

Rapid Communication

The invasive brown seaweed *Rugulopteryx okamurae* (Dictyotales, Ochrophyta) continues to expand: first record in Italy

Giancarlo Bellissimo¹, María Altamirano², Antonio Román Muñoz³, Julio De la Rosa⁴, Tin Hang Hung⁵, Gabriele Rizzuto⁶, Salvatrice Vizzini^{6,7} and Agostino Tomasello^{6,7}

¹Regional Agency for Environmental Protection of Sicily (ARPA Sicilia), Complesso Roosevelt, Lungomare Cristoforo Colombo snc, 90149 Palermo, Italy

²Universidad de Málaga, Departamento de Botánica y Fisiología Vegetal, Facultad de Ciencias, Campus de Teatinos s/n, 29080 Málaga, Spain

³Universidad de Málaga, Departamento de Biología Animal, Facultad de Ciencias, Campus de Teatinos, 29010 Málaga, Spain

⁴Universidad de Granada, Departamento Botánica, Facultad de Ciencias, Campus Fuente Nueva s/n, 18071 Granada, España

⁵Department of Biology, University of Oxford, South Parks Road, Oxford OX1 3RB, UK

⁶National Inter-University Consortium for Marine Sciences, CoNISMa, Piazzale Flaminio 9, 00196 Roma, Italy

⁷Department of Earth and Marine Sciences, University of Palermo, Viale delle Scienze, 90128 Palermo, Italy

Corresponding author: María Altamirano (altamirano@uma.es)

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Abstract

The brown seaweed *Rugulopteryx okamurae* (Dictyotales, Ochrophyta), native to the Pacific Ocean and widely distributed in Asia, has been recently recognized as an emblematic case of biological invasion by marine macroalgae in European waters. Since 2015 and from the Strait of Gibraltar, *R. okamurae* has rapidly spread towards Atlantic and Mediterranean coastal areas exhibiting an invasive behaviour with significant ecological and socio-economic impacts. Here, we report the first morphologically and genetically confirmed observation of this species in Italy, along the north-western coast of Sicily (Gulf of Palermo). Specimens were found as drifted material and as an established population on *Posidonia oceanica*, representing its new eastern distribution limit in the Mediterranean Sea, as the species was previously established in Marseilles (France). Furthermore, we performed a favorability distribution model with the current introduced distribution of the species for the Mediterranean, which shows most of the western Mediterranean, including the Balearic archipelago, Corsica and Sardinia, central Mediterranean, including Sicily, and the northern coast of Africa together with eastern Mediterranean basin, as highly favorable for *R. okamurae*. The most probable vectors for this introduction are sea currents and maritime traffic, including fishing activities. This hypothesis was supported by some of the ranked variables in the favorability model, i.e., current velocity, and the proximity of the introduced population to fishing ports. These results are a warning that the species can cover large sea distances via sea currents, thus also threatening the ecosystems and marine resources of the central and eastern Mediterranean Sea, as these areas are forecasted to be highly favorable regions for the species. We suggest coordinated actions at the European level with stakeholders in the fishing sector regarding prevention, both because it is a highly affected sector and because it potentially has a very important role in the dispersion of the species.

Key words: distribution modelling, favorability, fishing, macroalgae, marine bioinvasions, sea current, Sicily

Introduction

The introduction of alien species into new habitats, mediated by anthropic activities, is one of the greatest threats to natural and socio-economic systems, as some species can become invasive and can cause several-fold impacts (Simberloff et al. 2013; Anton et al. 2019; Díaz et al. 2019; Korpinen et al. 2019). Biodiversity is threatened by these species, which pose a continuous challenge for governments trying to minimize introduction events and the impacts they entail. The challenge is even greater in the case of the marine environment due to its spatial and ecological heterogeneity, the absence of physical barriers, as well as the difficulty in studying species invasions and controlling their spread all of which implies an increased difficulty of finding and controlling invasive alien species (Zenetos et al. 2022a).

The Mediterranean Sea is a hot spot for introductions of exotic species and invasion events, mainly due to the intense maritime traffic as well as its connection with two large bodies of water of a different biogeographical nature (Real et al. 2021). More than one thousand species are non-native in the Mediterranean Sea, and an upward trend has recently been detected in the rate of appearance of new exotic species, which for the period 2012–2017 has been estimated as an average of 21 species per year (Zenetos et al. 2022a, b). In a review of introduced species in Europe, it was estimated that 3 out of 4 exotic species that were introduced manage to establish themselves, and 3 out of 4 exotic species were identified for the first time in Mediterranean waters, mainly originating from the Indo-Western Pacific region (Tsiamis et al. 2018). Thus, the Mediterranean Sea may serve as a key indicator for species introductions across Europe. The major introduction vectors seem to be well identified for the marine environment – ballast water, biofouling and aquaculture activities (Tsiamis et al. 2018). However, the mechanisms of spread after a species has been introduced remain less clear, even though they play a crucial role in the successful colonization of species beyond their native distribution. These mechanisms are also vital in curbing their progression and reducing their effects when implementing control strategies. Thus, understanding the role of ocean currents and recreational aquatic and fishing activities in the dispersal area of species, once they have been introduced, may offer important clues on how to curb the expansion of invasive alien species.

Invasive species attract the attention of citizens and governments because of their sometimes highly visible impacts on natural ecosystems (Katsanevakis et al. 2014; Diagne et al. 2021). From the list of the 10 most invasive species in terms of their negative effects on biodiversity in the Mediterranean Sea, mainly due to competition for resources, the first six are macroalgae species (Tsirintanis et al. 2022) (in order): *Caulerpa cylindracea* Sonder, *Womersleyella setacea* (Hollenberg) RE Norris, *Lophocladia lallemandii*

(Montagne) F Schmitz (now *L. trichocladus* (C Agardh) F Schmitz), *Rugulopteryx okamurae* (EY Dawson) IK Hwang, WJ Lee & HS Kim, *Acrothamnion preissii* (Sonder) EM Wollaston and *Caulerpa taxifolia* (M Vahl) C Agardh. Seaweeds are important invasive species as they act as ecosystem engineers with a high capacity to homogenize native communities, with important consequences for trophic chains (Deudero et al. 2011). Of the six species mentioned above, the one most recently discovered—*R. okamurae*, fourth in terms of harmfulness—has also been the first and only species of macroalgae to be included in the list of invasive alien species of Union concern (EU 2022). This species has been added to the list not only for its detrimental effects on biodiversity, but also for the socio-economic implications of its fast spread, with estimated financial losses of several million euros (CIRCABC 2020; MITECO 2022).

Rugulopteryx okamurae (Dictyotales, Ochrophyta) is a subtropical brown seaweed species naturally distributed between the subtropical and temperate western Pacific Ocean (Lee and Kang 1986; Silva et al. 1987; Yoshida 1998). It exhibits a diplobiontic isomorphic life cycle, common to other morphologically similar species of the same family, as *Dictyota* or *Taonia* spp., which makes its introduction an excellent example of a cryptic invasion. Furthermore, in its native area, the species exhibits up to three different morphotypes throughout the year (Sun et al. 2006), which seem to occur in the newly introduced alien populations as well (Salido and Altamirano 2020). Similar to the native populations, the species maintains and establishes new populations through vegetative (propagules) and asexual (monospores) reproduction (Hwang et al. 2009; Altamirano et al. 2016, 2017), throughout the year (Salido and Altamirano 2020) and does not go through sexual reproduction. The first record outside its native range was in the Thau Lagoon in France in 2002 (Verlaque et al. 2009), where the species is still present but not invasive (Ruitton et al. 2021). The first record as an invasive species was on the Spanish coast of the Strait of Gibraltar in 2016 (Altamirano et al. 2016, 2017), on the African coast (Ceuta, Spain) and later on the European coast. Since then, the species has been spreading both towards the Mediterranean Sea and the Atlantic Ocean, being found in Spain (Altamirano et al. 2016; García-Gómez et al. 2020), Morocco (El Aamri et al. 2018; El Madany et al. 2024), Portugal (Liulea et al. 2023) and France (Ruitton et al. 2021). In addition, the species has even reached different Macaronesian islands such as the Azores (Faria et al. 2021), Madeira (Bernal-Ibáñez et al. 2022) and the Canary Islands (REDEXOS 2022). Its current eastern limit in the Mediterranean is on the coast of Marseille, in France (Ruitton et al. 2021). The species has a high capacity for vegetative proliferation as well as for dispersal, which gives it a high capacity to produce large amounts of biomass that can be moved by currents (García-Lafuente et al. 2023; Mateo-Ramírez et al. 2023) or helped by human vectors such as fishing or maritime transport (MITECO 2022),

and which can end up forming massive wracks on the coasts. The seaweed's ability to proliferate quickly is causing serious concern because it greatly impacts both native, natural communities (seagrasses and marine forests) by reducing their extension and diversity (Faria et al. 2022; García-Gómez et al. 2020, 2021), and human activities such as fishing and tourism, with losses of millions of euros across various sectors (CIRCABC 2020; MITECO 2022). The Mediterranean Sea has been identified as a highly favorable area for this species (Muñoz et al. 2019), which points to a potential increasing impact if its spread along European coasts is not halted. It is therefore essential to set up an early warning system for this species by identifying the dispersal vectors and entry points in new areas and extending coordinated protocols for its control.

In this context, the objective of this work is to report the first record of *R. okamurae* in Italy, on the island of Sicily, being the easternmost distribution of the species in the Mediterranean Sea to our knowledge and provide the current favorability for the species in the Mediterranean. This work provides additional evidence of the fast dispersal of this species in the eastern Mediterranean Sea, a very favorable area for its establishment, especially in the current climate scenario.

Materials and methods

Study site

The study area was the Gulf of Palermo (north-western coast of Sicily, Italy). A massive beached monospecific seaweed biomass was observed for the first time at the end of June 2023 on the beach of Aspra (38°06'17"N; 13°29'52"E), a small fishing village on the eastern side of the Gulf of Palermo (Figures 1, 2). The coastal zone has sandy bottoms with dead vermetid reefs and rocky blocks at shallow depths, while artificial submerged and subaerial breakwaters run parallel to the shoreline at about 80 m from the shoreline. Drifted material and detritus was also observed on rocks and concrete blocks scattered along the shore at the beginning of July 2023 in a locality adjacent to the main port area of Palermo (38°07'14"N; 13°22'18"E) and at the end of August 2023 on the shoreline of the Port of Bandita (38°05'54"N; 13°25'01"E) (Figure 2). An established dense population of *R. okamurae* was found in July 2023 in Aspra on *Posidonia oceanica* (Linnaeus) Delile meadows at 4 m depth. *In situ* observations and surveys of the relative abundance of *R. okamurae* were performed in an area of about 1 ha. Further monitoring for the species was performed in July and August 2023 along the coast of Sicily (Figure 2) and Lampedusa Island to study the distribution of the species (Figure 1 and Supplementary material Table S1).

Collection and identification

Specimens were collected at all discovery sites, put into ziplock bags, and stored under dark and cold conditions until morphological identification

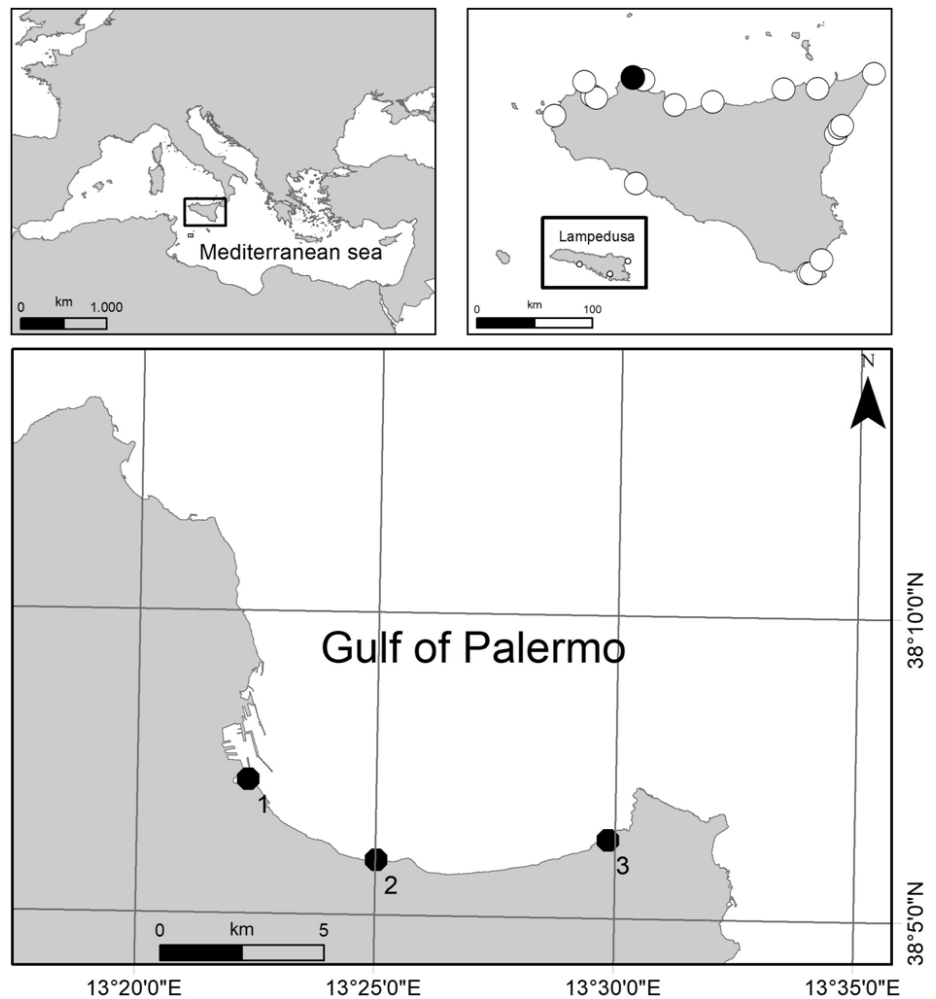


Figure 1. Distribution of *R. okamurae* along the coasts of Sicily and Lampedusa Island in summer 2023. Black dots indicate the presence of the species (1) Port of Palermo, (2) Port of Bandita, (3) Aspra beach, white dots indicate visited places where the species was not found.

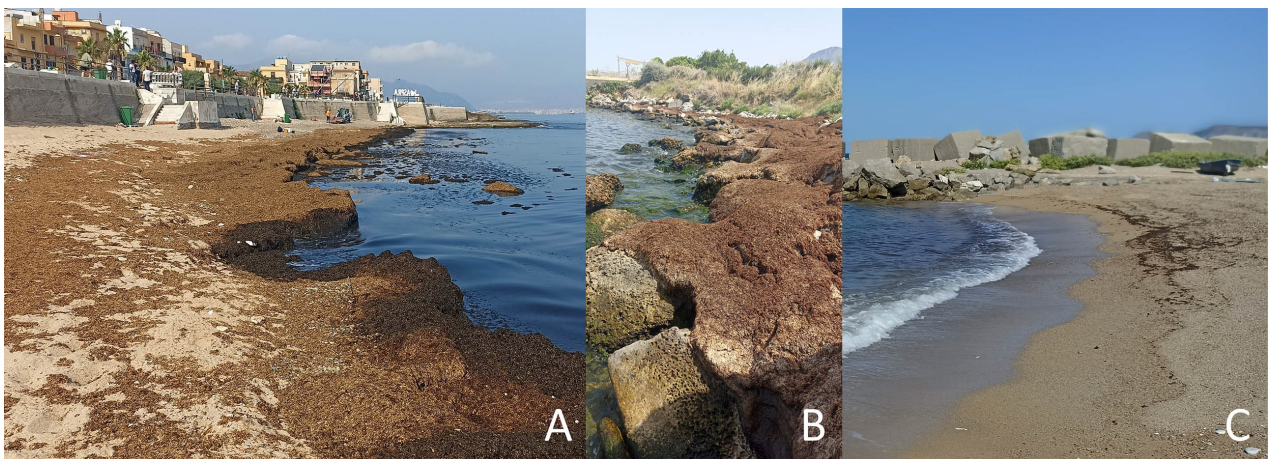


Figure 2. Drifted biomass of *R. okamurae* on the beach of Aspra (A), on artificial rocky blocks at Port of Palermo (B) and along the shoreline at Port of Bandita (C). Photographs by Giancarlo Bellissimo and Agostino Tomasello.

was performed in the laboratory. Samples from drifted material were collected from the outermost front of the mass, and those from established underwater populations were collected at depths ranging from 0.2 to 4 m. In the laboratory, thalli were observed using both a 3D Digital Microscope

(Hirox RH-2000) and a stereomicroscope (Discovery V20, Zeiss). Morphological identification was performed on material collected at all sites following reference literature (De Clerck et al. 2006; Sun et al. 2006; Hwang et al. 2009). Voucher specimens were deposited into the personal herbarium of GB at the Regional Agency for Environmental Protection in Palermo (ARPA Sicilia – UOC Area Mare) and into the University of Málaga herbarium (MGC-Phyc 5515).

Three samples were collected for species-level identification by DNA analysis and put into silica gel. Genomic DNA extraction was performed using a DNeasy Plant Mini Kit (Qiagen, United Kingdom), following the manufacturer's instructions. DNA yield and quality were assessed by using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific, United Kingdom). Only two samples passed the quality check and were retained for downstream work. Genes encoding chloroplast proteins *rbcL* and *psbA* were amplified by PCR using a ProFlex PCR System (Thermo Fisher Scientific). The primers were based on a previous study of Faria et al. (2021), with modifications to primers targeting the *rbcL* locus (see Table S2). The 25- μ l reactions contained: 12.5 μ l Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs, United Kingdom), 1.25 μ l forward primer (10 μ M), 1.25 μ l reverse primer (10 μ M), and 10 μ l genomic DNA (~ 50 ng). The thermal cycling profile was: 98 °C 30 s, 30 \times [98 °C 30 s, 60 °C 30 s, 72 °C 1 min], 72 °C 2 min. PCR product size and integrity were verified by electrophoresis on 1% agarose gel. Reactions were purified using a Monarch PCR & DNA Cleanup Kit (New England Biolabs) and sent to Source BioScience, Nottingham, United Kingdom for Sanger sequencing in both forward and reverse directions. Nucleotide sequences were inspected and trimmed to remove low base-call quality reads on Unipro UGENE v48.0. Finally, DNA sequences were aligned on MAFFT and the consensus sequence was uploaded on BLASTn for multiple pairwise comparisons with reference sequences stored at the NCBI database.

Distribution modelling

To model the distribution of *R. okamurae*, we used all known records in the Mediterranean Sea up to September 2023 (Figure 3). These records were restricted to the western Mediterranean until the appearance of the species in Italy reported here. In the modelling procedure, we used the Bio-ORACLE database (version 2.1 and 5 arc minutes resolution), which consists of a set of geophysical, biotic, and climatic data for all oceans (Assis et al. 2018). We also used two more variables, the distance to fishing harbours and the distance to commercial harbours. The distribution data of *R. okamurae* and the environmental layers were projected onto a hexagonal grid, with each hexagon representing Operative Geographical Units (OGU) covering an area of 7774 km². To control for multicollinearity among environmental



Figure 3. Current Mediterranean distribution of *R. okamurae*.

variables, we calculated Spearman correlation coefficients. For each pair of variables with $r > 0.8$, we only retained the variable with the highest individual predictive power (Chamorro et al. 2021; García-Carrasco et al. 2023). We limited the increase in type I error bound by the number of variables analyzed by addressing a False Discovery Rate control (Benjamini and Yekutieli 2001).

Considering that the risk of invasion derives from the degree to which the environmental conditions are favorable for the presence of the species, we built a risk model for *R. okamurae* invasion in the Mediterranean Sea by performing a multivariable forward-backward stepwise logistic regression of the current presence/absence data on the set of variables. In our model, variables were added to an initial null model if their inclusion significantly improved the regression. We used probability values from the logistic regression to obtain favorability values, ranging from 0 to 1, by applying the Favorability Function (Muñoz et al. 2005; Real et al. 2006). These values represent deviations from the expected chance of *R. okamurae* invasion and identify localities with environmental conditions associated with already invaded areas. We used the Wald test to assess the weight of each variable and the estimation of the parameters in the logit equation (Wald 1943). To evaluate the discrimination capacity of the model, we used the Area Under the Receiver Operating Characteristic Curve (AUC; Hosmer and Lemeshow 2000). This modelling approach complies with standard modelling protocols (Sillero et al. 2021).

Results

The morphology and anatomy of collected samples at all sites corresponded with the invasive brown seaweed *R. okamurae*. The external vegetative



Figure 4. *Rugulopteryx okamurae* specimen sampled in the established underwater population of Aspra. Scale bar = 3 cm. Photographs by Giancarlo Bellissimo.

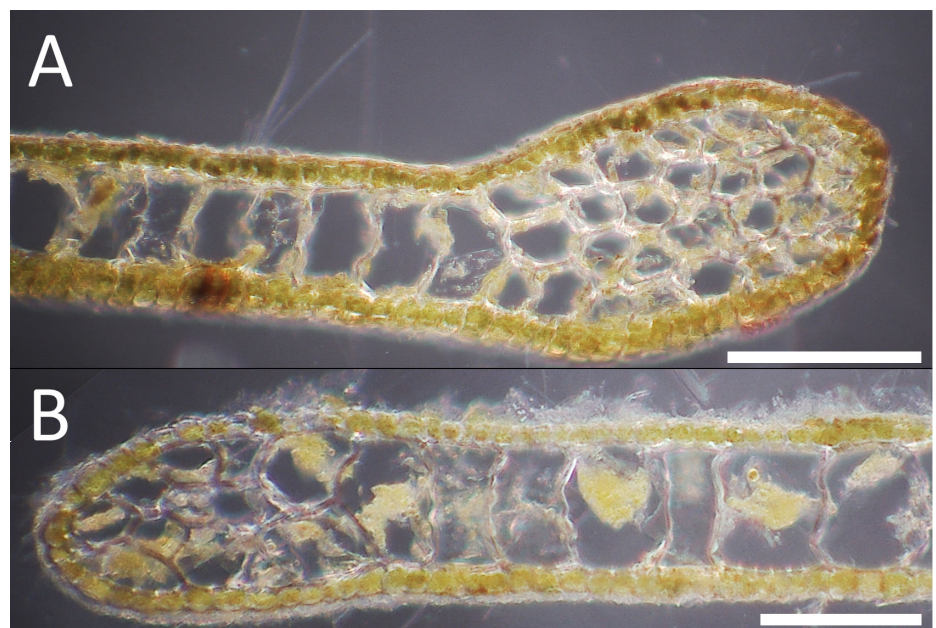


Figure 5. Cross-section of a specimen both beached (A) and from the settled population (B) showing a multi-layered medulla near the margin. Scale bar = 200 μ m. Photographs by Giancarlo Bellissimo.

morphology of thalli was flat, ribbon-like, corrugated and dichotomous, branched up to 18 cm height with a stoloniferous attaching system, and exhibited a yellow-brown colour without iridescence (Figure 4). The inner structure of the thalli was characterized by a monostromatic cortical, a monostromatic central medulla, and a pluristromatic medulla at the margin of the thalli (Figure 5). Two different morphotypes with this general anatomical pattern were observed in collected samples. Drifted material on the beach

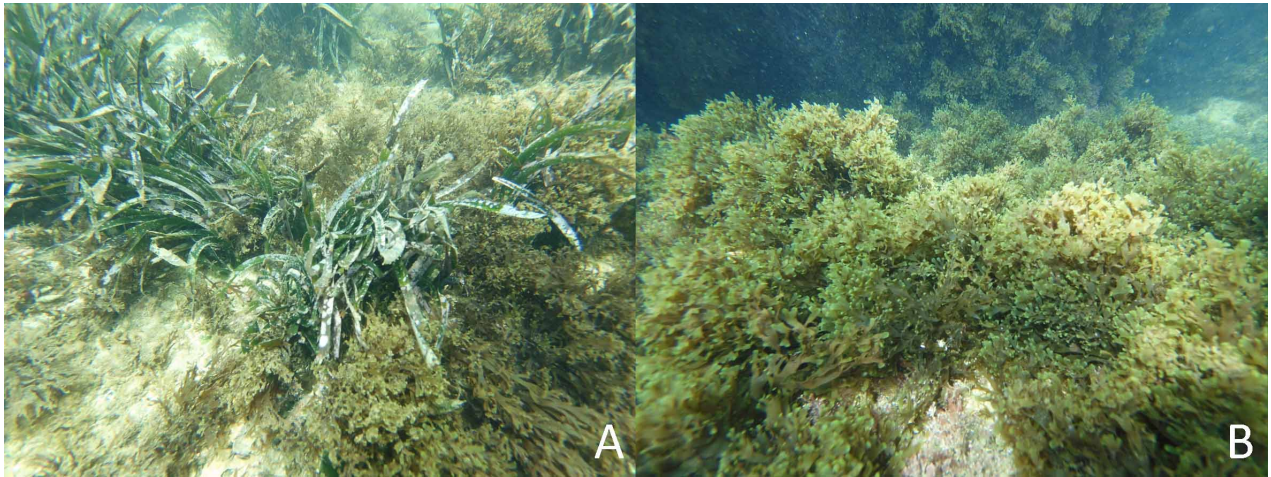


Figure 6. A) Established population of *R. okamurae* on *P. oceanica* and B) with native algal flora on artificial rocky blocks. Photographs by Giancarlo Bellissimo and Agostino Tomasello.

exhibited a thick morphotype, with more than 4 medullar cells at the margin of the thalli and wide branches up to 1 cm (Figure 5A). Thalli from established population exhibited a thin morphotype with 2–3 medullar cells at the margin of the thallus and narrow branches (< 0.5 cm) (Figure 5B). Vegetative propagules were observed in specimens from all sites, but no asexual monospores or sexual gametangia were found. Some epiphytic red algae, such as encrusting coralline *Hydrolithon farinosum* (JV Lamouroux) Penrose & YM Chamberlain, articulated coralline *Jania rubens* (Linnaeus) JV Lamouroux and filamentous Ceramiales were detected entangled or epiphytic on *R. okamurae*. No evidence of grazing was observed.

BLAST searches of *psbA* and *rbcL* gene sequences returned 99% to 100% identity scores compared to *R. okamurae* specimens collected around the world, including specimens from Madeira and Azores (Bernal-Ibáñez et al. 2022; Faria et al. 2022), Cádiz and Alicante (Spain), and the native areas of Korea and Japan (see Tables S3 and S4). Overall, these results confirmed the morphological identification of the samples collected along the Sicilian coast at the species-level as *R. okamurae* thalli. The newly obtained sequences were deposited in NCBI GenBank with the accession numbers OR578566–OR578567.

The established population of *R. okamurae* was found invading small *P. oceanica* patches extending about 100 m² at a depth of 3–4 m. The invasive seaweed was epiphytic on both rhizome and leaves of *P. oceanica*, competing with native flora (Figure 6A). Attached thalli of *R. okamurae* were also observed on breakwaters (Figure 6B), sharing habitat with other macroalgae species such as *Asparagopsis* spp. Montagne, *Laurencia* complex and the articulated corallines *Ellisolandia elongata* (J Ellis & Solander) KR Hind & GW Saunders and *Jania rubens*, exhibiting a coverage of approximately 60% with a depth range of 2–4 m. Water temperature at the time of collection was 27 °C.

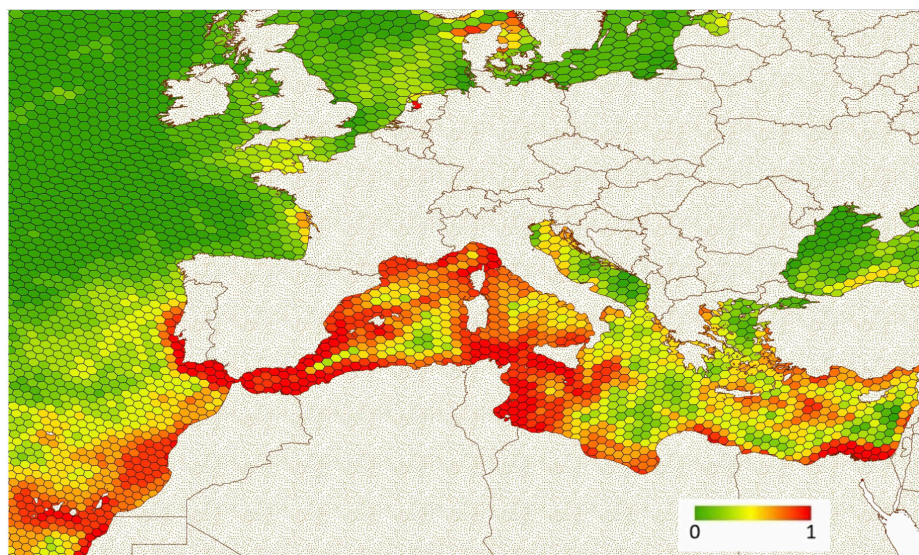


Figure 7. Cartographic representation of the current environmental favorability for *R. okamurae* in the Mediterranean in each operational geographic unit. Favorability color scale from 0 (unsuitable) to 1 (the most highly suited).

Table 1. Variables that entered the model via a forward–backward stepwise selection process, ranked by their order of entrance. β is the coefficient in the logit function, S.E. is the standard error of these coefficient, Wald is the Wald’s statistics value (representing the relative importance of the variable in the model) and p is the significance of the coefficients according to the Wald test.

Variable	β	S.E.	Wald	p
Maximum cloudiness	−0.962	0.299	10.338	0.001
Mean current velocity	26.334	3.410	59.631	< 0.001
Minimum velocity current	−10.617	2.380	19.892	< 0.001
Phosphate range	−7.012	2.649	7.006	0.008
Proximity to fishing port	0.027	0.015	3.385	0.066
Constant	−5.220	0.697	56.056	< 0.001

We obtained a significant environmental favorability model with high discrimination capacity ($AUC > 0.88$). This model showed that most of the western Mediterranean Sea is highly favorable for *R. okamurae* including the Balearic archipelago, Corsica and Sardinia, central Mediterranean, including Sicily, and the northern coast of Africa (Figure 7). Variables that entered and explained the model were more related to particular vectors than to factors determining the biological activity of the species and were ranked as follows: mean and minimum current velocity, cloud cover, phosphate, and distance to fishing harbours (Table 1 and Table S5).

Discussion

The morphological and genetic evidence provided in this work confirms the eastward expansion of the invasive seaweed *R. okamurae* in the Mediterranean Sea and its introduction in Italy. Chloroplast genome sequences are widely used for plant and algal species discrimination (Costa et al. 2016; Sun et al. 2020; Liu et al. 2022; Song et al. 2023). The genes coding for the photosystem reaction center protein D1 (psbA) and the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) are

molecular markers commonly used for brown seaweed phylogenetic studies (Saunders and Moore 2013). Here, we used primers targeting these chloroplast genomic regions to confirm morphological observations and obtained accurate identification at the species level (99%–100%) of the samples as the brown seaweed *R. okamurae*. This molecular analysis proved a close relationship with specimens previously collected in the Atlantic Ocean (Faria et al. 2021; Bernal-Ibáñez et al. 2022).

The species has once again shown its ability to cross great distances of open sea to reach islands, in this case the island of Sicily in Italy. Previously, the species managed to reach the Macaronesian islands, first the Azores (Faria et al. 2021, 2022), and afterwards Madeira and the Canary Islands (Bernal-Ibáñez et al. 2022; REDEXOS 2022), also covering great distances in open sea. The presence of *R. okamurae* in Sicily may have two explanations that are not exclusive, but rather, on the contrary, synergistic. On the one hand, the species may have arrived in the form of large accumulations of biomass transported by currents, as already observed in the Alborán Sea (García-Lafuente et al. 2023; Mateo-Ramírez et al. 2023) and suggested to explain the presence of the species in Madeira (Bernal-Ibáñez et al. 2022). In other words, marine currents may be facilitating the long-distance dispersal of the species, allowing for the arrival of large amounts of biomass that either have been detached from populations established in remote areas, or that have grown in suspension in the water column until they were dumped on the beach. The results of the present work show that this is what may have happened in Sicily too. In fact, wracks were found with thalli exhibiting the thick morphotype in July, while this morphotype is typical of cold months (Sun et al. 2006; Salido and Altamirano 2020), indicating that these thalli may have grown at another time and/or place with lower temperature, probably arriving from some other part of the western Mediterranean Sea, aided by a regime of marine currents that would be interesting to investigate. On the contrary, the thallus of the established population found in the study area presented a fine morphotype, typical of the warmer months (Sun et al. 2006; Salido and Altamirano 2020), coinciding with the time of its finding. The important role of sea currents as natural dispersal vectors for secondary introductions of *R. okamurae* can be inferred as well from the favorability model using the current introduced distribution of the species. Current velocity, expressed as mean and minimum values, were the variables that best explained *R. okamurae* distribution. This vector is worrying from the point of view of the management of the species, since it represents a dispersion that is difficult, if not almost impossible, to control and with an enormous geographical reach inside and outside the Mediterranean Sea.

The other explanation for the arrival of *R. okamurae* in Sicily, potentially synergistic and not exclusive with the previous one, is based on the hypothesis of the arrival of thalli mediated by vectors linked to anthropogenic

activities, potentially maritime transport or fishing or recreational activities. This argument was already used to explain the introduction of *R. okamurae* in the Azores islands (Faria et al. 2021), suggesting ballast water and/or fouling as potential vectors, since the species can survive up to three weeks in the dark (Rosas-Guerrero et al. 2018) and can adhere to different substrates (García-Gómez et al. 2018). The fact that the first records of *R. okamurae* in Sicily were very close to an important port strongly supports this explanation. The species could have been introduced through ballast water from other points in the Mediterranean Sea that share shipping routes with the port of Palermo or maybe the destination of fishing activities carried out in other invaded areas of the Mediterranean, like Marseilles, where *R. okamurae* is well established (Ruitton et al. 2021). The results of the favorability model also support this second hypothesis since proximity to fishing ports, although it was the last variable to enter the model, contributed to explaining the current distribution of the species. Transport-stowaways (attributed to maritime traffic) have been identified as responsible for around half of all Italian marine introductions of alien species, and Sicily is a hotspot due to its geographic location as a crossroad between Indo-Pacific and Atlantic waters (Servello et al. 2019). Furthermore, the port of Palermo is one of the major Mediterranean cruise ports, serving passenger ships mainly between Italy, France, and Greece, and linking the island to major Mediterranean cities (Civitavecchia-Rome, Genoa, Naples, Marseilles, Barcelona, Valencia, Athens and Tunis), besides the tourist marina serving yachts and catamarans.

In either possible introduction scenario, it would be interesting to know whether *R. okamurae* has reached the coasts of Corsica and Sardinia, as well as the coast of Tunisia, indicating a broad and fast expansion in the Mediterranean Sea, as already suggested by the environmental favorability model performed in this study. In any case, the presence of *R. okamurae* on the northern coast of Sicily should be considered as a warning that the species is at the gateway to the central Mediterranean Sea and is advancing towards the east, an area highly favorable for the establishment of this species.

Rugulopteryx okamurae has had unprecedented environmental and socioeconomic impacts at all sites where it has been introduced outside its native range since 2016 (Altamirano et al. 2016, 2017; El Aamri et al. 2018; García-Gómez et al. 2018; Faria et al. 2021, 2022; Ruitton et al. 2021; Bernal-Ibáñez et al. 2022; REDEXOS 2022; Liulea et al. 2023). The results of the present work do not allow us to assess the impacts of *R. okamurae* on the study area, as it seems that the seaweed is at the beginning of its invasion and the area was already highly degraded by anthropogenic pollution. However, it cannot be ruled out that the introduction of *R. okamurae* in Sicily could have similar impacts to those already observed in other areas, for example on the community associated with *P. oceanica* or on communities of macroalgae (García-Gómez et al. 2018; Faria et al.

2022) and coralligenous (Navarro-Barranco et al. 2021). The presence of the drifted biomass of *R. okamurae* near the port of Palermo should alert local councils that beach users may complain about macroalgae if it is not removed, and local councils would be remiss if they did not reserve budgetary items for this. Furthermore, we want to warn that the detection of *R. okamurae* can be difficult if the species first appears as thalli intermingled in banquettes of dead *P. oceanica* leaves or as wracks that are very similar in appearance to *P. oceanica* banquettes. Therefore, we need to inform and educate citizens and managers about this new invasion. In any case, the consequences of the introduction of *R. okamurae* in Sicily should be studied in detail as an example of what the species could cause if it spreads towards the eastern Mediterranean Sea.

In the central and eastern Mediterranean Sea, the most important invasive macroalgal species to date is *C. cylindracea*. This species was already present in the Strait of Gibraltar when and where *R. okamurae* began its invasion, together with *Asparagopsis armata* Harvey and *A. taxiformis* (Delile) Trevisan (Altamirano et al. 2019). It is possible that extant invasive species may support *R. okamurae* expansion through a process of invasional meltdown (Simberloff and Von Holle 1999), but later may gradually reduce their presence, although they do not completely disappear.

Management options for *R. okamurae* are challenging at the local, national, and European levels, but it should not be a reason for inaction. In this regard, the Spanish Government published the national control strategy for *R. okamurae* in 2022 (MITECO 2022). This document highlights that the prevention measures against the seaweed's introduction and spread are key to its control, pointing out the need to identify its dispersal vectors. In this context results of the favorability model performed in this study can be of much help. The favorability map indicated favorable areas for *R. okamurae* that have already been invaded, but also areas in which the species has not yet been recorded. Furthermore, the model has identified a variable that points towards a vector of anthropogenic origin, the proximity of fishing ports, which could be managed in such a way as to avoid or minimize the dispersal of the species. These two results extracted from the model represent an opportunity to work on preventing the introduction of the species, above all in areas of special interest for the conservation of marine habitats. In this context of opportunity to invest efficiently in preventing the introduction of *R. okamurae* into new areas, having the complicity and support of the fishing sector is crucial, and hence the need to inform them about the role they play in the management of this invasive species, not only as an affected sector but also as part of the solution. For this reason, the Spanish control strategy for *R. okamurae* points out the need to develop disinfection protocols for fishing gear, for example, as well as to work together with port authorities and the fishing sector (MITECO 2022). At the European level, coordination actions are

urgently needed to slow down the dispersal of the species and to minimize and mitigate its environmental impact on the affected sectors, such as fisheries.

Authors' contribution

GB, MA, ARM and AT performed the research conceptualization; GB, MA, and AT designed the research methods and sampling strategies, GB and AT carried out the field survey and collected samples; THH and GR carried out the molecular experiments and genetic analyses; ARM developed the distribution model; GB, MA, JDR, THH, GR and AT wrote the original draft of the manuscript; GB, MA, ARM, SV, JDR and AT reviewed and edited the manuscript; GR reviewed the English version of the manuscript; GB, MA, JDR, ARM and AT finalized the manuscript; SV and AT provided funding; All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Distribution records of *R. okamurae* in Italy.

Table S2. List of primers targeting the genetic markers used in this study.

Table S3. BLAST hit score table for psbA gene sequence searches against the NCBI.

Table S4. BLAST hit score table for rbcL gene sequence searches against the NCBI database.

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