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Eastward spreading of the invasive *Rugulopteryx okamurae* (Heterokontophyta, Dictyotales) in the Mediterranean: first record in the Adriatic Sea

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Abstract

The brown seaweed *Rugulopteryx okamurae* is currently included in the list of invasive alien species of European Union concern due to its rapid expansion that is causing both ecological and economic impacts. In this study, we report the first record of *R. okamurae* in the Southern Adriatic Sea, off the coast of Bari, Italy, currently representing the easternmost limit of its distribution in the Mediterranean Sea. Morphological observations combined with *rbc*L and *psb*A sequence analyses confirmed the taxonomic identification. Field surveys carried out between April 2023 and January 2024 highlighted the conspicuous occurrence of *R. okamurae* over a total surface of approximately 6.5 ha. Two main human-mediated pathways occurring near the sites of the first observation of the alien species may have been responsible for its introduction: the trade in living organisms for human consumption and maritime transport related to the proximity of the port of Bari to the invaded area. Future studies will aim to identify and implement practical, cost-effective management strategies to mitigate this alarming bioinvasion.

Keywords: alien species; biological invasions; Italy; Non-indigenous macroalgae; *psb*A; *rbc*L; coastal habitat monitoring; Mediterranean.

Introduction

The massive loss in marine biodiversity happening worldwide can be partially attributed to biological invasions due to Non-Indigenous Species (NIS), which represent part of the global changes occurring in the last decades (Bax *et al*., 2001; Galil *et al*., 2014). To date, more than 1,000 alien species have been reported in the Mediterranean Sea, over 200 of which are macroalgae (Verlaque & Breton, 2019). Evidence of ecosystem alteration at the Mediterranean scale due to invasive seaweeds has been observed in the case of several macroalgae, including the red algae *Asparagopsis armata* Harvey (Boudouresque & Verlaque, 2002; Guerra-García *et al*., 2012), *A. taxiformis* (Delile) Trevisan (Navarro-Barranco *et al*., 2018), *Womersleyella setacea* (Hollenberg) R.E. Norris (Bedini *et al*., 2015), *Lophocladia lallemandii* (Montagne) F. Schmitz (Ballesteros *et al*., 2007), *Melanothamnus japonicus* (Harvey) Díaz-Tapia & Maggs 2017 (Wolf *et al*., 2018; Rindi *et al*., 2020), as well as the green algae *Caulerpa cylindracea* Sonder (Alomar *et al*., 2016) and *C. taxifolia* (M. Vahl) C. Agardh (Bellan-Santini *et al*., 1996).

In the last decade, the brown alga *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim (Heterokontophyta, Dictyotales) has caused increasing concern in the Mediterranean Sea. It represents a serious threat to the conservation of coastal ecosystems with relevant social, tourist, and economic implications (García-Gómez *et al*., 2020). Originally described from the temperate western Pacific Ocean (Hwang, 1994), *R. okamurae* was spotted for the first time in 2002 in the Thau Lagoon (France) where it was most likely introduced through the import of the Japanese oyster *Magallana gigas* (Thunberg 1793) for mariculture purposes (Verlaque *et al*., 2009). Since 2015, a massive proliferation of *R. okamurae* has been observed on both sides of the Strait of Gibraltar and the Alboran Sea (Altamirano *et al*., 2020). Subsequently, the species has continued its expansion eastwards, along the French coast of Provence (Ruitton *et al*., 2021), and westwards, up to the Azores (Faria *et al*., 2022) and Madeira (Bernal-Ibáñez *et al*., 2022), in the northeastern Atlantic. In June 2023, *R. okamurae* was found in the Gulf of Palermo, along the northwestern coast of Sicily, Italy (Bellissimo *et al*., 2024).

Since its arrival in the Mediterranean Sea, this species has become dominant on rocky bottoms in several areas, replacing the native communities and affecting the conservation status of coastal ecosystems (García-Gómez *et al*., 2021a). For instance, in the Strait of Gibraltar *R. okamurae* is causing a significant decline in the coralligenous key species *Mesophyllum expansum* (Philippi) Cabioch & M.L. Mendoza, as well as the deterioration of the forests of the red gorgonian *Paramuricea clavata* (Risso 1826) (Navarro-Barranco *et al*., 2021; Sempere-Valverde *et al*., 2021). The success of *R. okamurae* is linked to its great ability to thrive under a wide range of depth, light intensity, and nutrient concentration (Rosas-Guerrero *et al*., 2020; García-Gómez *et al*., 2021b). Its invasiveness is enhanced by high rates of asexual reproduction by both vegetative propagules and asexual monospores, and by the presence of secondary metabolites involved in chemical defence against grazers and competitors (Casal-Porras *et al*., 2021).

Considering the expected expansion of the species, appropriate action should be taken to cope with new introductions and to control the spread of the established populations (Terradas-Fernández *et al*., 2023). Potential solutions involve using the biomass of *R. okamurae* in the production of biogas, bioplastics, aquaculture feed, and compost to try to slow down the spread of this NIS (Barcellos *et al*., 2023), although the effectiveness of these actions is still unknown. On the other hand, new records of *R. okamurae* and *ad hoc* studies on its populations can provide further information to support appropriate mitigation actions.

In this study, we report the first record of *R. okamurae* in the Southern Adriatic Sea. Species identification was confirmed by both morpho-anatomical observations and molecular analyses. After the first sighting of *R. okamurae* in April 2023, the presence and distribution of the species were monitored at the same site and in surrounding areas between May 2023 and January 2024 showing its massive spread along the coast of Apulia, which represents the easternmost evidence of the presence of this species in the Mediterranean Sea thus far.

Materials and Methods

Fieldwork and sample collection

Rugulopteryx okamurae was observed by chance for the first time in April 2023 in a shallow port area of the city of Bari (Italy, southern Adriatic Sea). Then, targeted surveys were carried out between May 2023 and January 2024 at three sampling sites (Sites A, B, and C) with three methodologies to assess the presence, distribution, coverage, and extension of *R. okamurae* (Fig. 1; Table 1). Site A was located in the southern port area where the first re-

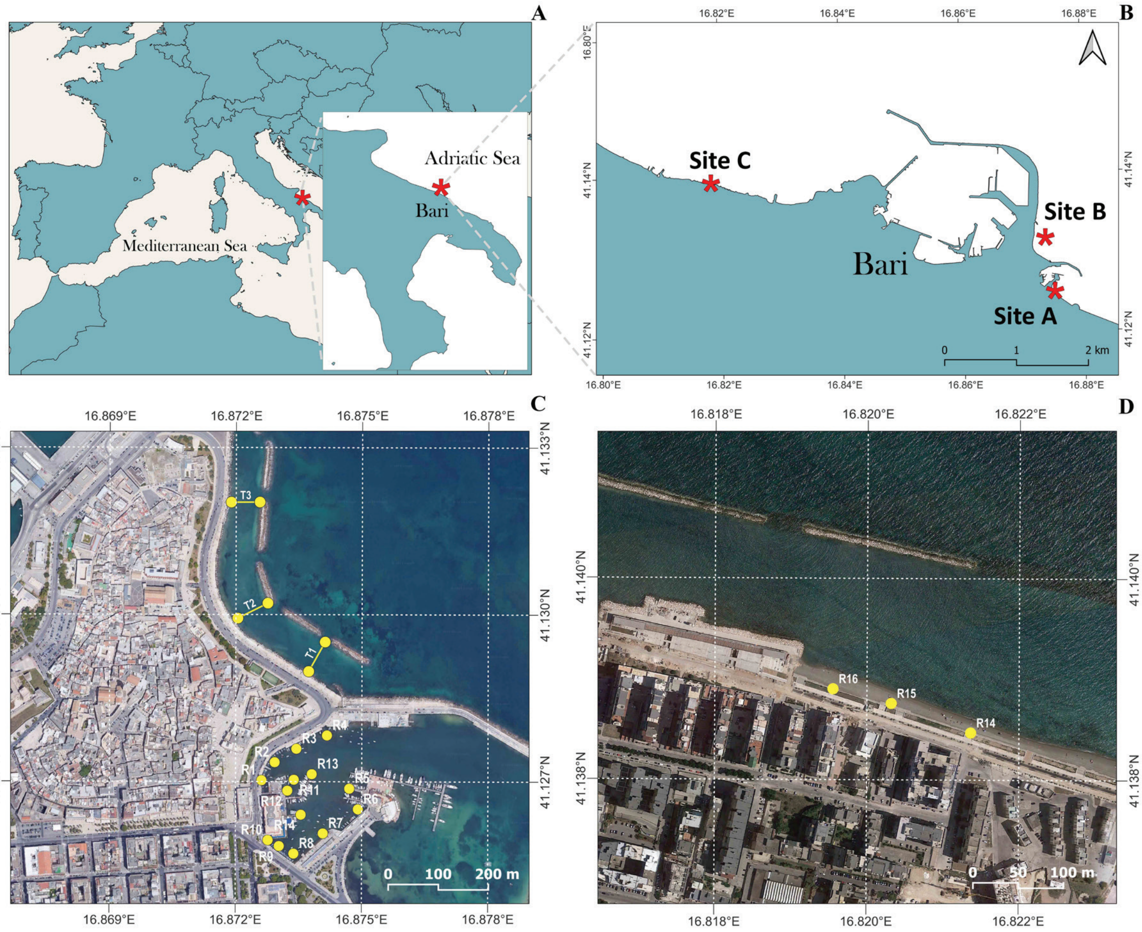

Fig. 1: Study area and survey sites. A) location of the city of Bari (Italy, Southern Adriatic Sea); B) the harbour area where *Rugulopteryx okamurae* was found with indication of sampling sites (Site A, B, and C); C, D) detail of the sampling sites with sampling stations (R1-R16, Sites A and C) and monitoring transects (T1-T3, Site B).

Table 1. Coordinates of each sampling point with indication of the investigation period, sampling procedure, and aims per each site investigated in this study.

Site	Point ID	Points coordinates		Investigation period	Sampling procedure	Sampling aims
		Lat. (DD)	Lon. (DD)			
Site A	R1	41.12703° N	16.87261° E	From May 2023 to January 2024	Specimens collected with a rake from moles and docks	Assessing presence, distribution, coverage, and extension of *R. okamurae* in a highly anthropized area mostly with artificial substrata
	R2	41.12735° N	16.87293° E			
	R3	41.12759° N	16.87344° E			
	R4	41.12783° N	16.87417° E			
	R5	41.12687° N	16.87470° E			
	R6	41.12650° N	16.87491° E			
	R7	41.12607° N	16.87408° E			
	R8	41.12571° N	16.87338° E			
	R9	41.12571° N	16.87338° E			
	R10	41.12595° N	16.87277° E			
	R11	41.12703° N	16.87338° E			
	R12	41.12684° N	16.87323° E			
	R13	41.12713° N	16.87381° E			
Site B	T1_start	41.12898° N	16.87374° E	November 2023	Underwater transects by SCUBA diving from the coastline to the breakwaters (2.5 m depth)	Assessing the presence, distribution, coverage, and extension of *R. okamurae* on a detrital seabed, as well as its habitus and behaviour underwater
	T1_end	41.12951° N	16.87412° E			
	T2_start	41.12994° N	16.87205° E			
	T2_end	41.13021° N	16.87276° E			
	T3_start	41.13202° N	16.87189° E			
	T3_end	41.13202° N	16.87257° E			
Site C	R14	41.13847° N	16.82136° E	January 2024	Visual surveys along the shoreline	Assessing the presence of beached thalli which could suggest the occurrence of *R. okamurae* in the nearby waters
	R15	41.13875° N	16.82031° E			
	R16	41.13890° N	16.81956° E			

cord occurred, in an area intensively urbanized and with several inputs of organic waste. Quantitative sampling of living thalli was carried out there at 13 sampling stations (R1-R13), on nearby piers and docks, using a rake. Site B was investigated by visual census to study the distribution of *R. okamurae* outside the port area, from the coastline to breakwaters placed at about 2.5 m depth (Fig. 1); three transects (T1, T2, and T3), each approximately 70 m long and 10 m wide, were carried out by SCUBA divers together with qualitative sampling for species identification. The presence of drift thalli was assessed at Site C, a few kilometres north of Sites A and B, where thalli were collected at three sampling stations (R14-R16) over a coarse-sandy beach along 200 m of coastline (Fig. 1; Table 1). All the thalli collected for morphological identification were preserved in a 4% formalin-seawater solution, while samples for molecular analysis were transported fresh to the laboratory.

Morphological identification

Morphological and anatomical observations were carried out using a Leica MZ 7.5 stereomicroscope (Leica, Wetzlar, Germany) and an Olympus BX-40 light microscope (Olympus America Inc., Center Valley, Pennsylvania, USA). Thalli were sectioned freehand or using a DSK-1000 Vibratome (Dosaka, Kyoto, Japan). Photomicrographs were taken using a DP21 digital camera (Olympus) equipped with DP2-Twain Software for measurements (Olympus). Newly collected voucher specimens were deposited in the Herbarium Horti Botanici Barensis (BI).

Molecular identification and phylogenetic analysis

Among morphologically identified and comparable thalli, specimen “1B” (transect T1, Site B) was selected for molecular analyses. The thallus was gently cleaned up of epiphytes and placed in silica gel (Carlo Erba Reagents S.r.l., Milano, Italy), according to the manufacturer’s recommendations. The dried sample was stored at -80±1°C for at least 48 hours to improve DNA extraction and homogenized in a Mixer Mill MM400 (RETSCH Mill, Verder Scientific GmbH & Co. KG, Haan, Germany). Total DNA was extracted from 75 mg of homogenized tissue using the Plant/Fungi DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada), modified by adding 2.5% of PVP-40 (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) to the Lysis Buffer. DNA purity and quantity were estimated using an Implen NanoPhotometer N60 (Implen

GmbH, München, Germany). Portions of the genes *rbc*L and *psb*A were PCR-amplified. The primer pairs were 543F/1381R (Bittner *et al*., 2008) and psbAF1/ psbAR2 (Saunders & Moore, 2013), respectively. The 25-µL PCR reactions were run in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA). Each reaction contained 12.5 µL Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.5 µL of each primer (10 µM), and 0.75 µL of genomic DNA (~ 50 ng/ µL); *psb*A reaction was amended with 0.75 µL of DMSO (Thermo Fisher Scientific). *Rbc*L amplification was carried out as follows: 98°C for 1 min, 35 cycles of 98°C for 10 sec, 50°C for 30 sec and 72°C for 30 sec, and 72°C for 7 min; the same conditions were used for the *psb*A amplification, except for the 45°C annealing temperature. An amplicon aliquot (5 µL) was run on 1.7% agarose gel in TBE buffer 1× (Bio-Rad), prestained with GelRed® (Biotium, Landing Parkway Fremont, CA, USA), and visualized using ChemiDoc XRS+ (Bio-Rad); 100 bp plus DNA ladder (Thermo Fisher Scientific) was used as marker. Amplicons were purified and sequenced by Macrogen Europe (Amsterdam, The Netherlands) external service.

The nucleotide sequence accuracy was assessed by FinchTV software (http://jblseqdat.bioc.cam.ac.uk/gnmweb/download/soft/FinchTV_1.4/doc/) and strand sequences were aligned using the free software MultAlin (http://multalin.toulouse.inra.fr/multalin/). Consensus sequences were analysed using the online NCBI BLAST search tool (https://www.ncbi.nlm.nih.gov/) and deposited in the GenBank database.

The sequences used for molecular phylogenetic analyses are listed in Table 2. Phylogenetic analyses of sequences were run in MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al*., 2021). *Stypopodium schimperi* (Kützing) Verlaque & Boudouresque was used as an outgroup. *rbc*L and *psb*A gene portions were concatenated, trimmed, and concatamers were further aligned according to ClustalW (Thompson *et al*., 1994). The evolutionary study of concatamers was obtained by the Maximum Likelihood (ML) method and the Tamura-Nei model (Tamura & Nei, 1993). The initial tree for the heuristic search was automatically achieved by Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model; the topology with superior log likelihood value was automatically selected. The analysis involved 17 nucleotide sequences and a total of 1615 positions was included in the final dataset. Bootstrap analyses consisted of 500 replications.

Table 2. GenBank sequences of Dictyotaceae species/isolate along with their *rbcL* and *psbA* accession numbers, used in phylogenetic analysis. Bold letters indicate newly generated sequences and accession numbers in this study. AZB: MAD21-01: Madeira; AZT: SMG-21-04: Azores; IK40: Korea.

Species/Isolate	**GenBank accession n°**	
	rbcL	***psbA***
Rugulopteryx okamurae/AZB:MAD-21-01 (Faria *et al*., 2022)	ON393999	ON677528
Rugulopteryx okamurae/AZB:SMG-21-04 (Faria *et al*., 2022)	MZ393480	MZ393489
Rugulopteryx okamurae/IK40 (Hwang *et al*., 2004)	AY422673	AY422635
***Rugulopteryx okamurae*/1B**	**PP335537**	**PP335536**
Rugulopteryx okamurae/Sicily (Bellissimo *et al*., 2024)	OR578567	OR578566
Rugulopteryx marginata	DQ472043	MW225832
Rugulopteryx suhrii	DQ472044	EU395617
Rugulopteryx radicans	DQ472045	EU395610
Dictyota dichotoma	DQ472051	GQ425205
Dictyota fasciola	GQ425110	GQ466074
Dictyota mediterranea	GU290254	GU265785
Dictyota spiralis	MW223307	GQ425220
Dictyota cyanoloma	MW223182	GU255590
Dictyota linearis	AY422669	AY422631
Stypopodium schimperi	MW225908	MW225870
Lobophora variegata	MW223403	MW225725
Dictyopteris polypodioides	DQ472042	MW224555

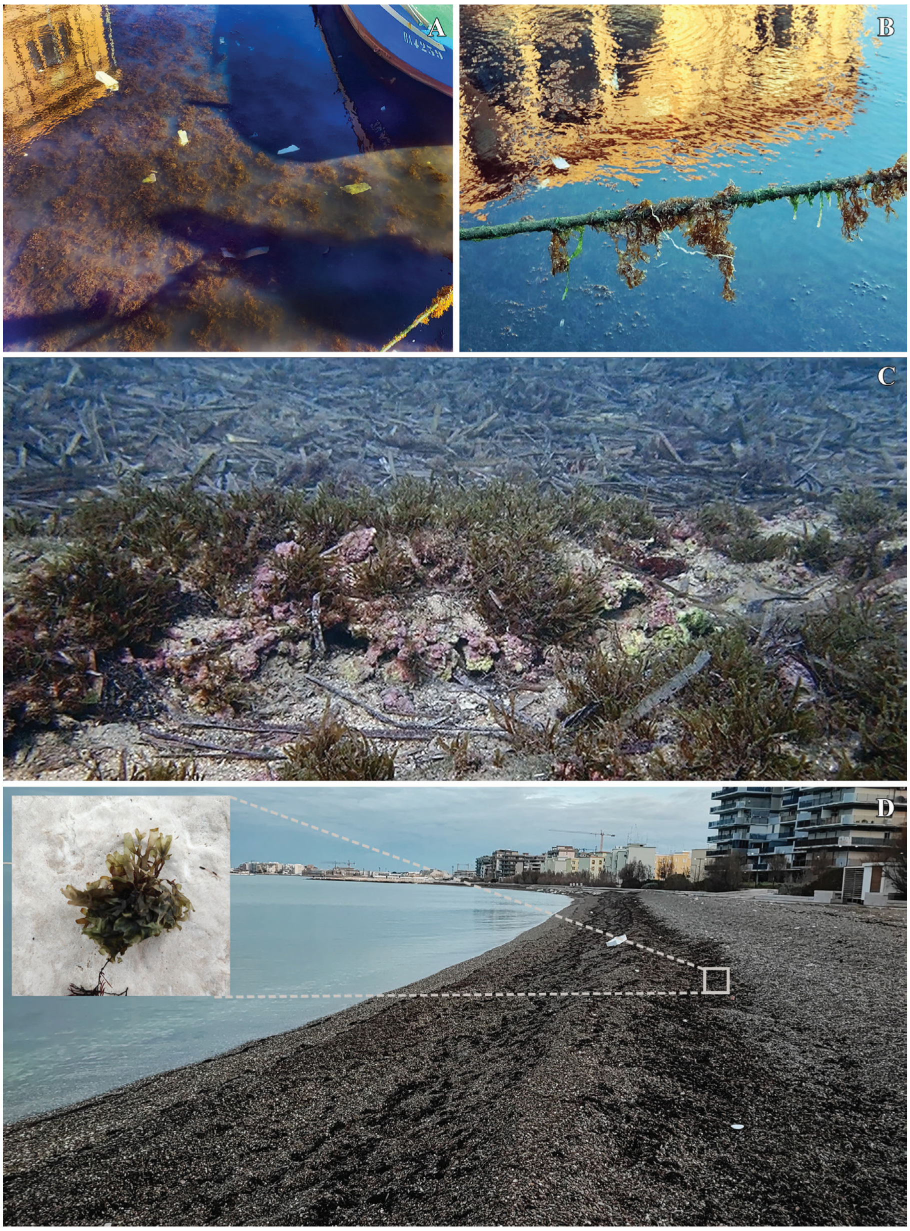

Fig. 2: A) *Rugulopteryx okamurae* forming dense rugs/mats and B) colonizing ropes at Site A; C) *R. okamurae* at Site B on a detritic seabed with interspersed dead *matte* of *Posidonia oceanica*; D) Thalli of *R. okamurae* beached along the shore at Site C.

Results

Field observations

Rugulopteryx okamurae was observed at all 13 sampling stations at Site A (R1-R13), being the dominant species on the majority of both natural and anthropic hard substrata, including piers, ropes, buoys, and boat hulls (Fig. 2), and forming thick mats covering up to 100% of the sea bottom over a surface of approximately 2.5 ha (Fig. 3). This NIS proved to be ubiquitous over the visual transects at Site B, where it usually covered 60-70%, and up to 100% of the seabed, over a surface of about 3.9 ha (Fig. 3; Fig. 4). In fact, *R. okamurae* was growing on pebbles, rocks and dead *matte* of the seagrass *Posidonia oceanica* (Linnaeus) Delile. Several drift thalli were also observed, floating in the water column and forming mats of loose thalli on the seabed. No signs of grazing were observed in any of the investigated area. Thalli of *R. okamurae* were also found at all the sampling stations of Site C, drifting all along the coastline (Fig. 2).

Morpho-anatomical analysis

Morpho-anatomical observations of samples collected at all sites confirmed the identification of the invasive brown alga *R. okamurae*. Membranous erect thalli, forming entangled bushy clumps up to 12 cm high, were attached to the substrate by multicellular, uniseriate rhizoids and stolon-like segments from which new fronds were formed. Fronds, not iridescent, yellow-brown to dark brown in colour, were flabellate and dichotomously branched (Fig. 5a), with adventitious branchlets arising both on the margins of the blade and at damaged parts of the thallus (Fig. 5b). Apexes were obtuse and the apical cell was lenticular and slightly protruding (Fig. 5c). The segments (3-10 mm long and 2-7 mm wide) expanded at

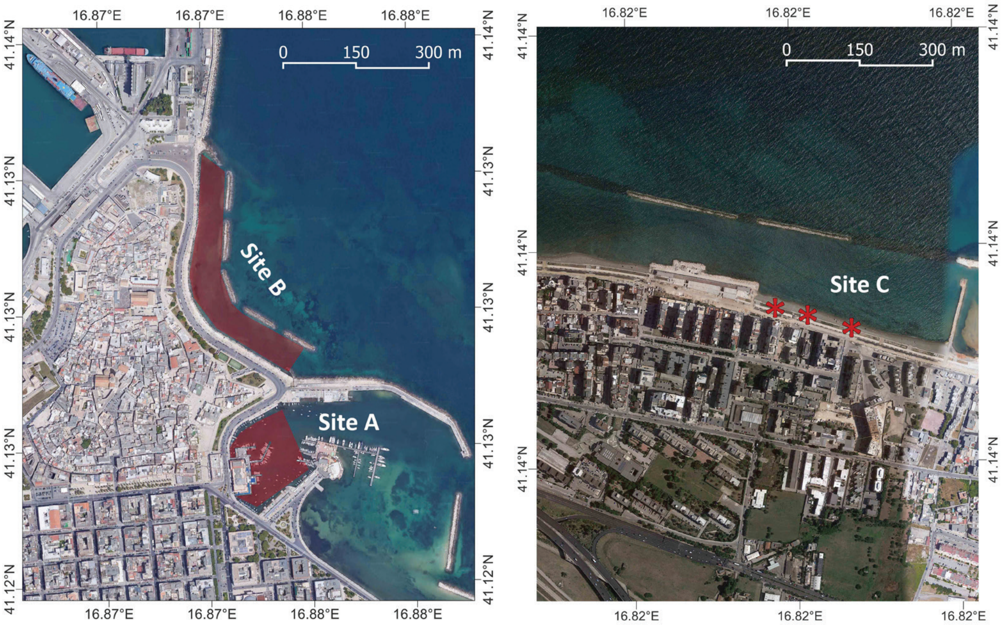

Fig. 3: Distribution of *Rugulopteryx okamurae* within the study area in the port of Bari. The polygons in red show the estimated extension of the species at Sites A and B, while the red asterisks represent the spots where beached thalli were occasionally found at Site C.

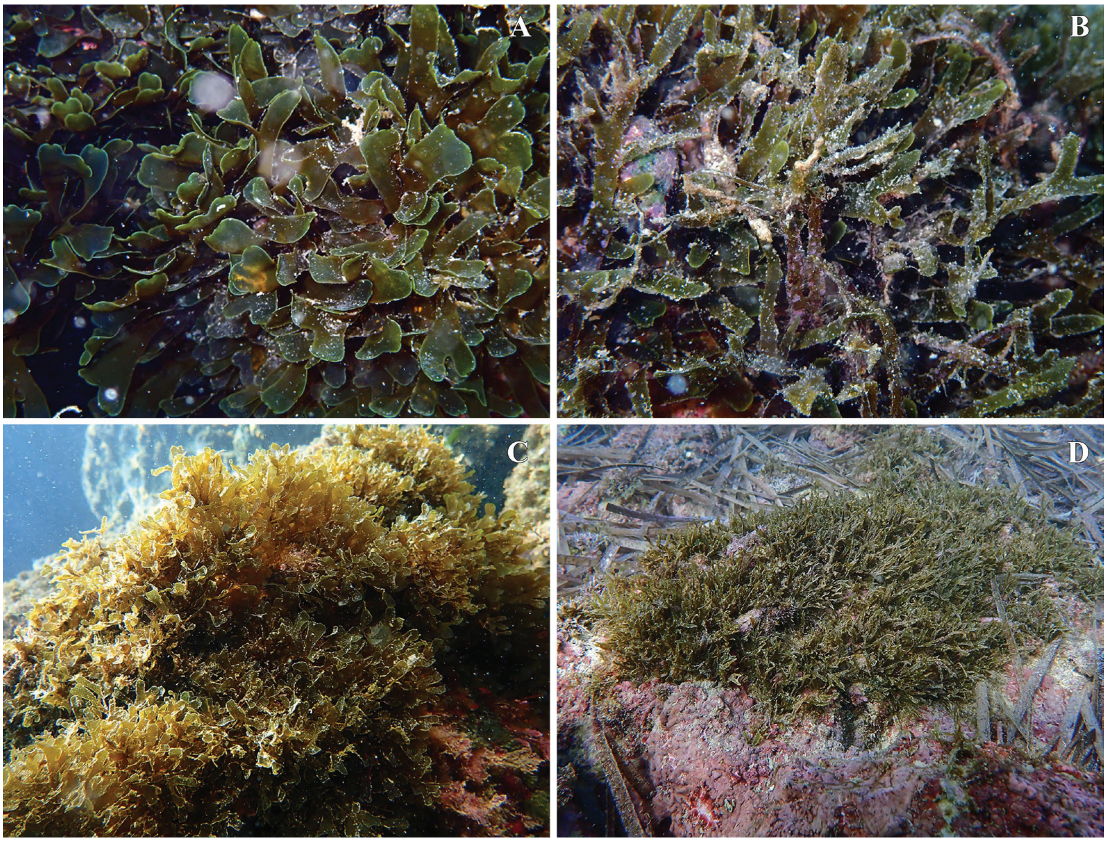

Fig. 4: Photos of *Rugulopteryx okamurae* taken during underwater surveys at Site B.

the dichotomies, assuming a cuneate form. The margin was smooth and considerably thickened. Cortical cells were rectangular in surface view, 30-40 × 10-12 µm, and regularly arranged in longitudinal rows (Fig. 5d). In the upper and intermediate portions of the thallus, the medulla was formed by a single layer of large anisodiametric cells (80-100 × 40-60 µm) (Fig. 2e), except for the portions near the margin where it was formed by 2-4 layers of cells (20-40 × 20-40 µm) (Fig. 5f). In the lower parts of the thallus, the medulla consisted of 3-4 layers of isodiametric cells (20-60 x 20-60 µm) with thickened cell walls (Fig. 5g). Reproductive structures were not observed.

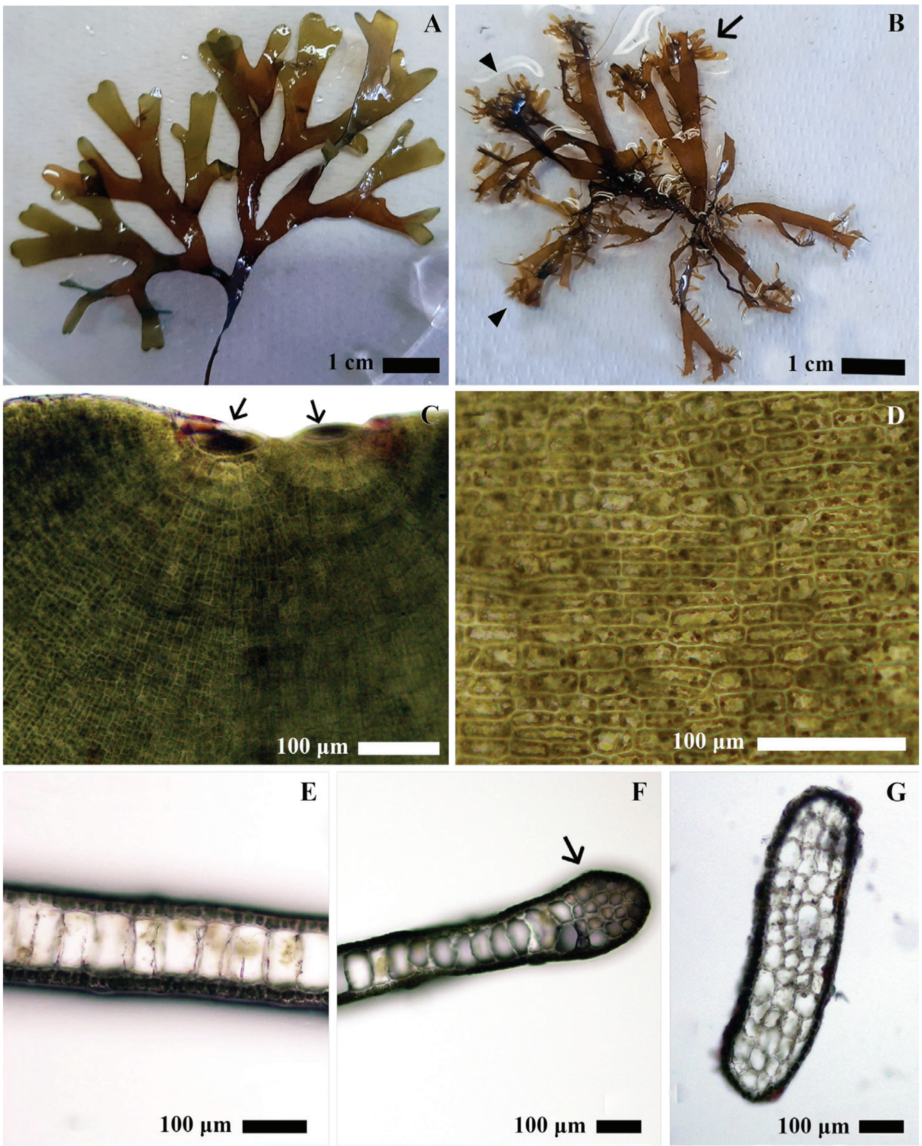

Fig. 5: Morphology of *Rugulopteryx okamurae*. (A) habit; (B) adventitious branchlets on margins (arrows) and damaged parts (arrowheads) of the thallus; (C) detail of two apical cells (arrows); (D) cortical cells in surface view; (E) cross-section of the mid part of the thallus showing the uni-layered medulla; (F) cross-section of the upper part of the thallus showing the multi-layered medulla (arrow) near the margin; (G) cross-section of the basal part of the thallus showing 3-4 layers of thick-walled medullary cells.`

Molecular analyses

Sequences of the two chloroplast protein-encoded genes *rbc*L and *psb*A generated in this study were 840 bp and 936 bp long, respectively, and have been deposited in GenBank with the accession numbers PP335537 for *rbc*L and PP335536 for *psb*A. The obtained sequences corresponded to *R. okamurae*, showing to be fully identical to sequences of Portuguese and native Pacific specimens, corroborating the morphological characterization.

According to phylogenetic analyses and branch lengths, specimen 1B forms a clade with other specimens belonging to *R. okamurae*, although it is separate from the European and native ones (Fig. 6). The genus *Rugulopteryx*, including *R. suhrii* (Kützing) De Clerck & Coppejans, *R. radicans* (Harvey) De Clerck & Coppejans and *R. marginata* (J.Agardh) De Clerck & Coppejans, consistently represents a monophyletic group (89% ML BP), as for *Dictyota* genus (99% ML BP). *Rugulopteryx* and *Dictyota* clades are sister groups with weak support (51% ML BP) (Fig. 6). According to MEGA11 analyses and among *R. okamurae* specimens, 90% of sites were conserved. Within the remaining percentage, there were 0.7% of variable sites and 0.7% of highlighted singleton sites.

Discussion

The Adriatic record of *R. okamurae* represents the easternmost limit of the species distribution in the Mediterranean Sea. Its arrival and establishment in the port of Bari highlight the ongoing expansion of this NIS in the Mediterranean Sea, as already assumed by recent distribution models (Bellissimo *et al*., 2024). The phylogenetic tree obtained in this study raises doubts about the origin of Adriatic specimens of *R. okamurae*. Based on the node bootstrap values, our specimen was separated from native (100% BP) and Portuguese and Sicilian records (98% ML BP) (Fig. 6). Nevertheless, the specimen from Ma-

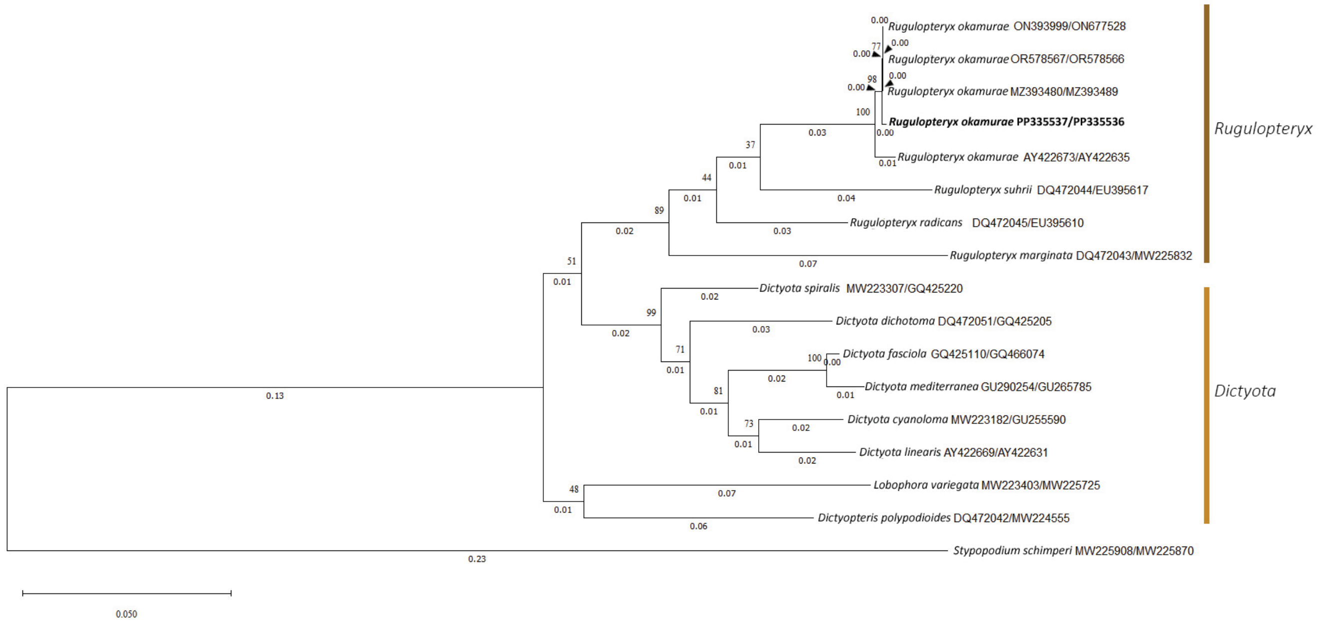

Fig. 6: Phylogenetic tree based on the concatenated sequences of gene portions of *rbc*L and *psb*A as estimated by Maximum Likelihood (ML). Bootstrap values are reported on the branches. Branch lengths are proportional to the number of nucleotide substitutions and are measured using the bar scale (0.05). All specimens are available in Table 2. Bold letters indicate newly generated sequences in this study.

deira (AZB: MAD-21-01 *Rugulopteryx okamurae*) and the Sicilian one (Sicily *Rugulopteryx okamurae*) clustered together displaying remarkable sequence similarity (Fig. 6). Schaffelke *et al*. (2002) described a comparable scenario regarding the spread of *C. taxifolia* in Australia. They found that specimens from different locations were clustered into distinct clades, indicating that they were derived from different source regions. Our record may be linked to multiple introduction events between the Mediterranean Sea and the Atlantic Ocean or putative unverified sexual cycles. To date, no sexual reproduction has been documented in the invaded areas. Thus, the high genetic variability shown by *R. okamurae* may be the result of repeated introductions that have taken place in the last decade, as already observed for other alien species (Dlugosch & Parker, 2008). Multiple invasions have been proved to favour the establishment success of alien species, enhancing their invasiveness (Paolacci *et al*., 2021).

In the case of Apulian waters, two main human-mediated pathways near the sites of the first observation of *R. okamurae* may be responsible for its introduction. The first one involves the import of living seafood for human consumption, which can often act as a vector of alien species. For instance, the trade in shellfish - mainly oysters and mussels - is known to be a major vector for the introduction of marine macrophytes in European waters and the Mediterranean Sea (44% and 46% of the total, respectively) (Zenetos *et al*., 2011; Petrocelli *et al*., 2013). The importation of Japanese oysters for aquaculture purposes (Verlaque *et al*., 2009), or live shellfish, and sea urchins for human consumption (Ruitton *et al*., 2021), has already been associated with the introduction of *R. okamurae* in France. Our study Site A is a slipway located in the innermost part of the old harbour of the city of Bari, where an open-air fish market has been active for decades, with raw seafood (especially mussels, oysters, and sea urchins) available for consumption on the spot and the accidental and/or intentional discharge of empty shells or damaged organisms into neighbouring waters by fishermen and consumers. Moreover, Apulia, along with Sardinia, Veneto and, to a lesser extent the Marche, are among the few Italian regions involved in the cultivation of Pacific oysters, which are currently farmed with seeds generally imported from French hatcheries (Wolf *et al*., 2018; Quaglia *et al*., 2023).

On the other hand, intense maritime traffic is thought to be a concomitant pathway for the introduction of alien species in the port of Bari. The new harbour, located very closely to our Site B, is one of the largest ports on the Adriatic Sea, with daily docking of several cruise and container ships, also providing direct connections for ferry services to Greece, Croatia, Montenegro, and Albania. This raises concerns about the potential for a further expansion of the species towards these south-eastern European countries. Transport stowaways through ship fouling and ballast waters are one of the main pathways for NIS introduction and expansion (Bailey *et al*., 2020). For instance, the massive invasion of *R. okamurae* along the Spanish coast has been linked to the ballast waters of Asian vessels anchored in the Strait of Gibraltar (García-Gómez *et al*., 2020).

The chances of successful establishment depend on the ecological plasticity of the species, which reflects strong adaptability to the recipient environment, as well as its capability to survive under stressed conditions. The finding sites in Bari are highly anthropized areas exposed to large organic and inorganic inputs, representing ideal sites for *R. okamurae* that can take advantage of eutrophic conditions thanks to a high nutrient storage capacity (Mercado *et al*., 2022). The degradation of these environments is also underlined by the occurrence of dead *mattes* of *P. oceanica* colonized by invasive species, including *R. okamurae*. The ability of *R. okamurae* to benefit from all kinds of substrata, including anthropogenic ones, as well as its fast vegetative reproduction and its survival as floating thalli, facilitate the dispersal of the species. The same ability to easily remain attached to nets and other artificial materials has already been observed in the Strait of Gibraltar (García-Gómez *et al*., 2021a, b). The floating fragments can maintain high photosynthetic rates (Figueroa *et al*., 2020) and reattach themselves to hard substrata (Mateo-Ramírez *et al*., 2023). Mainly within Site A, considerable amounts of unattached thalli flowed through an artificial channel under street level reaching Site B. Furthermore, free-drifted thalli could either accumulate on the foreshore at the northernmost Site C or be carried to the other two sites by the prevailing currents along this stretch of coastline.

Vegetative propagules consist of proliferous branchlets that arise on both thallus surfaces and can develop into new plants (Hwang *et al*., 2009). However, neither asexual monospores nor sexual reproductive structures were found. The inability of *R. okamurae* to complete its sexual life cycle has also been reported in Korean waters (Hwang *et al*., 2009) as well as in all Mediterranean and Atlantic areas of introduction (Verlaque *et al*., 2009; Altamirano *et al*., 2020; Ruitton *et al*., 2021; Bernal-Ibáñez *et al*., 2022; Faria *et al*., 2022; Terradas-Fernández *et al*., 2023; Bellissimo *et al*., 2024; El Madany *et al*., 2024). This enables the species to recruit new clonal individuals rapidly and abundantly throughout the year, thus maintaining constant propagule pressure. The lack of sexual reproduction with only vegetative processes for dispersal is also common in other invasive macroalgae within the Mediterranean Sea (Boudouresque & Verlaque, 2002), such as the red algae *Acrothamnion preissii* (Sonder) E.M. Wollaston (Piazzi & Cinelli, 2003; Klein & Verlaque, 2011) and *Womersleyella setacea* (Nikolić *et al*., 2010), as well as the green *C. taxifolia* (Meinesz *et al*., 1993; Žuljevic & Antolić, 2000).

In its native area, *R. okamurae* is widely distributed year-round but only in the form of dormant rhizoidal bases from January to April. Maximum growth and development of reproductive structures have been observed at seawater temperatures exceeding 15°C (Hwang *et al*., 2009). Although our observation period spanned from April 2023 to January 2024, the species did not seem to follow this seasonal growth pattern, and upright fronds were still persistent and healthy in January. In Bari, the mean seawater temperature is above 15°C all year around, with very few exceptions. This situation resembled what was observed in the waters of the Strait of Gibraltar (García-Gómez *et al*., 2020), where *R. okamurae* achieved high coverage even in the winter months (García-Gómez *et al*., 2021a).

As previously noted in Sicilian waters (Bellissimo *et al*., 2024), also in Bari *R. okamurae* was introduced in an environmentally degraded area. Therefore, we could not quantify the impacts on resident, well-preserved natural communities, although some adverse effects of its invasion cannot be excluded. Implementing monitoring programs and extending the investigated area is a first step towards the comprehensive assessment of the magnitude of the invasion and any possible action aiming at mitigating the impact of *R. okamurae* on Mediterranean coastal communities.

Conclusions

The invasive *R. okamurae* is an emerging NIS rapidly expanding its area of dispersal, with potentially negative implications for the conservation of marine life in the Mediterranean Sea, as well as negative effects on both economic activity and tourism. The finding of conspicuous populations along the Adriatic coast of Italy raises concerns about a possible ongoing eastward expansion in the Mediterranean Sea. For these reasons, monitoring programs and time- and cost-effective mitigation actions aimed at assessing the presence and distribution of this species along the Mediterranean coasts should be implemented, in order to improve the awareness of Mediterranean countries of this NIS.

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References

Alomar, C., Deudero, S., Andaloro, F., Castriota, L., Consoli, P. *et al.,* 2016. *Caulerpa cylindracea* Sonder invasion modifies trophic niche in infralittoral rocky benthic community. *Marine Environmental Research*, 120, 86-92.

Altamirano, M., De La Rosa, J., Kawai, H., Hanyuda, T., Carmona, R. *et al.,* 2020. Cryptic introduction and invasion of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) at the Strait of Gibraltar: a real threat to the Mediterranean ecosystems. *Phycologia*, 60, 8.

Bailey, S.A., Brown, L., Campbell, M.L., Canning-Clode, J., Carlton, J.T. *et al*., 2020. Trends in the detection of aquatic non-indigenous species across global marine, estuarine and freshwater ecosystems: A 50-year perspective. *Diversity and Distributions*, 26 (12), 1780-1797.

Ballesteros, E., Cebrian, E., Alcoverro, T., 2007. Mortality of shoots of *Posidonia oceanica* following meadow invasion by the red alga *Lophocladia lallemandii*. *Botanica Marina*, 50, 8-13.

Barcellos, L., Pham, C.K., Menezes, G., Bettencourt, R., Rocha, N. *et al.,* 2023. A Concise Review on the Potential Applications of *Rugulopteryx okamurae* Macroalgae. *Marine Drugs*, 21, 40.

Bax, N., Carlton, J.T., Mathews-Amos, A., Haedrich, R.L., Howarth, F.G. *et al.,* 2001. The Control of Biological Invasions in the World's Oceans. *Conservation Biology*, 15, 1234-1246.

Bedini, R., Bedini, M., Bonechi, L., Piazzi, L., 2015. Effects of non-native turf-forming Rhodophyta on mobile macro-invertebrate assemblages in the north-western Mediterranean Sea. *Marine Biology Research*, 11, 430-437.

Bellan-Santini, D., Arnaud, P.M., Bellan, G., Verlaque, M., 1996. The Influence of The Introduced Tropical Alga *Caulerpa Taxifolia*, on the Biodiversity of the Mediterranean Marine Biota. *Journal of the Marine Biological Association of the United Kingdom*, 76, 235-237.

Bellissimo, G., Altamirano, M., Muñoz, A.R., De la Rosa, J., Hung, T.H. *et al.,* 2024. The invasive brown seaweed *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) continues to expand: first record in Italy. *BioInvasions Records*, 13(2), 385-401.

Bernal-Ibáñez, A., Chebaane, S., Sempere-Valverde, J., Faria, J., Ramalhosa, P. *et al.,* 2022. A worrying arrival: the first record of brown macroalga *Rugulopteryx okamurae* in Madeira Island and its invasive risk. *BioInvasions Records*, 11, 912-924.

Bittner, L., Payri, C.E., Couloux, A., Cruaud, C., de Reviers, B. *et al.,* 2008. Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, 49 (1), 211-226.

Boudouresque, C., Verlaque, M., 2002. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin*, 44, 8-32.

Casal-Porras, I., Zubía, E., Brun, F.G., 2021. Dilkamural: A novel chemical weapon involved in the invasive capacity of the alga *Rugulopteryx okamurae* in the Strait of Gibraltar. *Estuarine, Coastal and Shelf Science*, 257, 107398.

Dlugosch, K.M., Parker, I.M., 2008. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecology letters*, 11 (7), 701-709.

El Madany, M., Hassoun, M., El Aamri, F., El Mtili, N., 2024. Recent occurrence and expansion of the non-indigenous alga *Rugulopteryx okamurae* in Morocco (Mediterranean and Atlantic shores). *Aquatic Botany*, 190, 103722.

Faria, J., Prestes, A.C., Moreu, I., Martins, G.M., Neto, A.I. *et al.,* 2022. Arrival and proliferation of the invasive seaweed *Rugulopteryx okamurae* in NE Atlantic islands. *Botanica Marina*, 65 (1), 45-50.

Figueroa, F.L., Vega, J.V., Valderrama, M.G., Flores-Moya, A., 2020. Invasión de La Especie Exótica *Rugulopteryx okamurae* En Andalucía I: Estudios Preliminares de La Actividad Fotosintética. *Algas*, 56, 35-46.

Galil, B.S., Marchini, A., Occhipinti-Ambrogi, A., Minchin, D., Narščius, A. *et al.,* 2014. International arrivals: Widespread bioinvasions in European Seas. *Ethology, Ecology and Evolution*, 26, 152-171.

García-Gómez, J.C., Sempere-Valverde, J., González, A.R., Martínez-Chacón, M., Olaya-Ponzone, L. *et al.,* 2020. From exotic to invasive in record time: The extreme impact of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in the strait of Gibraltar. *Science of the Total Environment*, 704, 135408.

García-Gómez, J.C., Florido, M., Olaya-Ponzone, L., Rey Díaz de Rada, J., Donázar-Aramendía, I. *et al.,* 2021a. Monitoring Extreme Impacts of *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) in El Estrecho Natural Park (Biosphere Reserve). Showing Radical Changes in the Underwater Seascape. *Frontiers in Ecology and Evolution*, 9, 639161.

García-Gómez, J.C., Florido, M., Olaya-Ponzone, L., Sempere-Valverde, J., Megina, C., 2021b. The Invasive Macroalga *Rugulopteryx okamurae*: Substrata Plasticity and Spatial Colonization Pressure on Resident Macroalgae. *Frontiers in Ecology and Evolution*, 9, 631754.

Guerra-García, J.M., Ros, M., Izquierdo, D., Soler-Hurtado, M.M., 2012. The invasive *Asparagopsis armata* versus the native *Corallina elongata*: Differences in associated peracarid assemblages. *Journal of Experimental Marine Biology and Ecology*, 416-417, 121-128.

Hwang, Z.G., 1994. *Marine Species and Their Distributions in China's Seas*, Vol.48. Beijing: China Ocean Press, 12 pp.

Hwang, I.K., Kim, H.S., Lee, W.J., 2004. Evidence for taxonomic status of *Pachydictyon coriaceum* (Holmes) Okamura (Dictyotales, Phaeophyceae) based on morphology and plastid protein coding *rbc*L, *psa*A, and *psb*A gene sequences. *Algae*, 19 (3), 175-190.

Hwang, I.K., Wook, J.L., Kim, H.S., De Clerck, O., 2009. Taxonomic reappraisal of *Dilophus okamurae* (Dictyotales, Phaeophyta) from the western Pacific Ocean. *Phycologia*, 48, 1-12.

Klein, J.C., Verlaque, M., 2011. Macroalgae newly recorded, rare or introduced to the French Mediterranean coast. *Cryptogamie Algologie*, 32, 111-130.

Mateo-Ramírez, Á., Iñiguez, C., Fernández-Salas, L.M., Sánchez-Leal, R.F., Farias, C. *et al.,* 2023. Healthy thalli of the invasive seaweed *Rugulopteryx okamurae* (Phaeophyceae) being massively dragged into deep-sea bottoms by the Mediterranean Outflow Water. *Phycologia*, 62, 99-108.

Meinesz, A., De Vaugelas, J., Hesse, B., Mari, X., 1993. Spread of the introduced tropical green alga *Caulerpa taxifolia* in northern Mediterranean waters, *Journal of Applied Phycology*, 5, 141-147.

Mercado, J.M., Gómez-Jakobsen, F., Korbee, N., Aviles, A., Bonomi-Barufi, J. *et al.,* 2022. Analyzing environmental factors that favor the growth of the invasive brown macroalga *Rugulopteryx okamurae* (Ochrophyta): The probable role of the nutrient excess. *Marine Pollution Bulletin*, 174, 113315.

Navarro-Barranco, C., Florido, M., Ros, M., González-Romero, P., Guerra-García, J.M., 2018. Impoverished mobile epifaunal assemblages associated with the invasive macroalga *Asparagopsis taxiformis* in the Mediterranean Sea. *Marine Environmental Research*, 141, 44-52.

Navarro-Barranco, C., Moreira, J., Espinosa, F., Ros, M., Rallis, I. *et al.,* 2021. Evaluating the vulnerability of coralligenous epifauna to macroalgal invasions. *Aquatic Conservation*, 31, 2305-2319.

Nikolić, V., Žuljević, A., Antolić, B., Despalatović, M., Cvitković, I., 2010. Distribution of invasive red alga *Womersleyella setacea* (Hollenberg) R.E. Norris (Rhodophyta, Ceramiales) in the Adriatic Sea. *Acta Adriatica*, 2, 195-202.

Paolacci, S., Bog, M., Lautenschlager, U., Bonfield, R., Appenroth, K.J. *et al.,* 2021. Clonal diversity amongst island populations of alien, invasive *Lemna minuta* Kunth. *Biological Invasions*, 23 (8), 2649-2660.

Petrocelli, A., Cecere, E., Verlaque, M., 2013. Alien marine macrophytes in transitional water systems: new entries and reappearances in a Mediterranean coastal basin. *BioInvasions Records*, 2 (3), 177-184.

Piazzi, L., Cinelli, F., 2003. Evaluation of benthic macroalgal invasion in a harbour area of the western Mediterranean Sea. *European Journal of Phycology*, 38, 223-231.

Quaglia, N.C., Capuozzo, F., Ceci, E., Cometa, S., Di Pinto, A. *et al.,* 2023. Preliminary survey on the occurrence of microplastics in bivalve mollusks marketed in Apulian fish markets. *Italian Journal of Food Safety*, 12, 10906.

Rindi, F., Gavio, B., Diaz-Tapia, P., Di Camillo, C.G., Romagnoli, T., 2020. Long-term changes in the benthic macroalgal flora of a coastal area affected by urban impacts (Conero Riviera, Mediterranean Sea). *Biodiversity and Conservation*, 29, 2275-2295.

Rosas-Guerrero, J., Loring, J., Carmona, R., Altamirano, M., 2020. Efecto de los nutrientes y la temperatura sobre el crecimiento y la fotosíntesis del alga invasora *Rugulopteryx okamurae* (Dictyotales, Ochrophyta). *Algas*, 56, 106-116.

Ruitton, S., Blanfuné, A., Boudouresque, C.F., Guillemain, D., Michotey, V. *et al.,* 2021. Rapid spread of the invasive brown alga *Rugulopteryx okamurae* in a national park in Provence (France, Mediterranean Sea). *Water*, 13, 2306.

Schaffelke, B., Murphy, N., Uthicke, S., 2002. Using genetic techniques to investigate the sources of the invasive alga *Caulerpa taxifolia* in three new locations in Australia. *Marine Pollution Bulletin*, 44 (3), 204-210.

Sempere-Valverde, J., Ostalé-Valriberas, E., Maestre, M., González Aranda, R., Bazairi, H. *et al.,* 2021. Impacts of the non-indigenous seaweed *Rugulopteryx okamurae* on a Mediterranean coralligenous community (Strait of Gibraltar): The role of long-term monitoring. *Ecological Indicators*, 121, 107135.

Saunders, G.W., Moore, T.E., 2013. Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae*, 28 (1), 31-43.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10 (3), 512-526.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38 (7), 3022-3027.

Terradas-Fernández, M., Pena-Martín, C., Valverde-Urrea, M., Gran, A., Blanco-Murillo, F. *et al.,* 2023. An outbreak of the invasive macroalgae *Rugulopteryx okamurae* in Alicante Bay and its colonization on dead *Posidonia oceanica matte*. *Aquatic Botany*, 189, 103706.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22 (22), 4673-4680.

Verlaque, M., Steen, F., De Clerck, O., 2009. *Rugulopteryx* (Dictyotales, Phaeophyceae), a genus recently introduced to the Mediterranean. *Phycologia*, 48, 536-542.

Verlaque, M., Breton, G., 2019. Biological invasion: Long term monitoring of the macroalgal flora of a major European harbor complex. *Marine Pollution Bulletin*, 143, 228-241.

Wolf, M.A., Buosi, A., Juhmani ,A.S.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.

Zenetos, A., Katsanevakis, S., Poursanidis, D., Crocetta, F., Damalas, D. *et al.,* 2011. Marine alien species in Greek Seas: Additions and amendments by 2010. *Mediterranean Marine Science*, 12 (1), 95-120.

Žuljevic, A., Antolić, B., 2000. Synchronous release of male gametes of *Caulerpa taxifolia* (Caulerpales, Chlorophyta) in the Mediterranean Sea. *Phycologia*, 39 (2), 157-159.