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First record of the genus *Paranebalia* Claus, 1880 from the Mediterranean Sea (Crustacea: Leptostraca)

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Abstract

Benthic monitoring of the marine shallow bottoms off Menorca (Balearic Islands, western Mediterranean) has yielded several specimens of the leptostracan genus *Paranebalia* Claus, 1880. This finding constitutes the first report of the genus from European latitudes and the Mediterranean Sea and therefore the third leptostracan genus known from the Mediterranean. Specimens are described, illustrated, and compared to other known species; they might represent a new species but their state of maturity and the lack of appropriate diagnosis for the type species of the genus, *Paranebalia longipes* (Willemöes-Suhm, 1875), did not allow confirmation of its taxonomic status.

Keywords: Distribution, Leptostraca, Mediterranean Sea, Paranebalia, Phyllocarida, subtidal.

Introduction

Diversity and ecology of extant leptostracans (Crustacea: Phyllocarida) is still poorly known in many parts of the world (Moreira et al., 2009); they can, however, be abundant in a number of marine habitats and are frequently found in benthic surveys and baited traps (e.g. Dahl, 1985; Haney & Martin, 2005; Lee & Morton, 2005; Moreira et al., 2009). Leptostraca comprises three extant families (Walker-Smith & Poore, 2001) of which Paranebaliidae Walker-Smith & Poore, 2001 is composed by three genera and a total of six species (Song et al., 2012): Paranebalia Claus, 1880 (three species and one subspecies), Levinebalia Walker-Smith, 2000 (two species), and Saronebalia Haney & Martin, 2004 (one species). To date, Levinebalia has only been found in New Zealand and Australia (Wakabara, 1976; Walker-Smith, 2000) and Saronebalia in the Caribbean Sea (Haney & Martin, 2004). On the contrary, Paranebalia is a pantropical genus (Haney & Martin, 2016) and has mostly been reported from temperate and tropical latitudes, usually in intertidal and shallow depths (< 100 m) associated with algae, seagrasses, sponges, corals, and sedimentary environments (e.g. Gamô & Takizawa, 1986; Modlin, 1996; Ledoyer, 2000; Song et al., 2012). Furthermore, undescribed species of Paranebalia from Australia still await formal description (Walker-Smith, 2000; Walker-Smith & Poore, 2001).

The type species of the family, P. longipes (Willemöes-Suhm, 1875), has been reported in the western Atlantic Ocean from the Bermudas (Willemöes-Suhm, 1875) to northern Patagonia (Roccatagliata et al., 2010, as cf.); other records include Japan (Thiele, 1904; Gamô & Takizawa, 1986), South Korea (Song et al., 2012), and across the Indian and Pacific oceans (Thiele, 1904; Ledoyer, 1994, 2001). Ledoyer (2000) described the subspecies P. longipes neocaledoniensis Ledoyer, 2000 from New Caledonia that mainly differs from the nominal species in the shape and number of eye denticulations and the number of comb-row 'spines' of the pleopod I exopod. Thus, P. longipes has been regarded as a cosmopolitan species (Song et al., 2012); this view was also held for species of Nebalia Leach, 1814 for long (Claus, 1888; Thiele, 1904) but in the last decades it has been proven wrong. Indeed, many new species of Nebalia have been described in the last 20 years and several species may coexist in the same geographic area even at small spatial scales (i.e. metres to a few kilometres) (see for instance Dahl, 1985; Martin et al., 1996; Vetter, 1996; Moreira et al., 2009; McCormack et al., 2016). In fact, the alfa-diversity of the extant Leptostraca still remains underestimated (Haney & Martin, 2016).

In 2007, benthic monitoring of the subtidal environment at Menorca in the Balearic Islands, western Mediterranean, has revealed the presence of several specimens of *Paranebalia*. This is the first report of the genus from European latitudes and the Mediterranean Sea and constitutes the third genus of Leptostraca ever reported from the Mediterranean. Specimens are described herein but were not nominated to the species level due to their state of immaturity and the lack of an accurate diagnosis for *P. longipes*.

Materials and Methods

Material examined was obtained during the research cruise 'CALMEN07', in the Menorca Channel, Balearic Islands, Spain, in July 2007. In total, three specimens of *Paranebalia* sp. were collected at only one station, D13 at Bahía de Pollensa, Menorca (39°47.19'N, 03°18.92'E), in muddy sediment at 28 m depth (8 July 2007). Benthic samples of that cruise were taken by means of a rectangular dredge, sieved on board (4, 1, and 0.5 mm circular mesh) and the retained material was fixed with 10% neutralized formalin. Specimens were then sorted in the laboratory and transferred to 70% ethanol.

Line drawings were made with the aid of a *camera lucida* connected to an Olympus BX51 microscope and provided with an ocular micrometre for measurements. Drawings were made from the largest dissected female specimen unless noted. Measurements included total length (TL), dorsal carapace length (DCL), lateral carapace length (LCL) and carapace height (CH), following Moreira *et al.* (2009). All specimens are deposited in the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN).

Α

Results

Systematics

Superorder Leptostraca Claus, 1880 Order Nebaliacea Calman, 1904 Family Paranebaliidae Walker-Smith & Poore, 2001 Genus *Paranebalia* Claus, 1880 Type species: *Nebalia longipes* Willemöes-Suhm, 1875 *Paranebalia* sp. (Fig. 1-5)

Material examined

Three specimens: one pre-ovigerous female (largest specimen, TL 4.75 mm, lacking rostrum, uropods broken, MNCN 20.04/11186) and two immature specimens (complete specimen, TL 3.25 mm, young male?, MNCN 20.04/11187; incomplete specimen lacking uropods, TL 2.57 mm, MNCN 20.04/11188). All specimens collected from Station D13, Bahía de Pollensa, Menorca (39°47.19'N, 03°18.92'E).

Description

Carapace: oval, reaching laterally pleonite 5 and covering laterally most of pleonites 3-4 (Fig. 1A). DCL 1.4-2.1 mm, LCL 2.0-2.9 mm, CH 1.4-1.7 mm.

Rostrum: present in one immature specimen only; long, extending beyond eyestalk, length about 2.4 times width, 0.45 times length of DCL; shape roughly triangular; apex provided with one subterminal spine.



Fig. 1: Paranebalia sp.: A, complete specimen, lateral view (immature specimen); B, eye, lateral view.

Compound eye: oval, length 3.4 times width (Fig. 1B); ommatidial part brown in colour, extending distally about 50-66% of eyestalk; anterior distal and dorsal surface armed with teeth: (1) anterior distal third with up to 14-15 teeth with rounded apex, more or less organised in two rows, decreasing in size backwards, (2) about 15 small teeth mid-dorsally.

Antenna I: peduncle composed of four articles (Fig. 2A). First article wider distally, shorter than second. Second article length 2.8 times width, with (1) cluster of six (four long, two short) smooth setae on distal posterior surface, (2) three short setae on lateral distal surface, (3) anterior subterminal cluster of about 15 short and long setae and one shorter thick seta. Third article shorter than second, wider distally, with cluster of about seven simple setae and one plumose seta on anterior distal margin, and two short simple setae on posterior margin. Fourth article about 0.5 times length of third, with lateral flange having 13-20 robust teeth with crenate margins (detail in Fig. 2A), with seven simple setae on distal and lateral margins (5+2). Antennular scale blade-like, length 2.9 times width, with numerous short and long setae along most of

anterior margin, increasing in length distally. Flagellum much shorter than peduncle, composed of 3-5 (6?) articles, proximal article at least two times longer than others combined; each article with (1) 0-3 aesthetascs; (2) 2-3 setae longer than aesthetascs, plumose along anterior distal half; distal article with additional short seta.

Antenna 2: peduncle of three articles (Fig. 2B). First article shorter than second. Second article almost rectangular. Third article longer than second, with two short acute cuticular projections on medial margin (cp, Fig. 2C): (1) proximal one larger, at about mid-length, (2) second one subdistal; several groups of setae along mediallateral anterior margin; most setae straight and distally curved, with comb of short teeth along distal third/half (dcs, Fig. 2B detail, 2C): (1) proximal group of 10-12 setae, (2) medial group of seven setae around first cuticular projection, (3) distal group of four setae; lateral internal row of 4-5 thinner slightly plumose setae (sps, Fig. 2C); one long plumose seta on posterior margin at mid-length. Flagellum well-developed, of 4-5 articles; first article longer, with three groups of three simple setae each; articles 2-4 with 2-4 subdistal setae: two setae similar to



Fig. 2: Paranebalia sp.: A, antenna I, lateral view (detail: tooth of lateral flange of peduncle fourth article); B, antenna II, lateral view, peduncle first article omitted (detail: seta of peduncle third article); C, antenna II, third article peduncle, detail (immature specimen); D, antenna II, flagellum second article (immature specimen). Abbreviations: cp, cuticular projection; dcs, distally curved seta; sps, sparsely plumose seta.

those of peduncle third article (i.e. dcs), one longer seta slightly plumose along proximal half, and one seta much shorter than others and curved (Fig. 1A, 2D).

Mandible: molar process well-developed, with proximal group of three rows of spines and two distal groups of several rows of shorter spines (Fig. 3A); incisor with two acute processes, a thinner distal one with ventral row of setae (Fig. 3B). Mandible palp composed of three articles (Fig. 3C); second article 1.75 times length of first, slightly curved dorsally; one long subterminal seta, medial margin with five setae plumose along distal half. Third article slightly shorter than second, posterior margin with several rows of setae: (1) two rows extending from ending of proximal quarter to distal end, one row with shorter setae than other, (2) row of shorter setae along distal quarter, (3) single terminal long seta.

Maxilla I: first endite (proximal) with rounded medial margin bearing long plumose setae (Fig. 3D). Second endite (distal) more rectangular in shape (Fig. 3D-E); distal margin with one plumose seta and two rows of five and seven thick spines respectively, with 3-4 teeth along distal inferior margin. Palp well-developed, about 4.5 times length of protopod, with about 15 long setae along its en-

tire length, more spaced along distal half.

Maxilla II: protopod with four endites (Fig. 3F); first endite the largest, second and third endites similar, fourth endite much shorter; first to third endites bearing 6-12 plumose setae; fourth endite with two long plumose setae, distal-most longer than protopod and endopod. Endopod composed of two articles, about 1.3 times length of exopod, proximal article 0.74 times length of distal; distal article with three terminal plumose setae of different length. Exopod 1.8 times length of endopod first article; lateral margins with about six plumose setae and row of many short setae, distal margin with three plumose setae.

Thoracopods: endopod longer than exopod (Fig. 4); with numerous plumose setae along anterior margin, less numerous and more spaced on posterior margin; endopod segmentation weak. Exopod with numerous plumose setae along posterior margin. Epipod small, reduced, with row of short setae along posterior margin (Fig. 4A); thoracopod VIII epipod comparatively longer than those of other thoracopods (Fig. 4H).

Pleonites: pleonites I-V with posterior border smooth (Fig. 1A); pleonites VI-VII bearing acute denticles along posterior dorsal and dorsolateral border (Fig. 5A).



Fig. 3: Paranebalia sp.: A, mandible, molar and incisor processes; B, mandible, incisor process, detail; C, mandible, palp; D, maxilla I; E, maxilla I, distal endite, detail; F, maxilla II.



Fig. 4: Paranebalia sp., thoracopods, lateral view: A, thoracopod V, showing setation; B, thoracopod I; C, thoracopod II; D, thoracopod III; E, thoracopod IV; F, thoracopod VI; G, thoracopod VII; H, thoracopod VIII. B-H, same scale.



Fig. 5: Paranebalia sp.: A, pleonites VI-VII, posterior dorsal and dorsolateral margins, denticles; B, pleopod I, protopod and endopod *appendix interna*, inner side; C, pleopod I, lateral view; D, pleopod I, bipectinate seta of comb-row (*sensu* Walker-Smith, 2000); E, pleopod II, lateral view (detail: serrations of protopod posterior margin); F, pleopod IV, protopod, lateral view; G, pleopod V, ventral view; H, pleopod VI, ventral view; I, anal scales, ventral view. G-H, same scale.

Pleopods I-IV: stenopodous, composed of protopod, endopod and exopod. Pleopod I protopod with 5-6 small serrations along posterior margin, short seta on proximal third (Fig. 5B). Exopod about 0.74 times length of protopod and 0.64 times length of endopod (Fig. 5C); with 'comb-row' of about 15-23 bipectinate setae (*sensu* Walker-Smith, 2000) along lateral border (Fig. 5D); five stout smooth spines on distolateral border, distal-most the longest; 6-7 long plumose setae along medial margin. Endopod bi-segmented; lateral and medial margins of distal article with about 15-20 plumose setae, distal margin with acute process at apex and terminal spine. Basal article much shorter, with *appendix interna* (Fig. 5B).

Pleopods II-IV similar. Protopod with 8-14 conspicuous serrations along posterior margin (Fig. 5E-F); three distal setae next to endopod base, plumose along distal third. Exopod 0.8 times length of protopod and 0.76 times length of endopod; lacking comb-row, instead with a row of 4-6 spine pairs, each pair consisting of one long and one shorter spine, with longer plumose seta in between; medial margin with long plumose setae; three distal spines, terminal one about two times length of others. Endopod bi-segmented; proximal article short, with *appendix interna*; lateral and medial margins of distal article each with about 15-20 plumose setae, ending in one stout spine.

Pleopods V-VI: pleopod V uniramous, length 3.2 times width (Fig. 5G), with two terminal stout conical spines, one much longer and thicker, one shorter curved distally; approximately ten simple setae along medial and distal border, appearing jointed at mid-length, a few sparsely plumose setae along distal half. Pleopod VI uniramous, length 3.0 times width (Fig. 5H); with two terminal stout conical spines; medial, lateral, and distal margins with about 12 'jointed' setae of different lengths, similar to those of pleopod V.

Anal somite, anal plates and uropods: anal somite slightly longer than pleonite VII (Fig. 1A). Anal plates triangular, lacking 'shoulder', with broad bases and acutely tapering distally (Fig. 5I). Uropods elongate, clearly longer than pleonite VII and anal somite combined (Fig. 1A). Each uropod with about 10-11 short setae along lateral margin and 3-5 long setae on distal margin, 12 plumose setae on medial margin (not drawn).

Distribution and ecology

The genus *Paranebalia* is distributed along the Western Atlantic, east Asia, Indian and Pacific oceans (Song *et al.*, 2012); Menorca (Balearic Islands, western Mediterranean; this work). Mediterranean specimens have been found in muddy sediments at 28 m depth with a high abundance of the serpulid polychaete *Ditrupa arietina* (Müller, 1776); other species collected were the bivalves *Nucula sulcata* Bronn, 1831, *Myrtea spinifera* (Montagu, 1803) and *Corbula gibba* (Olivi, 1792), the decapods *Ilia nucleus* (Linnaeus, 1758) and *Pagurus prideaux* Leach, 1815, and the echinoderms *Spatangus purpureus* Müller, 1776 and *Astropecten aranciacus* (Linnaeus, 1758).

Discussion

This record extends the known distribution of the genus Paranebalia eastwards from the Atlantic Ocean into the Mediterranean Sea. Furthermore, this is the fifth leptostracan genus known for European latitudes, the others being Nebalia, Nebaliopsis Sars, 1887, Nebaliella Thiele, 1904, and Sarsinebalia Dahl, 1985 (Mauchline & Gage, 1983; Dahl, 1985); several species of *Nebalia* and one of Sarsinebalia have been reported for the Mediterranean (e. g. Ledover, 1997; Koçak et al., 2011; Koçak & Moreira, 2015). Most records of leptostracans in the Mediterranean were initially attributed to N. bipes Fabricius, 1780 until a few decades ago; previous works have, however, shown that species other than N. *bipes* are present in those waters (e. g. Dahl, 1985; Ledoyer, 1997; Moreira et al., 2007; Koçak et al., 2011; Moreira et al., 2012; Koçak et al., 2015).

The specimens described here display the characters shared by members of the family Paranebaliidae, i.e., a short spine at the apex of the rostrum, two short cuticular projections on the third article of the antenna 2, the thoracopod epipod is reduced and shorter than the exopod, and the protopod of pleopods I-IV have a posterior margin that is serrated (Walker-Smith, 2000; Haney & Martin, 2004); on the contrary, in nebaliids the epipod is comparatively larger and longer than the exopod (Nebalia, Dahlella Hessler, 1984, Sarsinebalia) or lack the epipod (Nebaliella). In contrast, males of paranebaliids bear an antennular callynophore-like structure but this was not confirmed in these specimens because no male was available. In any case, these specimens belong to Paranebalia because of the presence of denticulate eyes; this feature is not present in Levinebalia and Saronebalia (Walker-Smith, 2000; Walker-Smith & Poore, 2001). Furthermore, Paranebalia is easily distinguished from Nebalia and Sarsinebalia because of the lateral flange of the antenna I peduncle, antennae, and thoracopods. Therefore, it seems unlikely that Paranebalia might have previously been mistaken in the Mediterranean for any other leptostracan. It may represent a new species (see below) or possibly a new record of a known taxon; if the latter, it might be a recently introduced species which would explain the lack of previous findings as many small exotic crustaceans have been found in the Mediterranean recently (Galil, 2011; Ros et al., 2014). Further sampling will be necessary to allow for a complete taxonomic description, and to assess its ecology and actual distribution.

The Mediterranean specimens were not fully mature and had some damaged body parts. In particular, there are broken cephalic appendages and uropods are missing. This prevented a full assessment of whether they may represent a new record or a new species. Most previous records of *Paranebalia* have been attributed to *P. longipes*; on the contrary, *P. belizensis* Modlin, 1991 (from Belize) and *P. ayalai* Escobar Briones & Alcocer Durand, 2004 (from Mexico) have never been found after their original description. Mediterranean specimens mostly differ from *P. ayalai* in having serrations on the protopod of pleopods I-IV and a pleopod I endopod that is clearly longer than the exopod; *P. belizensis* bears fewer teeth on the lateral flange of the antenna I fourth article (7-10), the proximal article of maxilla II endopod is comparatively much shorter than the distal one, and the endopod is only slightly longer than the exopod (Modlin, 1991).

To add to the confusion, there is no accurate diagnosis of *P. longipes*; the description provided by Sars (1887) after re-examination of type material mostly focuses on characters valid today only at the generic level (only the type species was known at that time). Descriptions of other specimens attributed to this species around the world differ in the number of teeth on the antennular lateral flange, the number of articles of the antennae I and II flagella, and proportions of the maxilla II endopod (see Song et al., 2012). Some characters (e.g. number of articles or setae) also show variation with age (Dahl, 1985) and therefore do not seem reliable for species differentiation unless mature females are available. Some real differences may, however, be noted between Mediterranean and type locality specimens of *P. longipes*: for instance, these specimens show 13-20 teeth on the antennular lateral flange. This number is greater than that of mature female and immature specimens of P. longipes from the type locality (12-14, redescription by Sars, 1887), while the upper limit of the range (20) is closer to that of Korean and Pacific specimens (19-23: Thiele, 1904; Song et al., 2012). However, the number and distribution of setae of the third peduncle article of antenna II differ from those of P. longipes sensu Song et al. (2012), mostly at the distal third of third article (cf. Song et al., 2012: fig. 2F) and the proximal article of the maxilla II endopod is 0.6 times the length of the distal one in Korean specimens; furthermore, Mediterranean specimens have an antenna II which is provided with setae distally curved with a comb of short teeth (dcs, detail in Fig. 2B) that apparently are not present in those from Korea. Female specimens from the Indian Ocean (south Indian Ocean: Ledoyer, 1994; Madagascar and Mauritius: Ledoyer, 2001) also differ from Mediterranean specimens in the number of teeth of antennular lateral flange (up to 13) and proportions in length of articles of maxilla II endopod (0.56). Finally, the subspecies *P. longipes neocaledoniensis* also bears fewer teeth (7-9) on the antennular lateral flange; furthermore, the maxilla II endopod is only slightly longer than the exopod. Therefore, our record of Paranebalia sp. in the Mediterranean strengthens what was already claimed by Roccatagliata et al. (2010), that a revision of the genus is needed in order to delineate the characters that may serve to separate species, and to assess whether P. longipes is a

cosmopolitan species or is a complex of several distinct, as yet undescribed species, as already found for *Nebalia* (Dahl, 1985; Moreira *et al.*, 2009).

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