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Grandidierella japonica (Amphipoda: Aoridae): a non-indigenous species in a Po delta lagoon of the northern Adriatic (Mediterranean Sea)

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Abstract

Background: The introduction and spread of non-indigenous species is one of the main threats to biodiversity of aquatic ecosystems and it is becoming an increasing problem for the international scientific community. Aquaculture and related activities are recognized as one of the most important drivers of non-indigenous species in the Mediterranean. *Grandidierella japonica* Stephensen, 1938 is an aorid amphipod species native of Japan. This species had previously only been reported a few times outside the Pacific region, in particular from coastal waters of England and French Atlantic coasts.

Results: A population of the non-indigenous amphipod *G. japonica*, has been detected in the Sacca di Goro, a Po delta lagoon of the northern Adriatic Sea (Italy), representing the first record of this species in the Mediterranean Sea. Adults of both sexes and juveniles were collected in muddy sediments reaching high densities. We examined 24 specimens: 8 adult males, 12 females, and 4 undifferentiated juveniles. Our specimens displayed a variability in the position of teeth of male gnathopod 1. Likely vectors for this introduction are the commercial shellfish transplants, mainly oyster farming.

Conclusions: The finding of a reproducing population of *G. japonica* suggests that the species has become well established in the Sacca di Goro. This finding also seems to be particularly relevant for the improvement on the knowledge of Mediterranean biodiversity and threats.

Keywords: Non-indigenous species, Amphipoda, Aoridae, Grandidierella japonica, Po delta lagoon, Mediterranean Sea

Background

Non-indigenous species introduction is one of the main threats to biodiversity of aquatic ecosystems; this is also an issue of growing concern in the marine and brackish contexts (Rosenthal 1980; Bax et al. 2003; Molnar et al. 2008). Aquaculture and related activities (e.g. sport fishing, fishery stock enhancement, ornamental trade) are recognized as important drivers of non-indigenous species in Europe (Olenin et al. 2008).

The Mediterranean Sea, represents a hot spot of biodiversity, but it is also one of the major recipients of non-indigenous species (Galil 2000; Streftaris et al. 2005; Gollasch 2006), many of which are considered established (Zenetos et al. 2005). The reasons of the exceptional susceptibility of the Mediterranean to biological invasions are: its long history of human occupation, crisscrossing shipping lanes, many major ports and innumerable marinas, lagoons and estuaries crowded with fish and oyster farms, and shores that are major tourist destinations (Galil 2000).

Apart from the opening of the Suez Canal, the most prominent vector for species introduction to Europe and the Mediterranean Sea is shipping, combining ballast water and hull fouling (Galil 2000; Gollasch 2006; Galil and Zenetos 2002). Aquaculture is the third most important means of introduction with unintentionally introduced species (e.g. fouling organisms on oyster) being more

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numerous compared with those introduced intentionally (Streftaris et al. 2005; Gollasch 2006). The north-western part of the Adriatic Sea is characterized by a large number of brackish coastal lagoons, that are recognized as highly vulnerable to species introduction as a consequence of their environmental instability, low number of species, exploitation for aquaculture and shellfish farming, and the presence of not saturated benthic communities (Munari and Mistri 2008).

Grandidierella japonica Stephensen, 1938 is an aorid amphipod species (Crustacea: Amphipoda: Aoridae) native of Japan (Chapman and Dorman 1975). Outside its native area, this amphipod was reported at first only in the Pacific region: it occurred in San Francisco Bay (California) in 1966 (Chapman and Dorman 1975), afterwards it was reported from intertidal and subtidal sediments of bays and estuaries of the western coast of North America from Mexico to Canada (Greenstein and Tiefenthaler 1997; Cohen et al. 1998; Cohen et al. 2002; Okolodkov et al. 2007), and it has also been found in Hawaii (Coles et al. 1999) and Australia (Myers 1981). Outside the Pacific region, reports of this species are from Southampton and the Orwell Estuary, in south-eastern England (Smith et al. 1999; Ashelby 2006; Noël 2011). Recently it has been reported for the first time from the Atlantic coast of France, specifically from Marennes-Oléron Bay (Jourde et al. 2013) and the Arcachon basin (Lavesque et al. 2014). G. japonica is not listed in the inventories for the Mediterranean Sea (Zenetos et al. 2005; Zenetos 2010; Zenetos et al. 2008; Zenetos et al. 2012; Galil 2008; Galil 2009), and EU databases (DAISIE -Delivering Alien Invasive Species Inventories for Europe. http://www.europe-aliens.org/. Accessed 22 Oct 2015; IMPASSE – Environmental impacts of alien species in aquaculture. http://www2.hull.ac.uk/science/biological_sciences/research/hifi/impasse.aspx. Accessed 22 Oct 2015) assess its absence from the Mediterranean.

This study reports of a dense population of the species *G. japonica* in the Sacca di Goro, a northern Adriatic lagoon. This represents the first record of the species in the Mediterranean basin. This is also the only member of the genus *Grandidierella* Coutière, 1904 to have been recorded, thus far, from the Mediterranean. Moreover, the finding of a reproducing population of this non-indigenous species seems to be particularly relevant for the improvement on the knowledge of Mediterranean biodiversity and threats. We give some morphological details related to the description of the species and some ecological notes about this species and a discussion about its possible vectors of introduction in the Mediterranean Sea.

Results

Sampling stations were characterized by muddy (silty-clay) sediments. Water (temperature, salinity and dissolved oxygen) and sediment (organic matter content and depth of the redox potential discontinuity layer) parameters at the 10 sampling stations are summarized in Table 1.

Systematics

Order AMPHIPODA Latreille, 1816 Suborder GAMMARIDEA Latreille, 1802 Family AORIDAE Stebbing, 1899 Genus *Grandidierella* Coutière, 1904 *Grandidierella japonica* Stephensen, 1938 (Figs. 1, 2 and 3)

Grandidierella japonica Stephensen, 1938: Nagata (1960); Chapman and Dorman (1975); Myers (1981); Greenstein and Tiefenthaler (1997); Cohen et al. (1998); Smith et al. (1999); Cohen (2002); Ashelby (2006);

Table 1 Main environmental characteristics of the ten sampling station during the sampling campaign of January 2015 (OM,
organic matter as percentage of dry weight; RPDL, depth of redox potential discontinuity layer)

	Lat	Long	Depth	O2	Temp	Sal	RPDL	OM
	DD	DD	m	mg/L	°C	PSU	mm	%DW
C1	44°49.061' N	12°19.395' E	1.7	6.6	6.8	34	1	6.80
C2	44°47.783' N	12°19.422' E	1.5	4.7	6.7	32	0	1.03
C3	44°47.717' N	12°20.620' E	1.5	5.8	6.8	35	0	4.49
C4	44°47.599' N	12°21.616′ E	1.2	5.8	6.2	35	0.5	3.37
C5	44°47.435′ N	12°22.328′ E	0.7	6.3	5.4	35	0.5	4.87
P1	44°49.758' N	12°18.105′ E	1.7	7.2	6.8	32	0.5	4.77
P2	44°48.676′ N	12°20.748′ E	1.3	6.5	5	34	1	5.26
P3	44°48.353′ N	12°21.223′ E	1	6.7	5.2	34	1	6.46
P4	44°48.079′ N	12°21.615 E	1	6.7	5.1	34	1	4.42
P5	44°47.793′ N	12°22.177' E	0.5	6.5	4.9	34	0.5	4.80



Fig. 1 Grandidierella japonica Stephensen, 1938: (a) adult male; (b) adult female. Scale bars: A-B, 1mm

Okolodkov et al. (2007); Ariyama (2007); Jourde et al. (2013); Lavesque et al. (2014).

From samples gathered in the Sacca di Goro on 13 January 2015, we collected 197 specimens of *Grandidierella japonica*, 56 out of these were in good condition. Among these we examined the best preserved specimens: 8 adult males (Fig. 1a), 12 females (Fig. 1b), and 4 undifferentiated juveniles. None of the specimens was complete; all were missing their antennae with the exception of two specimens; most of them were also missing pereopods 3-7.

The body of adult specimens was mottled grey to grey brown (Fig. 1); length up to 9.9mm in male and 13.2mm

in female (juvenile from 1.9mm), excluding antennae. All male specimens lacking antennae, with the exception of 1 with Antenna 1 (Fig. 1a) greater than one-half body length and flagellum with 20 articles; article 1 of peduncle with ventral spines (Fig. 2a). Specimens shows marked sexual dimorphism, particularly in the size and shape of the gnathopod 1. Male gnathopod 1 (Fig. 2b-d) massive, carpochelate; articles 2 and 5 greatly enlarged; article 5 with three distal posterior teeth (Fig. 2b), one tooth enlarged, forming a thumb, one tooth double (Fig. 2c-d), anterior edge with (about 40 in most specimens and more than 40 in someone; Fig. 2c) short transverse

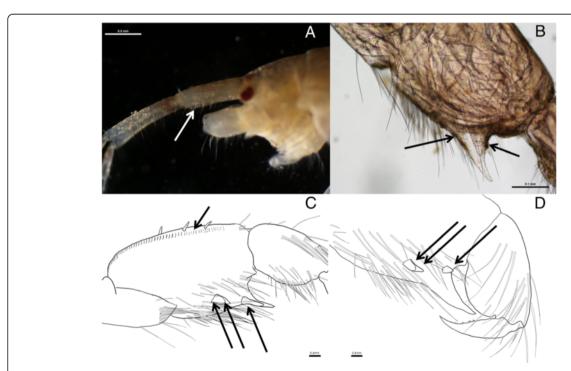


Fig. 2 *Grandidierella japonica* Stephensen, 1938, adult male: (a) antenna 1 with ventral spines; (b) article 5 of gnathopod 1, arrows indicate the three distal posterior teeth; (c) article 5 of gnathopod 1, arrows indicate the two smaller teeth (one of which is double), and the transverse grooves; (d) the double tooth of article 5 and the other small one with a different position are indicated by arrows. Scale bars: A, 0.5mm; B-D, 0.1mm

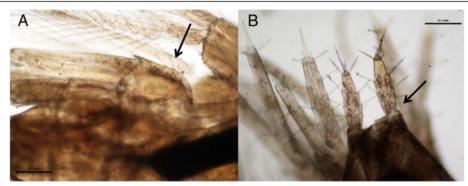


Fig. 3 Grandidierella japonica Stephensen, 1938: (a) urosomite 1, arrow indicates the three spines on antero-lateral surface; (b) uropod 3 indicated by arrow. Scale bars: A-B. 0.1mm

grooves/fine ridges ("stridulating organs" (Stephensen 1938)), and three/four anterior spines (Fig. 2c). Variability in the position of the two smaller teeth was observed among specimens (see Fig. 2c-d). Uropod 1 and 2 biramous; uropod 1 longer than uropod 2 and 3; urosomite 1 with three spines on antero-lateral surface (Fig. 3a); uropod 3 uniramous, ramus is 3 times peduncle (Fig. 3b); numerous spines on all uropods. Telson small, button-like, with pronounced medial groove.

Morphological characters of specimens recorded in the Sacca di Goro agree with the descriptions of G. japonica provided by Stephensen (1938), and Chapman and Dorman (1975); they are also similar to those observed in French waters by Jourde et al. (2013). Nevertheless, specimens from the Sacca di Goro differed from those described by Chapman and Dorman (1975) by the number of spines on urosomite 1 (2 spines according to these latter authors instead of 3 recorded in our specimens), and from those described by Stephensen (1938) by the number of transverse ridges (stridulating organs) on the gnathopod 1 carpus of males (18-20 transverse ridges according to Stephensen (1938), and 40 or more in specimens).

Overall, in the population from the Sacca di Goro, the number of females was approximately double that of males while the number of juveniles was comparable with that of males. Specimens were collected at all sampling stations except P3. Most records are from station C4 (up to 1998 ind. $\rm m^{-2}$ from a sampling replicate). The average density varied from 24.7 ind. $\rm m^{-2}$ at P4 to 1381.3 ind. $\rm m^{-2}$ at C4 (Table 2).

In the Sacca di Goro the species is found associated with the bivalve Arcuatula senhousia (Benson in Cantor, 1842), the gastropod Ecrobia ventrosa (Montagu, 1803) and the polychaete Streblospio shrubsolii (Buchanan, 1890); the species is found in silty-clay sediments reaching high densities in sediments close to seaweed (Ulvacea and Gracilariacea) beds. In addition to G. japonica, 11 other non-indigenous species were recorded from the present samples: Ruditapes philippinarum (Adams & Reeve, 1850), Anadara inaequivalvis (Bruguière, 1789), A. transversa (Say, 1822), Dyspanopeus sayi (Smith, 1869), Caprella scaura Templeton, 1836, A. senhousia, Desdemona ornata Banse, 1957, Podarkeopsis capensis (Day, 1963), Ficopomatus enigmaticus (Fauvel, 1923). These additional non-indigenous species have been previously recorded in the Adriatic area (Bianchi and Morri 1996; Occhipinti-Ambrogi 2000; Occhipinti-Ambrogi et al. 2011; Bevilacqua et al. 2015).

Remarks

In overall appearance and in the details of the male gnathopod 1, *G. japonica* is most similar to species of *Microdeutopus* (Costa, 1953) and it could be easily mistaken for a member of that genus by the non-specialist. It is, however, distinguishable from most of the Mediterranean aorids as it has uniramous 3rd uropods. The only additional aorid amphipod of Mediterranean Sea with uniramous uropods 3 and close to *G. japonica* is *Unciolella lunata* Chevreux, 1911, recorded from Algeria. The two species differ by: 1) the gnathopod 1, that is carpochelate in the former and subchelate in the latter; 2) the length of the uropod 3 rami which is in the latter almost 1.5

Table 2 Mean density (ind m⁻²) and standard deviation (SD) of *Grandidierella japonica* at the ten sampling sites

Station	P1	P2	P3	P4	P5	C1	C2	C3	C4	C5
Mean density (ind. m ²)	86.3	37	0	24.7	111	49.3	111	468.7	1381.3	160.3
SD	118.9	64.1	0	42.7	128.2	56.5	37	333.7	610.6	56.5

times the length of peduncle; 3) the ecology as the latter has been found on coralline bottom of the circalittoral.

Although in the genus *Grandidierella* further four species have transverse ridges on the carpus of the gnathopods 1, mature males of *G. japonica* can be identified by the three teeth on the carpus of gnathopod 1 (Fig. 2c).

Introduction of *G. japonica* into the Sacca di Goro prior to the 2015 seems unlikely because the macrobenthic community of the Sacca di Goro is periodically investigated by our laboratory (Mistri et al. 2001; Mistri et al. 2002; Mistri et al. 2004; Munari et al. 2006; Munari 2008a; Munari 2008b; Munari et al. 2009; Munari and Mistri 2010; Munari and Mistri 2014; Mistri and Munari 2015).

We exclude previous misidentification because G. japonica presents small but clear differences from Microdeutopus gryllotalpa Costa, 1853, which is the most common aorid amphipod recorded in the Sacca di Goro. These differences are: i) 3rd uropods uniramous in G. japonica and biramous in M. gryllotalpa; ii) different spinulation of uropod 3 rami; iii) ramus of uropod 3 much longer than peduncle in G. japonica whereas rami of uropod 3 subequal, as long as or longer than peduncle in M. gryllotalpa; iv) different position and shape of eyes (detectable at the stereomicroscope), which are oval in G. japonica and round in M. gryllotalpa; v) different position of teeth and further details (e.g. absence of stridulating ridges in M. gryllotalpa) of male gnathopod 1; vi) different shape and spinulation of carpus and propodus of female gnathopod 1 and 2.

Discussion

In Southampton water Grandidierella japonica often occurs with native aorids, particularly Aora gracilis (Bate, 1957) (Smith et al. 1999). Likewise, in the Sacca di Goro it was found together the native aorid Microdeutopus gryllotalpa Costa, 1853, which is a common amphipod living in shallow lagoons along the southern European coasts (Munari and Mistri 2010; Guelorget and Michel 1979), and Leptocheirus pilosus Zaddach, 1844. A strong similarity can be also found with community from the Orwell estuaries (British isles) reached by G. japonica. In fact, Ashelby (Ashelby 2006) reported the presence of G. japonica in communities dominated by the gastropod Peringia ulvae (Pennant, 1777), the polychaete Streblospio shrubsolii, with the presence of the bivalve Ruditapes philippinarum and the amphipods L. pilosus and M. gryllotalpa, and large amounts of seaweeds.

The ecology of this species is relatively well known. *G. japonica* builds U-shaped tubes in muddy sediments in which one male and one female often are found together (Chapman and Dorman 1975). Males have been observed wandering about in tide pools at low tide. The species occurs in the brackish and marine waters, in sandy-muddy sediments of lower intertidal and shallow

subtidal zone, but it can also be present in wild oyster reefs or in seagrass prairies (Chapman and Dorman 1975; Lavesque et al. 2014; Nagata 1960; Ariyama 2007), as well as in algal mats (Ashelby 2006). It is sensitive to high metal concentrations and it is also used in toxicity tests (Nipper et al. 1989). It seems to be sensitive to oil polluted sediments, and it may be favoured by moderate organic enrichment from sewage discharge (Smith et al. 1999).

Specimens of *G. japonica* recorded in the Sacca di Goro were of quite large size (12mm in the native area (Nagata 1960)). Their densities were quite comparable to those found in the brackish waters of France (1300 ind.m⁻² (Lavesque et al. 2014)) and British isles (1500-5800 ind m⁻² (Smith et al. 1999)). As the availability of food such as algae seems to have an important role on its reproduction (Aikins and Kikuchi 2002), we hypothesize that *G. japonica* have found a suitable habitat in the Sacca di Goro. The finding of ovigerous females from samples collected in the Sacca di Goro in summer 2015 seems to support our hypothesis.

Its appearance in the British Isles (Smith et al. 1999; Ashelby 2006) or Hawaii (Coles et al. 1999) was related to maritime transport activities (ballast waters and/or fouling). Conversely, Chapman and Dorman (1975) suggested that G. japonica arrived in America through commercial transplant of oyster spat from Japan. Similarly, oyster spats seem vector of introduction into Mexico (Okolodkov et al. 2007), Marennes-Oléron basin (Jourde et al. 2013) and Arcachon bay (Lavesque et al. 2014). A lack of intermediate records, suggest that a natural spread from the Atlantic coasts to the Adriatic Sea is unlikely. G. japonica is a fouling organism and it could have arrived via shipping to commercial locations close to the Sacca. The nearest commercial ports, namely Venice and Ravenna, are respectively 70 km upstream and 33 km downstream from the entrance to the Sacca di Goro. Moreover, an offshore gas terminal (LNG Adriatic, Porto Levante), receiving cargo ships from Arabian, Atlantic and Northern European coasts, is 50 km upstream from the Sacca. However, this benthic species has no planktonic larval dispersal phase (Ashelby 2006) and therefore can hardly colonize new environments by migration. For this reason, the most likely hypothesis of its introduction in the Sacca di Goro is via oyster farming. In Italy, oysters (Crassostrea gigas (Thunberg, 1793)) are farmed mostly in Apulia, Mar Piccolo di Taranto and Sacca di Goro, with an annual production of about 500 tons (Turolla 2006). However, such a production is not enough to meet the growing national consumption and market demand so that oyster population is supplied with an annual import (about 5000 tons) of non-indigenous oysters mainly from France and Holland (Turolla 2006). Recently oyster farming experiments using juveniles from France

(Arcachon) and England were performed in coastal waters of Veneto and Emilia Romagna, close to the Sacca di Goro (Turolla 2006; Grassia 2014). Moreover, prefattening tests using juveniles from USA, France and England were also performed in northern Adriatic coastal waters and Po delta lagoons (Sacca di Goro and Caleri Lagoon) (Turolla 2014).

Even though the presence of juveniles of G. japonica in the Sacca di Goro may provide indication of successful breeding, it remains to be seen whether their presence is transitory or permanent. Due to its ecological (sensitivity to oil and metal pollution, estuarine and brackish waters specialist) and biological (absence of dispersive planktonic phase) characteristics, we believe unlikely a natural spread of the population outside the Sacca. Conversely, aquaculture seems a more likely vector of spread of G. japonica towards other eutrophic Mediterranean lagoons. Considering that among all the introduction vectors aquaculture is the easiest to control, given its fixed locations and regular procedures (Nunes et al. 2014), in Mediterranean lagoons stringent control measures should be implemented to reduce or prevent further introductions of non-indigenous species.

It is difficult to predict the effect of this species on the benthic community of the Sacca di Goro, where it may interact with many other non-native species. Many species establish in a new habitat with few disruptions, whereas others alter entire ecosystems or put native species at risk of extinction (Molnar et al. 2008). With a high number of non-indigenous species recorded in this area, it is expected that related species will compete for resources also among themselves, and that species that are ecologically related in their native environment will facilitate establishment of each other (Simberloff and Von Holle 1999). Although in the Global Invasive Species Information Network (Global Invasive Species Information Network. http://www.gisin.org. Accessed 21 Oct 2015) *G. japonica* is reported as not invasive, it may

however compete with native species for resources. On the basis of our knowledge, we may hypothesize a possible competition with the native tube builder *M. gryllotalpa*, the most common and abundant amphipod of the study area. Given the frequent negative ecological and economic impacts resultant from invasions of non-indigenous species, a special attention to this species during all benthic monitoring programs must be paid. This would allow to improved evaluations on geographic spread, potential invasiveness in Mediterranean waters, and possible effects on native fauna.

Conclusions

This paper documents the introduction of the non-indigenous amphipod *Grandidierella japonica* Stephensen, 1938 along the Mediterranean coasts, showing the potential susceptibility of areas heavily exploited for aquaculture activities to biological invasions. Indeed, aquaculture seems a more likely vector of spread of *G. japonica* towards other Mediterranean lagoons.

The effects of the introduction of *G. japonica* on the native benthic community of the Sacca di Goro are unpredictable, but we may hypothesize a competition with *Microdeutopus gryllotalpa* Costa, 1853, the most common tube builder amphipod of the area.

The results of this study highlight the importance of monitoring not only the introduction of non-indigenous species, but also their distribution and persistence over time, as well as their possible effects on native fauna. The urgent need of stringent control measures in order to reduce or prevent further introductions of non-indigenous species in the Mediterranean lagoons appears evident.

Methods

The Sacca di Goro is a wide (26 km²) microtidal lagoon located in the southernmost Po Delta area (northern Adriatic Sea), and with a maximum depth of 2.0 m. It receives nutrient-rich freshwater, primarily from the Po



Fig. 4 Map of the study area with location of sampling sites

di Volano. The neighbouring Valle di Gorino (8 km²) is a cul-de-sac of the Sacca di Goro, with a maximum depth of 1.5 m, that receives freshwater from the Po di Goro through a gate. The Sacca di Goro is spatially enclosed by a long natural sandbank and is characterized by limited water circulation. Being an eutrophic ecosystem, it is an ideal environment for shellfish farming. The macrobenthic community of the Sacca di Goro has been thoroughly investigated through numerous research programs that we carried out between 1999 and 2015 (Mistri et al. 2001; Mistri et al. 2002; Mistri et al. 2004; Munari et al. 2006; Munari 2008a; Munari 2008b; Munari et al. 2009; Munari and Mistri 2010; Munari and Mistri 2014; Mistri and Munari 2015).

Previous studies showed that the soft-bottom benthic community of this lagoon is characterized by a relatively scarce complement of taxa well-adapted to perturbations, being the majority of species opportunist and tolerant detritivorous ones (Munari and Mistri 2010; Munari and Mistri 2014).

This study was carried out as part of the LIFE13 NAT/IT/000115 project (acronym: AGREE), whose overall objective is the study and the long-term conservation of Natura 2000 habitats and species in the Sacca di Goro.

Ten soft bottom stations (Fig. 4) have been sampled in January 2015, and at each station three replicates were collected. Water parameters (temperature, salinity and dissolved oxygen) were measured *in situ* with a probe, whereas sedimentary organic content was assessed in the laboratory through combustion and incineration. Sediments were taken with a Van Veen grab (area: 0.027 m²; volume: 4 l); the contents of the grab were sieved through a 0.5 mm sieve. Material retained on the sieve was fixed in 8 % buffered formalin. In the laboratory, macroinvertebrates were stained with Rose Bengal to facilitate sorting and identified at the species level whenever possible.

We based our identification at genus level on keys in Ruffo (Ruffo 1982) and Barnard and Karaman (1991).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CM noticed the specimens in the samples and partecipated in the identification of *Grandidierella japonica*, measured specimens, drew and prepared figures and drafted the manuscript. MM partecipated in the drafting of the manuscript and amended it for some important intellectual contents. NB made a substantial contribution to the identification of the species and counted specimens. All authors read and approved the final manuscript.

Authors' information

CM is a fixed term Researcher in ecology, who studies diversity, taxonomy and ecology of macrobenthic fauna from lagoon and marine habitats. Scientific interests of CM focus on the use macrobenthic community as indicators of ecological quality status, according to the 2000/60/EC; cryptic diversity of macrobenthic fauna in the Antarctic sea, vulnerability of coastal habitats to the introduction of non-indigenous species; response of the macrobenthic community to natural and anthropogenic impacts; CM was a

Ph.D. student in the laboratory of MM studying Macrobenthic biodiversity and the role of allochthonous species in aquatic transition environments. NB is a research fellow in the laboratory of MM, acquiring experience in the taxonomic identification of macrobenthic invertebrates from marine and lagoon environments.

MM is a Professor of Ecology, who studies: functionality of coastal ecosystems, natural and anthropogenic impacts on biological communities in marine and lagoon environments, resources management focusing on bivalve of commercial interests.

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