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The non-indigenous Paranthura japonica Richardson, 1909 (Isopoda: Anthuroidea: Paranthuridae) from the Mar Piccolo lagoon, Taranto (Italy, Mediterranean Sea

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Response to Reviewers:	All comments and suggestions have been accepted and the ms changed accordingly. The only suggested change we reject is the following "Figure 2: ... Replace tailfan by telson..." Since the tailfan include telson and uropods not only telson

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The non-indigenous *Paranthura japonica* Richardson, 1909 (Isopoda: Anthuroidea: Paranthuridae) from the Mar Piccolo lagoon, Taranto (Italy, Mediterranean Sea)

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Abstract

Several individuals of *Paranthura japonica*, a non-indigenous isopod species, recently recorded on Italian coasts, have been collected from the Mar Piccolo basin, Taranto (Italy). This finding extends the distributional range of the species southwards in the Mediterranean, including a semi-enclosed coastal basin, which is considered the second Italian hotspot for the introduction of alien species. The characteristics of the place reinforce the hypothesis that its introduction is linked to shellfish trade and farming. Remarks on the morphology and ecology are included.

Keywords: Anthuroidean isopod, Ionian Sea, Non-indigenous species, Shellfish trade, Coastal, *Paranthura japonica*

Introduction

Several isopod crustaceans belonging to the anthuroidean genus *Paranthura* have been found in the Mar Piccolo of Taranto (Ionian Sea, southern Italy) during a field survey aimed at collecting samples of wood for shipworm studies. The specimens showed the diagnostic characters of *P. japonica* Richardson, 1909, a species recently recorded from other Italian locations (Venice, Olbia and La Spezia) by Marchini et al. (2014).

Paranthura japonica has its native range in the Asian Western Pacific (Lee II and Reusser 2012) but it is spreading to other regions assumably as a result of human-mediated introductions (Ruiz et al. 2011; Lavesque et al. 2013; Marchini et al. 2014). In particular, the species was recorded from San Francisco Bay in 1993, initially as *Paranthura* sp. (Cohen and Carlton 1995), and is now considered a non- indigenous species (NIS) established in both Northern and Southern California (Lee II and Reusser 2012). *Paranthura japonica* has been discovered also on the Atlantic coast of France (Arcachon) (Frutos et al. 2011; Lavesque et al. 2013) where its presence has been linked to the farming of imported Pacific oysters (*Crassostrea gigas*).

Materials and methods

Study site and Sampling

The Mar Piccolo of Taranto is a wide semi-enclosed coastal basin consisting in two inlets, the first communicating with the sea through two channels. Taranto houses an important military base and a major industrial port. The Mar Piccolo is known as a shellfish trade center with a large farming of mussels.

Samples were collected in the Mar Piccolo, close to the "Mercato ittico galleggiante" (Fig. 1), in two times: on June 23rd 2013, when some individuals were found inside empty galleries of shipworms and gribbles drilled on the hull of a little boat wreck, lying just below the water surface (40° 29.194'N, 17° 13.841'E); on October 10th 2013, when some wood panels, previously deployed at the market pier (40° 29.192'N, 17° 13.886'E), were recovered.

The three panels deployed at the market pier were made in pine wood (*Pinus sylvestris*) and had a total surface exposed to water of 1200 cm^2. They remained immersed at a depth of about 1 meter from June 23rd to October 10th 2013. During this period, water temperature at the sampling site ranged from 23.4 °C to 28.4 °C, while salinity ranged from 38 to 40. On these panels, 17 individuals were collected among the overgrowing fouling. Samples were fixed in 70% ethanol.

Material examined

Material from Taranto. Five specimens of *Paranthura* ranging in body length from 9.0 to 10.9 mm were collected from the boat wreck: a larvigerous female with numerous mancae in the broodpouch (Fig. 2), a female with oostegites, and two adults lacking obvious external sexual attributes, herein designated as non-reproductive adults. The fifth individual showed characters of a submale in having an appendix masculina on the 2nd pleopod but a scarcely setose antennula. Other eleven specimens were sorted from the fouling of wood panels. They consisted of one female with oostegites, two mature males (featuring a fully developed appendix masculina and setose antennulae) in the size range 9-10.5 mm, two individuals about 5.5 mm long and 6 incomplete non-reproductive adults. Voucher specimens are lodged at the Center of Villa Dohrn-Benthic Ecology of the Stazione Zoologica di Napoli (ref. M. Lorenti).

Comparative material. Specimens identified as *Paranthura japonica* were kindly sent by Dr. Nicolas Lavesque (Université de Bordeaux, Station Marine d'Arcachon, France) and Dr. Andrew Cohen (Center for Research on Aquatic Bioinvasions CRAB, Richmond, CA), collected in the Arcachon Bay and San Francisco Bay, respectively. The material from Arcachon consisted of one ovigerous female and one non-reproductive adult 7.2 mm long. Specimens from San Francisco (18 individuals) comprised ovigerous females, mature males, non-reproductive adults and one juvenile. A substantial sample of about 30 specimens from Chioggia (Lagoon of Venice) comprising adults of both sexes and juveniles was kindly provided by Dr. Agnese Marchini (Department of Earth and Environmental Sciences, University of Pavia, Italy).

Specimens from all locations were examined with the aid of a Leica MZ12.2 stereo microscope equipped with a Canon S40 digital camera. Dissected parts were observed using a Leitz Diaplan microscope.

Results

***Abbreviated description of specimens from Taranto*.**

Density of *Paranthura japonica* collected on the panels was 142 ind/m^2, comparable to those found in other Italian sites (Marchini et al. 2014).

The main features of the individuals examined were as follows.

Presence of dark pigmentation in the form of scattered chromatophores or more or less regular patches. Relative proportions of posterior pereonal segments and pleon 5>6>7<Pln. Cephalon with anterolateral lobes extending beyond rostrum. Pereonites 4 to 7 each with a more or less marked transverse groove close to anterior margin. Pleonites 1 to 5 fused dorsally, but indicated by lateral incisions; pleonite 6 with slightly bilobed posterodorsal margin and a median slit extending about half the length of the segment. Telson linguiform, basally constricted, tapering to a broadly rounded, slightly truncated apex. Mandibular palp 3-segmented, second segment the longest, with a long subdistal seta, third segment with row of 9 to 11 robust setae. Maxillipedal palp with an obscure terminal article and about 10 setae on the distal half. Pereopod 1 with convex propodus palmar flange and a distinct thumb. Propodal palm of pereopod 2 with 9 stout setae in larger individuals; propodus of pereopod 7 with 2 stout setae in proximal half and single stout seta posterodistally (in two individuals, the setation of pereopods 2 to 7 varied between the two body sides, with one additional stout setae on propodus). Uropodal peduncle with inner margin sinuous and inner distal angle produced; exopod crenulated, setose, approximately 2.3 times as long as wide, notched sub-apically; endopod ovate, setose, barely reaching to telsonic apex.

The composition of the antennular flagellum varied among individuals. Females and non-reproductive adults, in the size range 9-11 mm, had either 6 or 7-articled flagella composed of one short ring-like proximal article, three or four longer articles, respectively, and two minute terminal ones. One individual had a 6-articled flagellum on the left antennula (Fig 3a) and a 7-articled flagellum on the right one (Fig. 3b) . Both the submale and the mature males presented a flagellum of 8 articles (Fig. 3c), the proximal ones being squatter in shape than in the female. The number of articles in the antennular flagellum varied in the other specimens depending on body size, with individuals < 8 mm in length featuring a 5-articled flagellum.

Sexual dimorphism was most evident in cephalic appendages and in tail-fan, with mature males having densely setose antennulae as well as broader uropodal peduncle and telson than the other stages (Fig 4a). The shape of the appendix masculina differed between the submale and the mature males, with the former having a rod-like appendix and the latter a spearhead-like one.

Comparison with specimens from other locations

The examined specimens from Taranto agreed with the comparative material from Arcachon, Chioggia and San Francisco of the same stage/size, as far as the major diagnostic characters were concerned, within a range of variability common in paranthurid species (Negoescu, 1984; 1999). The similarities in the body size, the variety of pigmentation patterns, the relative length and the grooving of posterior pereonites, the shape of the anterior cephalic margin, the demarcation of the pleonites, the shape of pleonite 6, the setation of the mandibular palp and the propodus of pereopods, the shape and setation of uropodal rami and of telson were most notable.

Gravid females from Arcachon and San Francisco had a rather regular dorsal pigmentation pattern similar to females from Taranto (less dense in the Arcachon specimen).

Also in the comparative material, a polymorphism of the antennular flagellum occurred in females and non-reproductive individuals. While specimens below 8 mm length featured a 5-articled flagellum, similar-sized individuals longer than 8 mm from San Francisco and Chioggia had either 6- or 7-articled flagella.

Discussion

The specimens from all four locations basically agree with the extant descriptions of *P. japonica* (Richardson 1909; Nunomura 1975; Kussakin 1982; Golovan and Malyutina 2010; Kwon 2012), although within the limits of these latter's thoroughness. A difference occurred in relation to the antennular flagellum of females, which will be discussed later on. The submale closely fits the description by Nunomura (1975) of a male individual of *P. japonica* while the distinguishing features of mature males correspond to the figures in Kussakin (1982) and to the individual described by Nunomura (1975) as *P. laticauda*, which in fact resembles a male of *P. japonica*.

Paranthura japonica can be distinguished from most of the other *Paranthura* species featuring dorsally fused pleonites by its larger size and differences in the setation of appendages and/or in the shape of uropods and telson. The boundaries within a group of species whose description is based on only a couple of individuals (e.g. *P. laticauda* Nunomura 1975, *P. kobensis* Nunomura 1975, *P. algicola* Nunomura, 1978) are more blurred and may be reconsidered in the light of intraspecific variability. In spite of the considerable number of records from different regions ascribed to *P. japonica*, this species is somewhat underdescribed, in particular as regards the distinction among different developmental stages. One issue concerns the structure of the antennular flagellum, a character that is often used in the identification of paranthurids. Female specimens from all the four locations examined by us do not conform to diagnoses by Richardson (1909), Kussakin (1982) and Golovan and Malyutina (2010) according to which *P. japonica* females have a 4-jointed antennular flagellum. This may be due to miscounting of the shortest antennular joints as well as to a variability in the number of flagellar articles, which has been observed in other paranthurid populations, even in the same individual (Negoescu 1984). The latter condition does occur in some of the observed specimens. The discrepancies found in literature and the variation displayed in the material we examined may point to a complex ontogenetic history of the species, which needs to be investigated.

The ecology and the habitat characteristics of the sampling location, highly impacted by alien introductions (Cecere and Petrocelli 2009), further support the identification of our specimens as *P. japonica*. The species has been frequently reported from Coastal Transitional Ecosystems, such as lagoons, estuaries and mangroves.

Anthuroidean species often inhabit soft-sediment in burrows or excavated by themselves or abandoned by other organisms. They can also be found among the rhizomes of seagrasses. Hard substrata anthuroids are component of the marine cryptofauna inhabiting small cracks, holes, crevices, shell beds and free spaces between colonial animals of biogenic reefs and fouling communities (Cadien and Brusca 1993).

Paranthura japonica seems to adapt quite well to different types of microhabitats, and it was found in the above mentioned habitats including oyster reefs, *Zostera marina* meadows, macroalgae and muddy sands (Golovan and Malyutina 2010; Lavesque et al. 2013). Actually, most of the microhabitat in which it was found offers tri-dimensional complex structures that favor the settlement of cryptofauna.

Interestingly, Kussakin (1982) recorded the presence of *P. japonica* inside burrows made by limnoriid borers, observation confirmed by our findings. There is growing evidence of the importance of the biogenic microhabitat of burrows in the establishment of non-indigenous species (Lorenti 2006; Davidson et al. 2010).

Anthuroid isopods have little ability for active dispersion (Poore 2001), and therefore their transfer and spreading between different regions may be largely due to passive transport via natural rafting or anthropogenic carriers (e.g. hull fouling, seafood trade).

The surface of the panels on which *P. japonica* was found was colonized by the non-indigenous colonial tunicate *Polyandrocarpa zorritensis* (Van Name, 1931). This tunicate, firstly described in Peru, was detected in the harbour of Taranto in 2001 (Brunetti and Mastrototaro 2004), the third site in the Mediterranean after La Spezia (Italy) in 1974 (Brunetti 1978-79) and the Ebro Delta (Spain) in 1986 (Turon and Perera 1988). There is probably a close association between shellfish trade, the introduction of *P. zorritensis,* which has been reported to overgrow farmed oyster (Perera et al. 1990) and the introduction of *P. japonica*. In fact, introduction of *P. japonica* has been associated with the planting or marketing of exotic oyster species (Lavesque et al. 2013; Marchini et al. 2014). Taranto is considered the second hotspot of introduction of marine alien species in Italy after Venice (Occhipinti-Ambrogi et al. 2010). This high occurrence of non-indigenous species is probably due to shipping and aquaculture (Cecere and Petrocelli 2004). The Mar Piccolo Lagoon has experienced shellfish farming of both mussels and oysters, being the main economic activity since the 1950s (Caffio 2009). Shellfish were imported since the 1980s to cope with the growing market demand. This demand increased even more after the suppression of the "First Inlet" mussel processing plants due to heavy contamination from the nearby iron industry (Petrocelli et al. 2013).

A close connection between the establishment of alien macrophytes and the import of shellfish for retail selling was suggested by Petrocelli et al. (2013), since in this case veterinary controls are less stringent than those for aquaculture. Petrocelli and Cecere (2010) attributed the unintentional introduction of alien seaweeds to the discarding of shells and packing material of *Crassostrea gigas*, usually imported from northern France, into the waters of the Mar Piccolo. The same way of introduction can be hypothesized for the specimens of *Paranthura*, especially because a number of them were found near the fish market. It is worth noting that even Marchini et al. (2014) found a high abundance of the species at the Chioggia fish market where seafood is traded and processed.

In conclusion, the most probable route to Taranto followed by *P. japonica* was through the import of shellfish from regions where the species is already present. Once delivered into the environment, individuals were able to occupy suitable cryptic habitats, such as tunnels made by woodborers. It remains to be assessed to what extent the species has spread and become established in the area.

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Figure captions

Fig. 1. Map of the Mar Piccolo of Taranto and its location on the Italian peninsula. The sampling site is evidenced by a circle.

Fig 2. *Paranthura japonica* from Taranto, dorsal view of female (body length: 10.9 mm).

Fig. 3. *Paranthura japonica* from Taranto. Drawing of the antennulae showing the 3-articled peduncle and the flagellum (most setae omitted) in a) female (body length 9.2 mm), left antennula, b) same individual, right antennula, c) mature male (body length 9.1 mm), Scale bar: 0.2 mm.

Fig 4. *Paranthura japonica* from Taranto, tail-fan in a) mature male (body length: 9.3 mm), b) female (body length: 9.2 mm). Scale bar: 0.5 mm.

Figure 1
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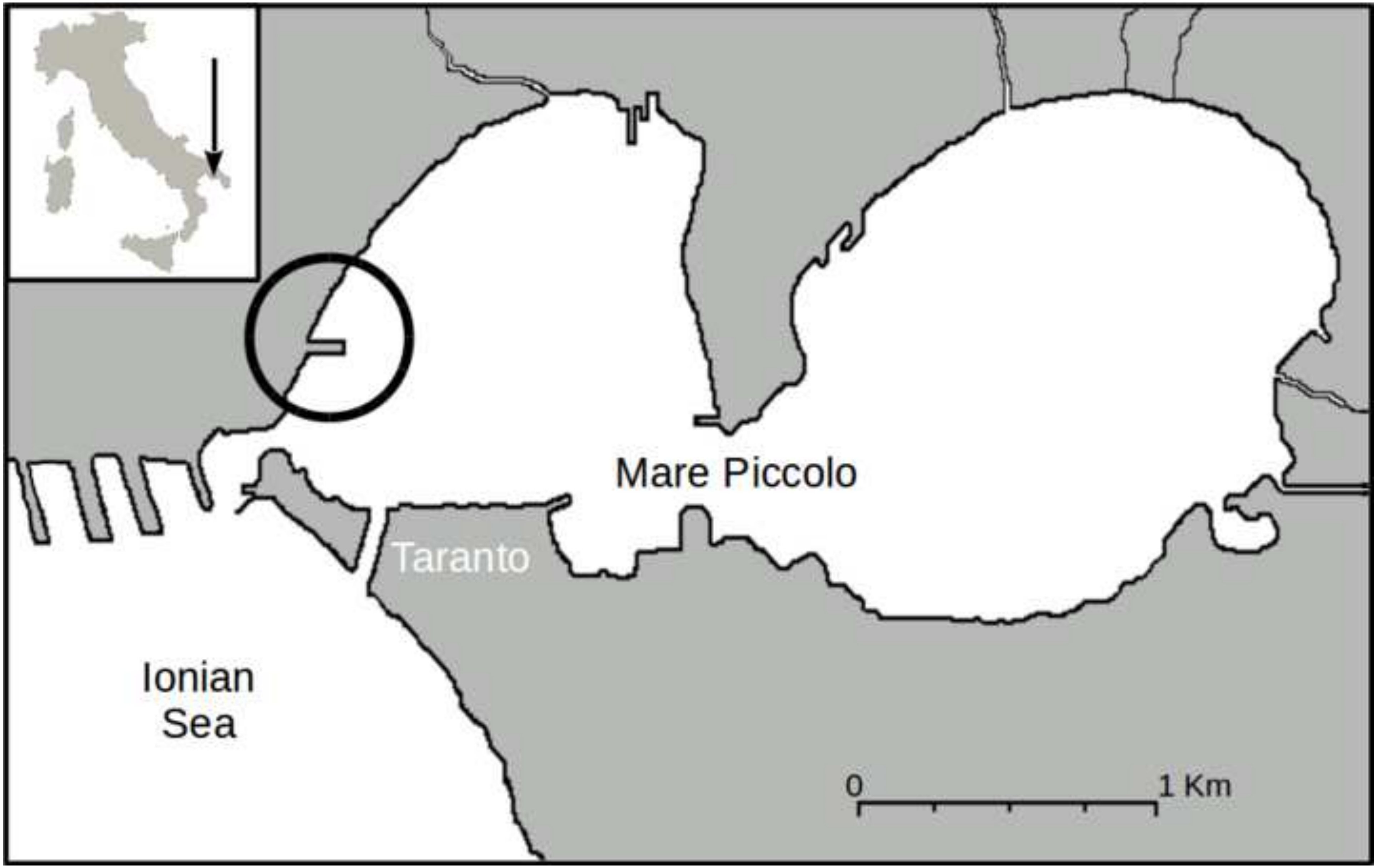

Figure 2
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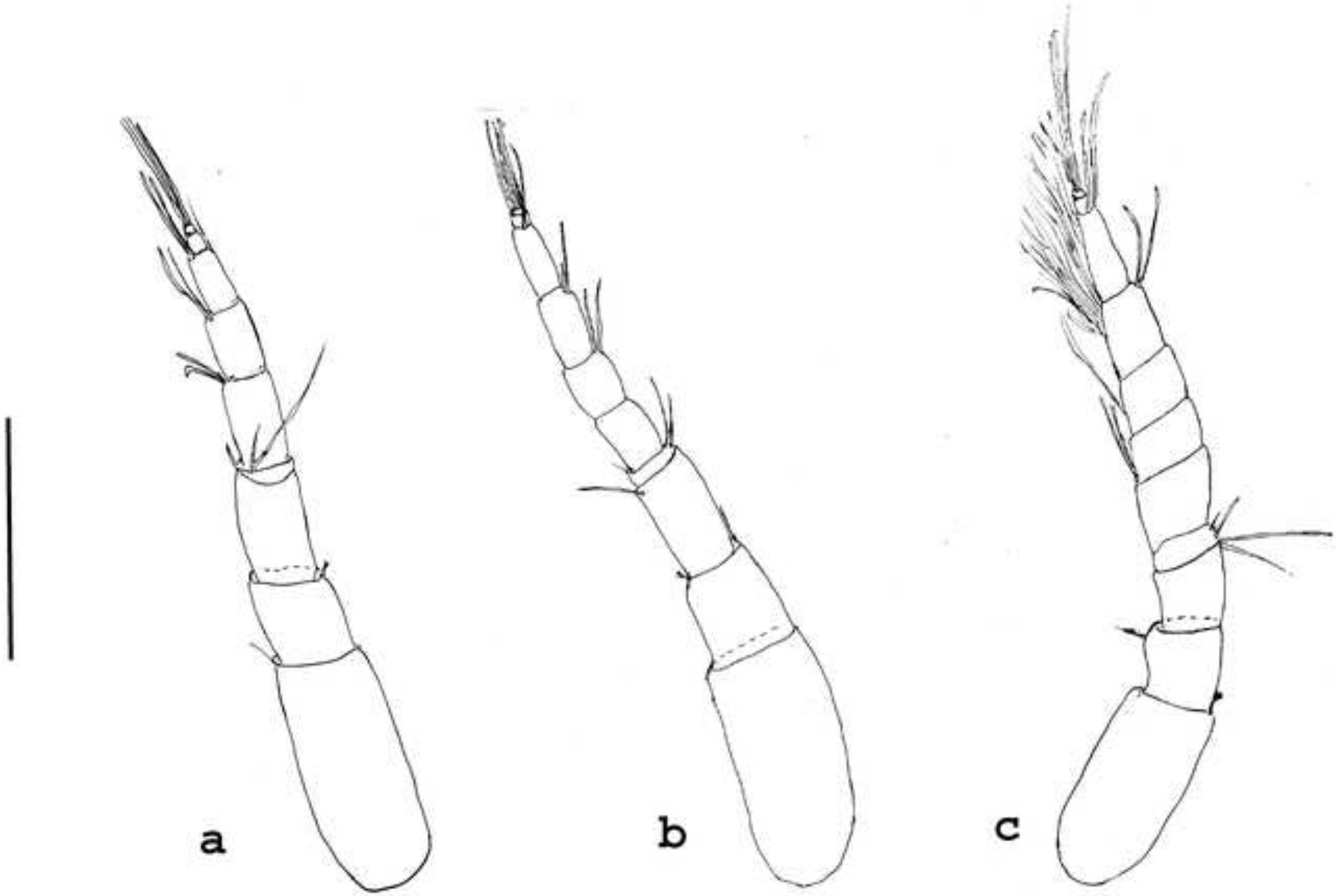

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