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Davide Iacofano & Sabrina Lo brutto

To cite this article: Davide Iacofano & Sabrina Lo brutto (2017) Parhyale plumicornis (Crustacea: Amphipoda: Hyalidae): is this an anti-lessepsian Mediterranean species? Morphological remarks, molecular markers and ecological notes as tools for future records, Systematics and Biodiversity, 15:3, 238-252, DOI: [10.1080/14772000.2016.1248519](https://doi.org/10.1080/14772000.2016.1248519)

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Research Article

Parhyale plumicornis (Crustacea: Amphipoda: Hyalidae): is this an anti-lessepsian Mediterranean species? Morphological remarks, molecular markers and ecological notes as tools for future records

DAVIDE IACIOFANO & SABRINA LO BRUTTO 

Department STeBiCeF, Section of Animal Biology, via Archirafi 18, University of Palermo, Palermo, Italy

(Received 21 September 2015; accepted 7 September 2016)

Hyalid amphipods living in coastal marine habitats are frequently included in ecological studies. The systematics of this taxon has been subject to profound changes, with an emphasis on the North Pacific fauna. Since a proper species delimitation is a prerequisite in taxonomic and ecological studies, *Parhyale plumicornis* (Heller, 1866) has been herein re-described, showing the criticisms and mismatches of various characters, which were previously used in dichotomous keys. This species was collected for the first time off the western coast of Sicily Island (Italy: central Mediterranean Sea). The male is peculiar, due to the second antennae heavily setose posteriorly and bearing long tufts of plumose ventral setae. In this paper, the species will be illustrated, and morphological polymorphism, molecular tags and ecological features will be reported. The species does not appear to be frequent in the Mediterranean Sea but it is important that marine biologists identify it accurately. A recent record, possibly ascribable to *Parhyale plumicornis* in the Red Sea, could indicate that this species is the first anti-lessepsian amphipod, which has migrated from the Mediterranean Sea towards the Red Sea.

<http://www.zoobank.org/urn:lsid:zoobank.org:pub:770BEDA1-3E06-464F-9D34-8AFE43592FCA>

Key words: Amphipoda, Hyalidae, Mediterranean Sea, mtDNA, *Parhyale plumicornis*

Introduction

The hyalids have been placed under the family Talitridae Rafinesque, 1815 for a long period of time (Barnard, 1972; Griffiths, 1974, 1976). This classification was maintained until 1993 by several authors (e.g. in Ruffo, 1993), although Bulycheva (1957) had revised the family Talitridae Rafinesque, 1815 and erected it to superfamily Talitroidea *sensu* Stebbing, 1906, moving part of it into two new families: Hyalidae Bulycheva, 1957 and Hyaellidae Bulycheva, 1957. The status of the family Hyalidae is now widely accepted under the superfamily Talitroidea *s.s.* Rafinesque, 1815. Hyalidae is distributed in the Mediterranean area together with other three talitroid families: Dogielinotidae Gurjanova, 1953; Phliantidae Stebbing, 1899; Talitridae Rafinesque, 1815 (Bousfield & Hendrycks, 2002; Lowry & Myers, 2013; Serejo, 2004). Bousfield and Hendrycks (2002) revised the family Hyalidae,

thereby creating new sub-families, new genera and new species from the northern Pacific Ocean; they also proposed new taxonomic keys, however lacking a focus on the Mediterranean region, where an updated specific key is currently absent.

In the Mediterranean Sea the hyalid genera mainly occur in intertidal (e.g. *Parhyale*) and shallow marine habitats (e.g. *Hyalé s.l.*). In particular, *Parhyale* Stebbing, 1897 includes three species: *P. plumicornis* (Heller, 1866), *P. eburnea* Krapp-Schickel, 1974 and *P. aquilina* (Costa, 1857) (Christodoulou, Paraskevopoulou, Syranidou, & Koukouras, 2013; Ruffo, 1993). Recently, a new species *Parhyale taurica* Grinstov, 2009 has been described from the Crimea coastal zone (Black Sea). However, the language (Russian) used to describe this species limited its acceptance, and the iconography displayed a *P. aquilina*-like dactylus of gnathopod-1, i.e., widened and strongly curved. This species should, therefore, be confirmed as a valid species.

The genus *Parhyale* Stebbing, 1897 has six main characters: the first antennae are longer than the

Correspondence to: Sabrina Lo Brutto. E-mail: sabrina.lobrutto@unipa.it

peduncle of the second antennae; the first maxilla with the one-jointed palp which does not extend beyond the distal margin of the outer plate; maxillipeds have a four-jointed palp; both pairs of gnathopods are subchelate, and these differ between the two sexes; the third uropods carry a minute inner ramus or small scale; and the telson is bipartite.

In their review of the family Hyalidae, Bousfield and Hendrycks (2002) described the new genus *Ptilohyale* as morphologically similar to *Parhyale*, and included two distinctive characters: (i) *Ptilohyale*: heavily plumose second antennae in both sexes, starting on the fifth peduncular segment; and a distomedial spine on the peduncle of the first uropods; (ii) *Parhyale*: when heavily plumose second antennae, setae starting on the fourth peduncular segment; and a distolateral spine on the peduncle of the first uropods. However the similarity between the two genera and various minor mistakes in Bousfield and Hendrycks (2002) (such as mentioning the species *Parhyale plumicornis* in *Ptilohyale*'s species-list, although *Parhyale plumicornis* was left in the *Parhyale* key to species) has caused confusion with some authors (Bakir, Sezgin, & Katagan, 2010; Bellan-Santini & Costello, 2001; Christodoulou et al., 2013; Lowry, 2015; Ruffo, 2010), who incorrectly placed *Parhyale plumicornis* in synonymy with *Ptilohyale plumicornis*, the latter *nomen dubium*.

Recently *Parhyale plumicornis* has been misidentified with *Parhyale explorator* Arresti, 1989 (Bakir, Katağan, & Sezgin, 2008; Bakir et al., 2010), and this collection was initially reported as a new record of alien species in the Mediterranean Sea. The case of the erroneous identification of *P. explorator*, now under the genus *Ptilohyale* (Bousfield & Hendrycks, 2002), subsequently corrected by the authors (Bakir, Katağan, & Sezgin, 2013), caused a cascade-effect throughout the literature. The following papers reported the invasive alien species in Mediterranean, although this assertion could not be substantiated: Bakir et al., (2010), Christodoulou et al. (2013) and Faasse (2014).

The dichotomous key to the *Parhyale* species, which was performed by Bousfield and Hendrycks (2002), and that previously performed by Arresti (1989), are mainly based upon characters which, in this study, have been ascribed to an intra-species polymorphism which was observed in *P. plumicornis*, species endemic to the Mediterranean Sea.

In order to clarify presumptive mismatches, a population from the central Mediterranean (southern Italy) of *Parhyale plumicornis* was examined, compared with specimens (which had been deposited at the Natural History Museum of Verona, Italy) and to specimens from the coast of Turkey (eastern Mediterranean), as sampled by Bakir et al. (2008, 2013). In support of morphological identification, COI and 16S mitochondrial genes were sequenced and observations relating to behaviour were reported.



Fig. 1. *Parhyale plumicornis* (Heller, 1866). Sampling localities of specimens examined, within Mediterranean Sea. (A) Lagoon of Venice (Italy); (B) Lagoon Stagnone di Marsala, and Trapani, western Sicily Island (Italy); (C) Iskenderun Bay (Turkey).

Materials and methods

Sampling

The study was based upon material which had been collected from the rocky, intertidal zone at the Stagnone of Marsala (37°55' 03"N, 12°28'11"E) and the coast of Trapani (38°3'6"N, 12°33'18"E) (Sicily, southern Italy), in March 2012, June 2013 and July 2014 (Figs 1 and 2). *Parhyale plumicornis* (Fig. 3) specimens were collected in association with *P. aquilina*. The samples were collected using hand-nets and were then carefully transferred into plastic containers and fixed in 95% ethanol. A male and a female specimen were requested from the collection of the Museum of Natural History of Verona (Italy); those specimens had been sampled in July 1999 in Venice (northern Adriatic basin; 45°26'38"N, 12°19'52"E) (Fig. 1). A further specimen, belonging to a population previously recorded by Bakir et al. (2008, 2013), was received by Prof. Murat Sezkin (Sinop University) from



Fig. 2. Habitat where *Parhyale plumicornis* (Heller, 1866) has been collected (western Sicily Island, Italy).



Fig. 3. Adult male of *Parhyale plumicornis* (Heller, 1866) collected at the Stagnone of Marsala (southern Italy, central Mediterranean Sea).

the Turkish coast (36°54'22"N, 35°58'05"E; eastern Mediterranean Sea) (Fig. 1). All the specimens were examined at the University of Palermo and then deposited at the Zoological Museum of the University of Palermo with the voucher number MZPA-AMPH-0001. During sampling, various aspects of the behaviour of *Parhyale plumicornis* were observed in the field, and specimens were collected in July 2014 for further observations in the laboratory. The specimens were transferred into plastic containers, using cool-bags, and relocated to an aquarium with marine water and stones; their behaviour was recorded on video, using Finepix S1800 (Fujifilm) (see online supplemental material, which is available from the article's Taylor & Francis Online page at <http://dx.doi.org/10.1080/14772000.2016.1248519>).

Iconography

One hundred and fifty-four (74 males, 80 females) specimens from the four Sicilian sites (three at the Stagnone of Marsala lagoon and one off the Trapani coast) were examined under a stereo-microscope. The specimens were then placed on graph paper and photographed (Finepix S1800, Fujifilm) in order for accurate measurements to be taken. Subsequently, the length of specimens was measured from head to the apex of the telson, using ImageJ software (Rasband, 2008). The flagellum articles of the first and second antennae were counted for all specimens. Finally, the setae arrangement on the ramous of the third uropods was observed. Three males and three females were selected for dissection. The appendages of the dissected specimens were examined, and drawings were executed using a Leica 4000B light microscope with camera lucida.

DNA extraction and amplification of the COI and 16S gene

Total genomic DNA extraction, performed using the DNeasy tissue kit (Qiagen, Valencia, CA, USA), was carried out on fixed specimens, after having dried them on paper. Two mitochondrial markers were amplified using universal primers. A 621-bp fragment of the mitochondrial (mt) cytochrome oxidase subunit I (COI) was amplified, using the LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGT-GACCAAAAAATCA-3') primer pairs (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). PCR was performed in a 25 μ L volume, containing: 1 \times reaction buffer (200 mM (NH₄) SO₄, 100 mM Tris HCl pH 8.8, 0.1% (v/v) Tween), 4 mM MgCl₂, 0.2 mM dNTP, 2.5 U *Taq* polymerase (Bioron GmbH, Germany), 1 μ M of each primer, and 80–100 ng of the DNA template. A 470-bp fragment of the target mitochondrial (mt) 16S ribosomal RNA (16S) was amplified using the 16sar-L (5'-CGCCTGTTTAT-CAAAAACAT-3') and 16sbr-H (5'-CCGGTCTGAACT-CAGATCACGT-3') primer pairs (Palumbi, 1996). PCR was performed in a 25 μ L volume, containing: 1 \times reaction buffer, 4 mM MgCl₂, 0.2 mM dNTP, 2.5 U *Taq* polymerase (Bioron GmbH, Germany), 1 μ M of each primer, and 80–100 ng of the DNA template.

Cycling conditions for PCR amplifications consisted of an initial 95°C denaturation step for 5 minutes, followed by 35 cycles of 60 s at 95°C, 60 s at 50°C (16S-rRNA) or 46°C (COI), and 60 s at 72°C, with a final extension at 72°C for 8 min and a final cooling at 4°C. The resulting amplified DNA fragments were purified with the QIAquick PCR Purification Kit (Qiagen, Germany); sequencing was performed by Macrogen Inc., Amsterdam, Europe, utilizing ABI 3730 XL automated sequencers (Applied Biosystems). The sequences were deposited in the GenBank database (Bilofsky & Christian, 1988) with the following accession numbers (A.N.): KU565875, KU565876, KU565877, KU565878 and KU565879.

The mitochondrial sequences of *Parhyale plumicornis* were then compared with sequences which had been downloaded from the GenBank (<http://www.ncbi.nlm.nih.gov>) database (see Tables 1 and 2 for details): two sequences of *Parhyale hawaiiensis* (Dana, 1853) A.N. AY639937 (Cook, Yue, & Akam, 2005), A.N. EF989709 (Browne, Haddock, & Martindale, 2007), and four sequences of *Parallorchestes cowani* Bousfield & Hendrycks, 2002 A. N. JX545443-44-70-71 (Best & Stachowicz, 2013).

The sequence analyses were performed with MEGA version 6 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Nucleotide sequences were aligned by using the ClustalW model (Thompson, Higgins, & Gibson, 1994) with default settings. A molecular analysis was performed using Kimura-2-Parameter distance model (K2P; Kimura, 1980). Unrooted Neighbour-Joining (NJ;

Table 1. Estimates of pairwise genetic divergence between 16S sequences of species downloaded and (*) herein sequenced. Analyses were conducted using the Kimura 2-Parameter model (K2P). Intra-species distance values in bold. (hap., haplotype; A.N., GenBank accession number.)

	<i>P. plumicornis</i> Sicily hap.2* A.N. KU565879	<i>P. plumicornis</i> Sicily hap.1* A.N. KU565878	<i>P. hawaiiensis</i> A.N. AY639937	<i>P. cowani</i> A.N. JX545443
<i>Parhyale plumicornis</i> Sicily haplotype 1* A.N. KU565878	0.005			
<i>Parhyale hawaiiensis</i> A.N. AY639937	0.291	0.291		
<i>Parallorchestes cowani</i> A.N. JX545443	0.343	0.352	0.335	
<i>Parallorchestes cowani</i> A.N. JX545444	0.351	0.351	0.319	0.052

Table 2. Estimates of pairwise genetic divergence between COI sequences of species downloaded and (*) herein sequenced. Analyses were conducted using the Kimura 2-Parameter model (K2P). Intra-species distance values in bold. (A.N., GenBank accession number.)

	<i>P. plumicornis</i> Venice* A.N. KU565875	<i>P. plumicornis</i> Sicily* A.N. KU565876	<i>P. aquilina</i> Sicily* A.N. KU565877	<i>P. hawaiiensis</i> A.N. EF989709	<i>P. cowani</i> A.N. JX545471
<i>Parhyale plumicornis</i> Sicily* A.N. KU565876	0.108				
<i>Parhyale aquilina</i> Sicily* A.N. KU565877	0.223	0.210			
<i>Parhyale hawaiiensis</i> A.N. EF989709	0.184	0.192	0.143		
<i>Parallorchestes cowani</i> A.N. JX545471	0.263	0.277	0.231	0.246	
<i>Parallorchestes cowani</i> A.N. JX545470	0.270	0.282	0.248	0.258	0.070

Saitou & Nei, 1987) trees were built and the nodes were supported by a high proportion (> 90%) of replicates in the bootstrap analysis (Felsenstein, 1985).

Terminology and abbreviations

The general terminology of amphipod morphology followed that commonly found in standard handbooks (Ruffo, 1993). Regarding the terminology of setae and spines, the descriptions of Watling (1989) and Bousfield and Hendrycks (2002) have been followed.

A1: first antenna (antennula). **A2:** second antenna (antenna). **Cx 1–7:** coxal plate of the first to the seventh pereopod. **Ep1–3:** first to third epimeral plate. **UL:** upper lip (labrum). **LL:** lower lip (labium). **Md:** mandible. **Mx1:** first maxilla (maxillula). **Mx2:** second maxilla (maxilla). **Mxp:** maxilliped. **Gn1:** first gnathopod. **Gn2:** second gnathopod. **P3–7:** third to seventh pereopod. **P11–3:** first to third pleopod. **U1–3:** first to third uropod. **T:** telson.

Acronyms for Museums: **MZPA**, Zoological Museum of the University of Palermo (Italy); **NHMW**: Natural History Museum of Wien (Austria).

Results

Shape variation in characters

The following re-description follows the original description by Heller (1866) and Krapp-Schickel (1974), and it focuses on the degree of variation during growth. A variation in the position of the setae on the third uropods and in

the arrangement of the plumose setae of the second antennae in male and female have been observed. In both cases, these characters were modified with the growth of this species. Younger male and female specimens only had apical setae on the third uropods, while the older specimens were characterized by an internal and external dorsal margin carrying setae (Fig. 4). A variation in the arrangement of plumose setae carried on the second antennae was observed. An abundance of plumose setae on the fourth and fifth peduncular segments increased during growth in male and female specimens. In hyper-adult males the articles appeared completely covered, whereas in juveniles the presence of setae on the fourth peduncular segment was not often observed.

Unfortunately, as different cohorts coexist in the same population, a statistically significant correlation between length or discrete size-classes and character shape was searched for, but not observed. However, it cannot be excluded that the transition from one morphotype to another requires more than one moult.

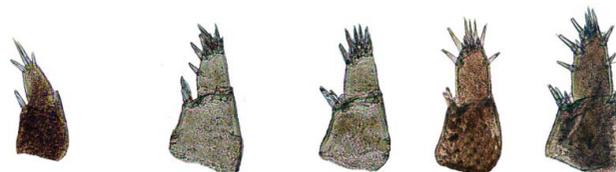


Fig. 4. *Parhyale plumicornis* (Heller, 1866). Different arrangement of setae observed on ramous of the third uropod (U3). Variation in third (U3) uropod, in male and female, from immature (left), to hyperadult (right).

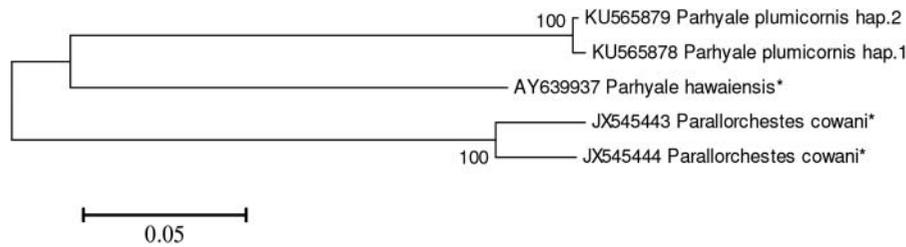


Fig. 5. NJ tree constructed on the K2P model performed with 470-bp 16S sequences, including sequences of the two *P. plumicornis* haplotypes and sequences reference from Genbank (shown with the A.N. and *). The values allocated to the nodes were those calculated on 1000 bootstrap replicates.

Overall, male body length ranged from 5.5 mm to 18.9 mm. The number of the flagellar articles of the first antennae ranged from 9 to 19; the number of the flagellar articles of the second antennae ranged from 13 to 26. The female body length ranged from 4.7 mm to 12.3 mm. The number of the flagellar articles of the first antennae ranged from 8 to 15; and the number of the flagellar articles of the second antennae from 7 to 21. Furthermore, two non-differentiated specimens, found in the marsupium of a pregnant female, measured 1.89 and 2.22 mm in length; they were observed with four flagellar articles in the first and second antennae and devoid of any type of setae. The smallest mature female (i.e., with oostegites) was 4.67 mm length, and the smallest ovigerous female was 5.43 mm length. The plumose setae on the fourth and fifth peduncular segments and on the flagellar articles of the second antennae became more abundant in males and females increasing with growth. The setae in large males (> 10 mm) and females (> 7 mm) were particularly abundant (brush-setae), while in the smallest females the fourth peduncular segment was devoid of plumose setae.

Molecular analyses

In order to support species delimitation, a total of six *P. plumicornis* specimens, five *P. plumicornis* specimens from the Sicily population and one *P. plumicornis* from Ruffo's collection, were sequenced; plus one *P. aquilina*.

With reference to the 16S-rDNA fragment, four Sicilian specimens were successfully sequenced: two juvenile *P. plumicornis* (one male and one female), both with only apical setae in the ramous of the third uropods and a paucity of setae on the second antennae; and two adult *P. plumicornis* (one male and one female), both with setae also on the margin of ramous of third uropods and an elevated abundance of setae on the second antennae (brush-setae). A total of 470 base pairs (bp) of 16S were aligned and compared with reference species; two haplotypes were detected from the Sicilian specimens (haplotype 1 and 2). The K2P model was applied to the dataset: the intra-species divergence was found to range from 0.5% to 5.2%, and the inter-species divergence varied from a minimum value of 29.1 to a maximum value of 35.2% (Table 1, Fig. 5).

A further Sicilian specimen and the one female from Ruffo's collection were successfully sequenced in order to analyse the mtCOI gene. A total of 621 bp of COI were aligned and compared with reference species (Table 2, Fig. 6). The K2P model was applied for all COI barcodes. The intra-species divergence ranged from 7.0 to 10.8%, and the inter-species divergence varied from a minimum value of 14.3 to a maximum value of 28.2%. The genetic distances demonstrated that the Sicilian *P. plumicornis* specimens are co-specific to the *P. plumicornis* from Venice (identified by S. Ruffo). It should be highlighted that the first sequence for *P. aquilina* was performed as part of the research outlined in this paper, and creates its own separate clade.

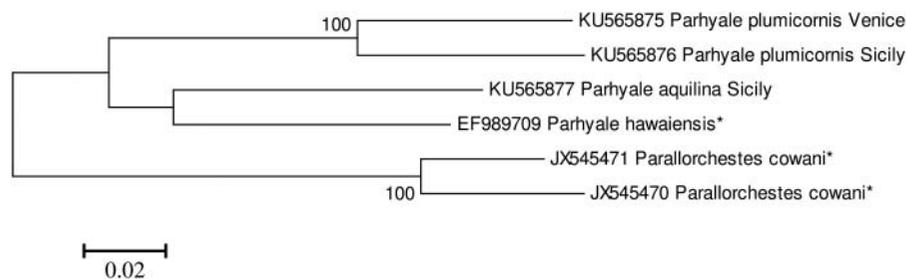


Fig. 6. NJ tree constructed on the K2P model performed with 621-bp COI sequences, including sequences of the *P. plumicornis* Sicily and Venice, *P. aquilina*, and sequences reference from GenBank (shown with the A.N. and *). The values allocated to the nodes were those calculated on 1,000 bootstrap replicates.

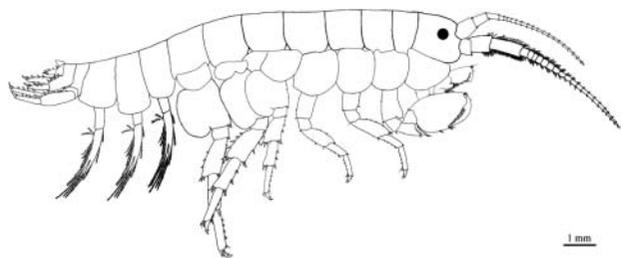


Fig. 7. Iconography of adult male of *Parhyale plumicornis* (Heller, 1866).

Systematics

Suborder Senticaudata Lowry & Myers, 2013
 Infraorder Talitrida Rafinesque, 1815 (Serejo, 2004)
 Superfamily Talitroidea s.s. Rafinesque, 1815
 Family Hyalidae Bulycheva, 1957
 Subfamily Hyalinae Bulycheva, 1957

Genus *Parhyale* Stebbing, 1897; *Parhyale plumicornis* (Heller, 1866) (Figs 7–9); *Nicea plumicornis* Heller, 1866: 5, pl. 1, figs 8–9; *Hyale prevostii* (part) Della Valle, 1893: 519, pl. 16, figs 39–42; *Allorchestes plumicornis* Stebbing, 1899: 412, pl. 33 C. –Stebbing, 1906: 583. –Chevreux & Fage, 1925: 291, fig. 302. –Krapp-Schickel, 1974: 326, pl. 3–4. –Zakaria & Farrag, 2012; *Parhyale plumicornis* –Ruffo, 1993: 757–758, fig. 518; *Parhyale explorer* –Bakir et al., 2008; *Ptilohyale plumicornis* –Bakir et al., 2010. –Ruffo, 2010. –Bellan-Santini & Costello, 2001. –Lowry, 2015. –Christodoulou et al., 2013.

Type material. Lectotype: *Nicea plumicornis* Heller, 1866, female, deposited at NHMW as *Nicaea plumosa* 'Heller Adria [coll.] Heller 1865' (NHMW 20536), subsequently dissected into two microslides (NHMW 21137, 21138) (P. C. Dworschak, personal communication) and drawn by Krapp-Schickel (1974). Further material is deposited at NHMW under *Nicea plumicornis* from Rovinj (northern Adriatic): NHMW 20534, 15 specimens, A.Nr. 1882.II.21 coll. Marenzeller; and NHMW 20535, 15 specimens, A.Nr. 1882.I.43, don. Steindachner.

Type locality. Dubrovnik (Croatia), Adriatic Basin, Mediterranean Sea.

Re-description. Based on material collected at the Stagnone of Marsala (Sicily, southern Italy) 37°55'03''N; 12°28'11''E; intertidal, 0 m, on the heavy substrate of the semi-closed beach; 22 July 2013; hand-collected. Four males and three females (MZPA-AMPH-0001).

Head without rostrum. Lateral cephalic lobe broad and somehow rounded, truncate vertically. Black eyes, medium size and kidney-shaped, or rounded. Body colour

pink, green, yellow or light green. A2 colour red-orange with dense setae brush-like. **Antenna 1** reaching well beyond the posterior margin of the 2nd pereomere, and about 2/3 of A2. Peduncle 3-segmented; flagellum 9–19 segmented, each article with short setae distally, arranged one pair of tooth setae with middle one plumose setae. **Antenna 2** of medium length, peduncle thick, colour red-orange; flagellum 13–26 segmented; in adult males articles 4 and 5 of the peduncle and first 9–12 flagellar articles posteriorly heavily setose, bearing long tufts of plumose ventral setae. In juveniles, article 4 of the peduncle scarcely setose, article 5 of peduncle weakly setose, posteriorly. In hyper-adults, setae much more abundant, and distributed on the ventral margin and internal margin of articles 4 and 5 of the peduncle, appearing as a complete covering of the articles; each article with short setae distally, arranged in one pair of tooth setae with middle one plumose seta. **Upper lip (labrum)** entire rounded, broad, unilobate, apical margin with hair-like setae (tooth setae). **Lower lip (labium)** bilobulate, with wide lobes and shoulders apically abundant tooth setae. Outer lobe truncated apically. **Mandible** without palp; molar process strong, triturative. Lacinia mobilis with 5 teeth; incisor with 5 teeth. **Maxilla 1**, inner plate short, with 2 long plumose apical setae; outer plate broad with 8 strong serrated spines. Palp 1-articulate, constricted in the middle, with 1 short apical seta (Fig. 10). **Maxilla 2**, inner plate with long apical setae; outer plate with 9 slender apical tooth setae and another 9 thin plumose setae; one long medio-ventral plumose seta on the external margin of the outer plates in continuous with spines. Plates sub-equal. **Maxilliped**, inner lobe reaching 1/3 of palp, with 3 apical teeth, and few subterminal setae. Present plumose setae on the inner margin and few setae on the surface; outer plate longer than the inner one, with 2 rows of setae extending from the apex to middle part of the inner margin where they become irregularly arranged and longer. Palp 4-segmented, articles 1 and 2 with few setae; article 3 with long setae on the apico-lateral margin and 2 plumose setae on the outer margin; 4th article unguiform, with terminal setae.

Peraeon. Coxal plate 1, subquadrate, rounded apically, as high as broad and posterior margin excavate with a shelf. **Gnathopod 1** basis broad distally with 2 short setae on the posterior margin and a postero-distal 3 slender setae, with a distal-medio-ventral process (middle part of distal margin), hydrodynamic lobe small; ischium short, with a rounded process on the anterior margin and another medio-ventrally, and a postero-distal tuft of tooth setae and 3 long-slender setae, a very reduced hydrodynamic lobe on the dorsal margin; merus, with postero-distal angle quadrate and slender setae; carpus triangular, with postero-distal hydrodynamic lobe and with 2 rows of strong plumose and serrated setae arranged distally and posteriorly; last 3

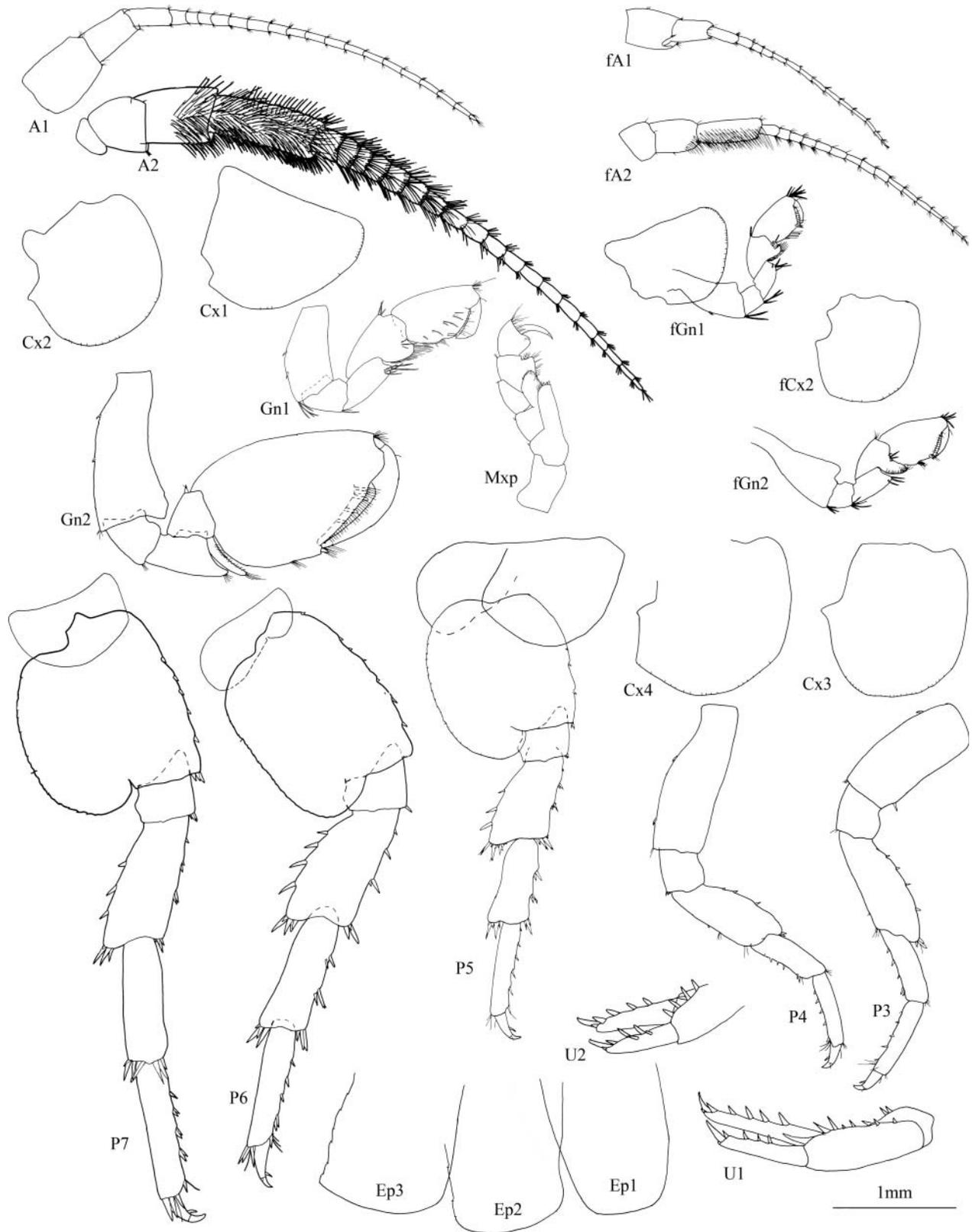


Fig. 8. *Parhyale plumicornis* (Heller, 1866). Iconography of first (A1) and second (A2) antenna; maxilliped (Mxp); first (Gn1) and second (Gn2) gnathopod; first (Cx1), second (Cx2), third (Cx3) and fourth (Cx4) coxae; third (P3), fourth (P4), fifth (P5), sixth (P6) and seventh (P7) pereopods; epimeral plates (Ep1,2,3); and first (U1) and second (U2) uropods in male. First (fA1) and second (fA2) antennae; first (fGn1) and second (fGn2) gnathopods; second (fCx2) coxae in female (f).

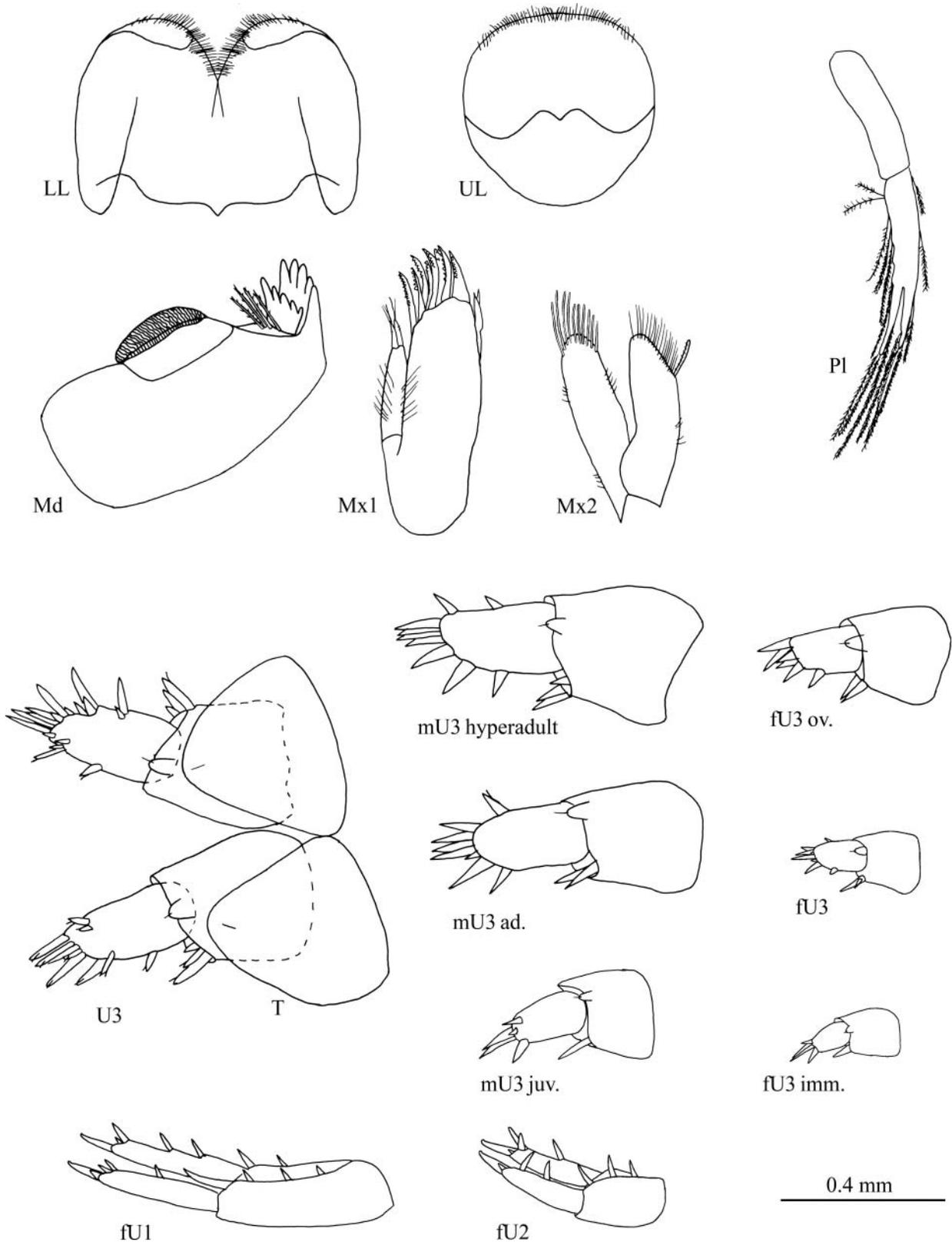


Fig. 9. *Parhyale plumicornis* (Heller, 1866). Mouthparts (LL, UL, Md, Mx1, Mx2), pleopod (Pl), first (U1) and second (U2) uropods in male (m) and female (f), telson (T). Variation in third (U3) uropod in male and female, immature (imm.), juvenile (juv.), adult (ad.), ovigerous (ov.), hyperadult.

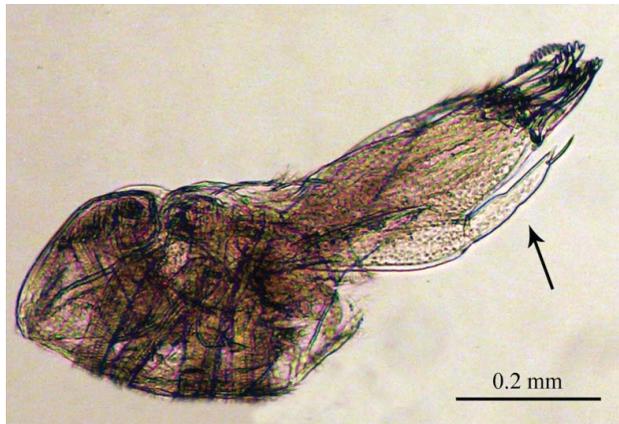


Fig. 10. *Parhyale plumicornis* (Heller, 1866). *Maxilla 1* with palp 1-articulate and constricted in the middle.

setae not plumose. Propodus broad, subquadrate, subchelate; anterior margin naked with a tuft of long setae on the anterodistal edge, the palm margin spiny with numerous short and thin setae, sub-transverse with 2 slightly enlarged setae in tandem at the defining corner, bearing individual plumose setae on the posterior edge, posterior margin with posterior setae which increase in length towards the dactylus. Dactylus short, strong and curved; inner margin with many setae, outer margin with only 1 plumose seta on the basis. **Coxal plate 2**, subquadrate, rounded apically, as high as broad and posterior margin excavate with a shelf. **Gnathopod 2**, basis broad distally, naked on the anterior margin and 2 curved setae on the posterior margin and with a medioventral process, reduced hydrodynamic lobe; ischium short with a rounded process on the anterior margin and another medioventrally; merus with sharp posterodistal angle and with thin and short setae; carpus, small triangular plate, with 2 strong spines distally on the anterior margin, and a pronounced process on the posterior margin, between the merus and the propodus (i.e. hydrodynamic lobe; Fig. 11); propodus ovoid, broad and well developed, anterior margin naked; small depression on anterior margin, near hinge of dactylus, with a few short setae; palm margin spiny with numerous short setae and bearing 2 rows of long and well defined setae, and numerous short setae. Dactylus strong, with short setae on the inner margin, and 1 plumose seta on the outer margin. **Coxal plate 3–4**, subquadrate, rounded apically, higher than broad and with a cusp on the posterior margin. **Peraeopod 3–4**, long and slender, subequal; basis long with naked anterior margin and thin setae on the posterior margin, with a rounded medioventral process; ischium with a rounded process on the anterior margin and another medioventrally; merus with plumose setae and tooth setae on the anterodistal margin, short setae on the anterior margin, and sharp posterodistal angle; carpus with posterodistal margin with row

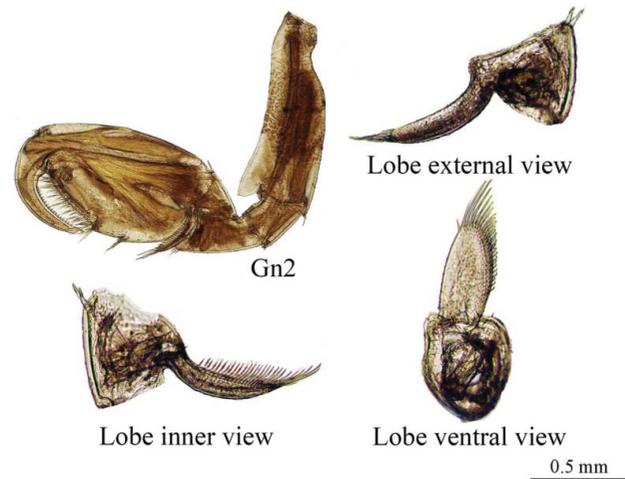


Fig. 11. *Parhyale plumicornis* (Heller, 1866). Male gnathopod 2 (Gn2), and detailed views of its hydrodynamic lobe.

of setae, anterior margin naked, apically a tuft of setae; propodus slender, naked on the anterior margin and with 4 setae on the posterior; dactylus with 1 plumose proximal seta and 1 antero-proximal seta on the anterior margin and 1 setae on the posterior margin. **Coxal plate 5–6**, big, bilobed unequally. **Peraeopod 5–6** robust and spiny on the anterior margin; P5 shorter than peraeopods 6 and 7; P5 basis broad and rounded, with rounded process posteroventral; P6 basis higher than broad with rounded process posteroventral; posterior margin crenulate and anterior one spiny; ischium with rounded process on the posterior margin and another medioventral; merus and carpus broad distally; merus spiny on both margins and especially on the distal part; carpus without setae on the posterior margin, with strong setae distally, and setae on anterior margin; propodus slender and naked on posterior margin, tuft of setae and setae on both distal sides, escalonated setae on the anterior margin; dactylus with only 1 short plumose seta on outer margin and 1 spine on posterior margin. **Coxal plate 7** hemispheric, not very high. **Peraeopod 7** robust and spiny on anterior margin; basis higher than broad; anterior margin spiny; with a rounded process posteroventral; small tubercles on the ischium, merus, carpus and propodus; ischium with rounded process on posterior margin, and another medioventral; merus spiny on both margins and especially on the distal part; carpus naked on posterior margin; propodus slender and naked on posterior margin, and spiny on that anterior with a tuft of long setae posterodistally; dactylus with only 1 short plumose seta on outer margin. **Peraeopod 5–7** basis posterior margin with a single small but distinct indentation or 'notch' where a short thick distally tufted seta ("surge seta" *sensu* Bousfield & Hendrycks, 2002), the surge seta is positioned at different heights among the three peraeopods.

Pleon. Epimeral plate 1–3 rounded antero-distally; crenulated posterior margin; epimeral plate 3 with subquadrate posterodistal angle. **Uropod 1**, peduncle robust and slightly longer than rami, with 2 to 4 dorsal setae on both sides and distolateral peduncular spine-like seta; rami subequal slender, with 1 to 4 dorsal spines, and with a group of spines apically of which the central longer and hooked. **Uropod 2** shorter than uropod 1; peduncle robust and spiny dorsally, 2 to 4 setae on outer and inner margin; rami subequal and spiny, 3 or 4 dorsal setae, and subequal to the peduncle; rami subequal present robust setae group apically. **Uropod 3** shorter than uropod 2; peduncle broad with group of setae apically on the outer side; outer ramus well defined and a little longer than the peduncle; 8–10 contiguous spines apically, also one or two spines in dorsal margin both internally and externally in adult size (Fig. 4); inner ramus poorly defined and fused to peduncle, with 1 apical seta. **Telson** cleft, with triangular lobes and each one bearing 1 dorsal seta.

Sexual dimorphism. All characters of the female overlap with those of the male, except for body size (smaller in female) and various details relating to A1, A2 and Gn1-2. **Antenna 1** reaches well beyond the posterior margin of the second peraeomere, and to approximately 2/3 of antenna 2. Peduncle 3-segmented; flagellum 8–15 segmented, each article with short, distal setae, arranged one pair of tooth setae with middle one plumose setae. **Antenna 2** of medium length, peduncle thick, colour red-orange; flagellum 7–21 segmented; in adult female articles 4 and 5 of peduncle and the first 6–8 flagellar articles heavily setose on the posterior, bearing long tufts of plumose ventral setae. In immature females, article 4 of peduncle without setae, article 5 of peduncle weakly setose on the posterior. **Gnathopods** equal in size and shape to gnathopod 1 of male. **Oostegites** have the characteristic shape (Fig. 12). Such a shape: i.e. interlocking female brood plates, with marginal elongate setae and interlocking with terminal hooks, is a specialized form of brood lamellae, which are present in saltating, intertidal



Fig. 12. *Parhyale plumicornis* (Heller, 1866). Female. The four oostegites, right side.



Fig. 13. *Parhyale plumicornis* (Heller, 1866). Adult female.

hyalid genera. They are a morphological-behavioural adaptation to ensuring safe mechanical retention of large numbers of relatively small eggs within the brood pouch (Bousfield & Hendrycks, 2002). The pre-amplexing notch *sensu* Bousfield and Hendrycks (2002) has been highlighted in the second pereonite after colouration in blue of toluene throughout all the female specimens (Fig. 13); the pre-amplexing notch in the anterodistal margin of the second peraeon segment can be visualized by the darker colouration. The pre-amplexing notch is an indentation only in mature females where the dactyl of gnathopod 1 of males is inserted during pre-copulatory behaviour. The notch in *Parhyale* is usually a shallow indentation in a slightly ventrally extended anterior lower lobe of the peraeon segment 2 (Bousfield & Hendrycks, 2002).

Habitat

Parhyale plumicornis has been reported in the intertidal on the rocky, gravel, and sandy semi-closed beaches, hidden from waves and direct exposure to solar radiation. The sampling sites reported in this paper included both categories of the biocoenoses on the intertidal soft substrates of Mediterranean shores, as described by Pérès and Picard (1964): (i) *rapidly drying sediments*, including sand, in which invertebrates can burrow, and (ii) *slow-drying sediments* where the sediment is covered by plant debris (*banquette* of *Posidonia oceanica*) or by cobbles and/or boulders and where desiccation is slow. When inhabiting on cobbles and boulders, it was observed that *P. plumicornis* positioned itself only under those stones which connected with a humid substrate (bottom-face).

Various aspects relating to the behaviour of *P. plumicornis* have been observed in the field and laboratory. *Parhyale plumicornis* was particularly abundant in a restricted space, ~100 specimens per dm² (pers. obs.). In the laboratory, the specimens were kept in an aquarium with seawater, stones and *P. oceanic* leaves, the latter the only source of organic



Fig. 14. Distribution of *Parhyale plumicornis* (Heller, 1866), with dates of record.

Mediterranean Sea: 1866, Dubrovnik (Croatia); 1893, Napoli (Italy); 1911, Annaba (Algeria); 1925, Sète, Cannes, Villefranche-sur-Mer, Beaulieu-sur-Mer (France); 1946, Rovinj (Croatia); 1950, Venice (Italy); 2010, Koukouras (Greece); 2013, Iskenderum Bay (Turkey); 2013, Stagnone of Marsala, Trapani (Italy). **Red Sea:** 2007, Hurghada and Safaja coast (Egypt).

matter. After four days, ~70% of individuals had died; the remaining specimens survived for four months, locating themselves predominately under stones.

In order to ascertain if the *P. plumicornis* species can move on dry sand, individuals were placed onto this substrate. The specimens were completely covered by dry sand, similar to breadcrumb meat; they were unable to move the pleopods and subsequently died (see supplementary material online – movie). This species is a primitive saltatory *sensu* Bousfield and Hendrycks (2002). In order to clarify this feature, we placed some specimens on wet rock, and observed if they were able to jump. The specimens did not often jump or, if they did, the leaps were very short (~2 cm). Finally, field and laboratory observations concluded that this *P. plumicornis* species prefer to swim laterally on one side on a water film over wet stones.

Distribution of *Parhyale plumicornis*

Parhyale plumicornis is considered to be endemic to the Mediterranean Sea (Fig. 14). It was described in 1866 at Dubrovnik (Croatia: northern Adriatic Sea) (Heller, 1866) and subsequently recorded off: the coast of Naples (Italy: Tyrrhenian Sea) by Della Valle (1893); the Annaba coast (Algeria) by Chevreux (1911); at Sète, Cannes, Villefranche-sur-Mer, Beaulieu-sur-Mer (France) by Chevreux and Fage (1925); Rovinj (Croatia) by Ruffo (1946); Venice (Italy: northern Adriatic Sea) by Giordani-Soika (1950); Greece by Koukouras (2010); Iskenderum Bay (Turkey) by Bakir et al. (2013); and more recently along Marsala and the coastline of Trapani (Italy: central Mediterranean). It is of note that a 2007 survey recorded a very high density of *Parhyale plumicornis* species in Hurghada and on the Safaja coast (Red Sea) under the name

Allorchestes plumicornis (Heller, 1866), senior synonymous of *P. plumicornis*, by Zakaria and Farrag (2012).

Discussion

Parhyale plumicornis shows abundant plumose setae on the second antennae and displays a polymorphism of their abundance, scarce to particularly abundant (brush-setae). During growth the species increases the number of setae on the peduncular and flagellar articles of the second antennae. It has also been observed that the setae on the third uropods have different positions. Some juveniles only display apical setae, while the adults possess setae which are apical and on the margins of the ramous of the third uropods. We contend that this polymorphism has established that the dichotomous key based upon the position and number of setae in third uropods (Arresti, 1989) is a weak scheme in the taxonomy of the genus.

The morpho-taxonomic identification of the *P. plumicornis* morphotypes was verified by screening COI and 16S mitochondrial DNA sequences. The 16S analysis showed low divergence values among specimens of analysed *P. plumicornis*, thereby indicating natural intra-specific variability. This thus confirms that the arrangement of setae varies with growth within this species. Further, the molecular analysis on the COI sequences discriminated *P. plumicornis* from other species, thereby supporting the genetic cohesion of the species. Few authors have performed COI sequences on hyalid species, focusing on species delimitation. Here, DNA barcoding has confirmed the co-specificity of diverse morphological forms and demonstrated its efficacy as a tool with which to integrate classical taxonomy for species diagnosis. Descriptions of species can benefit from such integrative taxonomy.

A careful examination of the literature has highlighted various minor incongruities with the morpho-anatomic features of the *P. plumicornis* species. Krapp-Schickel (1974) has drawn the second antennae of *P. plumicornis* with dorsal plumose setae while this species displays plumose setae on the ventral margin of second antennae. Bousfield and Hendrycks (2002) have described the *Parhyale* genus with (i) the ramous of third uropods only with apical setae, while this character has been shown to be variable in this paper (Fig. 4); and (ii) the carpal lobe of second gnathopods in males is weak or lacking, in contrast to the well-developed lobe in specimens mentioned in this paper (Fig. 11).

The *Parhyale* genus includes 15 species, eight of which have never been recorded elsewhere, excluding their *locus typicus*, and subsequently their description. Three species are distributed throughout the Mediterranean Sea and Table 3 lists all the *Parhyale* species. *Parhyale plumicornis* is a species which is endemic to the Mediterranean Sea, and any recordings of its presence outside this basin

Table 3. *Parhyale* species distribution

<i>Parhyale</i> species	Distribution	Reference
<i>Parhyale aquilina</i> (Costa, 1853)	Mediterranean Sea and NE Atlantic Ocean	Ruffo ed., 1993
<i>Parhyale basrensis</i> Salman, 1986*	Iraq Coast – Persian Gulf	Salman, 1986
<i>Parhyale darvishi</i> Momtazi & Maghsoudlou, 2016*	Persian Gulf and the Gulf of Oman	Momtazi & Maghsoudlou, 2016
<i>Parhyale eburnea</i> Krapp-Schickel, 1974	Mediterranean Sea (endemic)	Guerra-García, Cabezas, Baeza-Rojano, Espinosa, & García-Gómez, 2009
<i>Parhyale fascigera</i> Stebbing, 1897	Cosmopolitan in warm-temperate waters	Shoemaker, 1956
<i>Parhyale hachijoensis</i> Hiwatari, 2002*	Japan coast – NW Pacific Ocean	Hiwatari, 2002
<i>Parhyale hawaiiensis</i> (Dana, 1853)	Cosmopolitan in warm-temperate waters	Serejo & Sittrop, 2009 and references therein
<i>Parhyale inyacka</i> K.H. Barnard, 1916	Cosmopolitan in warm-temperate and tropical waters	Griffiths, 1974
<i>Parhyale iwasai</i> (Shoemaker, 1956)*	Formosa and Kyusyu – East China Sea	Iwasa, 1939; Shoemaker, 1956
<i>Parhyale kurilensis</i> Iwasa, 1934*	Urup Coast – Sea of Okhotsk	Iwasa, 1934
<i>Parhyale micromanus</i> Ren, 2012*	China Sea	Ren, 2012
<i>Parhyale multispinosa</i> Stock, 1987	Canarie Island coast – NE Atlantic Ocean	García, Palmero, del Carmen Brito, Núñez, & Worsaae, 2009 and references therein
<i>Parhyale penicillata</i> Shoemaker, 1956*	Baja California Sur – Gulf of California	Shoemaker, 1956
<i>Parhyale philippinensis</i> Hiwatari, 2002*	Philippines coast – NW Pacific Ocean	Hiwatari, 2002
<i>Parhyale plumicornis</i> (Heller, 1866)	Mediterranean Sea	This study

* Species recorded only for their Type Locality.

are probably due to inaccurate identification as a result of the morphological confusion mentioned in this paper.

Iwasa (1939) has recorded the presence of *Allorchestes plumicornis* on Japanese coasts (Pacific Ocean). And although the species is a senior synonym of *P. plumicornis*, the iconography of Iwasa describes setae only on the fifth peduncular segments of the second antennae, which is a diagnostic character for the *Ptilohyale* genus. There exist doubts about the observations of Ivanova, Belogurova, and Tsurpalo (2008): they do not include iconography or photographs of the specimens or any information relating to identification. However, the authors may have mistaken the identification of one of the *Ptilohyale* species with *P. plumicornis*. Another doubtful record is that by Christodoulou et al. (2013). They have reported a species list where *P. plumicornis* has been recorded in the Atlanto-Mediterranean area even if there is no mention made in the literature about this report.

Finally, a recent record of *Allorchestes plumicornis* (senior synonym of *P. plumicornis*) in the Red Sea (Zakaria & Farrag, 2012) can be confirmed and it does not conflict with any taxonomic evaluation. The authors have identified specimens in accordance with the dichotomy keys of Barnard (1971) and Chevreux and Fage (1925); here the *Parhyale hawaiiensis* species (already recorded in the Red Sea area) and *A. plumicornis* (a senior synonym of *P. plumicornis*) have been described. A hypothesis of an incorrect identification of *P. hawaiiensis* with *P. plumicornis* can be excluded as the former lacks brush setae on the peduncular segment of second antennae, which is a diagnostic character for *P. plumicornis*. It is difficult to extend this collection as ascribable to other species. Any confirmation of such an event would indicate *Parhyale plumicornis* as the first anti-lessepsian amphipod to have migrated from the Mediterranean Sea towards the Red Sea.

Conclusions

The Mediterranean Sea is a geographic area characterized by marked diversity. As the majority of the intertidal organisms experience strong thermal stress throughout the year, they can be used to study the structural complexity of this habitat (Helmuth et al., 2006). Recent studies have demonstrated the crucial role of intertidal communities in the evaluation of climate change (Sarà et al., 2014), and it is thus important to improve our assessment of the delimitation and distribution of the various species inhabiting this sea. *Parhyale plumicornis* is considered to be endemic to the Mediterranean Sea, albeit with a fragmented range. Consequently, this species may have been overlooked as an interest of research and its geographic pattern should be revised. Species identification is a central plank to ecology and conservation planning, and erroneous detections of species can cause a cascade effect on

databases, checklists, and papers. Thus, a continuous monitoring of habitats (and literature) is advisable in order to improve our understanding of the manifold ecological processes which characterize the Mediterranean Sea.

Acknowledgements

The authors are grateful to Dr Leonardo Latella, who provided laboratory facilities and access to the 'Sandro Ruffo' collection at the Museum of Natural History of Verona; to Professor Murat Sezgin for providing the authors of this paper with a specimen of *P. plumicornis* from Turkey; to Dr Peter C. Dworschak, Curator Crustacea collection of the Natural History Museum of Wien; and to the anonymous reviewers who provided valuable suggestions during revision of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the University of Palermo (Grant FFR 2012/2013).

Supplemental data

Supplemental data for this article can be accessed here: <http://dx.doi.org/10.1080/14772000.2016.1248519>.

ORCID

Sabrina Lo brutto  <http://orcid.org/0000-0002-9964-904X>

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Associate Editor: Elliot Shubert