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Contribution to the knowledge of *Gongolaria barbata* (Sargassaceae, Fucales) from the Mediterranean: insights into infraspecific diversity

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Abstract

Gongolaria barbata (Sargassaceae, Fucales) is a widespread species for which several infraspecific taxa have been described, indicating its polymorphism. This study contributes to the understanding of the molecular, nomenclatural, morphological and ecological aspects of *G. barbata* in the Mediterranean and sheds light on the infraspecific diversity and its implications for the taxonomy of this species. Molecular analyses were performed using sequencing of the mitochondrial *cox1* gene on both haptophytic and pleustophytic forms from different sites in the Adriatic and Tyrrhenian Seas. Vegetative and reproductive morphology was studied on thalli samples from the Adriatic. Our results showed that there are different morphotypes within *G. barbata* populations related to specific environmental conditions, suggesting infraspecific variation. In contrast, molecular analyses showed no differences between samples, regardless of whether individuals are growing “attached” to a substrate or “unattached”. We also discussed the taxonomic status and nomenclatural issues related to certain infraspecific taxa previously proposed for *G. barbata*. In particular, the confusion surrounding *Cystoseira aurantia* is clarified.

Key words: ecotypes, taxonomy, Northern Adriatic, *Cystoseira s.l.*

Introduction

In the Mediterranean, with the exception of the endemic *Fucus virsoides* J. Agardh, which is restricted to the Adriatic Sea (Orlando-Bonaca *et al.* 2013), the Fucales are characterized by the widely distributed *Sargassum* genus and the polyphyletic *Cystoseira sensu lato* (*s.l.*). The latter, following phylogenetic analysis made by Draisma *et al.* (2010), Bruno de Sousa *et al.* (2019) and Orellana *et al.* (2019), has been formally divided by Orellana *et al.* (2019) into three genera: *Cystoseira sensu stricto* (*s.s.*), *Carpodesmia* Greville and *Treptacantha* Kützinger. More recently, Molinari & Guiry (2020) re-instated *Gongolaria* Boehmer and *Ericaria* Stackhouse for *Carpodesmia* and *Treptacantha*, respectively, due to the priority principle. That, resulted in numerous new nomenclatural combinations.

Many studies have investigated the phenology of *Cystoseira s.l.* species and shown that they undergo both morphological and reproductive changes throughout the year (Gómez-Garreta *et al.* 1982, Hoffmann *et al.* 1992, Serio 1996a, Serio 1996b, Pizzuto 1998a, Pizzuto 1998b, Alongi *et al.* 1999, Marzocchi *et al.* 2003, Falace *et al.* 2005, Falace

& Bressan 2006, Savonitto *et al.* 2019, Medrano *et al.* 2020). Nevertheless, few studies have focused on morphological variation among populations of furoid species and have also reported significant differences in reproductive traits (De Paula & De Oliveira 1982, Silva *et al.* 2004, Savonitto *et al.* 2019, Sadogurska *et al.* 2021, Orlando-Bonaca *et al.* 2022).

In the Mediterranean Sea as well as in the Black Sea and the Sea of Azov *Gongolaria barbata* (Stackhouse) Kuntze [formerly *Cystoseira barbata* (Stackhouse) C. Agardh] is widespread and inhabits areas with different climatic and ecological conditions (Guiry & Guiry, 2023). Within its geographic range, *G. barbata* exhibits great morphological variability (Falace & Bressan 2006, Sadogurska *et al.* 2021). For this reason, numerous infraspecific taxa of this species are known in literature like:

Cystoseira barbata var. *hoppei* “*hoppii*” (C. Agardh) J. Agardh [afterwards reduced to the rank of a form by Woronichin (1908: 117) as *C. barbata* f. *hoppei* “*hoppii*” (C. Agardh) Woronichin] and *C. barbata* var. *turneri* J. Agardh, both reported by Sauvageau (1912) from Banyuls-sur-Mer (France) who considered them as seasonal morphologies of *G. barbata* (as *C. barbata*); *C. barbata* f. *insularum* Ercegović, *C. barbata* f. *punctata* Ercegović and *C. barbata* subsp. *tophuloidea* Ercegović [the latest later reduced to the rank of variety by Giaccone (in Amico *et al.* 1986: 906) as *C. barbata* var. *tophuloidea* (Ercegović) Giaccone], considered by Cormaci *et al.* (2012) as *Taxa Inquirenda* (TI) because they were based on morphological traits of low taxonomic value; *C. barbata* var. *flaccida* (Kützting) Woronichin whose basionym *C. flaccida* Kützting [considered by Woronichin (1908) as the basionym of a variety of *C. barbata*], was considered as the basionym of a variety of *C. crinita* Duby [= *Ericaria crinita* (Duby) Molinari *et Guiry*] by Schiffner (1933) who proposed the new combination *C. crinita* var. *flaccida* (Kützting) Schiffner. Both *C. barbata* var. *flaccida* and *C. crinita* var. *flaccida* were considered by Cormaci *et al.* (2012) as TI, while recently, Sadogurska *et al.* (2021) considered *C. barbata* var. *flaccida* and *C. barbata* f. *hoppei* × *flaccida* (sic!) as synonyms of *E. crinita* f. *bosphorica* (Sauvageau) Sadogurska, Neiva *et Israel* (= *E. bosporica* (Sauvageau) Serio *et Furnari*). It should be noted that erroneously Sadogurska *et al.* (2021) considered “*C. barbata* f. *hoppei* × *flaccida* Woronichin (1908: 118) as a synonym of *E. bosporica* (as *E. crinita* f. *bosphorica*) not realizing that because it is not a validly published name with no status under the art. 12 of ICN (Turland *et al.* 2018), it can’t be a synonym of any validly published name. However, they probably meant that the above specimen described by Woronichin shows the same features of *E. bosporica* (as *E. crinita* f. *bosphorica*). Finally, more recently, Giaccone (in Amico *et al.* 1986: 906) proposed the new combination *C. barbata* f. *aurantia* (Kützting) Giaccone, based on *C. aurantia* Kützting, a species described by Kützting (1843) from the Gulf of Trieste, for specimens that, as reported by Giaccone & Bruni (1973: 65) with reference to ecotypes of *C. barbata*, are pleustophytic and live in low-hydrodynamic, low-light and sediment-rich environments. It should be noted that the above form was considered by Gómez Garreta *et al.* (2000: 115) as a synonym of *C. barbata* f. *repens* Zinova *et Kalugina* [= *G. barbata* f. *repens* (Zinova *et Kalugina*) Sadogurska], a taxon described on specimens from the Black Sea. *G. barbata* f. *repens* is currently the only recognised infraspecific taxon according to Guiry and Guiry (2023). Finally, it should be noted that the molecular sequences of a free-swimming specimens from Cádiz (Atlantic Ocean, Spain) and from Tenerife (Atlantic Ocean) were referred to as *C. barbata* f. *aurantia* by Bruno de Sousa *et al.* (2019) and *C. aurantia* by Orellana *et al.* (2019), respectively. However, Tenerife’s specimen belongs to the clade of *Cystoseira s.s.* and not to that of *Treptacantha* (= *Gongolaria*) to which *C. aurantia* from the Adriatic Sea belongs (see discussion). On this based, *C. aurantia sensu* Orellana *et al.* (2019) was listed as a synonym of ‘*C. barbata* f. *aurantia*’ in AlgaeBase (Guiry & Guiry 2023) as well in following reports (e.g., Battelli & Catra 2021, Ramdani *et al.* 2021).

The recognition of such a large number of intraspecific taxa within *C. barbata* (= *G. barbata*) shows that researchers have considered *C. barbata* from the Mediterranean as a highly polymorphic taxon. Therefore, this research aims to investigate intraspecific variation in that species in the Adriatic Sea by combining molecular and morphological approaches and ascertain whether formal recognition of infraspecific taxa is justified. Additionally, we seek to determine the appropriate taxonomic rank that should be applied given the observed variation.

Materials & methods

Specimen collections

Haptophytic (= fixed to substrata) specimens were collected during the 2021 and 2022 reproductive seasons at three sites in the Gulf of Trieste (northern Adriatic Sea):

1) near Strunjan Landscape Park (45.53298, 13.64146), characterized by natural populations of *G. barbata* associated with *Cystoseira compressa* (Esper) Gerloff *et* Nizamuddin at depths of 1 to 3 m;

2) near Izola (45.543567, 13.676371), characterised by healthy and dense populations of *G. barbata* and *C. compressa* at depths of 1 to 3 m;

3) near Piran (45.5284, 13.5754), where belts of *G. barbata* occur in association with *C. compressa* and *Ericaria crinita* (Duby) Molinari *et* Guiry at depths of 1 to 3 m (Orlando-Bonaca *et al.* 2021b).

All sites have a shallow rocky seabed and are moderately exposed to wave action (Orlando-Bonaca *et al.* 2008).

Pleustophytic (= free-floating) specimens were collected in the Gulf of Trieste (Northern Adriatic, Eastern Mediterranean):

1) near the lagoon of Grado (45.681471, 13.434312). The area is connected to the lagoon by several flow-through channels and is characterised by a sedimentary bottom and shallow water depth (0–0.5 m)

2) in the Stjuža lagoon (Strunjan Stjuža Nature Reserve, 45.530556, 13.606389). The Stjuža lagoon is connected to the sea only by a flow-through channel, the water flow depends exclusively on the ebb and flow of the tide, and due to the shallow water depth (0.5 m) the water is subject to rapid heating and cooling.

For molecular comparison, both fixed and free-floating specimens of *G. barbata* were also collected in Sardinia (Tyrrhenian Sea, Western Mediterranean):

1) near Santa Giusta (39.864178, 8.57486), a lagoon area where pleustophytic specimens were collected;

2) near Portoscuso (39.205277, 8.37568), haptophytic specimens were collected in rock pools.

For molecular analysis, the specimens (TABLE 1) were kept in separate clean bags, dried and stored in silica gel and delivered to the Applied and Comparative Genomics Laboratory of the University of Trieste for DNA extraction.

TABLE 1. List of samples used for the molecular analyses.

ID sample	GenBank ID (first and second half of the Cox-1 gene portion)	Locations	Geographical coordinates	Morphotype
POZ	OR335716 OR343920	Portoscuso Sardinia, Italy	39.205277, 8.37568	haptophytic
LAG1	OR335717 OR343921	Santa Giusta, Sardinia, Italy	39.864178, 8.57486	pleustophytic
LAG2	OR335718 OR343922	Santa Giusta, Sardinia, Italy	39.864178, 8.57486	pleustophytic
LAG3	OR335719 OR343923	Santa Giusta, Sardinia, Italy	39.864178, 8.57486	pleustophytic
RAD	OR335720 OR343924	Grado, Italy	45.681471, 13.434312	haptophytic
F23	OR335721 OR343925	Grado, Italy	45.681471, 13.434312	pleustophytic
F24	OR343926	Grado, Italy	45.681471, 13.434312	pleustophytic, used only for “2 nd half alignment”
B3	OR335722 OR343927	Strunjan Landscape Park, Slovenia	45.681471, 13.434312	haptophytic
B6	OR335723 OR343928	Strunjan Landscape Park, Slovenia	45.681471, 13.434312	haptophytic
M2	OR335724 OR343929	Izola, Slovenia	45.543567, 13.676371	haptophytic
M7	OR335725 OR343930	Izola, Slovenia	45.543567, 13.676371	haptophytic
M8	OR335726 OR343931	Izola, Slovenia	45.543567, 13.676371	haptophytic
P1	OR335727 OR343932	Piran, Slovenia	45.5284, 13.5754	haptophytic
P2	OR335728 OR343933	Piran, Slovenia	45.5284, 13.5754	haptophytic
P4	OR335729 OR343934	Piran, Slovenia	45.5284, 13.5754	haptophytic
Str1	OR335730 OR343935	Strunjan Stjuža Nature Reserve, Slovenia	45.530556, 13.606389	pleustophytic
Str2	OR335731 OR343936	Strunjan Stjuža Nature Reserve, Slovenia	45.530556, 13.606389	pleustophytic

Reproductive morphology of Adriatic haptophytic samples

The length and width of randomly selected receptacles (N= 100) for each of the three haptophytic populations of the Gulf of Trieste (i.e., Strunjan, Piran, Izola) were measured under a stereomicroscope (Leica MZ 6, Leica Microsystems, Wetzlar, Germany) and photographed with a Nikon Coolpix 4500 camera (Nikon Corporation, Tokyo, Japan). Measurements were made on receptacles by randomly collecting 4 receptacles from 25 individuals in each population. The number of conceptacles was determined by counting the total number of ostioles on the outer surface of each receptacle using a stereomicroscope (Savonitto *et al.* 2019, Orlando-Bonaca *et al.* 2022).

Molecular analyses

Genomic DNA (gDNA) was extracted from 17 samples using the E.Z.N.A.® Plant DNA Kit (Omega Bio-tek, USA) and following the manufacturer's protocol for dried plant samples, with minor modification as a supplemental wash of ethanol 70% before DNA elution. DNA was checked for quality and concentration on Nanodrop 2000 (Thermo Fisher Scientific, USA).

All samples were amplified for two overlapping fragments of the mitochondrial gene *cox1* (Neiva *et al.* 2022) giving a sequence of 1255 bp (overlap of 34 nucleotides), primers are listed in TABLE 2. The amplification mix was prepared as follows: 1X AccuStart II PCR SuperMix (Quantabio, USA), 0.3 µM both forward and reverse primers and 20 ng gDNA in a final volume of 20 µL. PCRs were performed in a thermal cycler XT96 (VWR International) with the following parameters: 94°C for 5', 35 cycles: 94°C for 30", 49°C for 45", 72°C for 1' and finally 72°C for 5'.

TABLE 2. Sequences of primers used to amplify gDNA, alongside their annealing temperature and reference.

Locus [primers]	Primers 5' → 3'	Ta (°C)	Source	Note
cox1 [Gaz2]	F: 5'-CCAACCAYAAAGATATWGGTAC -3' R: 5'-GGATGACCAARAACCAAAA-3'	49	Lane <i>et al.</i> 2007	1st half of <i>cox 1</i> gene portion
cox1 [cox1-789F /cox1-1378R]	F: 5'-TNTAYCARCATTATTTTGGTT-3' R: 5'-TCYGGNATACGNCGNGGCATACC-3'	49	Silberfeld <i>et al.</i> 2010	2nd half of <i>cox 1</i> gene portion

PCR products were checked on TAE 1,5 % agarose gel to verify DNA amplification and then purified with 0,65X Mag-Bind® TotalPure NGS (Omega Bio-tek, USA) according to the manufacturer's instructions. The purified PCR products were sent to an external service to be Sanger sequenced (Eurofins Genomics, Ebersberg, Germany).

Phylogenetic analysis

The chromatograms representing each of our samples were manually cleaned at the 5' and 3' edges and the primer sequences were removed, resulting in two groups of sequences, one representing the first and the other the second half of the *cox1* sequence. Although the group of the second half consisted of a smaller number of sequences, we considered this comparison especially for the inclusion of the sequences of *C. compressa*, voucher TFC:15262, and *C. sp.*, voucher TFC:15276, both collected in Tenerife (Spain) (GenBank MH513832 and MH513833).

Sequences were aligned with MUSCLE (Edgar, 2004) within MEGA-X (version 10.1.1, Kumar *et al.* 2018), using as outgroup for the first tree *Himanthalia elongata* voucher GWS039908, GenBank MN184240, and for the second tree *Himanthalia elongata*, GenBank EU681409.

The phylogeny of our specimens was inferred together with other selected sequences (full list in Supp. Inf, Table S1) The analysis were run based on the Hasegawa-Kishino-Yano substitution model (Hasegawa *et al.* 1985) +G, which was evaluated as the best-fitting model for both the dataset with MEGA-X (version 10.1.1, Kumar *et al.* 2018). The trees were built using the software MrBayes 3.2.7_0 (Ronquist *et al.* 2012) on the web service of NGphylogeny.fr (Lemoine *et al.* 2019) with 100,000 generations. The trees of the two halves of the *cox1* gene portions were visualized and embellished by iTOL: Interactive Tree Of Life (Letunic & Bork 2021)

Results

Vegetative and reproductive morphology

Our haptophytic specimens from the northern Adriatic show the characters considered as diagnostic for the identification of the *G. barbata* (Gómez Garreta *et al.* 2000, Cormaci *et al.* 2012) viz.: a non-caespitose habit with a single cauloid, simple or branched, attached to the substrate by a basal disc 5–10 mm in diameter; the apex of the cauloid smooth and very pronounced in relation to the base of the primary branches. However, thalli from Izola had long fronds (with main branches up to 50 cm), while those from Piran and Strunjan were smaller and had shorter primary branches (up to 20 cm) and showed also reproductive structures with significant morphological differences between the three populations studied. The fertile specimens collected from both Strunjan and Piran had short, simple and cylindrical receptacles that were sparsely mucronated and had rare or no aerocysts (never in chain) (FIGURE 1, TABLE 3). The receptacles were predominantly smooth. In contrast, fertile specimens from Izola had long, terminal, fusiform, mucronate or pedicellate receptacles, numerous single or concatenated aerocysts with chains of up to 5 aerocysts. The conceptacles were prominent. In addition, a higher number of conceptacles was found in the receptacles of Izola specimens than in those of Strunjan (30.5 ± 1.23 conceptacles receptacle⁻¹ in Izola versus 25.33 ± 0.73 conceptacles receptacle⁻¹ in Strunjan) (TABLE 3).

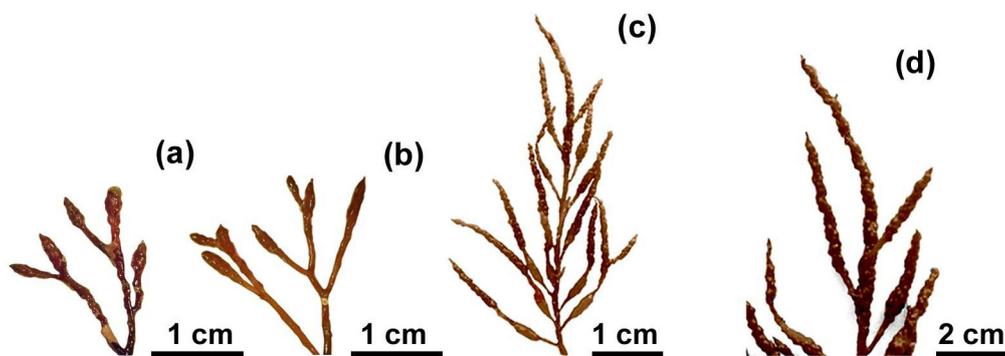


FIGURE 1. Fertile apical fronds of *G. barbata* populations from Piran (a), Strunjan (b) and (c), Izola (d)

TABLE 3. Reproductive morphology of the haptophytic *Gongolaria barbata* specimens from the Gulf of Trieste.

	Izola	Strunjan	Piran
Cryptostomata	numerous	numerous	numerous
Aerocysts	abundant, voluminous, oval to spindle shaped, isolated or in chains (up to 5 aerocysts)	absent or very rare (isolated)	absent or very rare (isolated)
Receptacle shape	simple, fusiform mucronate (long), peduncolate	simple or bifid, cylindrical, rarely mucronate (short)	simple or bifid, cylindrical, rarely mucronate (short)
Receptacle length	6.33 ± 0.33 mm, up to 19.79 mm	4.71 ± 0.18 mm, up to 9.97	4.55 ± 0.17 , up to 10.89 mm
Receptacle width	0.96 ± 0.02 mm	0.77 ± 0.02 mm	0.84 ± 0.02 mm
Conceptacle	prominent	smooth or slightly prominent	smooth or slightly prominent
Conceptacles receptacle ⁻¹	30.45 ± 0.45 conceptacles receptacle ⁻¹ (up to 93 conceptacles receptacle ⁻¹)	24.96 ± 0.25 conceptacles receptacle ⁻¹ (up to 65 conceptacles receptacle ⁻¹)	

The length of the receptacles differed significantly between the sites (Kruskal-Wallis, chi-square = 22.14, $P < 0.001$) with a significant difference between the population of Izola and Piran (pairwise Wilcoxon test, $P < 0.001$) and between the population of Izola and Strunjan (pairwise Wilcoxon test, $P < 0.001$) (FIGURE 2).

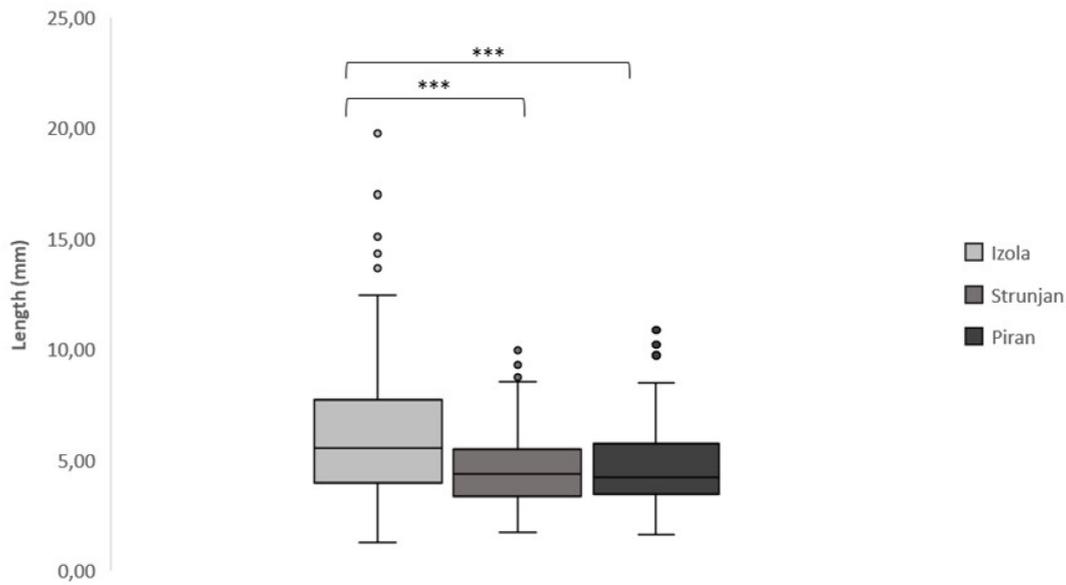


FIGURE 2. Boxplot of the length (mm) of the receptacles from Izola (light grey), Strunjan (dark grey) and Piran (black) sampling sites. The dark horizontal line is the median value (Q2), the extreme values of the box are the first (Q1) and third quartile (Q3), the distance between the two vertical segments is the range of the data and the dots are the extreme values. The stars represent the result of the Wilcoxon Pairwise test.

Overall, the thalli of the Izola population well-matched with *C. barbata* f. *hoppei* viz.: long fronds and long spindle-shaped receptacles with aerocysts ending in a mucron, while those of the Strunjan and Piran populations showed traits of *C. barbata* var. *turneri*, viz.: squat fronds, short cylindrical receptacles without aerocysts and not always mucronate.

The free-living thalli collected in the Gulf of Trieste lagoons are 20–30 cm long, always without anchorage and cauloid, floating on the bottom and on the surface. The primary branches show diffuse growth and can no longer be distinguished from the secondary ones; the branches are cylindrical, slender and oriented in all directions. Aerocysts may or may not be present (usually absent). The thalli collected were sterile (FIGURE 3).



FIGURE 3. Morphology of *Gongolaria barbata* f. *aurantia* comb. nov.

Phylogenetic analyses

The set of sequences representing the first half of the *cox-1* gene portion consisted of 81 sequences, including one sequence as outgroup (full list of sequences in TABLE S1).

Our samples, collected in Adriatic (Italy and Slovenia) and Tyrrhenian (Sardinia, Italy), were clustered with those of *Treptacantha barbata* (Stackhouse) Orellana *et* Sansón (MT978057-58), *Gongolaria barbata* (OK480438-39) and *Cystoseira barbata* (KY682970), regardless of their “attached” or “unattached” morphology (FIGURE 4). The group comprising *Cystoseira compressa* and *Cystoseira pustulata* (Ercegovič) Neiva *et* Serrão has been condensed, but the tree with all visible sequences is available in FIGURE S1.

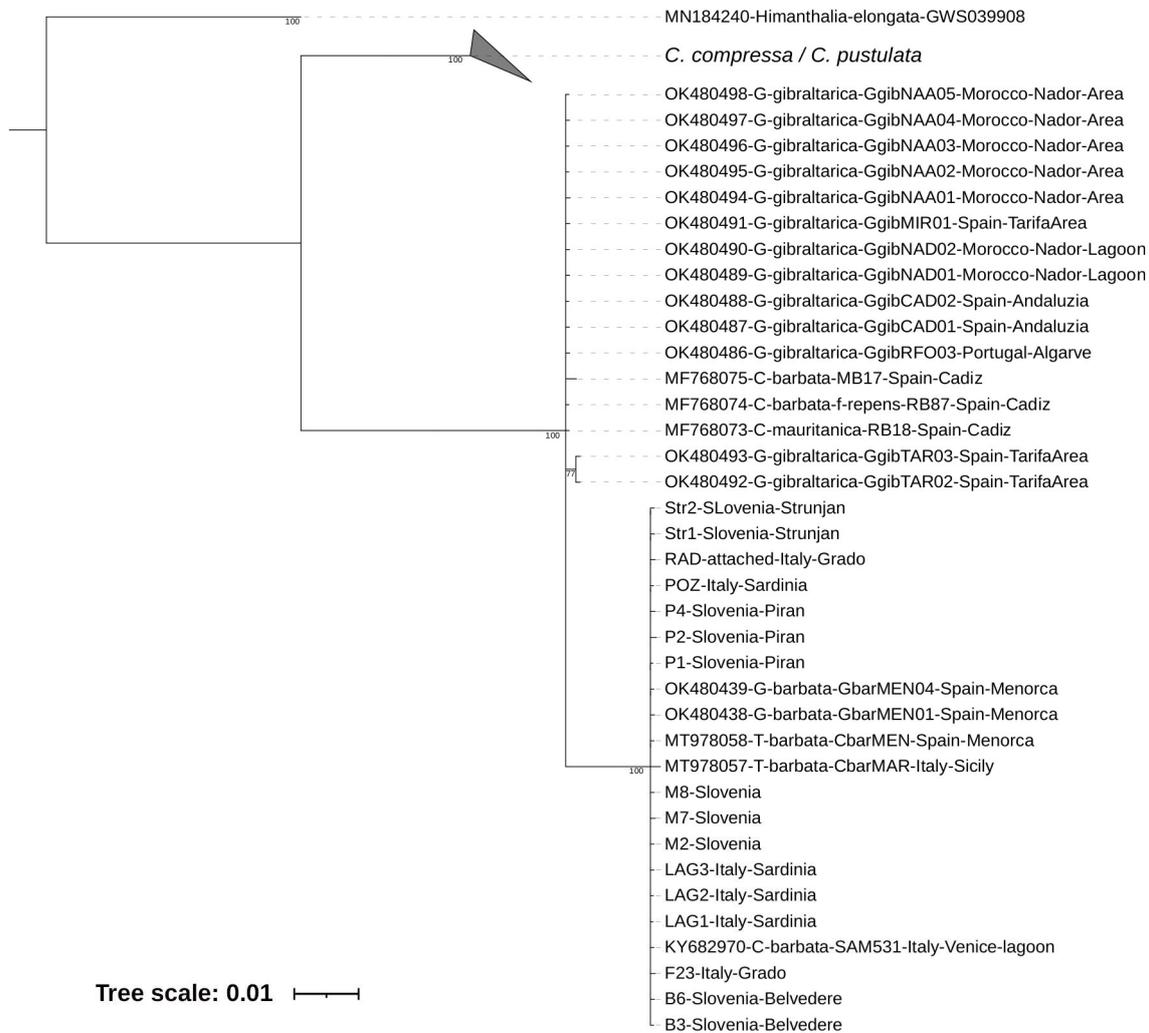


FIGURE 4. Phylogenetic tree of the 1st half of the *cox 1* gene portion. The cluster including all *C. compressa* and *C. pustulata* sequences were condensed. Numbers on branches represent the node support and the bar represents the scale for branches length.

The samples identified by the submitters as *C. barbata* (MF768074-75), both collected in Cádiz (Spain), were clustered with *Gongolaria gibraltarica* (Sauvageau) Neiva, Bermejo *et* Serrão.

The set of sequences representing the second half of the *cox 1* gene consisted of 67 sequences, including one sequence as outgroup (full list of sequences in TABLE S1).

Also considering the second half of the *cox 1* gene, the specimens collected in the Northern Adriatic and Sardinia (Italy) clustered with *G. barbata* voucher GbarMEN04 collected in Menorca (Spain) (GenBank OK480439) and among them with no regards of the “attached” or “unattached” morphology (FIGURE 5 with condensed branches and FIGURE S2 with all visible sequences). The sequence of the specimen *C. aurantia sensu* Orellana *et al.* (2019) (deposited in GenBank as *Cystoseira* sp., COI sequence: MH513833) were clustered with *Cystoseira compressa* (Ercegovič) Neiva *et* Serrão.

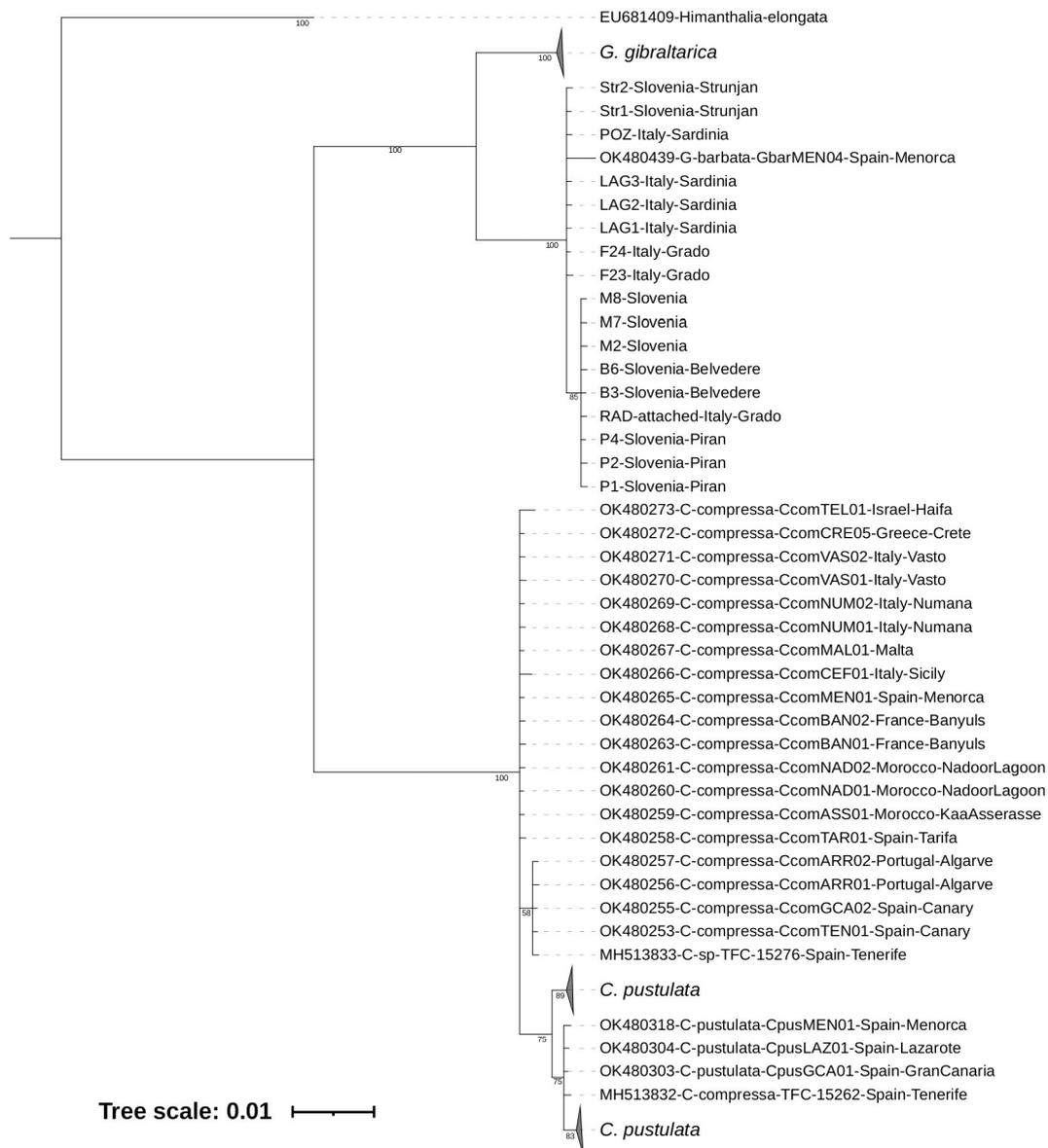


FIGURE 5. Phylogenetic tree of the 2nd half of the cox 1 gene portion. The cluster including all *G. gibraltaria* sequences were condensed. Numbers on branches represent the node support and the bar represents the scale for branches length.

Discussion

For *Gongolaria barbata*, a great morphological plasticity has been documented along the northern Adriatic Sea (Falace & Bressan 2006, Savonitto *et al.* 2019, Sadogurska *et al.* 2021, Orlando-Bonaca *et al.* 2022). It has been reported that *G. barbata* does not undergo a true dormancy period, as receptacles are present throughout the year, although their maximum development occurs in spring and early summer (Sauvageau 1912, Ercegović 1952, Gómez-Garreta *et al.* 1982). In contrast, for the Gulf of Trieste, Falace & Bressan (2006) indicated that *G. barbata* undergoes seasonal morphological changes in which fronds are lost, with a peak of fertility between March and May. On the other hand, Marzocchi *et al.* (2003) reported for the Venice Lagoon (northern Adriatic) that the fronds of *G. barbata* develop in autumn and winter and fall off in summer, although the cauloid is never completely bare.

Over the years, populations in the Gulf of Trieste (i.e., Izola, Strunjan and Piran) have been studied (Falace & Bressan 2006, Falace & Zanelli 2006, Savonitto *et al.* 2021, Orlando-Bonaca *et al.* 2021a, Orlando-Bonaca *et al.* 2021b, Orlando-Bonaca *et al.* 2022). The same observations for the same sites made in this work show the persistence of these morphological traits in the different populations, ascribable to *C. barbata* f. *hoppei* (i.e. Izola population) and

C. barbata var. *turneri* (i.e. Strunjan and Piran populations). This means that the differences between populations are not due to seasonal fluctuations, as Sauvageau (1912) and Giaccone & Bruni (1973) claim, but are rather related to different ecological conditions. The consistency of the morphological traits of these populations seems to argue for the assignment to separate intra-specific taxa, i.e., the occurrence of diverse visible traits due to minor genetic differences (Boudouresque, pers. comm. in Robvieux 2013) and especially for *hoppei* (i.e., Izola populations) and *turneri* (i.e., Piran and Strunjan) as described by J. Agardh (1842). Nevertheless, molecular analyses showed no differences between the Slovenian samples of *G. barbata* (both pleustophytic and haptophytic forms) and the Italian samples collected in both the Adriatic and in Sardinia, based on the mitochondrial *cox 1* gene sequencing data.

Although the three sampling sites on the Slovenian coast are very close, several studies have reported divergence over short distances between contrasting environments (Russell 1978, Sideman & Mathieson 1985, Kilar & Hanisak 1989). Izola is subject to greater riverine inputs from the Rižana and Badaševica rivers (Cozzi *et al.* 2012). In addition, wastewater discharge from the municipality of Izola increases nutrient levels (Cozzi *et al.* 2008). These factors lead to increased sedimentation and turbidity of the water. In contrast, the Strunjan and Piran sites are exposed to higher hydrodinamism but lower anthropogenic pressure (Orlando-Bonaca *et al.* 2015), as they are located on a coastline that is still in its natural state. In high turbidity environments, the development of aerocysts allows algae to stretch to the surface and improve access to light (Lüning 1991). The elongated shape of the receptacles could be another adaptation to increase the surface area exposed to light and thus improve photosynthesis. To further confirm the site-specific phenotypic plasticity of *G. barbata*, recruits from the Strunjan population (i.e., lower sedimentation, higher hydrodinamism), grown ex-situ and transplanted to the Miramare Marine Protected Area (Gulf of Trieste), which is exposed to a high sedimentation rate and lower hydrodinamism as in Izola (Ogorelec *et al.* 1991), developed long receptacles with concatenated aerocysts (Savonitto *et al.* 2019).

In summary, among the haptophytic forms, the morphological traits of the two described varieties *hoppei* [characterized by flexuous and elongated fronds; fusiform (up to 20 mm), simple, with long mucrons, pedunculate receptacles; numerous, voluminous, oval to fusiform aerocysts, solitary or more often in chains up to 5) and *turneri* [characterized by squat and short fronds; cylindrical receptacles (up to 10 mm), simple or bifid, not always mucronate (the mucron, when present, is short) and aerocysts absent or very rare, never in chains] are well recognizable as the extremes of a morphological variability due to well-identified, site-dependent ecological traits and that these variations remain constant throughout the year and in successive years (TABLE 5). In the absence of genetic differences, but in order to retain the ecological information associated with the two ecoforms, we retain the two infraspecific taxa mentioned above as distinct, for which we propose the following two new combinations under the genus *Gongolaria*: *Gongolaria barbata* f. *hoppei* and *G. barbata* f. *turneri*. In contrast, the descriptions of *G. barbata* f. *barbata* (Gómez Garreta *et al.* 2000, Cormaci *et al.* 2012), put into evidence a noticeable variability in reproductive characters (i.e. terminal, cylindrical or fusiform, slightly rough or smooth, pedunculate when exceeding an aerocyst, with variable length and a terminal mucron) and in thallus morphology (i.e. well-developed primary branches, especially in spring; secondary order branches bearing single aerocysts or arranged in chains) given as seasonal phenological aspects (TABLE 5).

As for the free-living form collected in lagoons, all analysed specimens from the Adriatic and Sardinia are morphologically similar and genetically correspond to *G. barbata*, with no variability between bases. This confirms the hypothesis that they are branches detached from the cauloid that continue to grow in calm environments (i.e., shallow and stagnant waters), such as lagoons. Loss of branches occurs in most *Cystoseira s.l.* and is related to seasonal periodism. In shallow and calm waters, these fronds can remain trapped in seagrasses or on the bottom and continue their growth. By remaining buoyant, they lose the apicality of the growth of the main branches, the spacing between the nodes also increases and they appear looser, so that it is no longer possible to distinguish between branches of different orders. It has even been shown that detached, sterile main branches of *G. barbata* can continue to grow and become fertile under favorable temperature and nutrient conditions (Kaleb-Sanchez-Pedro *et al.* 2023). Detached main branches of *G. barbata* were also observed by Iveša *et al.* (2022) in the Šćuza lagoon on the southern coast of Istria (northern Adriatic, Croatia).

Bruno de Sousa *et al.* (2019) placed *C. barbata* f. *aurantia* (COI sequence deposited in GenBank: MF768074, as *C. barbata* f. *repens*) in clade 2 by analyzing a free-living specimen from the Atlantic (Cádiz, Spain) that also showed high genetic affinity to *C. mauritanica* Sauvageau (COI sequence deposited in GenBank: MF768073) (Figures 3, 4 in Bruno de Sousa *et al.* 2019). For these two sequences, Neiva *et al.* (2022) proposed the reassignment under the names *Gongolaria gibraltarica* f. *lacunarum* Neiva *et* Serrão and *Gongolaria gibraltarica*, respectively, based on the comparison with Atlantic specimens (e.g., Nadar in Morocco, Algarve in Portugal).

A short time before, Orellana *et al.* (2019) include four taxa from the Canary Islands in the clade *Cystoseira s.s.* (= *Cystoseira* 1): *C. foeniculacea* (L.) Greville, *C. compressa*, *C. humilis* Schousboe ex Kützing and *Cystoseira aurantia*, all of which described as arborescent and epilithic cespitose species, although *C. aurantia* is shown in their figures as free-living branches (Figures 3 and 4 in Orellana *et al.* 2019). This taxon is characterized by cylindrical, smooth main branches and large aerocysts (up to 7 mm long) arranged mainly along the branches of the last order (Orellana *et al.* 2019). However, although it was renamed *C. aurantia* by Orellana *et al.* (2019), the molecular sequences of this taxon are curiously deposited in GenBank as *Cystoseira sp.* (COI sequence: MH513833) and were later genetically identified as *C. compressa* by Neiva *et al.* (2022). Orellana *et al.* (2019) also deposited in GenBank the COI sequence of a sample of *C. compressa* (MH513832), which was later described as *C. pustulata* by Neiva *et al.* (2022). It should be noted that Neiva *et al.* (2022: Table 4) incorrectly inverted the original ID of the two COI sequences deposited by Orellana *et al.* (2019), MH513832 and MH513833, and referred to the former as *Cystoseira aurantia* and the latter as *Cystoseira compressa*.

So far, free-living forms have been reported not only for *G. barbata*, but also for *C. foeniculacea* (Linnaeus) Greville f. *dubia* (Ercegovič) Bouafif *et Verlaque* (Bouafif *et al.* 2016), *G. gibraltaria* f. *lacunarum* (Neiva *et al.* 2022), *C. foeniculacea* f. *formosensis* Neiva *et Serrão* (Neiva *et al.* 2022). The ability to cut off branches that can continue to grow may be more widespread than previously documented and could be an adaptive growth form associated with a dispersal strategy that deserves further investigation.

Based on the above and our genetic analyses, the floating forms in the Atlantic do not seem to belong to *G. barbata*, so that the assignment of *C. aurantia* [= *C. barbata* f. *aurantia* (Kützing) Giaccone] by Ramdani *et al.* (2021) based on morphology alone should be reconsidered, also because Neiva *et al.* (2022) found *G. gibraltaria* f. *lacunarum* at the same location. Considering that the floating forms are morphologically convergent and poorly typified, their recognition based only on morphological traits alone is questionable, since the identification of *Cystoseira s.l.* is based mainly on diacritical features related to cauloid (e.g., caespitose or not, with smooth or spiny apex, prominent or not) or branching, which are absent or highly modified in the unattached forms. Finally, it should be noted that the identification keys of *Cystoseira s.l.* (e.g., Cormaci *et al.* 2012, Gómez-Garreta 2000) list *C. barbata* f. *aurantia* (or f. *repens*) as the only possible free-living ecotype, which has also led to misidentifications.

Therefore, we retain the form ‘*aurantia*’ for the free-living form, which refers to detached branches in lagoon environments that continue their biological cycle and also attain fertility. Such a form is here combined under the genus *Gongolaria* as *Gongolaria barbata* f. *aurantia*

Taxonomy

Gongolaria barbata f. *aurantia* (Kützing) Falace, Alongi *et* Kaleb *comb. nov.*

Basionym: *Cystoseira aurantia* Kützing, 1843: 357. Phycologia generalis.....Leipzig XXXII+458 pp, 80 pls.

Homotypic synonym:

Cystoseira barbata f. *aurantia* (Kützing) Giaccone in Amico *et al.* (1986: 906). Un nuovo approccio allo studio della sistematica del genere *Cystoseira* C. Agardh (Phaeophyta, Fucales). Bollettino dell'Accademia Gioenia di Scienze Naturali di Catania, 18(326): 887–988.

Note: in absence of molecular evidences, we here consider *G. barbata* f. *aurantia* from Mediterranean as a distinct taxon from *C. barbata* f. *repens* Zinova *et* Kalugina [= *G. barbata* f. *repens* (Zinova *et* Kalugina) Sadogurska] described for the Black Sea, due to its morphological characters rather different from those of Zinova *et* Kalugina's taxon as described and illustrated by Sadogurska (2021: 301, Fig 3), particularly due to the presence of short primary axis in f. *repens* (not present in f. *aurantia*) and both lateral and ultimate branches in f. *repens* (neither lateral nor ultimate branches distinguishable in f. *aurantia*) (see Sadogurska, 2021: 301, Fig 3a and Cormaci *et al.* 2012 tav 93 fig 1, respectively).

Gongolaria barbata f. *hoppei* (C. Agardh) Falace, Alongi *et* Kaleb *comb. nov.*

Basionym: *Cystoseira hoppei* “*hoppii*” C. Agardh, 1820: 59, Species algarum.....Volumen primum. Pars prima. pp. [i–iv], [1]–168. Lundae [Lund]: ex officina Berlingiana.

Homotypic synonyms: *Cystoseira barbata* var. *hoppei* “*hoppii*” (C. Agardh) J. Agardh 1842: 51, *Algae maris Mediterranei et Adriatici*, pp. [i]–x, 1–164. Parisiis [Paris]: Apud Fortin, Masson et Cie.
Cystoseira barbata f. *hoppei* “*hoppii*” (C. Agardh) Woronichin 1908: 117, *Buryya vodorosli* (Phaeophyceae) Chernago Morya [The brown algae of the Black Sea]. *Botanicheskii Zhurnal* (1–2): 19–46, 113–138, 11 figures, 2 tables. [in Russian with German summary]

Gongolaria barbata* f. *turneri (J. Agardh) Falace, Alongi et Kaleb *comb. et stat. nov.*

Basionym: *Cystoseira barbata* v. *turneri* J. Agardh 1842: 50, *Algae maris Mediterranei et Adriatici*, pp. [i]–x, 1–164. Parisiis [Paris]: Apud Fortin, Masson et Cie.

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