Marine Biodiversity Records

MARINE RECORD Open Access

Arrival of the invasive amphipod *Grandidierella japonica* to the Mediterranean Sea

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Abstract

Background: In the marine environment, shipping is globally acknowledged as the major vector of introduction of organisms outside their native range. We surveyed harbours and marinas in the Western Mediterranean Sea for occurrence of non-indigenous species.

Results: More than 200 specimens of the Japanese amphipod *Grandidierella japonica* were collected in 2013 from the docks of the marina of Viareggio (Tuscany, Tyrrhenian Sea). This is the first record of this species for the Mediterranean Sea.

Conclusions: *G. japonica* was previously introduced elsewhere by oyster trade and shipping; in the case of Viareggio, where no aquaculture facilities or international shipping occur, recreational boating is the only likely vector of introduction. In Europe, *G. japonica* is currently confined by a few localities, mainly estuaries, enclosed bays and brackish water areas, but its successful history of invasion in the Pacific coasts of North America suggests that a further spread can be expected in the Mediterranean Sea as well.

Keywords: Aorid amphipod, Crustacean, Introduced species, Fouling, Shipping, Recreational boating, Mediterranean Sea, Marina, Brackish water, *Ficopomatus enigmaticus*

Background

While analysing crustaceans collected in July 2013 in a marina in Tuscany (Italy), we noticed an unusual aorid amphipod, with a male gnathopod 1 presenting 18–20 transverse fine ridges on the anterior edge of carpus. The enlarged basis of male gnathopod 1 and the uniramous uropod 3 lead to the identification of the genus *Grandidierella*, which is not native to the Mediterranean Sea (Lo Brutto et al. 2016). Although *Grandidierella* is a rather speciose genus, with 43 currently accepted species (Lowry 2016), only 5 of them have the "stridulating ridges" (Stephensen 1938) on male gnathopod 1 carpus and, within them, only one has 3 teeth (one main tooth and two accessory teeth) at the posterodistal corner of the carpus: *Grandidierella japonica* Stephensen 1938.

This species was originally described from Japan. It is now widely distributed in the entire Pacific Ocean, also supported by anthropogenic dispersal (Nagata 1960; 1965; Chapman and Dorman 1975; Kudrjaschov and Tzvetkova 1975; Myers 1981; Hirayama 1984; Greenstein and Tiefenthaler 1997; Kim et al. 2005). More recently, it appeared in Europe: firstly in Britain (Smith et al. 1999; Ashelby 2006), and recently it was also reported from the Atlantic coasts of France (Jourde et al. 2013; Lavesque et al. 2014) and Sweden (Berggren 2015).

This article reports details on the first finding of this species in the Mediterranean Sea, an assessment of its likely vectors and pathways of introduction, and an analysis of the variations among the available descriptions of *G. japonica* from both the native and introduced ranges.

Results

Systematics

Order AMPHIPODA Latreille, 1816
Suborder SENTICAUDATA Lowry & Myers, 2013
Family AORIDAE Stebbing, 1899
Genus *Grandidierella* Coutière, 1904

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Grandidierella japonica Stephensen 1938
(Fig. 2)

Material examined

Areas A, B and C (marina of Viareggio, Italy; Fig. 1b; Table 1): *Grandidierella japonica* (Fig. 2), 245 specimens, maximum size 7.5 mm (ovigerous female) and 6.2 mm (male).

Diagnosis

The collected specimens correspond to the original description of *G. japonica* provided by Stephensen (1938). Diagnostic characters are: uniramous uropod 3 (Fig. 2), a short medio-ventral spine on peraeon segment 1, ovoidal basis of male gnathopod 1 (Fig. 3a, b), two accessory teeth on gnathopod 1 carpus (Fig. 3b), presence of stridulating ridges on the male carpus anterior margin (Fig. 3a, b).

Comparison

Descriptions of *G. japonica* were provided by several authors after records from various localities, sometimes highlighting variations, mainly related to male gnathopod 1. The short medio-ventral spine on peraeon segment 1, described by Stephensen (1938) and clearly visible in our specimens, was also drawn by Chapman and Dorman (1975), Ariyama (1996) and Smith et al. (1999), but was absent in Nagata (1960) specimens. Nagata (1960) also noticed that the basis of male gnathopod 1 is «*somewhat oblong oval rather than that in Stephensen's specimens*». The basis of male gnathopod 1 drawn in Stephensen (1938), Chapman and Dorman (1975), and photographed by Berggren (2015), is ovoidal, as well as in Viareggio specimens. Conversely, male gnathopods 1 drawn by Nagata (1965), Hirayama (1984) and Smith et al. (1999) are closer to Nagata (1960) description.

The two accessory teeth on gnathopod 1 carpus were indicated as a prominent and diagnostic character (Stephensen 1938; Chapman and Dorman 1975; Ariyama 1996). However, Myers (1981) described rather small male specimens from Australia lacking one of them. In our samples, the median tooth on the inner surface of carpus near posterior margin was always visible, but the small accessory tooth on inner surface of anterior side of the strong tooth was evident only in larger males (Fig. 3b). Also Morino and Dai (1990) observed that a small male had only a rudimentary process, whilst the accessory tooth was visible in a large male. Therefore, it is possible that this character varies with maturity. Number of stridulating ridges on the male carpus anterior

Fig. 1 Study area: (**a**) Location of Viareggio (Tuscany, Italy); (**b**) map of the marina, indicating the three sampling areas: A– Darsena della Madonnina, B– Burlamacca Canal, C– Nuova Darsena. Scale bar: 200 m

Table 1 GPS coordinates, temperature and salinity values recorded in the marina of Viareggio

Replicates	Longitude (E)	Latitude (N)	Temperature (°C)	Salinity (PSU)
A1	10.24355	43.86370		
A2	10.24348	43.86375	25	20.9
A3	10.2439	43.86337		
B1	10.24389	43.86481		
B2	10.24332	43.86423	26	15.8
B3	10.24206	43.86396		
C1	10.24173	43.85706		
C2	10.24165	43.85663	25	34.2
C3	10.24215	43.85587		

margin is also variable, from 18–20 ridges in Stephensen (1938), to 30 or more ridges counted from the drawings in Chapman and Dorman (1975) and Hirayama (1984), from a photo in Lavesque et al. (2014) and in our specimens (Fig. 3a, b), up to 40 ridges in Ariyama (1996).

Finally, populations described from different localities present a rather wide size range, from 4–5 mm (Myers 1981; Hirayama 1984; Ashelby 2006) to 9–12 mm (Stephensen 1938; Nagata 1960; 1965; Ariyama 1996), up to 22 mm (Chapman and Dorman 1975). The maximum observed sizes of our specimens were 7.5 mm (ovigerous female), and 6.2 mm (male).

Remarks

Specimens of *G. japonica* were especially abundant in the area A and B, where it was present in all the replicates, while just a single replicate was recorded in the area C. Among the total specimens collected, 74 specimens (out of which 21 males, 38 ovigerous females and 15 juveniles) were recorded in area A; 170 specimens (out of which 40 males, 96 ovigerous females and 34 juveniles) in area B; and 1 ovigerous female in area C.

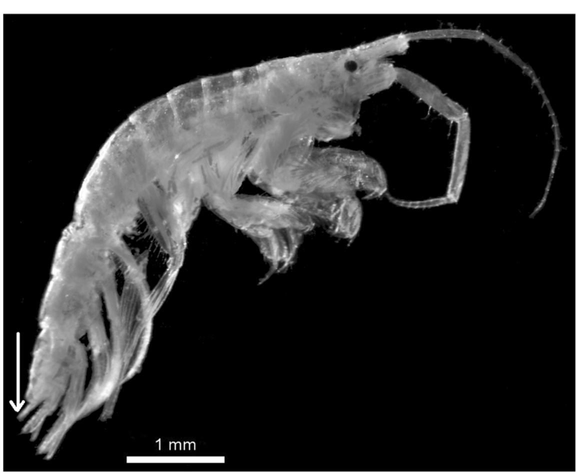

Fig. 2 *Grandidierella japonica* Stephensen 1938 from Viareggio (Italy, Tyrrhenian Sea): male specimen (5.4 mm). The arrow indicates uniramous uropod 3, a character of the genus *Grandidierella* which differs from other Mediterranean aorid amphipods

Salinity and temperature recorded in the three areas are shown in Table 1; areas A and B exhibited brackish water conditions, due to the freshwater inflow of an artificial canal, whereas salinity in area C approached fully marine conditions.

The benthic community characterising areas A and B were dominated by the non-indigenous polychaete *Ficopomatus enigmaticus* (Fauvel 1923), which formed conspicuous reefs along the docks. Flourishing colonies of another non-indigenous invertebrate, the bryozoan *Amathia verticillata* (Delle Chiaje 1822), characterised area C.

Discussion

Pathway of introduction

Previous records of *Grandidierella japonica* outside its native range have been explained from importation with the Japanese oyster *Crassostrea gigas* (Thunberg 1793; Chapman and Dorman 1975; Jourde et al. 2013; Lavesque et al. 2014), transport in ballast water, or from fouling associated with international shipping (Carlton and Eldredge 2009). The transport due to small recreational boats that travel between estuaries was defined "unlikely" by Pilgrim et al. (2013).

International shipping and oyster farming, however, do not take place in the marina of Viareggio, which is devoted to pleasure and fishing boats. Recreational boating has recently been regarded as an often overlooked vector of introduction and secondary spread of marine non-indigenous species (Clarke Murray et al. 2011; Davidson et al. 2010; Ros et al. 2013; Zabin et al. 2014). Sessile species (macroalgae, serpulids, barnacles, tunicates, bryozoans, and others) that attach on the hulls of sailing boats or motorboats can provide refuge and substratum for associated motile invertebrates, such as nudibranchs, peracarid crustaceans, pycnogonids (Brine et al. 2013; Mineur et al. 2012; Marchini et al. 2015a), thus facilitating their transport and accelerating their spread.

Several amphipod and isopod species are likely to be transported in the fouling by recreational boats, e.g. *Caprella mutica* Schurin 1935, *Caprella scaura* Templeton 1836, *Jassa marmorata* Holmes 1905, *Paracaprella pusilla* Mayer 1890, *Paranthura japonica* Richardson 1909, *Paracerceis sculpta* (Holmes 1904), as suggested by their frequent occurrence in marinas (Frey et al. 2009; Minchin et al. 2012; Ros et al. 2013; Ashton et al. 2014; McCollin and Brown 2014; Zabin et al. 2014; Marchini et al. 2015a; Ramalhosa and Canning-Clode 2015). *G. japonica* therefore adds to the list of peracarid crustaceans being spread by pleasure boats.

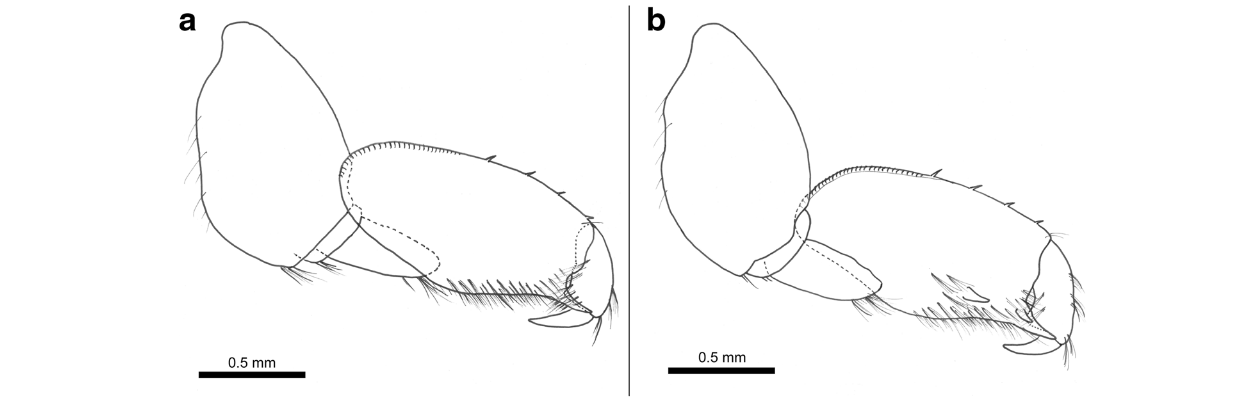

Fig. 3 *Grandidierella japonica* Stephensen 1938 from Viareggio (Italy, Tyrrhenian Sea). **a** *Right* male gnathopod 1, outer side; **b** *left* male gnathopod 1, inner side

While the vector of introduction is clarified, the source origin of the *G. japonica* population observed in Viareggio is still unknown. Whether a direct introduction event from the native region, or a secondary introduction from another European (or even Mediterranean) site of introduction, any hypotheses cannot be rejected or retained with certainty.

The analysis of samples collected in 2013–2015 during surveys of several French and Italian lagoon, harbour and marina sites of the Western Mediterranean Sea (localities: La Grande Motte, Genoa, Santa Margherita Ligure, La Spezia, Lerici, Leghorn, Viareggio, Orbetello, Olbia, Porto Torres, Castelsardo, Porto Rotondo; Ferrario et al., in preparation) allowed us to exclude the occurrence of *G. japonica* in localities other than Viareggio. A survey conducted by our research group in 2012 in the "sink and source" area of introductions in Italy, i.e. the Lagoon of Venice (Marchini et al. 2015b) was also negative as for the presence of *G. japonica*.

Salinity preference might partially explain this result: Viareggio was the only marina we examined that displayed brackish conditions, and within Viareggio, *G. japonica* only occurred in the most brackish areas (A and B), being substituted by another aorid amphipod, *Microdeutopus gryllotalpa* Costa 1853, in area C where salinity values were higher.

Another relevant characteristic of areas A and B in Viareggio marina is the coverage of the dock walls by dense reefs of the tubeworm *Ficopomatus enigmaticus*, a species not observed elsewhere in our survey. *F. enigmaticus* is an ecosystem engineer that creates novel, large, three-dimensional structures on the hulls of boats as well as in marinas, thus creating a suitable habitat for associated non-indigenous invertebrates which are favoured in their transfer and settlement (Heiman et al. 2008; Mineur et al. 2012; Zabin et al. 2014). An association between *F. enigmaticus* and *G. japonica* has already been observed in California (Elkhorn Slough estuary; Wasson et al. 2001).

At present, Viareggio marina therefore represents the first and only Mediterranean locality where *G. japonica* might be established. However, its successful history of invasion in North America (46 invaded estuaries along the Pacific coast; Pilgrim et al. 2013) suggests that a further spread can be expected in the future, particularly in brackish water areas such as lagoons and estuaries colonised by *F. enigmaticus* (which is known from 15 lagoons in Italy; Basset et al. 2006).

Distribution and ecology

The current distribution of *G. japonica* indicates it is a eurytopic species: in the Pacific Ocean, it ranges from Russia (Kudrjaschov and Tzvetkova 1975) to Canada (Pilgrim et al. 2013), California (Chapman and Dorman 1975; Greenstein and Tiefenthaler 1997), Australia (Myers 1981) and Hawaii (Muir 1997). Another record from Mexico (Okolodkov et al. 2007), is awaiting confirmation (Rodríguez-Almaraz and García-Madrigal 2014). In Europe, *G. japonica* ranges from the Swedish coasts (Berggren 2015) to Britain (Ashelby 2006), the Celtic-Biscay Shelf (Smith et al. 1999; Jourde et al. 2013; Lavesque et al. 2014) and the Mediterranean Sea (present study). It is able to colonise a large variety of habitats such as muddy and sandy bottoms in the lower intertidal zone (Ariyama 1996; Chapman and Dorman 1975; Smith et al. 1999), seagrass beds (*Zostera* spp., *Ruppia* spp.; Nagata 1960; Chapman and Dorman 1975; Lavesque et al. 2014), oyster beds (Chapman and Dorman 1975), *F. enigmaticus* reefs (Wasson et al. 2001; present study), on different algal species, on experimental artificial substrates (Aikins and Kikuchi 2001), brackish waters, estuaries and lagoons (Stephensen 1938; Ashelby 2006; Lavesque et al. 2014), harbours and marinas (Chapman and Dorman 1975; Muir 1997; present study), and near sewage treatment plants (Chapman and Dorman 1975). It was one of the animals able to tolerate the highest variations in salinity during experiments of ballast tanks treatment with concentrated sodium chloride brine solutions: *G. japonica* had 65 % survivorship after 48 h at 45 ppt sodium chloride solution (Santagata et al. 2009). Such widespreadness and generalism lead Pilgrim et al. (2013) to suspect the existence of cryptic diversity and a complex introduction history of *G. japonica*. They conducted molecular analyses on populations of *G. japonica* from several estuaries along the Pacific North American coasts and discovered the co-occurrence of two different lineages: a clade A, that has spread from San Francisco Bay northward, and a clade B, only found from San Francisco Bay southward. This result raises questions about the taxonomic identity of the two evolutionarily divergent clades introduced to North America. Unfortunately, Pilgrim et al. (2013) did not provide a taxonomic description of the two clades, suggesting that no clear morphological differences were observable.

However, a few morphological differences appear in the various descriptions of *G. japonica* (see the Results section). Such minute but constant morphological differences occurring within putative "cosmopolitan" amphipods have revealed the existence of species complexes (e.g. Krapp-Schickel and De Broyer 2014; Krapp-Schickel 2015). As Pilgrim et al. (2013) pointed out, a taxonomic revision based on comparative material and combined with ecological observations and genetic analysis of *G. japonica* populations are necessary to verify the existence of constant morphological differences between clades, the relevance of the observed variability in ecological tolerance, and to have more insights on the nature, source and pathways of this invasion.

Conclusions

The recent appearance of *Grandiderella japonica* in the Mediterranean Sea confirms the ability of this amphipod to spread and colonise a large range of coastal habitats, therefore a further spread can be expected in the Mediterranean Sea in the next future.

Methods

The marina of Viareggio is located in the north of Tuscany (Tyrrhenian Sea, Italy; Fig. 1a). It hosts six docks, for a total number of 2000 berths. We collected benthic invertebrates from three distinct areas in the marina (Fig. 1b): the dock called "Darsena della Madonnina", a mooring area for resident leisure craft (area A); the canal harbour called "Burlamacca", a mooring area for transient leisure craft (area B); the dock called "Nuova Darsena", a fishing boats transit area (area C). Three replicates were collected from each area. The sampling was carried out on June 27, 2013 by scraping the artificial hard substrate, i.e. the main dock walls, with a hand-held rigid net (25 × 20 cm), over an area of approximately 0.23 m^2. Samples were subsequently fixed in formalin (5 %) and sorted under a stereoscopic microscope. Specimens of *Grandidierella japonica* were separated from the community, examined under a dissecting microscope, and photographed with an AXIO CAM ERc5s camera, using the Software ZEISS- AXIO VISION 4 for total length measures.

Acknowledgments
The authors gratefully thank Alan Myers for suggestions and support in the initial phase of identification process, and one anonymous reviewer for comments which improved the paper.

Funding
JF was supported by a PhD grant from the University of Pavia.

Availability of data and materials
Specimens of *Grandidierella japonica* are deposited in the Natural History Museum of the University of Pavia, Italy (code: MSNPV-2016/05). The record has also been registered in the online information system on Aquatic Non-Indigenous and Cryptogenic Species AquaNIS (AquaNIS Editorial Board, 2015. Information system on Aquatic Non-Indigenous and Cryptogenic Species. World Wide Web electronic publication. www.corpi.ku.lt/databases/aquanis. Version 2.36+. Accessed 2016-05-12).

Authors' contributions
AM conceived the study, partecipated in the identification of *Grandidierella japonica* and wrote the manuscript. JF planned and carried out field activities and contributed to writing, drawings and figures. EN participated in fieldwork activities, counted specimens, contributed to taxonomic identification and drawings. All authors read and approved the final manuscript.

Authors' information
AM and JF are postdoc researchers at University of Pavia; EN is an undergraduate working on amphipods in marinas.

Competing interests
The authors declare that they have no competing interests.

Received: 12 May 2016 Accepted: 3 June 2016
Published online: 18 July 2016

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