi Miladi	10/08/2015	Tunisia		Sousse	GB# MF614785 BOLD# TUGRE005-17
PHL	RM0270	*Ulva laetevirens*	*Ulva lacinulata*	Ramzi Miladi	12/04/2015	Tunisia		Sousse	GB# MF614790 BOLD# TUGRE011-17
PHL	RM0296	*Ulva compressa*	*Ulva compressa*	Ramzi Miladi	26/03/2016	Tunisia		Sousse	GB# MF614784 BOLD# TUGRE006-17
PHL	RM0190E	*Ulva laetevirens*	*Ulva lacinulata*	Ramzi Miladi	04/04/2015	Tunisia		Tabarka	GB# MF614791 BOLD# TUGRE010-17
PHL	RM0190S	*Ulva compressa*	*Ulva compressa*	Ramzi Miladi	04/04/2015	Tunisia		Tabarka	GB# MF614782 BOLD# TUGRE001-17
PHL	RM0203	*Ulva compressa*	*Ulva compressa*	Ramzi Miladi	04/04/2015	Tunisia		Tabarka	GB# MF614788 BOLD# TUGRE002-17
PHL	RM000A	*Ulva flexuosa*	*Ulva aragoënsis*	Ramzi Miladi	12/03/2014	Tunisia		Zarat	GB# MF614789 BOLD# TUGRE008-17

3.5 *Ulva lacinulata* (Kützing) Wittrock

The presence of *U. lacinulata* (Kützing) Wittrock (Figure 4) is confirmed in the marine sites ST09 and ST10 of Torre Faro and in the channel connecting Lake Ganzirri to the sea (ST08). The presence of *U. lacinulata* from the Lagoon of Venice is reported in Porto S. Leonardo and Bocca di Porto di Lido. These samples had been previously identified as *U. laetevirens* by Armeli Minicante (2013), being genetically conspecific with *U. laetevirens* from Australia (type area, Port Phillip, South Australia) (Kirkendale et al. 2013).

According to Hughey et al. (2021), *U. lacinulata* is the correct name to apply to the globally [excluding European] distributed species that was previously but incorrectly known as *U. rigida*. In Europe, both the genuine *U. rigida* and *U. lacinulata* occurs; however, according to the distribution based on DNA sequences reported by Hughey et al. (2021), at present, *U. rigida* appears to be restricted to the European north-eastern Atlantic Ocean, whereas *U. lacinulata* – variously reported either as *U. rigida* or *U. laetevirens* – is distributed further in the Mediterranean Sea.

In the Lagoon of Venice, *U. lacinulata* was already reported as *U. lactuca f. lacinulata* by Schiffner and Vatova (1938) and Pignatti (1962). Sfriso (1987) and later (Sfriso 2010b; Sfriso and Curiel 2007), referring to Bliding (1968), listed *U. lactuca f. lacinulata* as a synonyms of *U. rigida.*

Furthermore, the comparison of the sequences from Tunisia published by Miladi et al. (2018) showed that *U. lacinulata* was previously labelled as *U. laetevirens* in Monastir, Sousse and Tabarka sites (Figure 4) for the same reason stated above (Kirkendale et al. 2013).

3.6 *Ulva lactuca* Linnaeus

Samples of *U. lactuca* Linnaeus (Figure 4) were collected in stations ST1, ST2, and Porto San Leonardo, all three falling within the inner part of the Lagoon of Venice, a poor-quality area according to the MaQI index. In Lake Ganzirri, the species was collected in ST05.

According to Hughey et al. (2019), the holotype of *U. lactuca* is the species previously called *U. fasciata* in the subtropical area and *U. lobata* in the eastern Pacific Ocean. The current distribution of *U. lactuca* confirmed by DNA sequences is in the eastern (Australia) and northern (India) Indian Ocean, central (Hawai'i, USA) and temperate south-east (Chile, Peru), southwest (Australia), and northwest (South Korea, Japan) Pacific Ocean, warm temperate eastern (Azores) and western Atlantic Ocean (including the Gulf of Mexico and Atlantic Florida, USA) and the eastern (Egypt, Israel) and western (Italy) Mediterranean Sea (Hughey et al. 2019). The presence of this species in the two sites in Italy, in Capo Peloro Lagoon and the Lagoon of Venice (Miladi et al. 2018), might be due to recent introductions as both sites have anthropogenic impacts, also according to Hughey et al. (2019). However, their introduction date might be older. The first record of *U. lactuca* in the Lagoon of Venice dates to 1987 as *U. fasciata* (Sfriso 1987); in 1938, Schiffner reported as *"U. fasciata Delile […] has also been collected in the Adriatic at Makarska (Dalmatia); is rare and is often confused with U. lactuca"* (Schiffner and Vatova 1938).

3.7 "*Ulva linza/procera/prolifera*"

Samples of "*U. linza/procera/prolifera*" (Figure 4) were collected in spring at the ST10 in 2018 and at Celestia station in 2010 (Table 1), with a poor and good MaQI Index, respectively. The latter specimen had previously been identified by Armeli Minicante (2013) as *U. linza* (Supplementary Table 2).

ITS sequence data obtained by Brodie et al. (2007) showed *Ulva procera* (Ahlner) Hayden, Blomster, Maggs, Silva, Stanhope *et* Waaland as conspecific with typical *Ulva linza* Linnaeus. Furthermore, Kang et al. (2014) note that several authors regard *U. procera* as a separate species from *U. linza* (Heesch et al. 2009; Kirkendale et al. 2013; Saunders and Kucera 2010).

The lectotype of *U. prolifera* O. F. Müller is a drawing in Müller (Müller 1778), the epitype was designated and sequences for the ITS-2 and 5S rDNA spacer regions generated. The ITS-2 sequence of this specimen was not made publicly available but was reported to be identical to a previously published sequence with GenBank accession number AJ012276 (Kuba et al. 2022).

To date, there are two major opinions (Cui et al. 2018) on the classification of *U. prolifera* based on molecular markers. One point of view is that the true *U. prolifera* is included in the linza–procera–prolifera (LPP) complex clade (consisting of *U. linza* Linnaeus, *U. procera* (Ahlner) Hayden, Blomster, Maggs, Silva, Stanhope *et* Waaland, and *U. prolifera*), and the other is that *U. prolifera* forms a separate clade that includes specimens from Scotland and Ireland, while the entire LPP complex clade is *U. linza*.

Using ITS and 5S rRNA gene spacer region as barcode markers on *Ulva* LPP complex, *U. procera* was recently reduced into synonymy under *U. prolifera* by Cui et al. (2018).

Based on this synonymy, Kuba et al. (2022) identified *U. procera* specimens from San Juan Islands (USA) on the basis of ITS-2 sequences, that were 1–2 base pairs different (0.47–0.93 %) from that of the *U. prolifera* epitype. However, the San Juan Islands specimens molecularly identified as *U. prolifera*, were not branched tubes, the characteristic morphology of the species, but distromatic blades that became tubular where they were basally narrow near the point of attachment. This latter morphology has been identified as *U. linza* in the Northeast Pacific (Kuba et al. 2022). In the light of these observations, the authors suggest that the concept of *U. prolifera* needs to be expanded to include blade-form thalli.

The LPP-clade has been a troublesome group, and morphology does not appear to be helpful because this clade is known to have wide-ranging morphologies from *linza*-like to *prolifera*-like. Additionally, only *tuf*A sequence data was obtained for our samples since this has shown to be an excellent barcoding marker for green algae, and therefore, we were not able to compare this sequence data with the 5S rRNA and ITS sequence data from the epitype. For these reasons, we choose to call the clade "*U. linza/procera/prolifera*" as the designation for the samples from the Lagoon of Venice identified in this study. Multi-gene or genomic analysis should be considered in the future.

3.8 *Ulva ohnoi* Hiraoka *et* Shimada

Samples of *U. ohnoi* Hiraoka *et* Shimada (Figure 4) were found in human-impacted sites (ST01 and ST06) of Lake Ganzirri, and its introduction was probably attributable either to maritime traffic, particularly relevant in the Strait of Messina, or to mollusc trading, similarly to what was proved for *Agardhiella subulata* (C. Agardh) Kraft *et* M. J. Wynne in the same site (Manghisi et al. 2010). In the Mediterranean, the first detection of *U. ohnoi* had been by Mineur et al. (2007) in the algal fouling biomass on ships arriving at the commercial harbour at Sète (Mediterranean coast of France); similarly, a few years later, Flagella et al. (2010) recorded the species in ballast water from ships, coming from Singapore, in the harbour of Naples (Italy).

However, colonization by this species had not been detected in the area (Flagella et al. 2010). *U. ohnoi* is commonly regarded as a NIS, having a disjointed distribution and being recorded in highly disturbed sites. However, its presence in the Mediterranean Sea, as well as in other sites, might be underestimated and its actual distribution, as well as its native range, might be far different from what presently known (Miladi et al. 2018).

3.9 *Ulva* sp. 1 and *Ulva* sp. 2

Of the 52 *tuf*A sequences generated, two have remained unresolved at the species level.

The sample SAM1166, collected in the Lido station in the Lagoon of Venice, falls into the *Ulva* sp. 1 clade (Figure 4) with a sample from Tasmania (Australia), reported as *Ulva* sp.10GWS (GenBank Accession number JN029338, Kirkendale et al. 2013).

Additionally, three samples from Lake Ganzirri (CB174, CB175 and CB164) fall into the *Ulva* sp. 2 clade (Figure 4) with a sample from Aransas Pass, Texas, USA (GenBank Accession number MT859901, Melton and Lopez-Bautista 2021). Clarifying the taxonomic identity of these spp. requires further work.

3.10 *Ulva chaugulei* M. G. Kavale *et* M. A. Kazi

Ulva chaugulei M. G. Kavale *et* M. A. Kazi (Figure 4) was reported for first time in Pacific Ocean (type locality: Vayangani Maharashtra, India) by Kazi et al. 2016. To date, the distribution of *U. chaugulei* is reported by molecular data in Brazil (Carneiro et al. 2023) and Persian Gulf (Pirian et al. 2016). In Mediterranean Sea, the first record of *U. chaugulei* was reported by Krupnik et al. (2018) through molecular analyses of herbarium samples collected in 2015/2016 from the Israel coasts. In this study, we record the presence of a sample of *U. chaugulei* (RM0287) also on the Tunisia coast (Gabes) and previously reported as *Ulva* sp. by molecular analyses.

4 Discussion

Ulva species play a considerable role in marine ecosystems, providing significant advantages and additional services (Cotas et al. 2023; Israel and Shpigel 2023). Edible *Ulva* species hold considerable nutritional value (Peñalver et al. 2020; Xu et al. 2023) and are extensively used in aquaculture to feed and enhance the growth of marine organisms (Abdel-Warith et al. 2016). *Ulva* species contain bioactive compounds with potential pharmaceutical applications (Guo et al. 2022; Yang et al. 2021) or as a source of plant growth regulators (Spagnuolo et al. 2022).

Given all these properties and benefits, these green algae have been identified as the most suitable candidate and model organism for a novel kind of European mariculture and included in the COST Action CA20106 (SeaWheat, https://seawheat.ussl.info/), with the aim to improve knowledge in the biology of the most promising *Ulva* spp., capitalize on their economic potential, and explore commercial applications in the human food, animal feed, pharmaceutical industries, and ecosystem service.

In addition to the numerous positive aspects highlighted, it is essential to acknowledge that *Ulva*, like many other prolific macroalgae, can have unintended consequences in certain environments. Notably, in eutrophic conditions, *Ulva* species have been known to undergo rapid and extensive growth, often resulting in the formation of large-scale accumulations, commonly referred to as "green tides" (Blomster et al. 2002; Kang et al. 2014; Xie et al. 2020). These events can have detrimental effects on local ecosystems, impacting water quality, marine biodiversity, and aquaculture operations. In this context, the correct identification of *Ulva* species and a comprehensive review of past efforts, especially if based only on morphological investigations, is becoming increasingly crucial.

To date, 23 species of *Ulva* are currently reported in the Mediterranean Sea, of which molecular data are available for 12 taxa, whereas 18 species are reported for the Italian coasts, but molecular data are still lacking or under investigation for half of these (Table 2). Based on molecular analyses conducted by Hughey et al. (2021), we excluded *U. rigida* C. Agardh from the Mediterranean list. This species has only been confirmed by DNA sequences from Ireland, Portugal and the Atlantic coasts of Spain, and *U. pseudorotundata* Cormaci, G. Furnari *et* Alongi, for which the holotype from Italy was identical to *U. lactuca* (Hughey et al. 2021). Furthermore, as already discussed above, we currently consider "*U. linza/procera/prolifera*" as a single form, until further investigations are made.

For this first time in this study, we added molecular data for *U. lactuca*, "*U. linza/procera/prolifera*" and *U. flexuosa* subsp. *flexuosa* from the Lagoon of Venice; molecular data were also obtained for *U. lacinulata*, previously sequenced and labelled as *U. rigida* by Wolf et al. (2012). The specimens identified as "*U. linza/procera/prolifera*" exhibit blade forms that became tubular near the basal attachment. This morphology was also observed by Kuba et al. (2022) in specimens from the San Juan Islands (USA). Lastly, one unidentified species designated as *Ulva* sp. 1., was resolved in a clade with samples from Australia.

In the last 85 years, the list of data reported for the different species of *Ulva* has changed considerably in the Lagoon of Venice (Supplementary Table 2). The most exhaustive work, in terms of geographical coverage, has been conducted by Schiffner and Vatova (1938). However, the number of species reported in this study was overestimated, as often happens for the work at the time based on morphological characters. Over time, updates and reports of new species have been obtained by morphological observations and/or bibliographic revisions; conversely, this high diversity is not supported in the last molecular studies (see Supplementary Table 2 and the references therein). Furthermore, the contemporary published works focus on localized areas of the Lagoon of Venice, and massive studies based on large sampling areas and long-term series (including the preservation of herbarium specimens) have reduced over time.

Regarding the sampling sites in Sicily, molecular data for *U. lactuca* and *U. ohnoi* were obtained from the brackish basin of Lake Ganzirri, whereas data for *U. lacinulata* and *U. compressa* came from the marine sampling sites in the Strait of Messina. Specimens of an unidentified species, referred to as *Ulva* sp. 2., formed a clade with the sequences of a specimen from the Gulf of Mexico (Texas, USA).

Of the 23 *Ulva* species recorded in the Mediterranean Sea, five taxa – *U. ohnoi* Hiraoka et Shimada, *U. australis* Areschoug, *U. chaugulei* M. G. Kavale et M. A. Kazi, *Ulva tepida* Y. Masakiyo et S. Shimada and *U. californica* Wille – are non-indigenous and cryptogenic introductions, with a point-like distribution thus far in the Mediterranean Sea (Supplementary Figure 1); however, their distribution needs to be studied further. With the exception of *U. tepida* and *U. chaugulei*, reported for the southeastern Mediterranean Sea (Bartolo et al. 2022; Krupnik et al. 2018), all the other alien species are present in Italy, in the investigated area of Lake Ganzirri (*U. ohnoi*) and the Lagoon of Venice (*U. australis* and *U. californica*).

According to van der Loos et al. (2023), *U. californica* and *U. ohnoi* are NIS native to the Northeast Pacific Ocean; whereas the origin of *U. australis* would be identified in the Northeast Asia (Hanyuda et al. 2016). Among the NIS, these authors also report *U. chaugulei* as a cryptogenic taxon, native to the Indo Pacific Ocean/Red Sea.

In this study, molecular analyses showed the presence of a *U. chaugulei* voucher among the *Ulva* samples hosted in the PHL Herbarium (Table 1; Figure 4; Supplementary Figure 2). The species was collected in Tunisia (Gabes), in a highly impacted area, close to commercial and fishing harbours, chemical and oil industries, and tourist pathways, in the same site where the Japanese bloom-forming *U. ohnoi* was recorded (Miladi et al. 2018).

The first record of *U. chaugulei* in the Mediterranean Sea was reported by Krupnik et al. (2018) based on molecular analyses of herbarium samples collected in 2015/2016 from the Israel coasts. Despite the recent report of this species, the authors suggest that this species could have been previously misidentified as *U. linza* (Einav and Israel 2008; Krupnik et al. 2018). Being easily confused with other tube-forming *Ulva* species and due to the paucity of molecular data in the Mediterranean Sea, it is currently difficult to speculate on the native or non-indigenous nature of this species.

The difficulty of identifying *Ulva* species makes determining between indigenous, pseudo-indigenous (see Carlton

Table 2: Species of *Ulva* reported for the Mediterranean Sea and along Italian coasts (marked in bold).

Species	Morphotype	Type locality	Molecular data				
			Type specimens sequenced	Sequences from the Mediterranean	References	Sequences from Italy	References
Ulva aragoënsis (Bliding) Maggs	Tube-forming	Syntype localities: various, in Atlantic France and Mediterranean Sea	✗	✓	Krupnik et al. (2018); this study	✗	–
***Ulva australis* Areschoug**	Blade-forming	"In caulibus Cauliniae antarcticae parasitica, ad oram Novae Hollandiae australem in sinu Port Adelaide." Areschoug (1854)	✓	✓	–	✓	Manghisi et al. (2011); Wolf et al. (2012); Miladi et al. (2018); this study
***Ulva californica* Wille**	Blade-forming	La Jolla, California Collins et al. (1899)	✗	✓	Bartolo et al. (2022)	✓	Wolf et al. (2012); this study
Ulva chaugulei M. G. Kavale *et* M. A. Kazi	Blade-forming	Vayangani, Maharashtra, India Kazi et al. (2016)	✓	✓	Krupnik et al. (2018); this study	✗	–
***Ulva clathrata* (Roth) C. Agardh**	Tube-forming	Fehmarn, SW Baltic Berger et al. (2003)	✗	✗	–	✗	–
***Ulva compressa* Linnaeus**	Tube-forming	"Habitat in Europae mari & tectis maritimis" [probably Bognor, Sussex, England] Hayden et al. (2003)	✗	✓	Miladi et al. (2018); Bartolo et al. (2022)	✓	Wolf et al. (2012); Miladi et al. (2018); this study
***Ulva curvata* (Kützing) De Toni**	Tube-forming		✗	✗	–	✗	–
***Ulva flexuosa* Wulfen**	Tube-forming	Duino (near Trieste), Adriatic Sea [marine localtion] Womersley (1984)	✗	✓	Miladi et al. (2018)	✓	–
***Ulva flexuosa* subsp. *flexuosa* Wulfen**	Tube- or blade-forming		✗	✓	This study	✓	This study
***Ulva intestinalis* Linnaeus**	Tube-forming	Woolwich, London, England Hayden et al. (2003)	✗	✗	–	✗	–
***Ulva kylinii* (Bliding) H. S. Hayden, Blomster, Maggs, P. C. Silva, Stanhope *et* Waaland**	Tube-forming	Kristineberg, Sweden Silva et al. (1996)	✗	✗	–	✗	–
***Ulva lacinulata* (Kützing) Wittrock**	Blade-forming	–	✓	✓	Miladi et al. (2018)	✓	This study
***Ulva lactuca* Linnaeus**	Blade-forming	"In Oceano" [Atlantic Ocean] Linnaeus (1753)	✓	✓	Miladi et al. (2018)	✓	This study
***Ulva linzoides* Alongi, Cormaci *et* G. Furnari**	Tube-forming	–	✗	✗	–	✗	–
***Ulva ohnoi* M. Hiraoka *et* S. Shimada**	Blade-forming	Tosa Bay, Tosa, Kochi Prefecture Hiraoka et al. (2004)	✓	✓	Miladi et al. (2018)	✓	Miladi et al. (2018); this study
***Ulva paradoxa* C. Agardh**	Tube-forming	Bangor, Wales Berger et al. (2003)	✗	✗	–	✗	–
***Ulva pilifera* (Kützing) Škaloud *et* Leliaert**	Tube-forming	Tennstedt, Thüringen, Germany (Silva et al. 1996)	✗	✗	–	✗	–
***Ulva polyclada* Kraft**	Tube-forming	Sylphs Hole, Lord Howe Island, Australia Kraft (2007)	✗	✗	–	✗	–
"***Ulva linza/procera/prolifera***"	Tube-forming	*U. linza*: "In Oceano" Linnaeus (1753) *U. procera:* Syntype localities: various in SwedenSilva et al. (1996) *U. prolifera*: "U. p. tubulosa simplex teres, adultior compressiuscula. In fossa ad Nebbelund Lalandiae" [Lolland, Denmark]	✓	✗	–	✓	This study
Ulva pseudolinza (Koeman et Hoek) H. S. Hayden, Blomster, Maggs, P. C. Silva, Stanhope *et* Waaland	Tube-forming	Den Helder, Netherlands Koeman and Hoek (1982)	✗	✗	–	✗	–
***Ulva ralfsii* (Harvey) Le Jolis**	Tube-forming	Lectotype locality: Bangor, North Wales Silva et al. (1996)	✗	✗	–	✗	–
Ulva torta (Mertens) Trevisan	Tube-forming	Norderney, East Frisian Islands, Germany Silva et al. (1996)	✗	✓	Miladi et al. (2018); Bartolo et al. (2022)	✗	–
Ulva tepida Y. Masakiyo *et* S. Shimada	Blade-forming	Enoshima Island, Fujisawa, Kanagawa Prefecture, Japan Masakiyo and Shimada (2014)	✓	✓	Krupnik et al. (2018)	✗	–

2009) and non-indigenous species even more complicated. In this context, different aspects are needed in the approach to the study of the biodiversity of *Ulva*.

Traceability: An accurate biodiversity assessment is essential for monitoring biological introductions, and also critical for providing a baseline to detect evidence for short- and long-term trends in biodiversity and human-induced environmental changes.

Temporal surveys allow to obtain data on vegetation and ecosystem structure, as well as will allow to uncover the trends in non-indigenous species introduction rates. According to van der Loos et al. (2023), diversifying the type of survey – from punctual to comprehensive surveys, including morphological-based and DNA-based assessments – is a need for early detection and to monitor trends over time in new species introductions.

Furthermore, the molecular identifications allow us to trace and update the nomenclatural changes over time, helping to "solve" the twisted cases of the *Ulva* species.

Accuracy and caution: To compare sequences from field-collected specimens with those in GenBank, accuracy and caution are required; in fact, the name applied to a GenBank accession does not always come from a comparison with the sequences from type material.

The importance of herbarium specimens: Extensive molecular investigations of *Ulva* have been performed around the world, revealing major taxonomic revisions also thanks to historical herbarium specimens and the type material. In this context, preserved specimens of institutional herbaria, and their associated metadata, are a fundamental tool for monitoring environmental variations and taxonomic changes. Furthermore, as reported by van der Loos et al. (2023), the herbaria play an important role in documenting spatio-temporal patterns of non-indigenous seaweeds.

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