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RESEARCH ARTICLE

DNA barcode, taxonomic and ecological notes for the identification of the invasive amphipod *Monocorophium acherusicum* (Costa, 1851)

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ABSTRACT
We report the occurrence of the invasive amphipod *Monocorophium acherusicum* in Mar del Plata port, Argentina, using morphological and molecular analysis and estimated mean abundance to detect monthly variations of this population. We compared *M. acherusicum* morphology with that of *M. insidiosum* and *Crassicorophium bonelli*, the two most similar locally occurring species, to establish the diagnostic characteristics for the correct identification of them; moreover, we provide some taxonomic notes about others corophiids regionally distributed. *M. acherusicum* were collected in a subtidal biofouling community and its mean abundance was maximum in summer. In the molecular analysis, the maximum-likelihood tree showed that specimens from Mar del Plata were clustered with *M. acherusicum* specimens from GenBank and Boldsystems. We support and confirm the species identity in Mar del Plata port using DNA barcoding and with taxonomic methods.

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Introduction

Biological invasions are a common phenomenon in marine ecosystems and their frequencies have increased in recent time, particularly in environments associated with international trade ports (eg Cohen and Carlton 1995; Reise et al. 1998; Ruiz et al. 2000; Orensanz et al. 2002; Hewitt et al. 2004; Kerckhof et al. 2007; Schwindt et al. 2014). The main pathway for the introduction of marine invasive species to ports around the world has been shipping through biofouling on the hull of the vessels, ballast water and the sea chest (ie intake chambers in vessel hulls) (Gollasch 2002; Godwin 2003; Hewitt et al. 2004; Coutts and Dodgshun 2007). Invasive species may homogenise the global biodiversity (Olden and Poff 2004), favour the introduction of parasites and diseases (Kuris and Culver 1999), compete with native organisms for food and space, and in turn may act as easily accessible food items for a range of predatory fish and invertebrates (Griffiths et al. 2009).

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One of the major consequences of coastal development and urbanisation is the addition of an artificial hard-bottom structure to coastal areas, including seawalls, dykes, breakwaters, jetties and offshore platforms (Airoldi et al. 2009). Port areas concentrate a variety of such structures that support many different organisms (Glasby 1999; Connell 2001), and it is known that artificial and natural habitats are not equally colonised by fouling species (Connell 2001). Artificial structures often provide habitat for exotic species (Floerl and Inglis 2003) and, consequently, ports may act as recipients and dispersal areas for these organisms. Monitoring these areas constitutes an early detection mechanism of these species.

Amphipods are peracarid crustaceans widely represented in coastal and shelf communities throughout the oceans, being an important link between benthic and demersal food webs (De Broyer and Jażdżewski 1996; Dauby et al. 2003). Among them, corophiids (Corophiidae) are tube-dwelling amphipods that inhabit soft sediments and hard surfaces. As part of biofouling they are easily transported great distances, either synanthropically or naturally, via their settlement on ship bottoms or drifting debris, respectively (Thiel and Gutow 2005; Noël 2011). Consequently, a number of species are now virtually cosmopolitan, at least within their range of environmental tolerance, and it is often difficult to determine their original distributions. An additional problem affecting the accuracy of the known distributions for these taxa lies in the fact that they are superficially quite similar to one another and many misidentifications exist in the literature (LeCroy 2004). The invasion of exotic amphipods is considered an important threat to biodiversity because the explosive growth in their population size may affect community composition and ecosystem processes (Conlan 1994). An accurate identification of invasive species is essential to determine the potential effects on local populations. In this context, *Monocorophium acherusicum* (Costa, 1951) is considered a global invasive species due to their wide distribution around the world (Griffiths et al. 2009; Ruiz et al. 2011). This species is most probably originated in the eastern North Atlantic and was introduced, by shipping and other means, to all protected marine coasts between 50°N and 50°S latitude (LeCroy 2004; Chapman 2007). It is a dorsoventrally flattened and yellowish-brown species that occurs subtidal on sediments or where silt and detritus accumulate among biofouling organisms such as algae, ascidians and bryozoans, and man-made installations (eg wharf pylons, rafts and buoys). It feeds by grazing on bacteria, on sediment particles, or on organic matter suspended in the water column. It builds conspicuous, fragile U-shaped tubes of silk, mud and sand particles, and can reach high abundances and tolerate a wide range of salinities (Inglis et al. 2006).

In Argentina, several species of corophiids have been recorded (López Gappa et al. 2006), including *M. acherusicum*, *M. insidiosum* (Crawford 1937) and *Crassicorophium bonellii* (Milne Edwards, 1830). The superficial morphological similarities of these species (see Crawford 1937; Chapman 2007) made the identification of specimens difficult. *M. acherusicum* was recorded as an exotic species in various ports of Argentina (Albano et al. 2013; Schwindt et al. 2014). However, none of these studies has focused on both morphological and molecular analyses to confirm the presence of this invasive species. Furthermore, the identification difficulties mentioned have led to an erroneous report of *M. acherusicum* as *M. insidiosum* (see Albano and Obenat 2009). In recent years, the use of molecular taxonomy has proved to be a powerful tool for solving the misidentification of morphologically similar species (eg Bucklin et al. 2007; Hou et al. 2007; Bilgin et al. 2015; Rumbold et al. 2015a). It was also has been used to document species introductions (Bourlat et al. 2013).

The population dynamics of amphipods *M. acherusicum* and *Ericthonius punctatus* from Mar del Plata harbour has been done by Rumbold et al. (2016). They found that both species showed a seasonal pattern characterised by high densities in warmer months, related to the highest reproductive activity and the increase of recruitment in summer and early autumn, as well as lower densities in the cold season.

As part of an ongoing project for monitoring and identification of native and exotic benthic invertebrates within the Mar del Plata port (Argentina), using DNA barcoding, in this paper we report the occurrence of the invasive amphipod *M. acherusicum* using both morphological and molecular analysis. Also, we estimated mean abundance in order to detect monthly variations of this population. Finally, we compared *M. acherusicum*, *M. insidiosum* and *C. bonellii*, in order to highlight the taxonomic characters that serve as tools for the correct identification of these species, and also we provide some taxonomic notes about other corophiids distributed regionally.

Material and methods

Study area

Specimens of *Monocorophium acherusicum* were collected from marinas of the Club Naútico, Mar del Plata port, 38°02′29″S, 57°32′16″W, Argentina, SW Atlantic (Figure 1). Mar del Plata is a seaport where different products are exported overseas and touristic activities are developed. The port is a semi-enclosed area, protected by two artificial breakwaters (North and South) made of orthoquartzite blocks that delimit an approximately 300-metre wide mouth. The mean water depth is 5 m, ranging between 3 and 12 m. A navigational channel is maintained to a depth of 10 m (Rivero et al. 2005). Mean monthly water temperature ranges from 9.3°C in July (austral winter) to 20.9°C in February (austral summer), (data obtained from Argentine Oceanographic Data Center, CEADO). In the inner sector of the port, silty-clay sediments with higher levels of organic matter and phytopigments, are dominated by opportunistic nematodes and *Capitella* sp., usually associated worldwide to polluted habitats (Albano et al. 2013).

Sampling

Field sampling and laboratory procedures

In order to determine the mean abundance of *M. acherusicum*, from April 2007 to March 2008, the macrofouling community living on wooden piles on the inner side of the marinas was extracted using SCUBA diving at a depth of 3–4 m. Each month, six independent replicates ($N = 6$) were collected at random using an epibenthic net (mesh 0.5 mm) galvanised wire frame 25 × 25 cm ($0.0625\ m^2$) and scraping with metal spatula, from different vertical wooden piles. Samples were fixed in 96% alcohol. In the laboratory, samples were sieved through a 0.35 mm mesh and organisms were sorted and counted using a stereomicroscope.

Morphological and genetic identification

The material used for taxonomic identifications were collected among the biofouling community of the marinas using spatula, during April, July and November 2014 and February

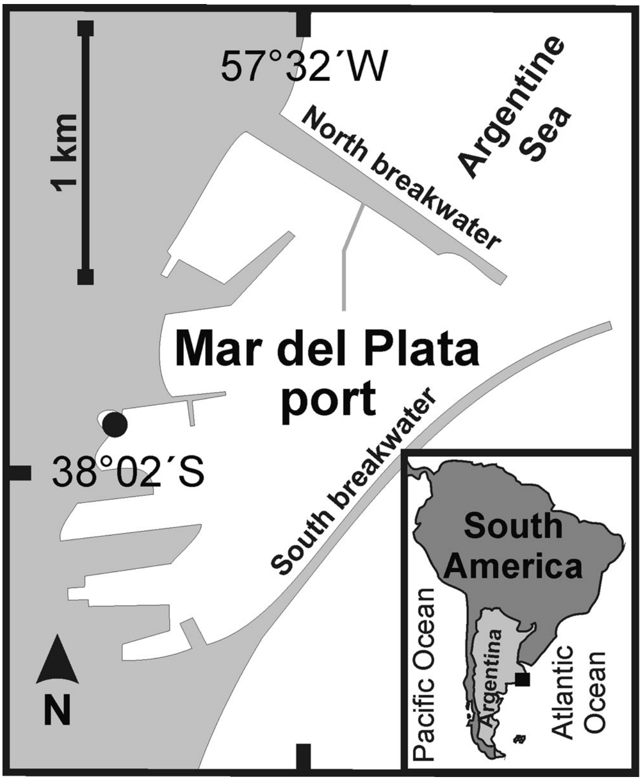

Figure 1. Map of the Mar del Plata port, Argentina, showing the sampling site (●).

2015. Amphipods of genus *Monocorophium* Bousfield and Hoover, 1997 were separated from the extracted material using a stereoscopic microscope and identified with taxonomic guides (LeCroy 2004; Chapman 2007). Adult specimens of *M. acherusicum* were measured and dissected in order to determine morphologically the sexes. To confirm the species identification through genetic analysis, a tissue sample was obtained from three adult specimens (1 male, 1 non-ovigerous and 1 ovigerous female) collected on 21st November, from which a partial fragment of the barcode gene cytochrome c oxidase I was sequenced. DNA extraction, PCR amplification, PCR product purification and sequencing were conducted following the protocols of the Canadian Centre for DNA Barcoding (CCDB protocols http://ccdb.ca/docs/; see also Ivanova et al. 2006, using the universal primers HC02198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′), and LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTGG-3′) (Folmer et al. 1994).

Statistical and molecular analysis

To assess whether there were differences in monthly variation in mean abundances (no. of individuals per m^2) in the population, of adult males and females, a univariate analysis were performed. The null hypothesis of no difference in the monthly average abundances of *M. acherusicum* was assessed by one-way analysis of variance (ANOVA; eg Underwood 1997) considering ‘Month’ as the fixed factor. Data were previously log-transformed to carry out the statistical analysis. Following Underwood (1997), *post-hoc* comparisons were performed with Student Newman Keuls (SNK) tests. ANOVA was performed using SigmaPlot 11.0.

To carry out the molecular identification of specimens, the public COI sequence obtained from Barcode of Life (BOLD, http://www.barcodelife.org) were used to make the BIN assignment (barcode index number) and were compared against GenBank (http://www.ncbi.nlm.nih.gov/GenBank) using the BLAST algorithm. BLAST provides a statistical value for each sequence, called the E-value, which describes the number of hits one can 'expect' to see by chance when searching a database. E-values closer to zero indicate a significant match. A phylogenetic analysis based on the construction of a maximum-likelihood tree was conducted to validate the identity of our material and the *Monocorophium* spp. from GenBank. For this phylogenetic analysis, additional COI sequences of *Monocorophium* spp. were retrieved from GenBank and BOLD (for species name and accession numbers, see Figure 5). The COI sequence of the isopod *Sphaeroma serratum* (Fabricius, 1787) was used as outgroup. The COI sequences were aligned using BioEdit v.7.08.0 software (Hall 1999) through the Clustal W method (Thompson et al. 1994) and were verified by sight. A phylogenetic tree was inferred in MEGA v6 (Tamura et al. 2013); http://www.megasoftware.net/) and was evaluated with a bootstrap test with 1000 replications.

Results

Systematics

Order Amphipoda Latreille, 1816
Suborder Senticaudata Lowry and Myers, 2013
Family Corophiidae Leach, 1814
Subfamily Corophiinae Leach, 1814
Tribe Corophiini Leach, 1814
Monocorophium acherusicum (Costa 1853)
(Figure 2)

Examined material

Club Náutico de Mar del Plata; among biofouling community, <1 m deep; April 2014, 8 males and 10 ovigerous females; July 2014, 1 male; November 2014, 1 male, February 2015, 1 male; specimens collected by M. J. Albano.

Diagnosis

Male of *M. acherusicum* (Figure 2A–F) has a minute, deeply recessed rostrum; antenna 1, peduncle articles 1 and 2 with several long distal setae on dorsomedial margin (also present in female), peduncle article 1 without robust setae on dorsomedial margin and ventromedial margin without process (may have irregular crenulations in large male); antenna 2, enlarged in adults, peduncle articles sparsely setose, peduncle article 4 without ventromedial processes, ventrolateral distal angle with 1 large acute process, article 5 with proximal small process on ventromedial margin and broad distal process;

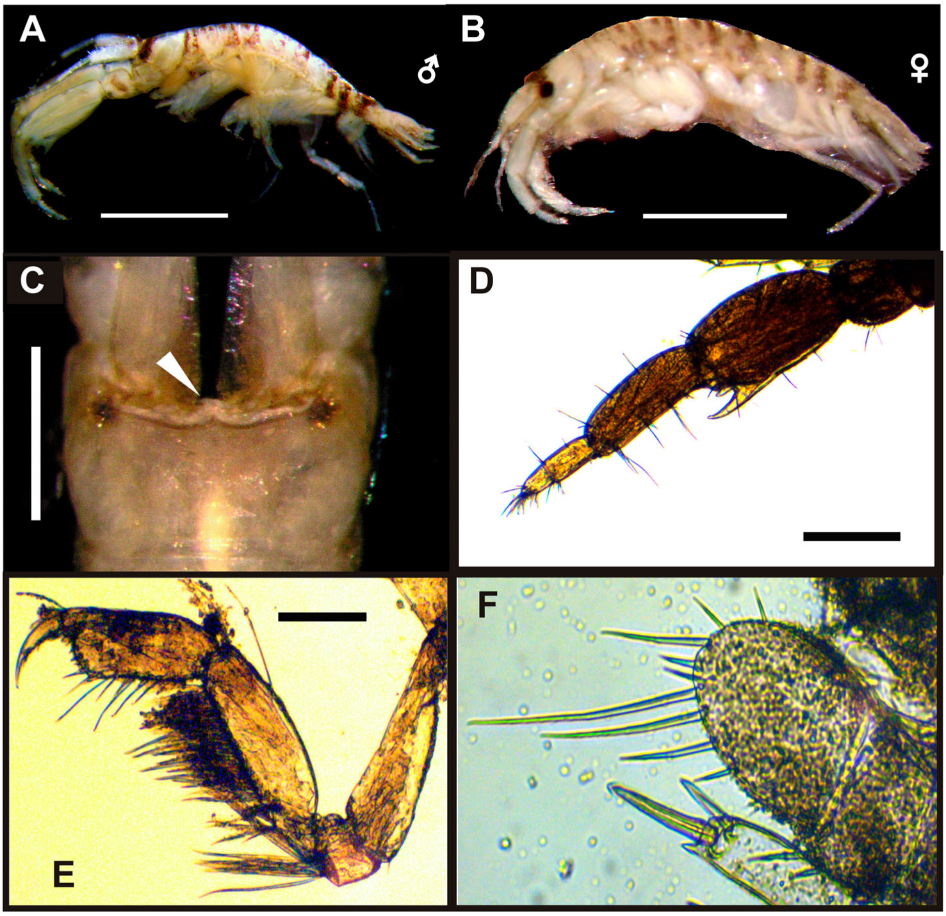

Figure 2. *Monocorophium acherusicum* from Mar del Plata port. **A**–**B**, lateral view of adult male and female; **C**, dorsal view of head of male; **D**, male antenna 2; **E**, male gnatophod 1; **F**, uropod 3. Scale bars: **A**–**B**, **C** and **F**, 0.05 mm; **D**, 0.5 mm; **E**, 0.1 mm.

gnathopod 1, dactyl extending well beyond palmar angle; gnathopod 2, dactyl with 2 teeth on posterior margin; pereopod 3 and 4, anterior margin of basis lacking long setae; pereopod 7 not elongate; urosomites fused, with distinct lateral notch; uropod 1, peduncle with 2–5 robust setae on medial margin, and 6–7 on outer margin, rami short; uropod 2, outer margin of inner ramus with robust setae; uropod 3, ramus sub circular, approximately as long as wide. Female, antenna 1, peduncle article 1 with several robust setae on dorsomedial and ventral margin; antenna 2 peduncle articles 3–5 with several robust setae on ventral margin, article 5 not distally produce, with several long simple setae. See Bousfield (1973); Lincoln (1979); Bousfield and Hoover (1997) and LeCroy (2004).

Distribution

M. acherusicum is virtually cosmopolitan in temperate and tropical waters (LeCroy 2004). It was first described from Italy (Type locality: lake Fusaro, near Naples) by Costa (1851). Bousfield and Hoover (1997) suggested that it most probably originated in the eastern North

Table 1. First records of *Monocorophium acherusicum* according to country or region, site where it was found and the status.

Country or Region	Site	First record	Status	References
North America (Atlantic coast)	Unspecified	1818	N	Say (1818) (probably described as *Podocerus cylindricus*, Shoemaker (1934))
Europe / North Africa (Mediterranean sea)	Unspecified	1851	NIS	Costa (1853); Della Valle (1893); Stebbing (1906)
New Zealand	Harbour	1880	NIS	Chilton (1921) (as *C. crassicorne*)
North Africa (Atlantic coast)	Unspecified	1892	C	Chevreux (1911, 1935)
United States (Pacific coast)	Harbour	1905	NIS	Cohen and Carlton (1995)
China	Unspecified	1906	NIS	Stebbing (1906)
Red Sea	Unspecified	1909	NIS	Walker (1909)
South Africa	Harbour	1915	NIS	Barnard (1916)
Australia	Unspecified	1921	NIS	Hayes et al. (2005)
Europe (Atlantic coast)	Unspecified	1928	C	Schellenberg (1928); Crawford (1937)
Eastern Africa (Tanzania)	Unespecifiesd	1928	NIS	Schellenberg (1928)
Brazil	Unspecified	1934	NIS	Shoemaker (1934)
Hawaiian Is.	Unspecified	1947	NIS	Shoemaker (1947)
Japan	Artificial rocks outcrops	1955	NIS	Irie (1956)
Argentina	Unspecified	1969	NIS	USNM #127701
India	Harbour	1969	NIS	Sivaprakasam (1970)
Chile	Aquaculture facility	2009	NIS	Schultheiss (2009)

NIS: non-indigenous species; N: native; C: cryptogenic.

Atlantic: France, Belgium and England. It has been recorded in Argentina (Albano et al. 2013; Schwindt et al. 2014, Rumbold et al. 2016; Nuñez Velazquez et al. 2017), Chile (Thiel and Hinojosa 2009), Australia (Chilton 1921; Hayes et al. 2005), New Zealand (Chilton 1921), Japan (Irie 1956; Hirayama 1995), South Africa (Barnard 1916), the Pacific coasts of North America (Wasson et al. 2001; Ruiz et al. 2011), and Black Sea, North Pacific Ocean, Indian Ocean, Eastern and South China Seas (Molnar et al. 2008). Other authors have suggested that it is cryptogenic (crypt-, Greek, Kryptos, secret; -genic, Latin, genic, origin), as species that is not demonstrably native or introduced (Carlton 1996) in Brazil (Neves et al. 2007; Neves and Rocha 2008), Atlantic Europe (UK to Portugal) (Noël 2011), and the east and northwest coasts of the USA (Ruiz et al. 2011). On the other hand, Neves and Rocha (2008) hypothesised that *M. acherusicum* could have originated from the southern hemisphere in general, and it have been moved to the northern hemisphere by the sailing vessels of the Europeans from the 16th to 18th centuries. Table 1 and Figure 3 show the global distribution of *M. acherusicum*. However, due to the tendency for misidentifications among corophiid species, as well as the age of some records, this distribution should be taken with caution.

Abundance

The mean abundance of *M. acherusicum* showed statistical differences (ANOVA, $F = 2.78$, $df = 11$, $P = .006$). Subsequent analyses revealed that the maximum average abundance of *M. acherusicum* was during the austral summer, with a peak in December (SNK test). The mean abundance was 9209 ± 142,004 ind/m^2 and varied seasonally, the lowest values were recorded during winter–spring (1 ± 0.34 ind/m^2) while from the rest of the summer the mean density remained unchanged (between 15,000 and 30,000 ind/m^2; SNK test, $P > .05$; Figure 4).

Figure 3. *Monocorophium acherusicum* world distribution.

Molecular analysis

The 658 bp COI sequences obtained from the three specimens showed between 98.37% and 99.85% similarity with a mitochondrial COI sequence of *M. acherusicum* from Atlantic and Pacific coast of United States (n: 15), Germany (North Sea, n: 5) and Portugal (n: 1) (for accession numbers see Figure 5; in all cases, E-value: 0), confirming the species identity (BIN assignment: BOLD AAU1637). The maximum-likelihood (ML) tree showed that specimens from Mar del Plata port were clustered with *M. acherusicum* specimens from GenBank and Boldsystems (Figure 5). In addition, all the available sequences of *Monocorophium* spp in BOLD and Genbank were also segregated by species, registering a clear differentiation between *M. acherusicum* (n: 24), *M. sextonae* (n: 5) and *M. insidiosum* (n: 9); while the cryptic species *Crassicorophium bonelli* (n: 1) revealed genetic differences with *Monocorophium* spp.

Comparison with other corophiids distributed regionally

The males of *Monocorophium insidiosum,* the other species belonging to this genus present in Mar del Plata port, are recognisable by the very elongate and slender rostrum, which is minute in *M. acherusicum* (see Figure 2C and Figure 6). In addition, *M. acherusicum* differs from *M. insidiosum* by the following features (those of *M. insidiosum* in parentheses): antenna 1 of males, peduncle article 1 without process (ventromedial margin with a short and blunt proximal process); antenna 2 of males, peduncle article 5 with proximal process on ventromedial margin (without proximal process); gnathopod 1, dactyl extending well beyond the palmar angle (barely extending); gnathopod 2, dactyl with 2 teeth on posterior margin (with 3 teeth); pereopod 3 and 4, anterior margin of basis without long setae (moderately to strongly setose); uropod 2, outer margin of inner ramus with robust setae (lacking setae) (see Bousfield and Hoover 1997; LeCroy 2004).

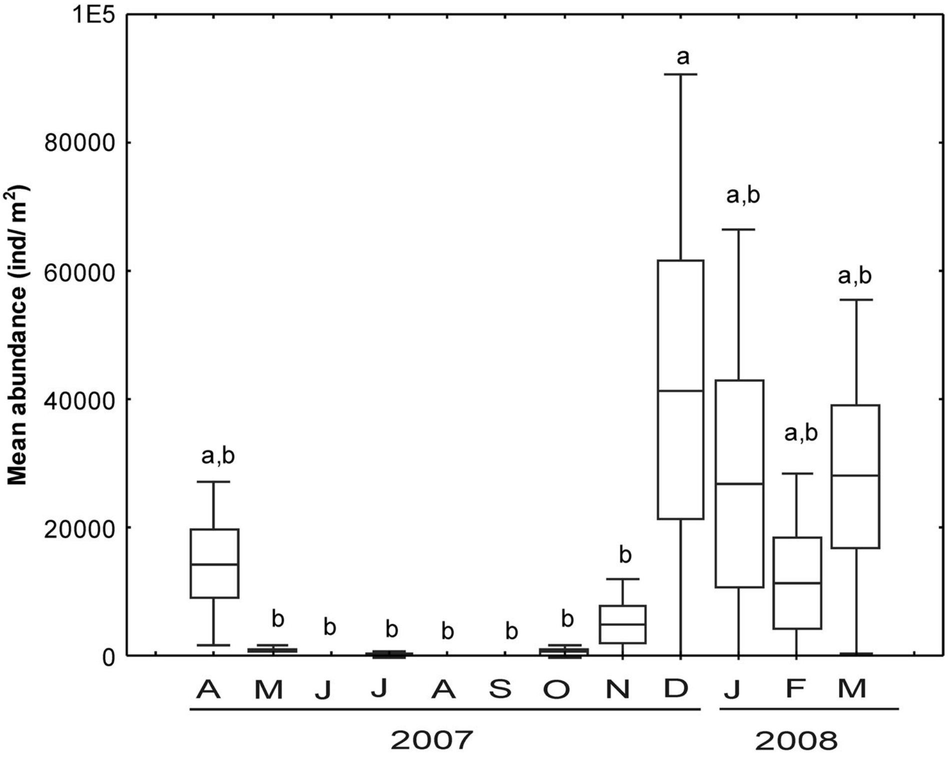

Figure 4. Monthly variations in the mean abundance of the exotic amphipod *Monocorophium acherusicum* in the Mar del Plata port during April 2007 to March 2008. Boxes indicate the ±SE, vertical lines are ±SD and the horizontal line the average. Different letters indicate differences between months according to SNK test (ANOVA: $p < .05$).

In the Argentine Sea, *M. insidiosum* has been recorded from the coast of Buenos Aires Province (Ieno and Bastida 1998), including Mar del Plata port (Rivero et al. 2005; Lopez Gappa et al. 2006; Albano et al. 2013; Carcedo et al. 2015), as well as other ports of Patagonia: San Antonio Este, Puerto Madryn, Puerto Deseado, San Julián and Ushuaia (Sueiro et al. 2012; Schwindt et al. 2014; Martin et al. 2015).

Besides *M. insidiosum*, two other species of this genus are distributed regionally: *M. cylindricum* (Say, 1818) has been cited from Malvinas/Falkland Islands, and *M. josei* Valério-Berardo and de Souza, 2009 was described based on material collected in southern Brazil (see López Gappa et al. 2006; Valério-Berardo and de Souza 2009).

After reviewing the specimens of *M. cylindricum* deposited in the U.S. National Museum, Shoemaker (1934) found that all were identical with *M. acherusicum* (see also Crawford 1937, p. 591; Shoemaker 1947, p. 53). Later on, based on their overlap distributions, and although the type material of *M. cylindricum* had been apparently lost, Bousfield and Hoover (1997) suggested that *M. cylindricum* was synonymous with *M. insidiosum*. However, the material of *M. cylindricum* from Malvinas/Falkland Islands (Stebbing 1914, p. 372; Barnard 1932, p. 244) has apparently never been revised (see Crawford 1937, p. 612, 617), and *M. cylindricum* is currently considered as a valid species for this area (De Broyer et al. 2007, p. 241).

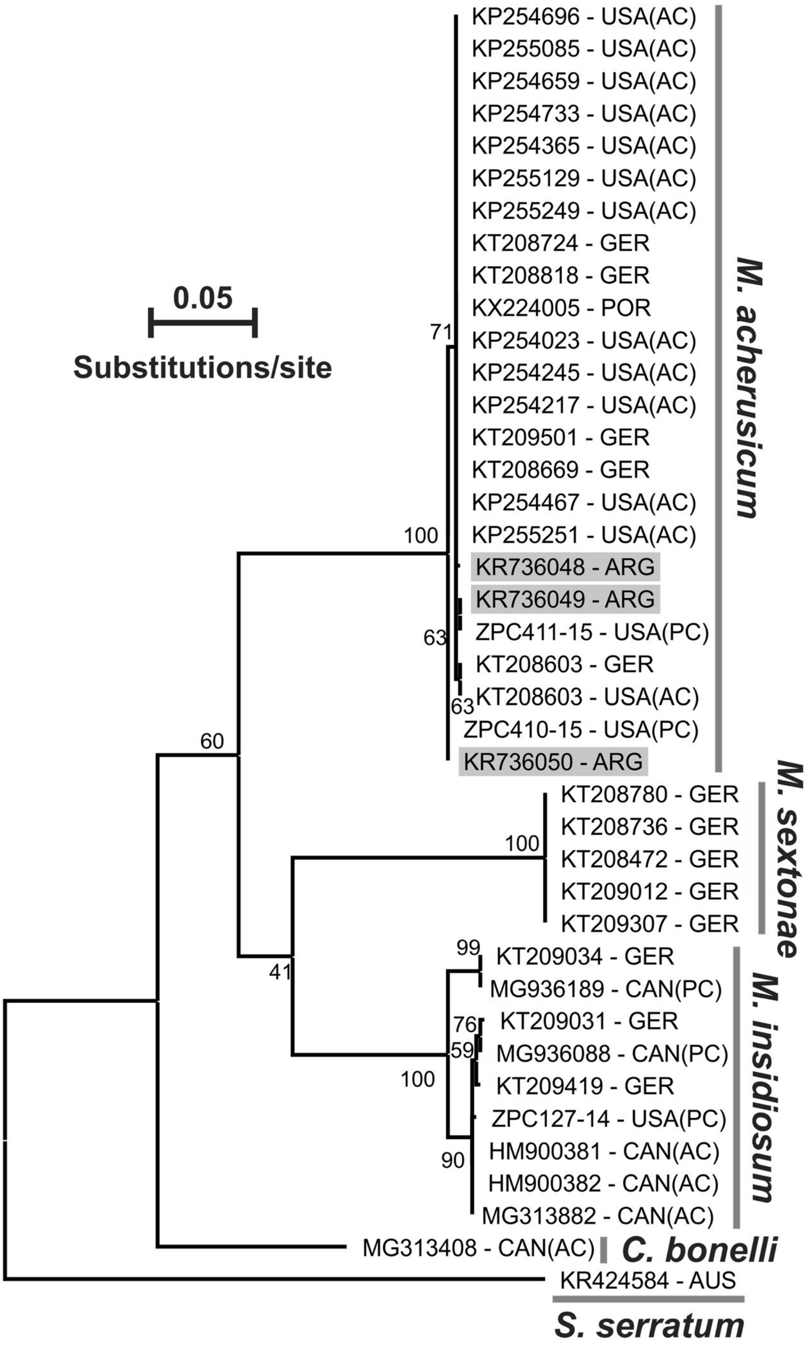

Figure 5. Maximum-likelihood phylogenetic tree of *Monocorophium* spp. Cytochrome c oxidase I. Specimens of *Monocorophium acherusicum* collected in the Mar del Plata port are marked with grey bars. *Sphaeroma serratum* outgroup. Abbreviations: USA, United States of America; GER, Germany; POR, Portugal; ARG, Argentina; CAN, Canada; (AC), Atlantic coast; (PC), Pacific coast.

Figure 6. Rostrum of *Monocorphium insidiosum*, specimen collected in the Mar del Plata port. Scale bar: 0.05 mm.

The other regional species, *M. josei,* differs from *M. acherusicum* by the following features (those of *M. acherusicum* in parentheses): antenna 1 of male, peduncular article 1 with 1 small proximal robust seta (without seta); antenna 1 of female, peduncular article 1 with 2 robust setae on dorsomedial and ventral margins respectively (3–4 robust setae on dorsomedial and 4–5 on ventral margin).

The genera *Crassicorophium* and *Monocorophium* were established by Bousfield and Hoover (1997) mainly on the basis of characters states of mandibles and gnathopod 2: *Crassicorophium* bearing a mandibular palp article 1 with distal process (reduced in *Monocorophium*), and the dactyl of gnathopod 2 with 1 tooth on posterior margin (with 2–3 teeth in *Monocorophium*). Despite these differences, the genera are superficially similar (Bousfield and Hoover 1997, p. 102) and certain species, especially *C. bonellii*, *M. acherusicum* and *M. insidiosum*, have frequently been confused (see Crawford 1937, p. 593). Some of the antennal and uropod characters normally used to identify the species of *Crassicorophium* and *Monocorophium* are closely similar in *M. acherusicum* and *C. bonellii* (see LeCroy 2004, p. 447), and the gnathopod 2 dactyl teeth numbers is too variable for species distinctions (see Chapman 2007, p. 573). In addition, males of *C. bonellii* have not been yet recorded (Alonso 2012), making it even more difficult the identification of both species. However, the female of *M. acherusicum* can be distinguished by the relative length of the dactyl of gnathopod 1, which it extends well beyond the palmar angle (Figure 2E), while it is not extending in *C. bonellii* (see Bousfiled and Hoover 1997; LeCroy 2004).

In the Magellan region, *C. bonellii* was first recorded in Punta Arenas and in the Beagle Channel by Schellenberg (1931), and although it has been recently mentioned from three Patagonian Atlantic ports (Comodoro Rivadavia, Puerto Deseado and Ushuaia), as well as in open waters of Rada Tilly (Chubut Province) and the Beagle Channel (see Alonso 2012;

Schwindt et al. 2014), De Broyer et al. (2007) had already highlighted that the status of Magellan material of this species should be confirmed (see Crawford 1937, p. 612).

Discussion

Previous work on crustaceans found DNA barcoding to be a useful tool for specimen determination in both marine and estuarine amphipod species (Costa et al. 2009; Radulovici et al. 2009; Rumbold et al. 2015a). Most genetic analyses of species boundaries, especially in crustaceans, reveal the existence of cryptic species, some of which are distinguished by surprisingly large genetic differences given their morphological similarity (Palumbi and Benzie 1991; Bucklin et al. 1995; Knowlton and Weigt 1998; Sarver et al. 1998) such as corophiids (eg *Monocorophium acherusicum, M. insidiosum* and *Crassicorophium bonellii*). In the present study, we support and confirm the species identity of the invasive amphipod *M. acherusicum* in Mar del Plata port, using DNA barcoding.

In several studies, it has been shown that molecular analyses could be used as a tool to determine the geographic origin of invasive species and to understand the patterns and processes implied in their dispersion (Cristescu et al. 2001; Miura 2007, Makino et al. 2010). Knox et al. (2011) have shown that in their native habitat, some corophiid amphipods from discrete geographical areas presented markedly differences in their genetic lineages related with higher variation in their COI sequences. In the case of *M. acherusicum* we recorded a lower variation in this sequences between Argentina and the three widely spread locations (ie United States, Germany and Portugal), suggesting a recent introduction from a single source. However, more molecular analyses are needed in order to establish the specific geographic origin of this invasion, using not only other molecular markers (eg RNA and microsatellites), but also including specimens from other important populations, such as Japan, Australia, India, South Africa and Chile, among others.

The first record of *M. acherusicum* in Argentina dates from 1969 in La Lucila (36°39′ S, 56°45′ W, Buenos Aires province, USNM # 127701), which suggests that the species has been established in Argentina for 50 years. In the port areas, its first registry corresponds to the port of Mar del Plata in 2013 by Albano et al. (2013) and Quequén port (Albano 2012). Schwindt et al. (2014) extended the range of this species to several ports of Patagonia, indicating a rapid expansion of this species to southern South America. So far, this species has been reported mainly in port environments, suggesting that its introduction has been mainly by human-mediated vectors (eg ballast water and ship hull biofouling). Modern human-mediated dispersal of this species around the world appears to continue, *M. acherusicum* is found in ballast tanks after 116 days of confinement (Gollasch 2002), but also in biofouling: over 136,000 individuals, including ovigerous females were calculated to be associated with the barge fouling community, in the sub Antarctic Macquarie Island (Lewis et al. 2006). Although *M. acherusicum* has been recorded worldwide, due to the tendency for misidentifications among corophiid amphipods this wide distribution should be confirmed by molecular tools.

According to Albano and Obenat (2009), in the aggregates (mats) of the ecosystem engineer *Phyllochaetopterus socialis* Claparède, 1869, the invasive amphipod *M. acherusicum* (erroneously reported as *M. insidiosum*) showed higher densities mainly during summer. In this study, we registered the same pattern. Warmer temperatures can possibly accelerate growth and sexual maturity and thus encourage recruitment

(Wilson and Parker 1996; Lee et al. 2005; Scinto et al. 2007; Beermann and Purz 2013; Rumbold et al. 2016). In colder periods, the low densities may be explained by an increased mortality and a reduced recruitment (Alonso 1984; Prato and Biandolino 2006). This pattern was similar to the observed in other peracarid species of Mar del Plata port (Kittlein 1991; Rumbold et al. 2015b, 2016). The presence of *M. acherusicum* in wood piles has been registered not only in Mar del Plata but also in biofouling communities of other port areas. In Los Angeles port, Barnard (1958) found that *M. acherusicum* dominates the biomass of the biofouling community on wooden pilings, suggesting that high density of mat-forming biofouling organisms such as corophiids (eg *M. acherusicum* and *M. insidiosum*) might deter the settlement of marine borers on wooden pilings. In a large-scale study in the Port of Tauranga, New Zealand, Inglis et al. (2006) recorded the introduced *M. acherusicum* from wooden piles. Also, it was recorded in the biofouling from Japan (in and around Fukuyama Harbor), commonly found at any submerged structures such as piles, pontoons, buoys, ropes of set-nets, ships' bottoms and shells of cultivating oysters or pearl-oysters (Onbe 1966).

Our results suggest that, due to the morphological similarities of *M. acherusicum* with other corophiids, the use of the DNA barcode is a significant tool for the confirmation and identity of this species in the Mar del Plata port. The combination of genetic, ecological and morphological data, allowed meaningful progress in our understanding of this ecologically important non-indigenous species. Although the ecological impacts of these species have not been considered in Southwestern Atlantic, they may compete with native organisms for food and space and in turn may act as easily accessible food items for a range of predatory fish and invertebrates, as has been shown for other invasive species (see Katsanevakis et al. 2014; Katsanevakis et al. 2016). However, more ongoing project, monitoring programmes and biological identification of native and exotic benthic invertebrates using DNA barcoding, are necessary to determine a possible impact in marine biofouling communities within the ports. Also, further studies will need in order to establish, for example, on how the origins and dispersions of these groups of crustaceans might be in the South West Atlantic ports.

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