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Dasysiphonia adriatica sp. nov. (Delesseriaceae, Rhodophyta), a new red algal species from the North Adriatic Sea (Mediterranean Sea)

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Abstract

Dasysiphonia is a genus of the family Delesseriaceae (Rhodophyta) including 9 taxonomically accepted species, among which only the non-indigenous *Dasysiphonia japonica* has been documented from the Adriatic Sea (Mediterranean). This invasive species is native to Hokkaido Island (Japan) and was introduced to Europe and the Mediterranean Sea through imports of the commercial Pacific oyster *Magallana gigas*. In this study, we describe a new species belonging to the genus *Dasysiphonia,* collected in the Mediterranean Sea. This new taxon was sampled in Slovenian coastal waters; its thalli were analysed using both molecular and morphological approaches and it was compared with the other known species. Moreover, samples of the invasive *D. japonica* were collected from different Venice Lagoon (Italy) sites and used for comparison with the new taxon. The phylogenetic reconstruction, based on the plastid *rbc*L gene, clearly distinguished the new Slovenian entity from all the known *Dasysiphonia* species*,* including the ones recently transferred from the sister genus *Dasya.* These results indicate that the Slovenian samples represent a new species, hereby named *Dasysiphonia adriatica* sp. nov.

Keywords: *Dasysiphonia*; Delesseriaceae; North Adriatic Sea; new macroalgal species; *rbc*L.

Introduction

The genus *Dasysiphonia* I.K. Lee & J.A. West (Delesseriaceae, Rhodophyta) was established in 1979 with the description of the type species *Dasysiphonia chejuensis* from Korea. The type species described by Lee & West (1979) is characterized by a typical *Polysiphonia*-type life history, with isomorphic gametophytes and sporophytes, although for some species of the genus *Dasysiphonia* only the tetrasporophytic phase is known (Schneider, 1989). Recently, a molecular study on the systematics of the sister genus *Dasya* C. Agardh transferred four species from Bermuda to the *Dasysiphonia* genus: *Dasysiphonia clavigera* (Womersley) M.M. Cassidy, C.W. Schneider & G.W. Saunders, *D. collinsiana* (M. Howe) M.M. Cassidy, C.W. Schneider & G.W. Saunders, *D. naccarioides* (Harvey) M.M. Cassidy, C.W. Schneider & G.W. Saunders and *D. sessilis* (Yamada) M.M. Cassidy, C.W. Schneider & G.W. Saunders (Cassidy *et al*., 2022). Therefore, this small red algal genus currently includes nine taxonomically accepted taxa (Guiry & Guiry, 2023); however, other *Dasya* species are phylogenetically close to the *Dasysiphonia* lineage, but additional studies are required to transfer them officially to the latter genus (Cassidy *et al*., 2022).

Among the currently accepted taxa, *Dasysiphonia japonica* (Yendo) H.-S. Kim is so far the only one reported from the Adriatic Sea (Mediterranean Sea) (Sfriso *et al*., 2020). This invasive macroalgal species is native to Hokkaido Island in Japan (Kim, 2012), an area that represents one of the major oyster production sites of the country (around 700 tonnes per year; Hasegawa *et al*., 2015). In Europe, this invasive red seaweed was first recorded in Brittany (France) in 1984, near an oyster farming site, and in 1988 in Galicia (Spain) (Sjøtun *et al*., 2008). In 1998, it was also recorded in the Thau Lagoon along the French Mediterranean coast (Verlaque, 2001). Its introduction and dispersal along the European and Mediterranean coasts is due, most probably, to its association with the Pacific oyster *Magallana gigas* (Thunberg, 1793) (Sjøtun

et al., 2008). According to recent studies, *D. japonica* has been spreading massively to a number of coastal areas, with blooms that have been associated with fish die-offs (Fofonoff *et al*., 2018).

In this study, we describe a new species belonging to the genus *Dasysiphonia* collected in the North Adriatic Sea (Mediterranean Sea), herein named *Dasysiphonia adriatica* M.A. Wolf, K. Sciuto, A. Buosi, M. Orlando-Bonaca, A. Fortič & A. Sfriso sp. nov. Thalli of the new species were analysed using a molecular approach based on the *rbc*L marker as well as morphological methods, and the data were compared with all the other known taxa of the genus, including the invasive *D. japonica*.

Materials and Methods

Sampling

Sampling was carried out in Slovenian coastal waters, as part of the national monitoring of non-indigenous species in the greater area of Izola Marina (45.539386° N, 13.656258° E). Specimens were collected in January 2023 in the lower mediolittoral and upper infralittoral zone (approx. 1 m depth), using a scraper hand net. The sampling site was characterized by scattered limestone rocks, densely covered with macroalgae. Three non-indigenous algal species, *Asparagopsis armata* Harvey, *Codium fragile* (Suringar) Hariot and *Colaconema codicola* (Børgesen) Stegenga, J.J.Bolton & R.J. Anderson, were recorded at this site during the period 2021-2023 (Mavrič *et al*., 2023).

Moreover, several thalli of the invasive species *D. japonica* were sampled, for purposes of comparison, from three areas of the Venice Lagoon (Italy), during different monitoring campaigns within the framework of the Mo.V.Eco V regional project, developed to assess the ecological status of the lagoon (European Water Directive 2000/60/EC).

The Venice Lagoon samplings were performed during winter 2023 at different sites: Station ENC1_1 (45.321303° N, 12.312681° E Santa Maria del Mare, Pellestrina island), Station ENC1_9 (45.358467° N, 12.263336° E San Leonardo, Venice), Station ENC2_1 (45.442394° N, 12.408675° E; San Nicolò, Lido of Venice island).

Morphological analyses

A 4% formaldehyde/seawater solution was used as tissue fixative for the preservation of all the collected specimens, which were successively observed using a light microscope (alternatively, an Optika B-510PH, Ponteranica, Italy or an Olympus SZX16, Tokyo, Japan) equipped with a digital image acquisition system. The final figure plate was assembled with GIMP v. 2.8.22 (https://www.gimp.org) and Inkscape v. 0.92 (www.inkscape.org).

The holotype of the new species was deposited at PAD Herbarium, Botanical Garden Padova (Italy).

Molecular analyses

The specimens were preserved in silica gel for the molecular analyses. The Genomic DNA purification kit (Thermo Fisher Scientific™) was used to purify the DNA from both the Italian and Slovenian samples. A fragment of the plastid *rbc*L gene (*rbc*L-5P, about 700 bp long) was amplified using the primer pair F57-R753 (Freshwater & Rueness, 1994) and following the PCR parameters reported in Wolf *et al.* (2018). The obtained PCR products were purified with an enzymatic method using the HT ExoSAP-IT (Applied Biosystems™), and sequencing was performed at the Eurofins Genomics Sequencing Service (Germany). The GeneStudio software (http://genestudio.com) was used to assemble the final consensus sequences. One sequence for each sampling site was deposited in the International Nucleotide Sequence Database Collaboration (INSDC) repositories, through the GenBank platform, with the following accession numbers: PP133045 - PP133047 (for *D. japonica* sampled at three different sites of the Venice Lagoon) and PP133048 (for the new Slovenian taxon).

Through the BLAST program, available at the USA National Center for Biotechnology Information (NCBI) web server (http://www.ncbi.nlm.nih.gov), the obtained sequences were compared with those already deposited in the INSDC archives.

A dataset was created including the newly obtained sequences and other *rbc*L-5P sequences available in GenBank, following the most recent classifications for the genera *Dasysiphonia* and *Dasya* (Cassidy *et al*., 2022). Sequences of representative species of both the genera of the family Delesseriaceae were included. One sequence of the genus *Ceramium* (INSDC accession: GQ252456) was used as the outgroup for tree orientation.

For the phylogenetic analyses, a multiple sequence alignment was created using the MUSCLE (Edgar, 2004) software; it included 28 sequences for a total of 660 aligned positions. Phylogenetic analyses were based on the Neighbour joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) methods and were obtained with the MEGA 11 program (Tamura *et al*., 2021). For ML, the model that best fit the data was GTR+G, as suggested by the "Find best DNA Models" tool implemented in MEGA under the BIC criterion (Schwarz, 1978). A non-parametric bootstrap re-sampling (Felsenstein, 1985) of 1000 replicates was performed to test the robustness of the tree topologies.

Bayesian Inference (BI) analyses were carried out with MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). The analyses included two independent MCMC runs, each composed of four chains (three heated and one cold); each MCMC ran for 1 x 10^6 generations, sampling trees every 100 generations. The sampling of the posterior distribution was considered to be adequate if the average standard deviation of split frequencies was ≤0.01. The first 2500 trees were discarded as burn-in, as determined by stationarity of log likelihood assessed using Tracer version 1.5 (Rambaut & Drummond, 2007). The consensus topology and posterior probability values were then calculated from the remaining trees. The final tree

figure was created with Inkscape version 0.92.

Alignments of the *rbc*L-5P sequences of the genera *Dasya* and *Dasysiphonia* were also obtained with MUSCLE to calculate the sequence percent identities within these groups.

Results and Discussion

In the phylogenetic reconstruction, based on the plastid *rbc*L gene (Fig. 1), the Italian samples collected from different sites of the Venice Lagoon were all included in a well-supported clade (100ML/100MP/100NJ/1.00BI) with another sequence of *D. japonica* from Massachusetts (USA) (INSDC accession: MW698722). The Slovenian sequence, instead, was separated and well distinct from all the other species included in the genus *Dasysiphonia*, as well as from those that were recently transferred to this taxon from the sister genus *Dasya*.

The *rbc*L-5P interspecific range of nucleotide percent identities, among the sequences of clades identified at genus level and including different species of the corresponding genera, were: 91.96–96.97% for *Dasya* and 88.47–98.18% for *Dasysiphonia* (Table 1). For both the genera, all the specimens attributed to a same species showed 100% *rbc*L-5P sequence identity (Table 1). With respect to the other recognized species of the genus *Dasysiphonia*, the sequence representing the Slovenian specimens showed a minimum percent identity of 90.29% with *D. hutchinsiae* and a maximum percent identity of 94.23% with *D. corymbifera* (Table 1).

The above molecular results demonstrate that the investigated Slovenian specimens represent a distinct lineage, separated from all the other species included in the genus *Dasysiphonia*, even if they are morphologically attributable to this taxon (Fig. 2 and below description). This finding is supported by a detailed morphological analysis of the new taxon diagnostic characters and by the morphological comparisons with the other so far accepted species of *Dasysiphonia* (Table 2).

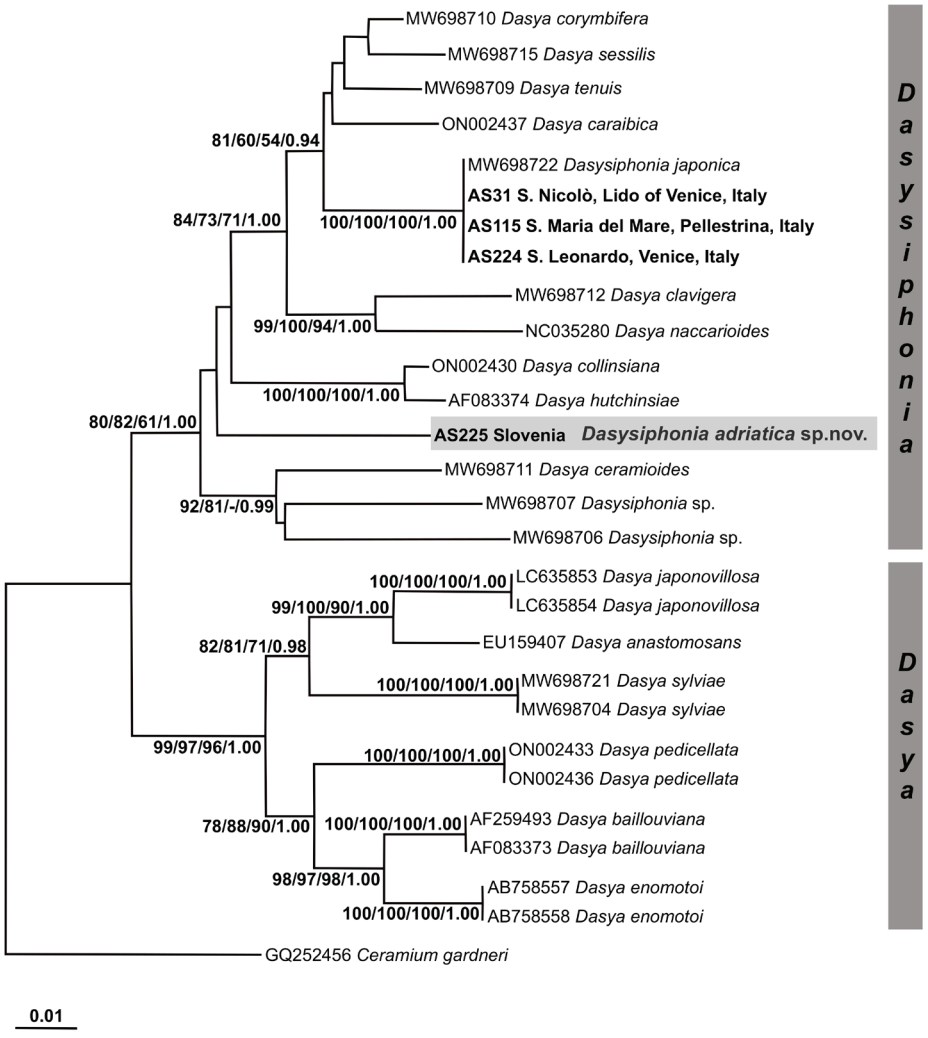

Fig. 1: Phylogenetic tree based on the partial *rbc*L gene of the genera *Dasya* and *Dasysiphonia*. The reconstruction obtained with the NJ method is depicted and the support values from NJ bootstrap, MP bootstrap, ML bootstrap and BI posterior probabilities are reported, respectively. Only bootstrap supports ≥ 50% and posterior probabilities ≥ 0.70 are shown. Values for nodes supported in only two of the phylogenetic analyses were omitted. For each downloaded sequence, the INSDC accession number and the species name are reported. The sequences obtained in this work are in boldface font. The scale bar represents the expected number of nucleotide subs per site.

Table 1. Percent identity matrix for the *rbc*L-5P gene fragment (660 aligned positions) of the genera *Dasya* and *Dasysiphonia*. Grey boxes highlight genus borders.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24	25	26	27
1: CN002436 D. pedicellata	100.00	100.00	94.08	94.08	94.69	94.69	92.87	92.87	93.32	93.32	93.47	89.98	91.20	90.29	89.83	87.41	89.38	89.83	89.83	89.83	89.83	90.59	89.68	89.98	89.83	88.47	88.92
2: CN002433 D. pedicellata	100.00	100.00	94.08	94.08	94.69	94.69	92.87	92.87	93.32	93.32	93.47	89.98	91.20	90.29	89.83	87.41	89.38	89.83	89.83	89.83	89.83	90.59	89.68	89.98	89.83	88.47	88.92
3: AB758558 D. encmotoi	94.08	94.08	100.00	100.00	96.97	96.97	92.26	92.26	92.41	92.41	91.96	90.14	90.90	90.74	91.05	88.92	89.38	90.59	90.59	90.59	90.59	89.98	89.68	89.98	90.14	89.07	89.98
4: AB758557 D. encmotoi	94.08	94.08	100.00	100.00	96.97	96.97	92.26	92.26	92.41	92.41	91.96	90.14	90.90	90.74	91.05	88.92	89.38	90.59	90.59	90.59	90.59	89.98	89.68	89.98	90.14	89.07	89.98
5: AF259493 D. baillouviana	94.69	94.69	96.97	96.97	100.00	100.00	92.56	92.56	92.72	92.72	92.87	89.53	89.68	89.53	91.05	88.92	89.83	90.90	90.90	90.90	90.90	90.44	90.29	90.29	90.44	88.92	89.83
6: AF083373 D. baillouviana	94.69	94.69	96.97	96.97	100.00	100.00	92.56	92.56	92.72	92.72	92.87	89.53	89.68	89.53	91.05	88.92	89.83	90.90	90.90	90.90	90.90	90.44	90.29	90.29	90.44	88.92	89.83
7: MW698721 D. sylviae	92.87	92.87	92.26	92.26	92.56	92.56	100.00	100.00	93.63	93.63	93.93	89.38	89.38	90.14	89.98	88.01	89.53	90.14	90.14	90.14	90.14	89.68	89.83	89.83	90.29	88.32	88.47
8: MW698704 D. sylviae	92.87	92.87	92.26	92.26	92.56	92.56	100.00	100.00	93.63	93.63	93.93	89.38	89.38	90.14	89.98	88.01	89.53	90.14	90.14	90.14	90.14	89.68	89.83	89.83	90.29	88.32	88.47
9: LC635854 D. japonovillosa	93.32	93.32	92.41	92.41	92.72	92.72	93.63	93.63	100.00	100.00	96.66	90.44	90.90	90.59	90.29	87.41	89.98	89.83	89.83	89.83	89.83	89.38	89.23	89.53	89.38	87.71	88.62
10: LC635853 D. japonovillosa	93.32	93.32	92.41	92.41	92.72	92.72	93.63	93.63	100.00	100.00	96.66	90.44	90.90	90.59	90.29	87.41	89.98	89.83	89.83	89.83	89.83	89.38	89.23	89.53	89.38	87.71	88.62
11: EU159407 D. anastomosans	93.47	93.47	91.96	91.96	92.87	92.87	93.93	93.93	96.66	96.66	100.00	90.44	90.59	91.05	89.98	87.41	89.98	90.44	90.44	90.44	90.44	90.29	90.44	90.44	90.29	89.53	89.53
12: MW698706 Dasysiphonia sp	89.98	89.98	90.14	90.14	89.53	89.53	89.38	89.38	90.44	90.44	90.44	100.00	93.47	93.93	91.81	88.47	92.41	91.81	91.81	91.81	91.81	91.35	91.50	90.74	91.35	90.59	91.20
13: MW698707 Dasysiphonia sp	91.20	91.20	90.90	90.90	89.68	89.68	89.38	89.38	90.90	90.90	90.59	93.47	100.00	94.08	91.81	89.07	92.26	91.96	91.96	91.96	91.96	92.72	91.65	91.20	92.11	90.59	92.11
14: MW698711 D. caramicoides	90.29	90.29	90.74	90.74	89.53	89.53	90.14	90.14	90.59	90.59	91.05	93.93	94.08	100.00	92.56	90.14	93.17	92.72	92.72	92.72	92.72	92.72	92.41	92.56	93.17	91.65	91.50
15: CN002430 D. collinsiana	89.83	89.83	91.05	91.05	91.05	91.05	89.98	89.98	90.29	90.29	89.98	91.81	91.81	92.56	100.00	96.05	93.32	93.17	93.17	93.17	93.17	93.63	93.78	93.32	93.63	91.65	92.26
16: AF083374 D. hutchinsiae	87.41	87.41	88.92	88.92	88.92	88.92	88.01	88.01	87.41	87.41	87.41	88.47	89.07	90.14	96.05	100.00	90.29	90.74	90.74	90.74	90.74	91.20	91.05	91.05	91.20	89.23	89.38
17: AS225 Dasysiphonia sp. nov	89.38	89.38	89.38	89.38	89.83	89.83	89.53	89.53	89.98	89.98	89.98	92.41	92.26	93.17	93.32	90.29	100.00	92.72	92.72	92.72	92.72	93.17	92.87	93.02	94.23	92.26	92.72
18: AS31 D. japonica	89.83	89.83	90.59	90.59	90.90	90.90	90.14	90.14	89.83	89.83	90.44	91.81	91.96	92.72	93.17	90.74	92.72	100.00	100.00	100.00	100.00	95.90	96.21	96.05	96.05	93.17	93.47
19: AS115 D. japonica	89.83	89.83	90.59	90.59	90.90	90.90	90.14	90.14	89.83	89.83	90.44	91.81	91.96	92.72	93.17	90.74	92.72	100.00	100.00	100.00	100.00	95.90	96.21	96.05	96.05	93.17	93.47
20: AS224 D. japonica	89.83	89.83	90.59	90.59	90.90	90.90	90.14	90.14	89.83	89.83	90.44	91.81	91.96	92.72	93.17	90.74	92.72	100.00	100.00	100.00	100.00	95.90	96.21	96.05	96.05	93.17	93.47
21: MW698722 D. japonica	89.83	89.83	90.59	90.59	90.90	90.90	90.14	90.14	89.83	89.83	90.44	91.81	91.96	92.72	93.17	90.74	92.72	100.00	100.00	100.00	100.00	95.90	96.21	96.05	96.05	93.17	93.47
22: CN002437 D. caraibica	90.59	90.59	89.98	89.98	90.44	90.44	89.68	89.68	89.38	89.38	90.29	91.35	92.72	92.72	93.63	91.20	93.17	95.90	95.90	95.90	95.90	100.00	96.81	96.36	97.27	94.08	93.47
23: MW698709 D. tamuis	89.68	89.68	89.68	89.68	90.29	90.29	89.83	89.83	89.23	89.23	90.44	91.50	91.65	92.41	93.78	91.05	92.87	96.21	96.21	96.21	96.21	96.81	100.00	96.81	98.03	94.54	93.93
24: MW698715 D. sessilis	89.98	89.98	89.98	89.98	90.29	90.29	89.83	89.83	89.53	89.53	90.44	90.74	91.20	92.56	93.32	91.05	93.02	96.05	96.05	96.05	96.05	96.36	96.81	100.00	98.18	94.08	94.08
25: MW698710 D. corymbifera	89.83	89.83	90.14	90.14	90.44	90.44	90.29	90.29	89.38	89.38	90.29	91.35	92.11	93.17	93.63	91.20	94.23	96.05	96.05	96.05	96.05	97.27	98.03	98.18	100.00	94.69	94.39
26: NC_035280 D. naccarioides	88.47	88.47	89.07	89.07	88.92	88.92	88.32	88.32	87.71	87.71	89.53	90.59	90.59	91.65	91.65	89.23	92.26	93.17	93.17	93.17	93.17	94.08	94.54	94.08	94.69	100.00	95.45
27: MW698712 D. clavigera	88.92	88.92	89.98	89.98	89.83	89.83	88.47	88.47	88.62	88.62	89.53	91.20	92.11	91.50	92.26	89.38	92.72	93.47	93.47	93.47	93.47	93.47	93.93	94.08	94.39	95.45	100.00

***Dasysiphonia adriatica* M.A. Wolf, K. Sciuto, A. Buosi, M. Orlando-Bonaca, A. Fortič & A. Sfriso sp. nov. (Fig. 2)**

Description

Vegetative structures

Plants grow in the lower mediolittoral and upper infralittoral coastal zone, on limestone rocks. Thalli are small, 1.5-2 cm high, dark red in colour and bushy (Fig. 2A). Thalli are composed of main polysiphonous indeterminate axes, 0.15-0.25 mm in diameter, irregularly alternately to pseudo-dichotomously branched (Fig. 2B), corticated only at the base and distally tapering. The main axes bear shorter determinate monosiphonous laterals with a spiralled disposition (Fig. 2A, B). Few pseudolaterals can be polysiphonous at the base (Fig. 2C). Pseudolaterals are 0.5-0.9 mm in length, pseudo-dichotomously branched (Fig. 2C) 2-6 times, with upper portions unbranched and slightly tapering (Fig. 2D). Basal and median cells of pseudolaterals are ellipsoidal, longer than wide (Fig. 2D), 40-50 µm in diameter and 130-150 µm long; apical cells are globose to slightly elongated, 30-40 µm in diameter and 30-120 µm long, elongating more centrally and reaching greatest lengths distally (Fig. 2E). Apices of pseudolaterals are covered by hair-like filaments (Fig. 2E).

Reproductive structures. Tetrasporangial stichidia developing singularly or in pairs, borne from one basal cell (Fig. 2F), lanceolate and often curved in outline (Fig. 2F, G), 60-110 µm in diameter and 250-500 µm in length at maturity, composed of 10-14 fertile segments and 1-3 terminal sterile cells (Fig. 2G). Sporangia globose, 40 µm in diameter, tetrahedrally divided (Fig. 2G). Only tetrasporophytes were collected.

Diagnosis: The species is clearly distinct from all the other species of the same genus, based on *rbc*L-5P phylogenetic analyses. Morphologically, it differs also from the other small species as regards the cortication pattern and pseudolateral structure.

Holotype (here designated): Voucher A001046 at PAD Herbarium, Botanical Garden Padova (Italy).

Type locality: Izola Marina, Slovenia, North Adriatic, Mediterranean.

Etymology: the specific epithet (fem. adj.) refers to the area (i.e., the Adriatic Sea) where the new species was found for the first time so far.

Molecular voucher: PP133048 (*rbc*L-5P gene).

The new *Dasysiphonia* entity is one of the smallest species (1.5-2 cm in height) of the genus, together with *D. collinsiana*, *D. concinna*, *D. doliiformis*, and *D. okiensis* (see Table 2). It differs from all the aforementioned taxa as regards the cortication pattern; in fact, the new entity is characterized by cell cortication restricted to the basal part of the thallus, whereas *D. concinna* and *D. doliiformis* are completely ecorticated (Schneider, 1989) and *D. collinsiana* and *D. okiensis* are almost completely corticated (Kajimura, 1992; Schneider *et al*., 2021). Moreover, the Adriatic specimens can be distinguished

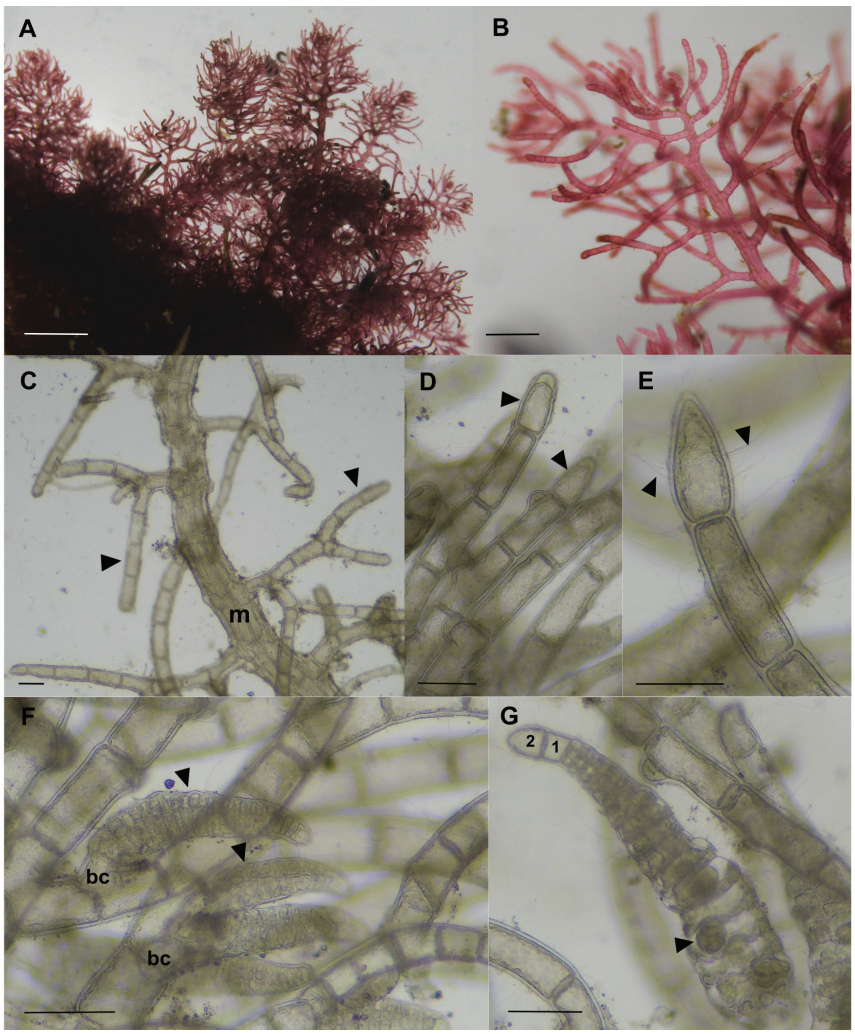

Fig. 2: *Dasysiphonia adriatica* sp. nov. from Slovenia. (A) Habit of vegetative thallus. (B) Detail showing alternate to pseudo-dichotomous branching. (C) Main polysiphonous axes (m) with monosiphonous pseudolaterals (arrowheads). (D) Detail of pseudolateral apices (arrowheads). (E) Detail of apex covered by hair-like filaments (arrowheads). (F) Tetrasporangial stichidia (arrowheads) developing from one basal cell (bc). (G) Detail of a tetrasporangial stichidium showing a mature tetraspore (arrowhead) and two apical sterile cells (1, 2). Scale bars represent: A, 2 mm; B, 200 µm; C–G, 100 µm.

from the other species by the structure of the pseudolateral branches. In fact, *D. concinna*, *D. doliiformis, D. collinsiana* and *D. okiensis* have only monosiphonous pseudolaterals, whereas the new Adriatic specimens have polisiphonous pseudolaterals at the base of the thalli. The new species can also be easily distinguished from *D. japonica*, the other taxon occurring in the Adriatic Sea. Indeed, this invasive species is characterized by larger sized thalli (up to 15 cm) and a larger diameter of the main axes (0.5-0.8 mm), which bear shorter determinate monosiphonous laterals with a planar disposition instead of a spiral one (Sfriso *et al*., 2020). Moreover, in the Asian specimens of *D. japonica* the tetrasporangial stichidia are bigger in size (the diameter and length are three times larger than in the Adriatic *Dasysiphonia* sp. samples) (Choi, 2001).

Conclusion

In this study, we report on a new *Dasysiphonia* species recorded for the first time in the Adriatic Sea (Slovenia). In the phylogenetic reconstruction based on the plastid *rbc*L gene, this new taxon was clearly distinct from all the other known *Dasysiphonia* species. Moreover, the nucleotide percent divergence found between the new Adriatic entity and the other *Dasysiphonia* species was comparable with the interspecific range calculated for the genus. From a morphological point of view, the new putative species can be distinguished from all the other small taxa of the genus by the cortication pattern and the pseudolateral structure. In addition, it is well distinct from the other invasive species present in the Adriatic Sea, *D. japonica*, for the smaller sized thallus, the diameter of the main axes and the spiral disposition of the pseudolaterals.

In light of these molecular and morphological findings, we can conclude that the Adriatic specimens from Slovenia reported in this study represent a new species of the genus *Dasysiphonia*, herein named *Dasysiphonia adriatica* sp. nov.

Table 2. Morphological comparison of *Dasysiphonia* species.

	***D. adriatica* sp.nov.**	***D. chejuensis***	***D. clavigera***	***D. collinsiana***	***D. concinna***	***D. doliiformis***	***D. japonica***	***D. naccarioides***	***D. okiensis***	***D. sessilis***
Plant height (cm)	1.5-2	3-5	2-20(35)	1-3	1.5	1.5	2.5-15	Up to 50	0.5-4.0	(3)5-25(28)
Branching pattern	Alternate, irregular, to pseudo-dichotomous	Sympodial, bilateral/ dorsiventral	Sympodial	Alternate, irregular, to pseudo-dichotomous	Pseudolateral, 3-5 times pseudo-dichotomously branched	Pseudolateral, 1-2 times pseudo-dichotomously branched	Alternate to pseudo-dichotomously in one plane	Irregularly and radially branched	Sympodial, densely alternate-pinnated, tuffled with decumbent tendency	Dichotomous to pseudo-dichotomous
Main axes cortication	Only at the base	Lightly corticated	Thickly corticated at the base	Mostly complete	Ecorticated	Ecorticated	Rhizoidal, complete at the base	Densely corticated	Densely in the lower part, lightly in middle to upper part.	Densely corticated throughout
Main axes diameter (mm)	0.15-0.25	0.13-0.23	Up to 2.0	0.5-0.75	0.08-0.26	0.1-0.22	0.5-0.8	Up to 3.0	0.17-0.66	0.1-2.0
Apices of main axes	Tapering distally	Tapering slightly to tips	From slightly tapering to truncated	Tapering distally	Obtuse to conical	Short to long conical	Tapering distally	Tapering distally	Tapering to obtuse	Gradually tapering distally
Pseudolaterals	Monosiphonous, few polysiphonous at the base	Monosiphonous and polysiphonous	Monosiphonous	Monosiphonous	Monosiphonous	Monosiphonous	Monosiphonous, few polysiphonous at the base	Monosiphonous	Pseudo-dichotomously branched	Monosiphonous
Disposition	Spiralled	-	Spiralled	Spiralled	-	-	Random	Spiralled	Pseudo-dichotomous	Spiralled
Axial coverage	Dense coverage, denuded only proximally	Densely covered distally, naked proximally	Densely covered distally, naked proximally	Dense coverage up to the last 2-3 dichotomies, lighter above	Dense bushy coverage	Dense coverage	Dense bushy appearance	Dense coverage, denuded only proximally	Rhizoidal coverage only in the lowermost cells.	Densely covered distally, naked proximally
Branching	2-6 pseudo-dichotomous	Dichotomous	Dichotomous to pseudo-dichotomous	5-8 times divaricately pseudo-dichotomous	Alternate, distichously arranged	Alternate, distichously arranged	Alternate to pseudo-dichotomous; sympodial at the apex.	Irregularly	3-6 pseudo-dichotomous	Dichotomous to pseudo-dichotomous
Overall length (mm)	0.5-0.9	0.5-1	0.38-1.15	To 0.9	-	-	-	To 3.0	0.7-1.7	1.5-3
Basal cell diameter (µm)	40-50	-	60-100	100-130	-	-	50-70	80-100	-	(40) 45-65 (75)
Basal cell lenght (µm)	130-150	-	60-180	Wider than long	-	-	150-200	40-75	-	(65) 70-75 (80)
Median cell diameter (µm)	40-50	-	-	To 75	40-80	75-100	25-30	-	40-80	-

Continued

Table 2 continued

	***D. adriatica* sp.nov.**	***D. chejuensis***	***D. clavigera***	***D. collinsiana***	***D. concinna***	***D. doliiformis***	***D. japonica***	***D. naccarioides***	***D. okiensis***	***D. sessilis***
Median cell length (µm)	130-150	-	-	To 2 diameters	80-100	100-170	70-100	-	50-130	-
Apical cell diameter (µm)	30-40	15	15-30	45-55	-	-	7-10	6-10	7	15-20
Apical cell length (µm)	30-120	15	10-15	90-110	-	-	Wider than longer	15-20	17	15-55
Tetrasporangium diameter (µm)	40	35-40	24-25	30-40	22-30	25-48	30-60	30-45	20-30	35-45
Tetrasporangial stichidium	Lanceolate, often curved	Lanceolate	Lanceolate	Ovate-lanceolate, often curved	Lanceolate, replacing a branchlet at the 2nd-4th pseudolateral dichotomy	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Ovate or ovate-lanceolate to elongate-cylindrical
Diameter (µm)	60-110	100-130	120	90-130	50-75	90-125	300-350	125	80-100	(100) 110-130 (150)
Length (µm)	250-500	700-800	450	300-500	120-200	220-310	600-850	To 500	160-280	(350) 400-900 (950)
Fertile segments	14	20	12-15	-	-	-	-	10	10	10-25 (27)
Sterile terminal cells	1-3	1	1-2	3	-	-	-	1-3	-	-
Type locality	Slovenia	Korea	South Australia	Bermuda	Carolina (USA)	Carolina (USA)	Japan	Georgetown (Tas.)	Oki Islands. Sea of Japan	Japan
References for morphological data	This study	Lee & West, 1979	Womersley, 1946; Parsons, 1975	Schneider et al. 2021	Schneider, 1989; Schneider *et al.,* 1994	Schneider, 1989	Choi, 2001	Parsons, 1975	Kajimura, 1992	Yamada, 1928; Peña & Bárbara, 2006

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References

Cassidy, M.M., Schneider, C.W., Saunders, G.W., 2022. The *Dasya baillouviana* and *D. cryptica* complexes (Delesseriaceae, Rhodophyta) in Bermuda with three additional new species from the archipelago. *Journal of Phycology*, 58, 731-745.

Choi, H.G., 2001. Morphology and reproduction of *Heterosiphonia pulchra* and *H. japonica* (Ceramiales, Rhodophyta). *Algae,* 16, 387-409.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*., 32, 1792-1797.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution,* 39, 783-791.

Fofonoff, P.W., Ruiz, G.M., Steves, B., Simkanin, C., Carlton, J.T., 2018. *National Exotic Marine and Estuarine Species Information System*. http://invasions.si.edu/nemesis (Accessed 13 September 2023).

Freshwater, D.W., Rueness, J., 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. *Phycologia*, 33, 187-194.

Guiry, M.D., Guiry, G.M., 2023. *AlgaeBase*. https://www.algaebase.org (Accessed 13 September 2023).

Hasegawa, N., Onitsuka, T., Takeyama, S., Maekawa, K., 2015. Oyster Culture in Hokkaido, Japan. *Bulletin of Fisheries Research Agency,* 40, 173-177.

Kim, H.-S., 2012. *Algal flora of Korea. Volume 4, Number 6 Rhodophyta: Florideophyceae: Ceramiales: Ceramiaceae II (Corticated Species), Dasyaceae*. National Institute of Biological Resources, Incheon, 191 pp.

Kajimura, M., 1992. A new species of *Dasysiphonia* (Dasyaceae, Rhodophyta) from the sea of Japan. *Botanica Marina*, 35, 407-413.

Lee, I.K., West, J.A., 1979. *Dasysiphonia chejuensis* gen et sp. nov. (Rhodophyta, Dasyaceae) from Korea. *Systematic Botany*, 4, 115-129.

Mavrič, B., Orlando-Bonaca, M., Trkov, D., Zamuda, L. L., Kajtna, U. *et al.,* 2023: *Spremljanje vrstne pestrosti in abundance tujerodnih vrst v slovenskem morju v obdobju 2021-2023 (eng: Monitoring the species richness and abundance of alien species in the Slovenian coastal sea in the period 2021-2023). Final report, October 2023.* Marine Biology station Piran, National Institute of Biology, Piran, 117 pp.

Parsons, M.J., 1975. Morphology and Taxonomy of the Dasyaceae and the Lophothalieae (Rhodomelaceae) of the Rhodophyta. *Australian Journal of Botany*, 23 (4), 549-713.

Peña, V., Bárbara, I., 2006. Revision of the genus *Dasya* (Ceramiales, Rhodophyta) in Galicia (NW Spain) and the addition of a new alien species *Dasya sessilis* Yamada for the European Atlantic coasts. *Anales del Jardín Botánico de Madrid*, 63, 13-26.

Rambaut, A., Drummond, A.J., 2007. *Tracer, version 1.5.* http://beast.bio.ed.ac.uk/Tracer.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572-1574.

Schneider, C.W., 1989. Two new species of *Dasysiphonia* (Dasyaceae, Rhodophyta) from the southeastern United States (Carolinas). *Botanica Marina,* 32, 521-526.

Schneider, C.W., Cassidy, M.M., Saunders, G.W., 2021. The pseudodichotomous *Dasya sylviae* sp. nov. (Delesseriaceae, Ceramiales) from 60-90 m mesophotic reefs off Bermuda. *European Journal of Phycology*, 751, 24-37.

Schneider, C.W., Walde, R.E., Morelli, R.A., 1994. L-systems computer models generating distichous from spiral organisation in the Dasyaceae (Ceramiales, Rhodophyta). *European Journal of Phycology*, 29, 165-170.

Schwarz, G., 1978. Estimating the dimension of a model. *The annals of statistics*, 6, 461-464.

Sfriso, A., Buosi, A., Wolf, M.A., Sfriso, A.A., 2020. Invasion of alien macroalgae in the Venice Lagoon, a pest or a resource? *Aquatic Invasions,* 15, 245-270.

Sjøtun, K., Husa, V., Peña, V., 2008. Present distribution and possible vectors of introductions of the alga *Heterosiphonia japonica* (Ceramiales, Rhodophyta) in Europe. *Aquatic Invasions,* 3, 377-394.

Tamura, K, Stecher, G, Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution,* 38, 3022-3027.

Verlaque, M., 2001. Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. *Oceanologica Acta*, 24, 29-49.

Wolf, M.A., Buosi, A., Juhmani, A. F., Sfriso, A., 2018. Shellfish import and hull fouling as vectors for new red algal introductions in the Venice Lagoon. *Estuarine, Coastal and Shelf Science*, 215, 30-38.

Womersley, H.B.S., 1946. Studies on the marine algae of southern Australia. No 2 A new species of *Dasyopsis* (family Dasyaceae) from Kangaroo Island. *Transactions of the Royal Society of South Australia*, 70, 137-144.

Yamada, Y., 1928. Report on the biological survey of Mutsu Bay. 9. Marine algae of Mutsu Bay and adjacent waters. II. *Science Reports of the Tohoku University, Fourth Series (Biology)*, 3, 497-534.