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The *Hyalella* species flock of Lake Titicaca (Crustacea: Amphipoda): perspectives and drawbacks of DNA-based identification

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Abstract

Lake Titicaca, in the High Andes of Perú and Bolivia, harbours the world's third most speciose ancient-lake amphipod radiation on record. A minimum of nineteen species of *Hyalella* derived from at least five independent colonization episodes concentrate in this high altitude water body, although the actual species number present has not yet been established and could be much higher. Herein, we take advantage of the description of three new species (*H. krolli*, *H. gonzalezi*, and *H. hirsuta*) and the re-description of other two (*H. solida* and *H. nefrens*) to assess the feasibility of adopting a DNA-based identification approach to

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resolve the magnitude of this highly speciose amphipod assemblage. A Bayesian phylogenetic analysis of the evolutionary relationships among South American *Hyalella cox1* haplotypes, including those of four out of the five species dealt with herein, shows a great disagreement between taxonomic units delimited under morphological and genetic data, hampering species identification exclusively based on *cox1* DNA barcode sequences.

Keywords

ancient lakes – Altiplano – Andes – South America – Titicaca – DNA barcoding – integrative taxonomy

Introduction

At 3,810 m elevation in the Andean Altiplano of Perú and Bolivia, Lake Titicaca harbours one of the few cases of a large endemic species flock on record among lacustrine amphipod crustaceans. Only the Caspian "Sea" – placed at the southeast boundary between Europe and Asia – and Lake Baikal in southern Siberia, show higher levels of species richness, whereas other two ancient lakes, Lake Ohrid in the Balkans and Lake Fuxian in Junnan, China, show comparable numbers. All these lakes depart from the ordinary condition of continental waters, where amphipods do not reach the diversity and ecological disparity found in marine habitats.

In the Caspian (371.000 km^2, 1.025 m maximum depth), the amphipod assemblage comprises 91 species distributed among 41 genera and ten families (Sars, 1894, 1896; Grabowski, 2014; Krapp-Schickel & Sket, 2015; Copilas-Ciocianu & Sidorov, 2021). This faunistic array is very heterogeneous in terms of its phylogenetic composition and includes, aside primarily freshwater Gammaroids, representatives of at least five well-established and highly divergent marine families: Uristidae Hurley, 1963 (*Onisimus* Boeck, 1871); Melitidae Bousfield, 1973 (*Melita* Leach, 1814); Pontoporeiidae Dana, 1852 (*Monoporeia* Bousfield, 1989); Corophiidae Leach, 1814 (*Corophium* Latreille, 1806; *Chelicorophium* Bousfield & Hoover, 1997); and Gammaracanthidae Bousfield, 1989 (*Gammaracanthus* Spence Bate, 1862). Furthermore, two additional families – Caspicolidae Birstein, 1945 and Behningiellidae Kamaltynov, 2002 – are of uncertain phylogenetic affinity, although it is very likely that they are nothing but highly specialized Gammarids phylogenetically nested within the Ponto-Caspian clade (see Copilas-Ciocianu & Sidorov, 2021). In any event, most of the Lake's species diversity (70 species in 29 genera) corresponds to primarily freshwater forms (Gammaridae Leach, 1814; Pontogammaridae Bousfield, 1977; Niphargidae Bousfield, 1977).

In the Baikal (31.722 km^2, 1.642 m maximum depth) – where no amphipods of direct marine derivation occur – there are around 354 species recorded thus far distributed among 39 genera and seven families (Takhteev, 2019). This remarkably high diversity represents about 5% of the grand total of freshwater amphipods known worldwide (Takhteev, 2000). Despite their broad morphological and ecological disparity (see below), molecular phylogenies suggest that all Baikalian taxa derive from *Gammarus* Fabricius, 1775 (Gammaridae) and appear nested within this genus (Hou & Sket, 2016).

Lake Ohrid (358 km^2, 289 maximum depth), between Macedonia and Albania,

harbours at least 17 taxa belonging to three primarily freshwater families: Crangonyctidae Bousfield, 1973 (one species of *Synurella* Wrzesniowski, 1877), Niphargidae (five species of *Niphargus*) and Gammaridae (11 morpho-species of *Gammarus*). All these taxa except two of the *Gammarus* are endemic to the lake and its shore springs (Grabowski et al., 2017).

Finally, Lake Fuxian (212 km², 150 m maximum depth) in Yunnan (SW China) harbours a (modest) cluster of five Anisogammaridae Bousfield, 1977, including the monotypic *Eurypodogammarus helobius* Hou, Morino & Li, 2005 and *Fuxiana yangi* Sket, 2000, plus three species of *Fuxigammarus* Sket & Fiser, 2009. Both *Fuxiana* and *Fuxigammarus* are endemic to the lake, whereas the *Eurypodogammarus* species has been recorded also in neighbouring areas (Sket & Fiser, 2009).

The Titicaca (8.300 km², 281 m maximum depth) shows lower amphipod diversity than the aforementioned two larger lakes since it is geologically younger and occupies a considerably smaller surface. All taxa found here are unambiguously assignable to *Hyalella* S.I. Smith, 1874 (Fam. Hyalellidae Bulycheva, 1957), which otherwise is the only genus of epigean amphipod known to occur in continental waters of South America. Furthermore, ecological disparity among these species has not proceeded as far as in the Caspian or the Baikal. No truly gigantic or dwarf forms, commensals of other invertebrates, brood parasites of other amphipods, permanent burrowers, bentho-pelagic migrants, or fully nektonic pelagic species similar to those found in the two aforementioned lakes, have been recorded thus far in the Titicaca (Barnard & Barnard, 1983; Takhteev, 2000).

The number of species present in the Titicaca has not been precisely established, although estimations by different specialists suggest it should not exceed of a hundred species (Bousfield, 1982; Hoffman, 2006; Crawford et al., 1993). Nineteen species have been recorded thus far, of which 15 are apparently endemic (Faxon, 1876; Chevreux, 1904, 1907; Weckel, 1910; González & Watling, 2001, 2003; González & Coleman, 2002; Coleman & González, 2006). This number represents about one-quarter of the total *Hyalella* found in the Neotropics, where about 74 species are known to occur.

The *Hyalella* of the Titicaca are remarkable in their morphological disparity, largely due to the presence of species with a strongly armoured body integument. These "processiferous" forms – with spines, keels and/or flanges developed on body tergites and pereiopodal coxal plates – recall the aspect of members of some typical marine families such as the Epimeriidae Boeck, 1871, Iphimediidae Boeck, 1871, Dexaminidae Leach, 1814 or Atylidae Lilljeborg, 1865, but are rarely reported among epigean freshwater amphipods. Only some Baikal species (Takhteev, 2000, 2019), some Caspian Gammaroids (Sars, 1894, 1896) and oddities such as *Fuxiana yangi* Sket, 2000, from Lake Fuxian (China; Sket, 2000) and *Issykogammarus hamatus* Chevreux, 1908, from Lake Issyk-Kul (Kazakhstan; Chevreux, 1908) display such a comparable armoured condition. In the case of the species of the Titicaca, the development of this armature might be related to the predation pressure exerted by the Cyprinodontid killifish endemic to the Lake (*Orestias* Valenciennes, 1839; up to 24 spp.; Lauzanne, 1992), but this is still not firmly established (González & Coleman, 2002).

Recently, Adamowicz et al. (2018) showed based on molecular markers that the *Hyalella* of the Titicaca are not monophyletic but derive from at least five independent colonization episodes. Furthermore, Jurado-Rivera et al. (2020) and Zapelloni et al. (2021) disclosed

a high discordance between morpho-species and Molecular Operational Taxonomic Units (MOTUs) within this assemblage. The derivation from multiple colonization events and the incongruence between morpho-species and MOTUs seem to be a hallmark of the species radiations arisen in ancient lakes and oceanic islands (Monaghan et al., 2006; Critescu et al., 2010).

It is high time to address the true magnitude of the Titicacan *Hyalella* species diversity. Increasing levels of eutrophication due to urban sewage discharge in populated bays, and the industrial pollution derived from mining across the lake watershed, pose significant threats to the conservation of this remarkable species assemblage (Archundia et al., 2017). An even greater menace is represented by two predatory fish species introduced into the lake: *Onkorhynchus mikiss* (Walbaum, 1792) and *Odontesthes bonariensis* (Valenciennes, 1835), with the former being extensively cultured in net-cages (Vila et al., 2007).

Here we contribute to the revision of the *Hyalella* amphipods of Lake Titicaca introducing the description of three new species while re-describing other two. One of the new taxa corresponds to a shallow water form with a smooth, compact body, shortened antennules and antennae, and strongly spinose, shortened posterior pereiopods that remind the condition exhibited in some typically fossorial marine forms such as the Haustoriidae Stebbing, 1906, or the Phoxocephalidae G.O. Sars, 1891. This new taxon is remarkable also in the display of hardly sexually dimorphic second gnathopods. The second new species is a deeper water form that also shows an unarmoured body but displays a transverse dorsal hump along the posterior margin of body tergites. The third new species corresponds again to a smooth body, deep-water form that seems to be limited to occur on unconsolidated murky sediments. It is characterised by the display of numerous long setae along the medial margin of anterior pereiopods, a feature reminiscent to the condition of some limnic Gammaridae. Furthermore, we redescribe *H. solida* Chevreux, 1907, a species characterised by displaying a hispid integument and the presence of five dorsal flanges; it was originally described based on a single specimen and had not been reported again since its discovery. Finally, we redescribe *H. nefrens* González & Watling, 2003, a species with a peculiar, untoothed mandible and three pointed dorsal processes, emphasising the variable expression of the latter feature, otherwise profusely used in *Hyalella* taxonomy.

We have obtained DNA sequences of the mitochondrially-encoded cytochrome c oxidase subunit I (= *cox1*) of four out of the five aforementioned species in addition to morphological descriptions. We take advantage of these data and of all previous molecular information gathered on the Titicacan *Hyalella* (Adamowicz et al., 2018; Jurado-Rivera et al., 2020) to explore the feasibility of adopting an integrative approach – combining morphological and molecular information – to unveil the actual species diversity present in the lake.

Material and methods

Specimens were collected directly with a hand-held plankton net or with a small dredge thrown either from the shore of the lake or from a boat and preserved in the field in 96% ethanol immediately after collection. Specimens were treated in the laboratory with lactic acid to remove internal tissues to facilitate observation. Drawings were prepared using a camera lucida on a Leica DM 2500 microscope equipped with Nomarski differential interference contrast. Body measurements were derived from the sum of the maximum dorsal dimensions of head,

pereionites, pleonites and urosomites, including telescoped portions, and exclude telson length. Type material is deposited in the invertebrate collection of Naturalis Biodiversity Center, Leiden [RMNH].

Cox1 sequences and molecular phylogenetic analyses were obtained and performed following the protocols described in our previous study on the *Hyalella* from the Titicaca (Jurado-Rivera et al., 2020). The new DNA sequences introduced herein were merged with the large dataset used in the aforementioned study and are freely available via GENBANK (see corresponding accession numbers in the corresponding sections on material examined below).

Taxonomy

Order AMPHIPODA Latreille, 1816
Family Hyalellidae Bulycheva, 1957
Genus *Hyalella* S.I. Smith, 1874

Hyalella krolli Jaume sp. nov.
(figs 1A, 2–6)

Material examined. Bolivia: Escoma (northern basin of Lake Titicaca) [15.73621S; 69.08813W]. Between 0 and 1 m depth; sandy/muddy bottom among Totora (*Schoenoplectus californicus*), *Myriophyllum*, *Zannichellia*, *Isoetes*, *Nostoc*. Collected by O. Kroll, 14/12/2009. SYNTYPES: single male (penile papillae present and well developed) and 9 females [RMNH.CRUS.A. 5092]. Single male and two brooding female syntypes partially dissected, each preserved in single 70% ethanol vial; rest of syntypes preserved all together in single 70% ethanol vial. Additional 11 female specimens preserved at IMEDEA collection [IMEDEA/4816A]. One specimen used to sequence mitogenome [GENBANK access. no. MT672031] and another one to sequence *cox1* [GENBANK access. no. MN582347].

Bolivia: Chua (southern basin of Lake Titicaca) [16.19953S; 68.74745W]. At 1.5 m depth; clay; gravel; macrophytes (*Ruppia* or *Zannichellia*); sponges. Collected by O. Kroll, 18/05/2007. Five specimens [IMEDEA /11-6B].

Diagnosis. Body smooth, compact, with somites comparatively much broader than long. Eye ellipsoid. Antennules, antennae and pereiopods V–VII strongly shortened. Five pairs of sternal gills, one on each of pereionites III–VII. Mandible incisor toothed, left 5-denticulate, right 6-denticulate. Maxillule (= Maxilla 1) basal endite (= outer plate) with nine rake-like spines; endopod (= palp) comparatively elongate (i.e., surpassing midway of distance between base of segment and distolateral angle of basal endite), with single serrulate seta on tip; seta as long as half segment length. Maxilla (= Maxilla 2) coxal endite (= inner plate) with single hipertrophied pappose seta proximally on medial margin. Male gnathopod II propodus hardly sexually-dimorphic, slender, subrectangular, hardly expanded, about 1.5 times longer than broad, with palm margin much shorter than posterior margin. Coxal plate IV comparatively slender, much longer (1.5 ×) than broad. Pereiopods V–VII bristled with spines. Male uropod I endopod lacking modified curved spine. Uropod III ramus much shorter than protopod. Telson rimmed with numerous (up to 10) slender setae, but devoid of spines.

Etymology. Species name refers to the German malacologist Oliver Kroll, who collected all the material of the new taxon known.

Distribution. Endemic to Lake Titicaca.

Brooding female. Body (fig. 1A) up to 8.16 mm long, smooth, compact, stout, with all limbs shortened and with body somites much broader than long compared to rest of members of genus (e.g., compare fig. 1A and 1B). Head ~80% length of pereionites I–II

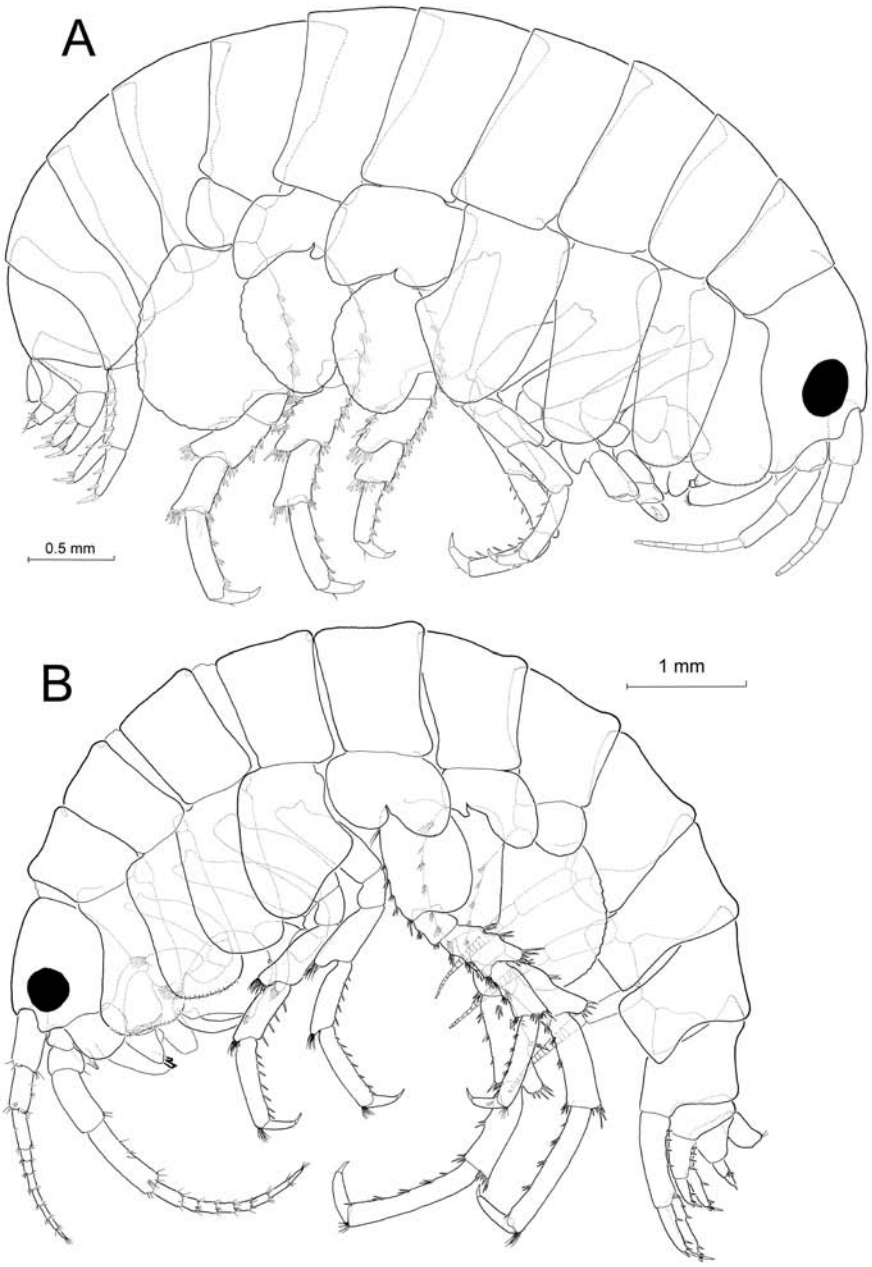

FIGURE 1 A, *Hyalella krolli* Jaume sp. nov., habitus of brooding female 9.2 mm, lateral; B, *Hyalella gonzalezi* Jaume sp. nov., male 11.0 mm habitus, lateral. Notice figures not at same scale.

combined; head lobe hardly produced. Eye ellipsoid. Sternal gills present on pereionites III to VII, finger-like (fig. 6D). Epimeral plates (fig. 3D) unarmed; plate I rounded; plates II–III posterodistally acuminate.

Antennules and antennae strongly shortened, both hardly longer than head (fig. 1A). Antennule (= Antenna 1) (fig. 5A) much shorter than antenna (= Antenna 2); peduncle shorter than head, segments 1–3 relative

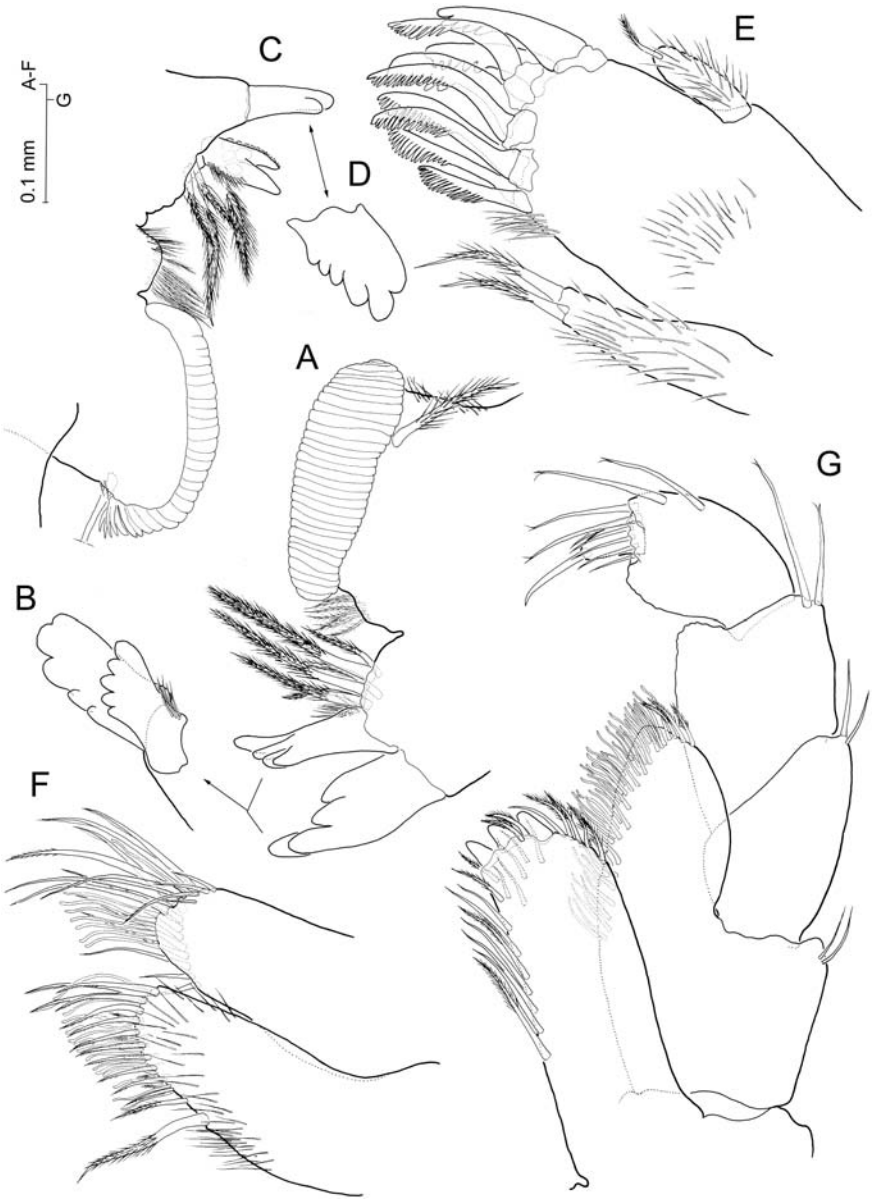

FIGURE 2 *Hyalella krolli* Jaume sp. nov., brooding female. A, left mandible, medial; B, inset of incisor and lacinia of latter; C, right mandible, lateral; D, inset of incisor of latter; E, maxillule; (F) maxilla; G, left maxilliped with armature on medial margin of distal segments omitted, posterior view.

length as: 1: 0.63: 0.40; main flagellum shortened (shorter than two distal segments of peduncle combined); accessory flagellum absent; three penultimate articles of main flagellum with one or two short simple aesthetascs.

Antenna (= Antenna 2) (fig. 5B) peduncle about as long as head, segments 4–5 combined short, shorter than peduncle of antennule; relative length of peduncle segments 4–5 as 0.86: 1; flagellum about equal in length to peduncle segments 4–5 combined.

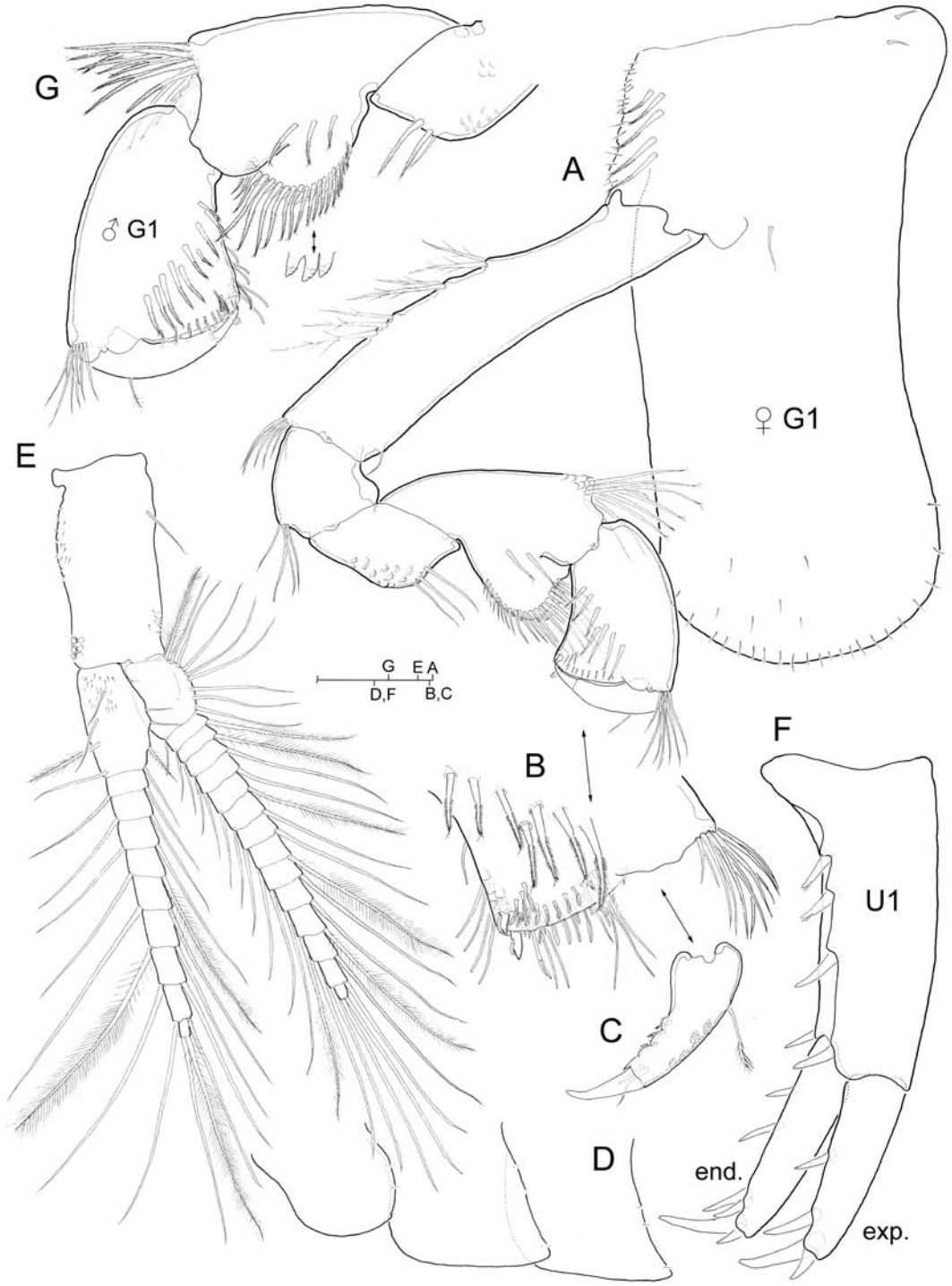

FIGURE 3 *Hyalella krolli* Jaume sp. nov. A, brooding female left gnathopod I, medial; B, inset of distal portion of propodus of latter, medial; C, inset of dactylus, medial; D, left epimeral plates, lateral; E, right pleopod I, posterior; F, right uropod I, lateral; G, distal portion of male right gnathopod I, medial. Scale bars: 0.1 mm (B, C, F, G); 0.2 mm (A, D, E).

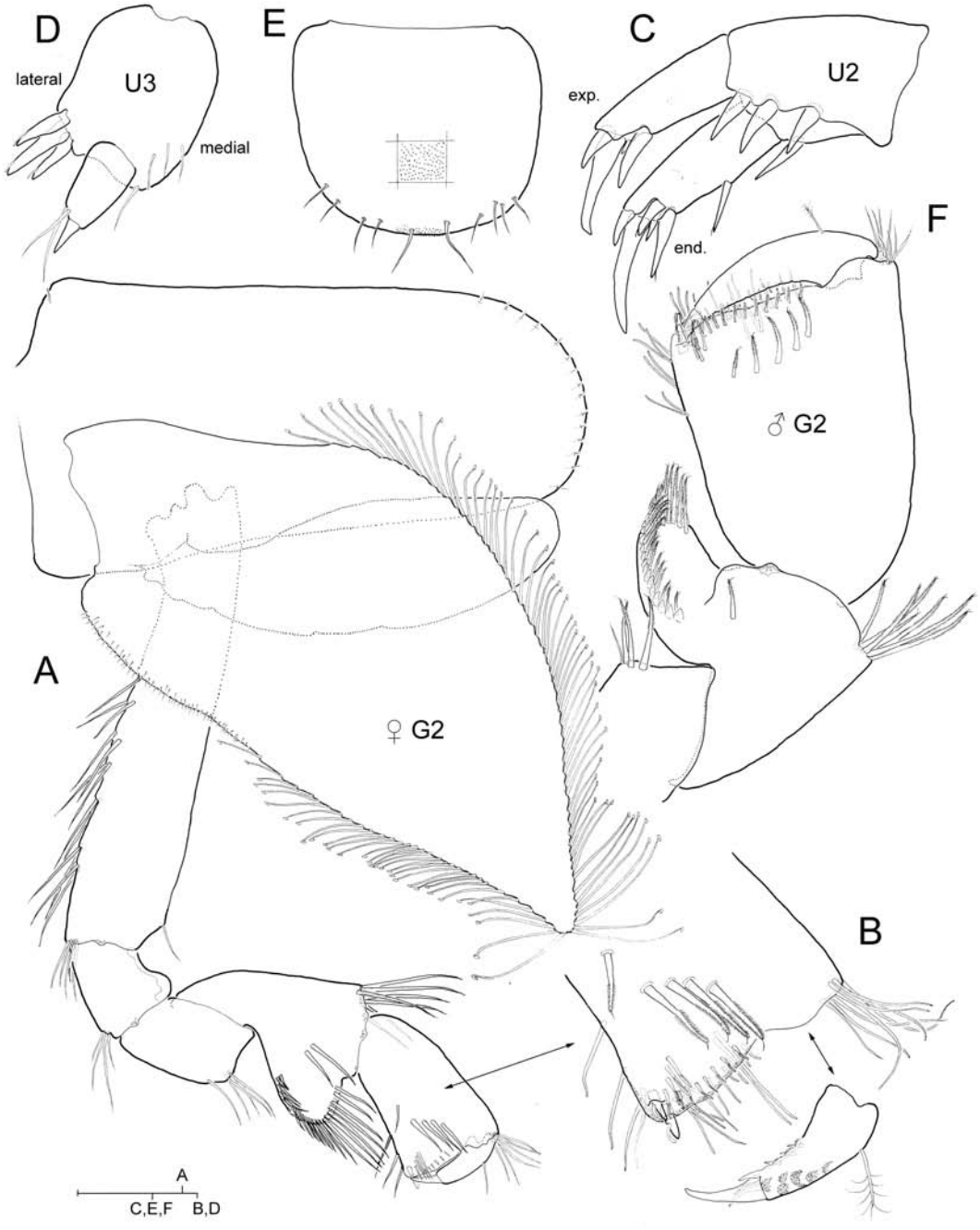

FIGURE 4 *Hyalella krolli* Jaume sp. nov. A, brooding female left gnathopod II, medial; B, inset of distal portion of propodus and disarticulated dactylus, medial; C, left uropod II, lateral; D, right uropod III, ventral (= posterior); E, telson, dorsal; F, inset of distal portion of male gnathopod II, medial. Scale bars: 0.1 mm (B, D); 0.2 mm (A, C, E, F).

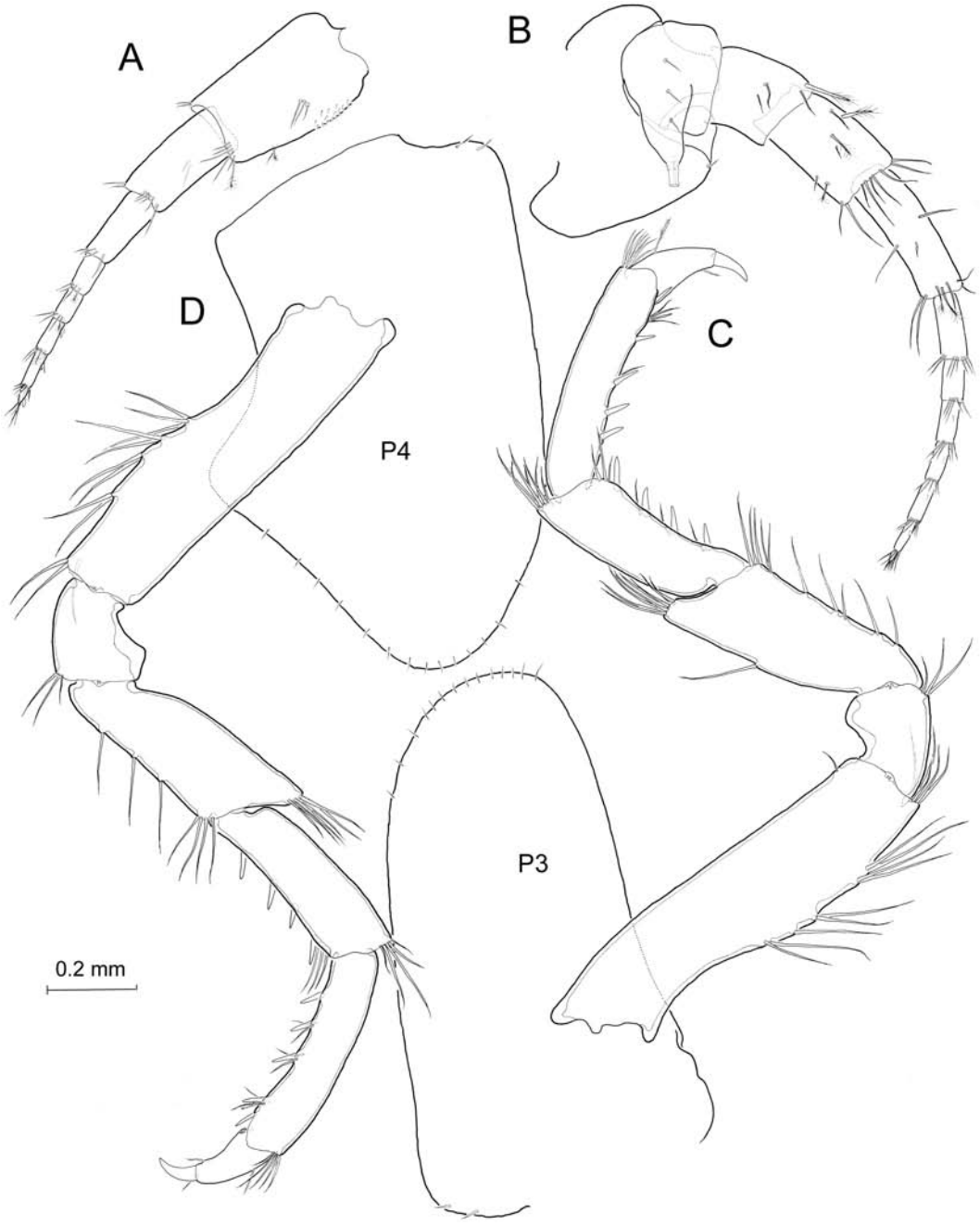

FIGURE 5 *Hyalella krolli* Jaume sp. nov., brooding female. A, left antennule, lateral; B, left antenna, medial; C, left pereiopod III, medial; D, left pereiopod IV, medial.

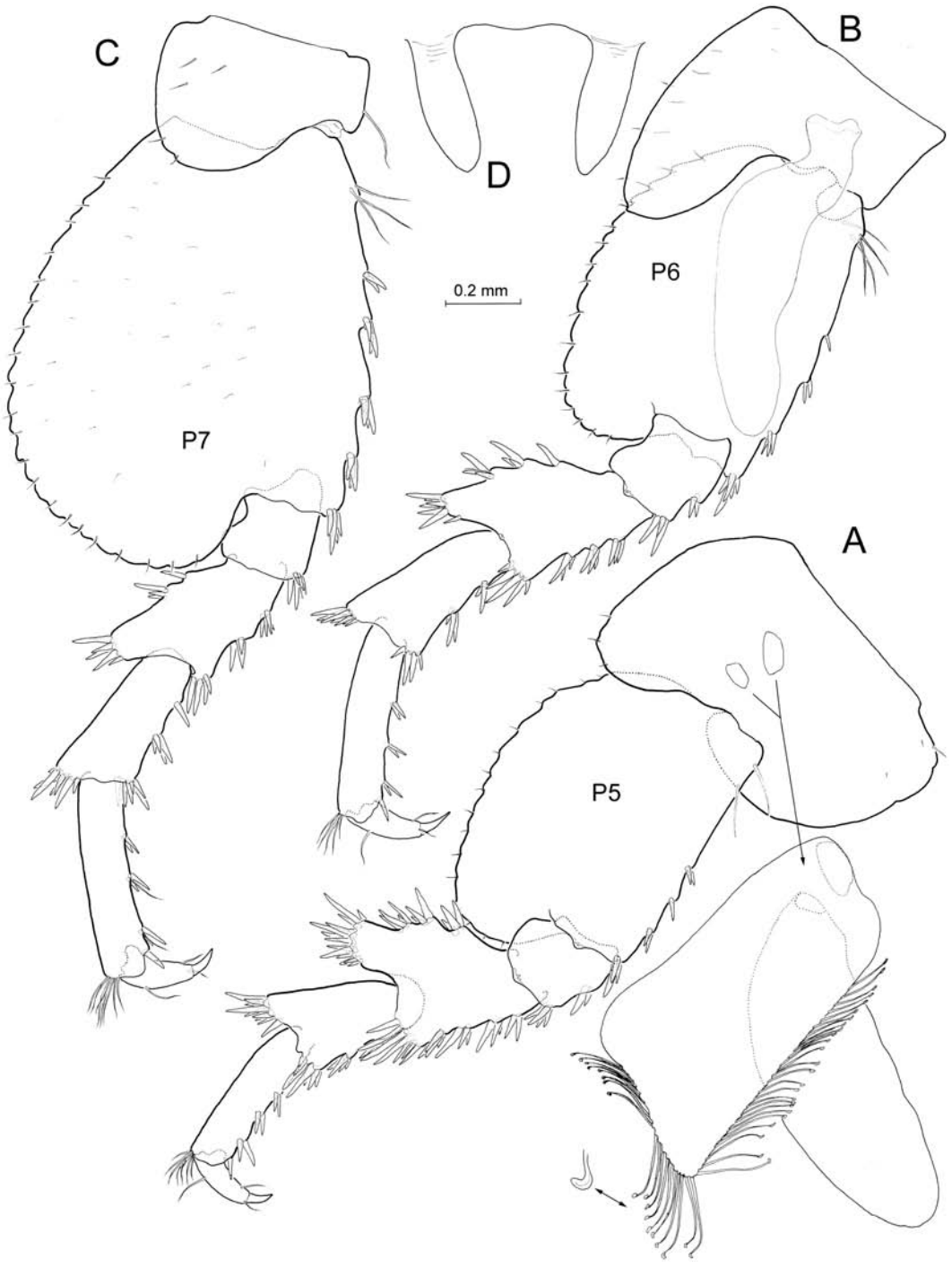

FIGURE 6 *Hyalella krolli* Jaume sp. nov., brooding female. A, left pereiopod V with ooöstegite and coxal gill detached, medial; B, left pereiopod VI, medial; C, right pereiopod VII, lateral; D, pair of sternal gills on sternite of pereionite VII, anterior.

Labrum (= upper lip) and paragnaths (= lower lips) not figured, ordinary, latter with inner lobes not developed.

Both mandibles with toothed incisor and triturative columnar molar; molar seta well developed on both; palp lacking. Left mandible (fig. 2A) incisor and lacinia both 5-denticulate (fig. 2B), latter with patch of long setules proximally; mandible setal row comprising three main setae and three accessory setae disposed in two parallel rows, all setae pappose; molar with patch of slender setae at base on distal margin as figured.

Right mandible (fig. 2C) incisor 6-denticulate (fig. 2D); lacinia bifid with patch of setules disposed proximally, one of branches multi-tuberculate as figured. Mandible setal row consisting of 2 + 2 pappose setae.

Maxillule (= Maxilla 1) (fig. 2E) coxal endite (= inner plate) narrow and tapering, with two pappose setae on tip. Basal endite (= outer plate) with nine stout rake-like spines, three of which stouter and more coarsely denticulate than rest. Endopod (= palp) unsegmented, not tapering, blunt, comparatively elongate (i.e., surpassing midway of distance between base of segment and distolateral angle of basal endite), with single serrulate seta as long as about mid length of segment on tip.

Maxilla (= Maxilla 2) (fig. 2F) coxal endite (= inner plate) with hipertrophied pappose seta proximally on medial (= inner) margin.

Maxilliped (fig. 2G) ordinary; basal endite (= inner plate) with three short cuspidate spines on distal margin; rest of limb as figured.

Pereiopodal coxae I–III (figs 1B; 3A; 4B; 5C) of similar length, each much longer than broad. Coxa I with anteroproximal angle produced into lobe; anterior margin of plate concave; posterior margin straight. Coxa IV (fig. 5D) rhomboid, ~1.5 times longer than broad and about same length as preceding coxae, with deeply excavate posterior margin. Coxa V (fig. 6A) bilobed, both lobes of similar length. Coxa VI with reduced, finger-like anterior lobe and strongly overhanging posterior lobe (fig. 6B). Coxa VII with only posterior lobe developed (fig. 6C).

Coxal gills (figs 4A, 6A, B) on gnathopod II to pereiopod VI, smooth, sac-like. Oöstegites on gnathopod II to pereiopod V, that on gnathopod II (fig. 4A) ellipsoid and tapering, rest subrectangular in outline (fig. 6A).

Gnathopod I (fig. 3A–C) subchelate with carpus 1.3 times longer than propodus, latter about 1.5 times longer than broad, with convex palm margin; palm angle marked with two unequal flagellate spines; up to seven bipectinate setae present on medial surface of propodus as figured.

Gnathopod II (fig. 4A, B) subchelate with carpus also longer than propodus, but latter comparatively more elongate than gnathopod I counterpart, about 1.7 times longer than broad.

Pereiopods III–IV (fig. 5C, D) about similar in length, ordinary.

Pereiopods V–VII (fig. 6A–C) strongly spinose, especially pereiopod V, resembling in some way condition exhibited by fosorial marine amphipods such as some members of the Haustoriidae or the Phoxocephalidae. Distal portion of pereiopods comparatively short (i.e., each with basis longer than corresponding ischium-merus combined). Basis of periopod VII broadest, only 1.2 times longer than broad; basis of pereiopods V–VI more slender, each 1.4 times longer than broad.

Pleopods I–III (fig. 3E) ordinary, unmodified, each with protopod much shorter than corresponding rami, about twice longer than broad, with 3, 3 and 4 retinacles, respectively.

Uropod I (fig. 3F) both rami much shorter than protopod; latter with 3–4 spines along posterolateral margin and one spine on each posterolateral and posteromedial distal angle of segment. Exopod with one spine along

posterolateral margin; endopod with two spines along posteromedial margin.

Uropod II (fig. 4C) shortened, total length not surpassing length of protopod of uropod I. Rami slightly shorter than protopod; latter with two spines along posterolateral margin and single spine on each posterolateral and posteromedial distal angle of segment; posteromedial margin naked. Exopod with four unequal spines distally. Endopod with five unequal spines distally and single slender spine about midway on posteromedial margin.

Uropod III (fig. 4D) with ramus much shorter than protopod; latter with three spines on distolateral angle and simple seta on distomedial angle; three more slender simple setae placed on medial surface of segment as figured. Ramus with spine and pair of simple setae on tip; spine shorter than half ramus length.

Telson (fig. 4E) subrectangular, slightly broader than long, with about 10 simple setae along distal margin, median pair of setae longer than rest; integument densely microspinulose as figured.

Male. Body 9.59 mm long. As female in all respects except for presence of penile papillae, up to ten bipectinate setae (vs. up to seven in female) on medial surface of propodus of gnathopod I (fig. 3G), and propodus of gnathopod II. Latter (fig. 4F) slender, 1.4 times longer than carpus, subrectangular, hardly expanded, about 1.5 times longer than broad, with palm margin much shorter than posterior margin; palm angle marked with two flagellate spines; medial surface of segment with transverse row of five bipectinate setae.

Remarks. This species is easily distinguished from the rest of representatives of the genus based on its compact aspect with shortened antennules and antennae, and shortened spiky pereiopods V–VII. As stated above, its aspect resembles in some way the condition exhibited in fossorial marine amphipods such as some members of the Haustoriidae or the Phoxocephalidae, but we are uncertain as regard the life habits of the new species. In any event, the two known localities where the species was collected correspond to shallow waters (up to 1.5 m depth) covered with macrophytes.

The hardly developed sexual dimorphism on male gnathopod II is also a remarkable feature of the new taxon, not found in any other *Hyalella* species, and reminds the condition exhibited in some talitrids (e.g., *Talitrus* Latreille, 1802).

As it will be stated below, this species arises in molecular phylogenetic analyses together with *H. gonzalezi* Jaume sp. nov. – described below – as the closest relative of *Hyalella armata*, perhaps the most bizarre of all Titicacan species, characterised by the display of an extremely elongate, laterally-directed spine on each pereiopodal coxal plate I–IV (see González & Coleman, 2002). Whereas in *H. gonzalezi* Jaume sp. nov. the close kinship with *H. armata* (Faxon, 1876) could be at the base of the common display of peculiar morphological features such as the presence of body dorsal humps, the truncate endopod (= palp) of maxillule with a comparatively long distal seta, the male gnathopod II propodus of triangular outline and with the two spines on palm angle comparatively elongate, and a third uropod with shortened ramus, there is nothing in the morphology of *H. krolli* Jaume sp. nov. denoting its closeness to *H. armata* except perhaps the truncate endopod of maxillule with a comparatively long distal seta and the short ramus of uropod III.

Hyalella gonzalezi Jaume sp. nov.

(figs 1B; 7–12)

Material examined. Perú: Coata Bay (northern basin of Lake Titicaca), to the lee of Capachica Peninsula [15.726904S; 69.805414W]. Black

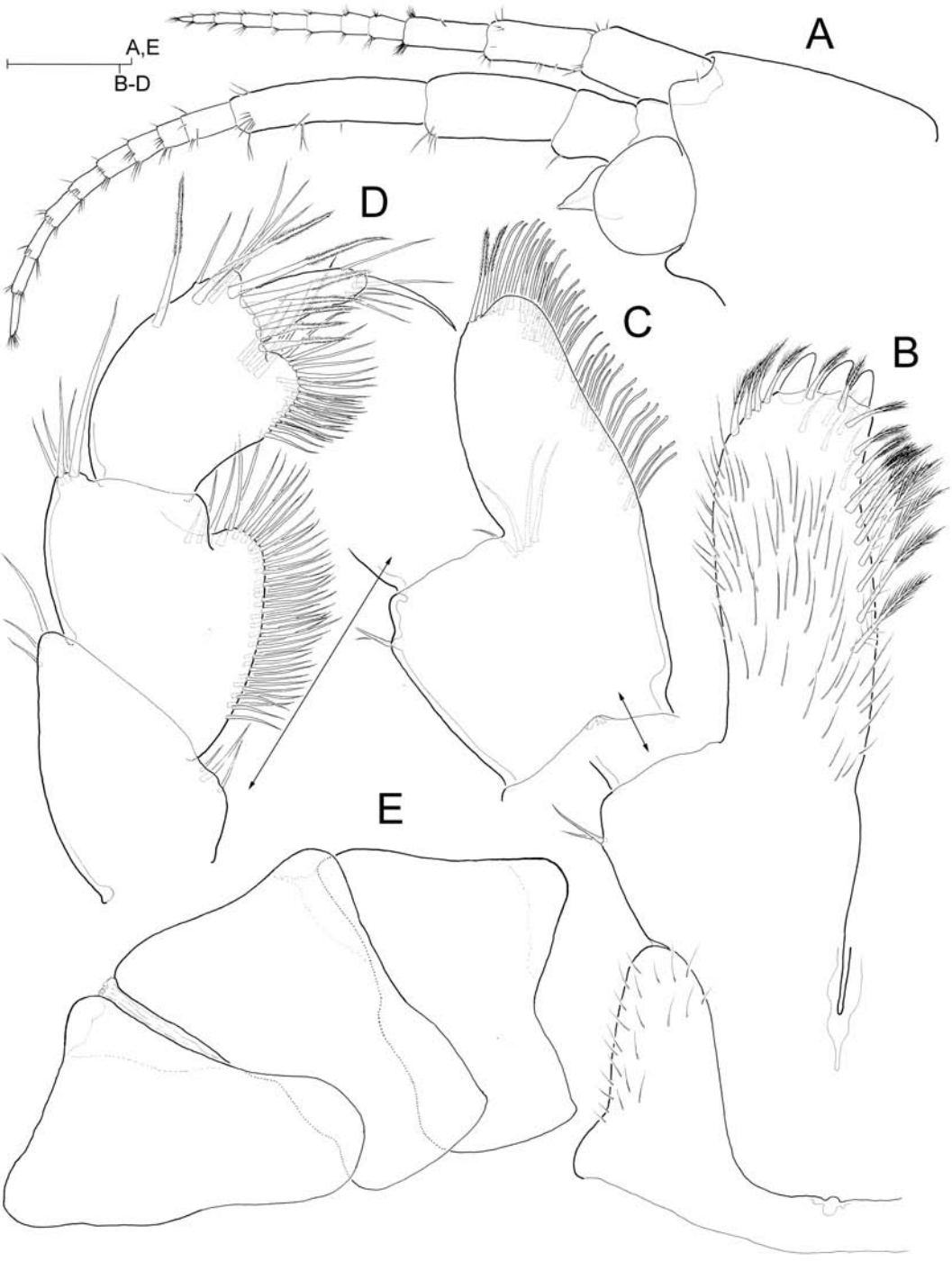

FIGURE 7 *Hyalella gonzalezi* Jaume sp. nov., male. A, head with left antennule and antenna, lateral; B, coxa and basis+basal endite of left maxilliped, anterior; C, ischium+ischial endite of latter, anterior; D, carpus-dactylus of latter; E, left epimeral plates I–III, lateral. Scale bars: 0.5 mm (A, E); 0.1 mm (B-D).

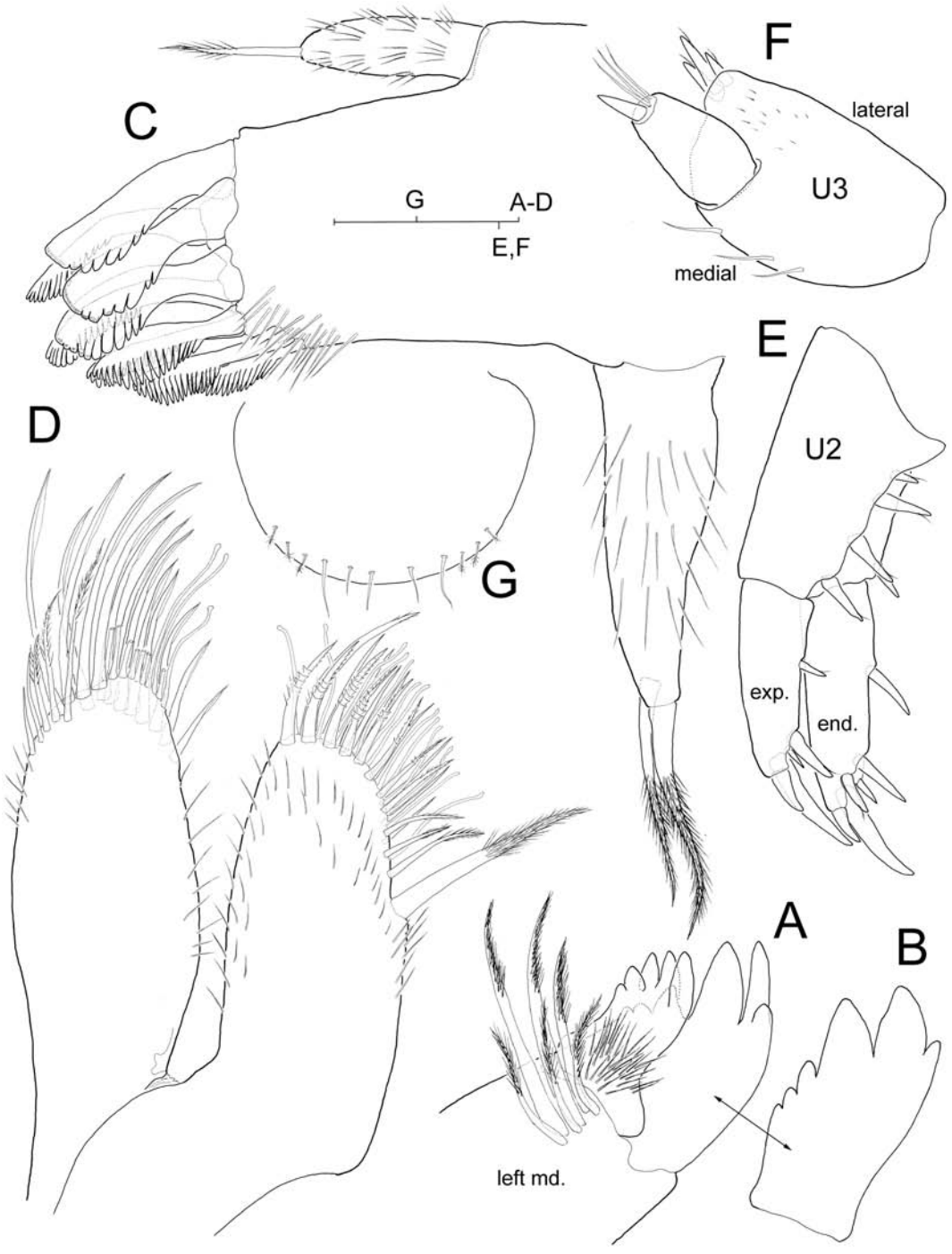

FIGURE 8 *Hyalella gonzalezi* Jaume sp. nov., male. A, left mandible; B, inset of incisor of latter; C, maxillule; D, maxilla; E, left uropod II, lateral; F, right uropod III, dorsal; G, telson, dorsal. Scale bars: 0.1 mm (A–D, F, G); 0.2 mm (E).

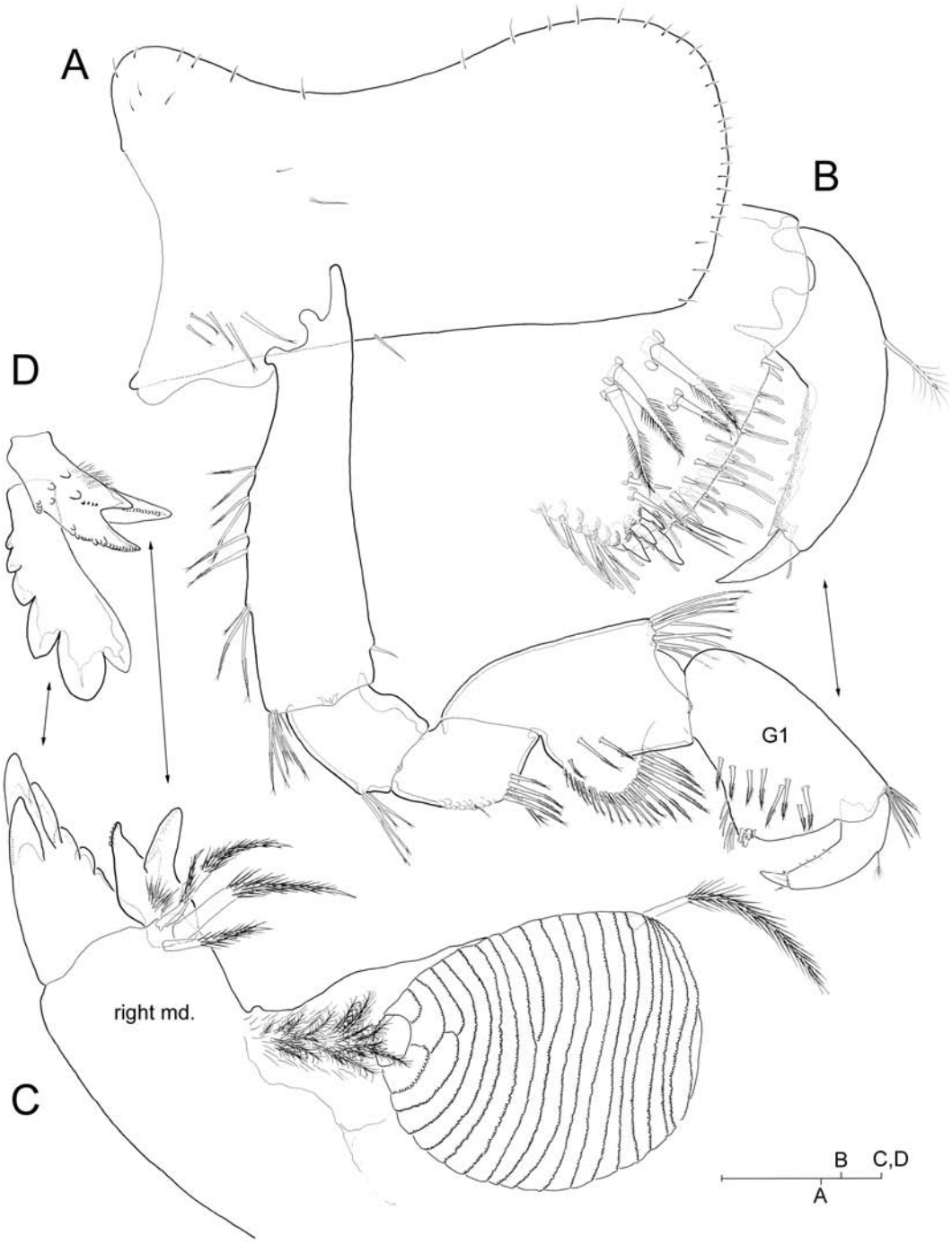

FIGURE 9 *Hyalella gonzalezi* Jaume sp. nov., male. A, left gnathopod I, medial; B, inset of palm margin of propodus+dactylus of latter, medial; C, right mandible; D, inset of incisor and lacinia of latter. Scale bars: 0.2 mm (A); 0.1 mm (B–D).

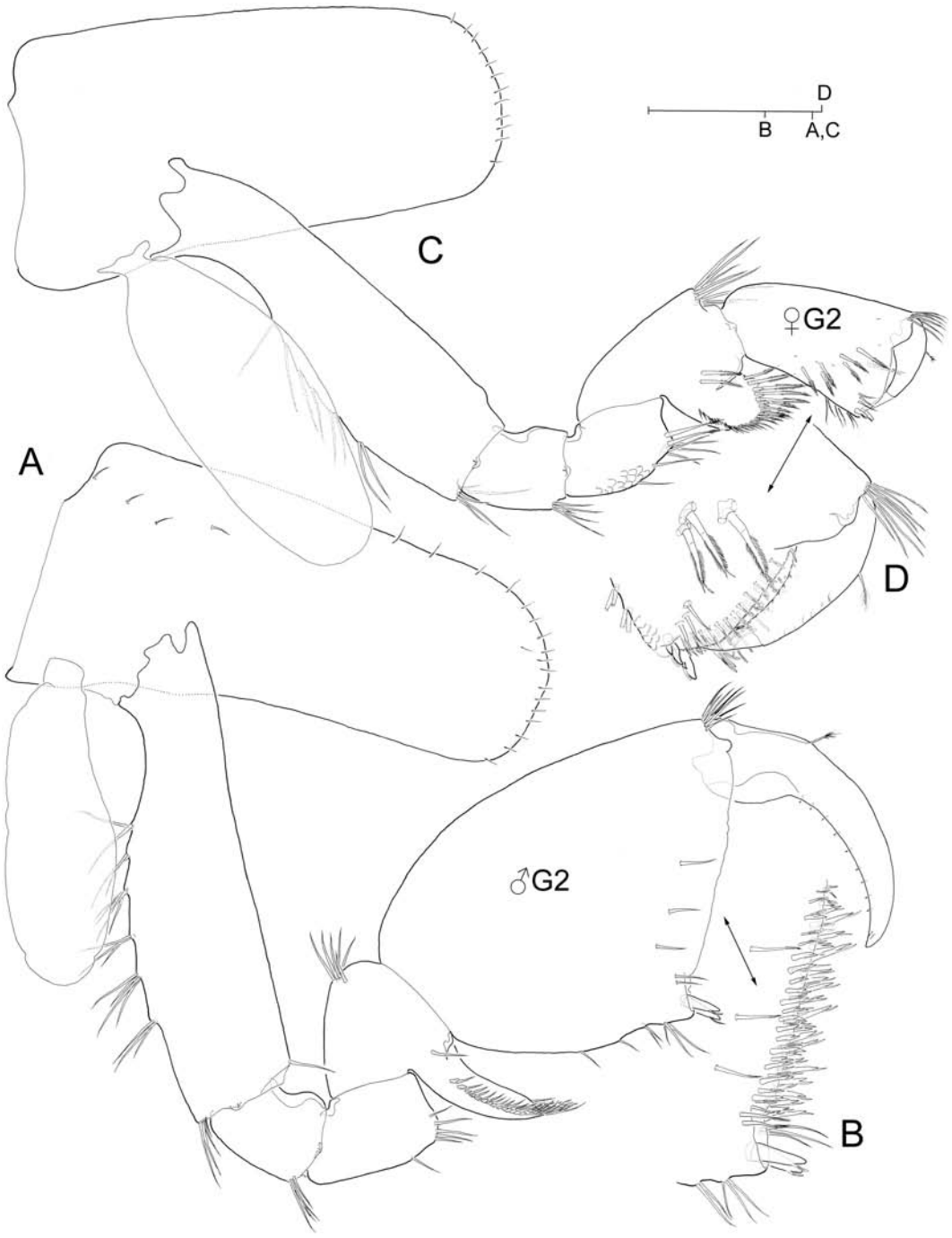

FIGURE 10 *Hyalella gonzalezi* Jaume sp. nov. A, male left gnathopod 11, medial (armature along palm margin of propodus omitted); B, inset of palm margin of propodus of latter, medial; C, female left gnathopod 11, medial (oöstegite and armature along palm margin of propodus both omitted); D, inset of distal portion of propodus+dactylus of latter, medial. Scale bars: 0.4 mm (A, C); 0.2 mm (B, D).

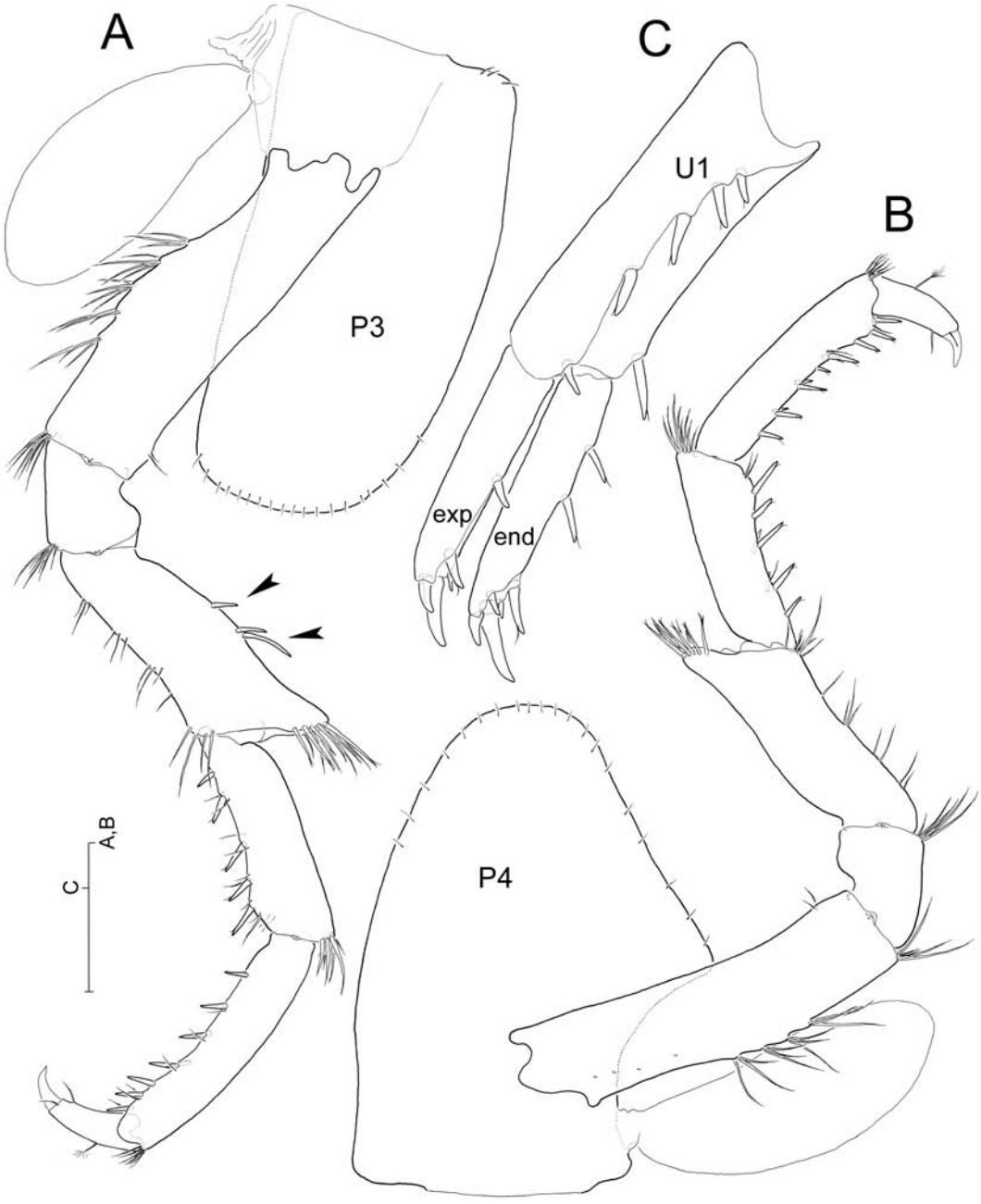

FIGURE 11 *Hyalella gonzalezi* Jaume sp. nov., male. A, left pereiopod III, medial (arrowheads pointing to teratological armature present on anterior margin of merus); B, left pereiopod IV, medial; C, left uropod I, lateral. Scale bars: 0.4 mm (A, B); 0.2 mm (C).

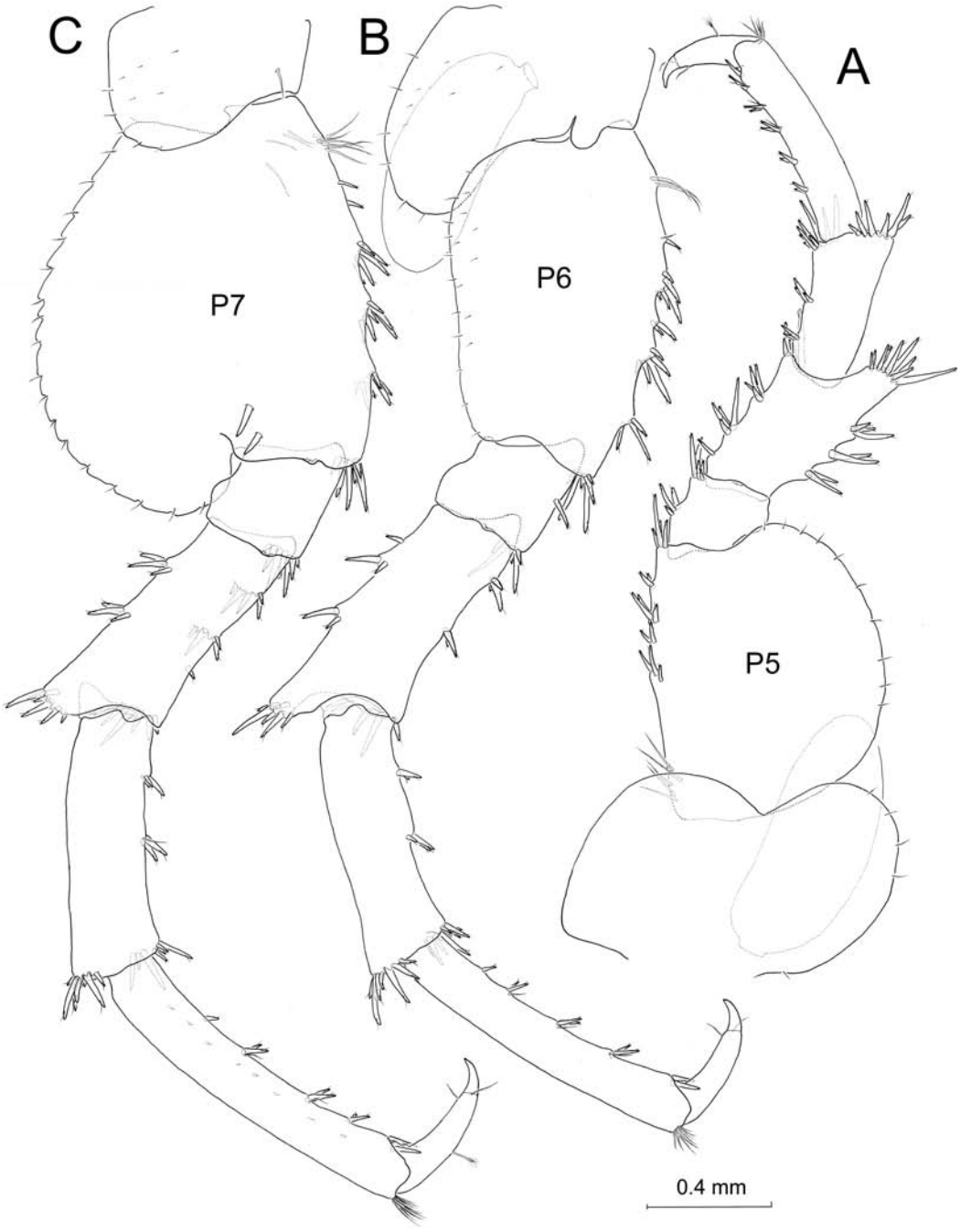

FIGURE 12 *Hyalella gonzalezi* Jaume sp. nov., male. A, right pereiopod V, lateral; B, right pereiopod VI, lateral; C, left pereiopod VII, medial.

conchiferous mud, 18 m depth. Collected by M. Alonso & D. Jaume, 30/03/2019. SYNTYPES: 15 males and 15 females preserved separately in two 95% ethanol vials [RMNH.CRUS.A. 5093; ex IMEDEA/4TK6B].

Perú: Escallani (northern shore of Capachica Peninsula; northern basin of Lake Titicaca), ~150 m offshore [15.478119S; 69.88840W]. On *Chara* bottom, ~10 m depth. Collected by M. Alonso & D. Jaume, 27/03/2019. Twenty specimens, both sexes [IMEDEA/4TK1A5]. Two specimens used to sequence *cox1* [GENBANK access. nos. MW714604 and MW714605].

Perú: Isla Soto (northern basin of Lake Titicaca) [15.54300S; 69.49700W]. On *Chara* bottom, 12 m depth. Collected by O. Kroll, 15/07/2007. Two males [IMEDEA/2316E1].

Perú: Isla Amantani (northern basin of Lake Titicaca) [15.66700S; 69.698800W]. Stony bottom, < 1 m depth. Collected by O. Kroll, 11/07/2007. Many specimens, both sexes [IMEDEA/29-7B].

Bolivia: Puerto Pérez (southern basin of Lake Titicaca) [16.31800S; 68.62800W]. On *Chara* bottom, <1.5 m depth. Collected by O. Kroll, 19/05/2006. Single specimen [IMEDEA/13-1A1].

Bolivia: Escoma (northern basin of Lake Titicaca) [15.73621S; 69.08813W]. <1 m depth; sand; mud; Totora, *Myriophyllum*, *Zannichellia*, *Isoetes*, *Nostoc*. Collected by O. Kroll, 14/12/2009. Three specimens [IMEDEA/4816D].

Diagnosis. Body smooth, with faint but distinct transverse hump along posterodorsal margin of pereionites and pleonites; body integument strongly tinged dark when alive. Eye circular. Five pairs of sternal gills, one on each of pereionites III–VII. Incisor of both mandibles toothed, 6-cuspidate. Maxillule (= Maxilla 1) basal endite (= outer plate) with nine rake-like spines, three of them spatulate; endopod (= palp) comparatively elongate (i.e., surpassing midway of distance between base of segment and distolateral angle of basal endite), with single serrulate distal seta; seta comparatively elongate, about as long as segment. Maxilla (= Maxilla 2) coxal endite (= inner plate) with distal margin lined with series of modified slender spines provided with series of transverse lamellae; only one hypertrophied pappose seta present proximally on medial margin of endite. Male gnathopod II propodus massive, triangular; palm margin as long as posterior margin. Coxal plate IV comparatively slender, much longer (1.4 ×) than broad, with deeply excavate posterior margin. Male uropod I endopod lacking modified curved spine. Uropod III ramus much shorter than protopod. Telson devoid of spines, with about five simple setae along distal margin.

Etymology. Species name refers to the Chilean *Hyalella* specialist Prof. Exequiel González Balbontín, one of the main contributors to the study of the species of Lake Titicaca.

Distribution. Endemic to Lake Titicaca.

Male. Body (fig. 1B) up to 11 mm long, smooth except for faint but distinct transverse hump developed along posterior margin of tergites of pereionites and pleonites; body integument strongly tinged dark when alive. Eye circular. Head attaining ~72 % length of pereionites I–II combined; head lobe hardly produced, triangular (fig. 7A). Five pairs of sternal gills (not figured), one on each of pereionites III–VII. Epimeral plates (fig. 7E) unarmed; distal margin of plate I evenly rounded; plates II–III posterodistally acuminate but with angles hardly produced.

Antennule (= Antenna I) (figs 1A, 7A) attaining about two-thirds length of antenna. Peduncle longer than head; relative length of peduncle segments 1–3 as 1: 0.78: 0.61. Flagellum shorter than peduncle.

Antenna (= Antenna II) (figs 1A, 7A) less than half body length, peduncle longer than

head; relative length of peduncle segments 4–5 as 1: 0.77. Flagellum about as long as peduncle.

Labrum (= Upper lip) and paragnaths (= lower lips) not figured, ordinary, latter with inner lobes not developed.

Both mandibles with toothed, 6-cuspidate incisor and triturative columnar molar; latter with patch of short slender pappose setae and long setules at base of anterior margin as figured; molar seta well developed on both branches; palp lacking. Left mandible (fig. 8A, B) lacinia 5-cuspidate with proximal portion of inner surface covered with densely set short spinules. Mandible setal row comprising three main pappose setae and three accessory pappose setae disposed in two parallel rows (3 + 3).

Right mandible (fig. 9C, D) lacinia trifid, with series of round marginal denticles on each cusp distributed as figured, and with patch of densely set short spinules on proximal portion of inner surface. Mandible setal row comprising 2 + 2 pappose setae.

Maxillule (= Maxilla 1) (fig. 8C) coxal endite (= inner plate) narrow and tapering, with two pappose setae on tip. Basal endite (= outer plate) with nine stout rake-like spines, larger three spatulate. Endopod (= palp) unsegmented, not tapering, blunt, comparatively elongate (i.e., surpassing midway of distance between base of segment and distolateral angle of basal endite), with single serrulate distal seta; seta not reduced, about same length as segment.

Maxilla (= Maxilla 2) (fig. 8D) with single hypertrophied pappose seta proximally on inner margin of coxal endite (= inner plate); latter with distal row of modified slender spines provided with series of transverse lamellae.

Maxilliped (fig. 7B-D) ordinary; basal endite (= inner plate) with three short cuspidate spines on distal margin; rest of limb as figured.

Pereiopodal coxae (fig. 1B) I–III of similar length, each much longer than broad (fig. 10A, 11A); coxa I with anteroproximal angle produced into lobe and with excavate, concave anterior margin (fig. 9A). Coxa IV (fig. 11B) rhomboid, ~1.4 times longer than broad, with deeply excavate posterior margin. Coxa V (fig. 12A) bilobed, both lobes of similar length. Coxa VI with reduced, finger-like anterior lobe and strongly overhanging posterior lobe (fig. 12B). Coxa VII with only posterior lobe developed (fig. 12C).

Coxal gills present on gnathopod II (fig. 10A) and pereiopods III–VI (figs 11A, B, 12A, B), sacciform.

Gnathopod I (fig. 9A) subchelate; propodus about equal in length to carpus, ~1.5 times longer than broad, expanded distally; up to eight bipectinate setae distributed on medial surface of segment as figured; palm angle marked by four unequal flagellate spines; palm margin slightly oblique, convex, armed as in fig. 9B.

Gnathopod II (fig. 10A) subchelate; propodus massive, triangular, 1.3 times as long as broad with posterior margin about as long as palm margin; palm angle placed at about 54% length of segment, marked with two strong flagellate spines; palm margin strongly armoured (fig. 10B).

Pereiopods III–IV (fig. 11A, B) about similar in length, ordinary; both with posterior margin of carpus and propodus lined with spines.

Pereiopods V–VII (fig. 12A-C) elongate, each with basis about as long as corresponding ischium-merus combined. Basis of pereiopod VI not as strongly expanded posteriorly nor with posterodistal angle so strongly overhanging as in pereiopods V and VII, with straight posterior margin.

Pleopods I–III (fig. 1B) each with protopod shorter than rami.

Uropod I (fig. 11C) rami shorter than protopod, latter armed with four spines along

posterolateral margin and one spine on each posterolateral and posteromedial distal angle of segment. Exopod with one spine midway on posterolateral margin. Endopod lacking modified distal spine, with two spines along posteromedial margin.

Uropod II (fig. 8E) rami shorter than protopod, latter armed with three spines along posterolateral margin and one spine on each posterolateral and posteromedial distal angle. Exopod with spine midway on posterolateral margin. Endopod with spine on posteromedial margin.

Uropod III (fig. 8F) with ramus much shorter than protopod, latter with three short spines on distolateral angle and three simple setae along medial margin. Ramus with short spine and three simple setae on tip; spine less than half ramus length.

Telson (Fig. 8G) broader than long, with evenly rounded posterior margin. Armature consisting on about five slender simple setae along distal margin and three short penicillate setae subdistally at each side.

Brooding female. Body up to 10.7 mm long. As male in all respects except for presence of oöstegites on gnathopod II and pereiopods III–V, and structure of gnathopod II. Latter (fig. 10C) with propodus slender, slightly longer (1.1 ×) than carpus and 1.7 times longer than broad; palm angle marked with two flagellate spines; palm margin slightly transverse, convex, armed as in fig. 10D; medial surface of segment with row of six bipectinate spines as figured.

Remarks. This new species is remarkable for the display of a faint but distinct transverse hump along the posterior margin of tergites of pereionites and pleonites, in an otherwise smooth body. Furthermore, it displays a peculiar ornamentation composed of transverse lamellae on the spines of one of the series present on the coxal endite (= inner plate) of maxilla. Similar body dorsal humps occur in other two species, both also endemic to Lake Titicaca: *H. armata* (Faxon, 1876) and its closely related sibling *H. longispina* González & Coleman, 2002. The latter two species share the display of a long transverse spine on each of pereiopodal coxal plates I–IV, conferring them one of the more peculiar aspects among the Titicacan species, and readily tell them apart from the new species. As previously stated above in the remarks on *H. krolli* Jaume sp. nov., the latter species and *H. gonzalezi* Jaume sp. nov. arise as the closest relatives of *H. armata* in molecular phylogenetic analyses. The last two species share the display of body dorsal humps, a truncate endopod (= palp) of maxillule with a comparatively long distal seta, a male gnathopod II propodus of triangular outline and with the two spines on palm angle comparatively elongate, and a third uropod with shortened ramus.

With regard the row of spines provided with transverse lamellae present on the coxal endite (= inner lobe) of maxilla, similar structures have been reported to occur only in *H. nefrens* (see redescription below) aside the new species.

The gut contents of one of the specimens analysed included remains of a large anomopod branchiopod crustacean (*Simocephalus* sp.).

Hyalella hirsuta Jaume sp. nov.

(figs 13A; 14–19)

Material examined. Perú: Coata Bay, to the lee of Capachica Peninsula (northern basin of Lake Titicaca) [15.726904S; 69.805414W]. Black conchiferous mud, 18 m depth. SYNTYPES: six males and six females preserved in etanol vial [RMNH.CRUS.A. 5094]. Collected by M. Alonso & D. Jaume, 30/03/2019. Additional 130 specimens, both sexes, preserved at IMEDEA collection [IMEDEA/4TK6D]. One specimen

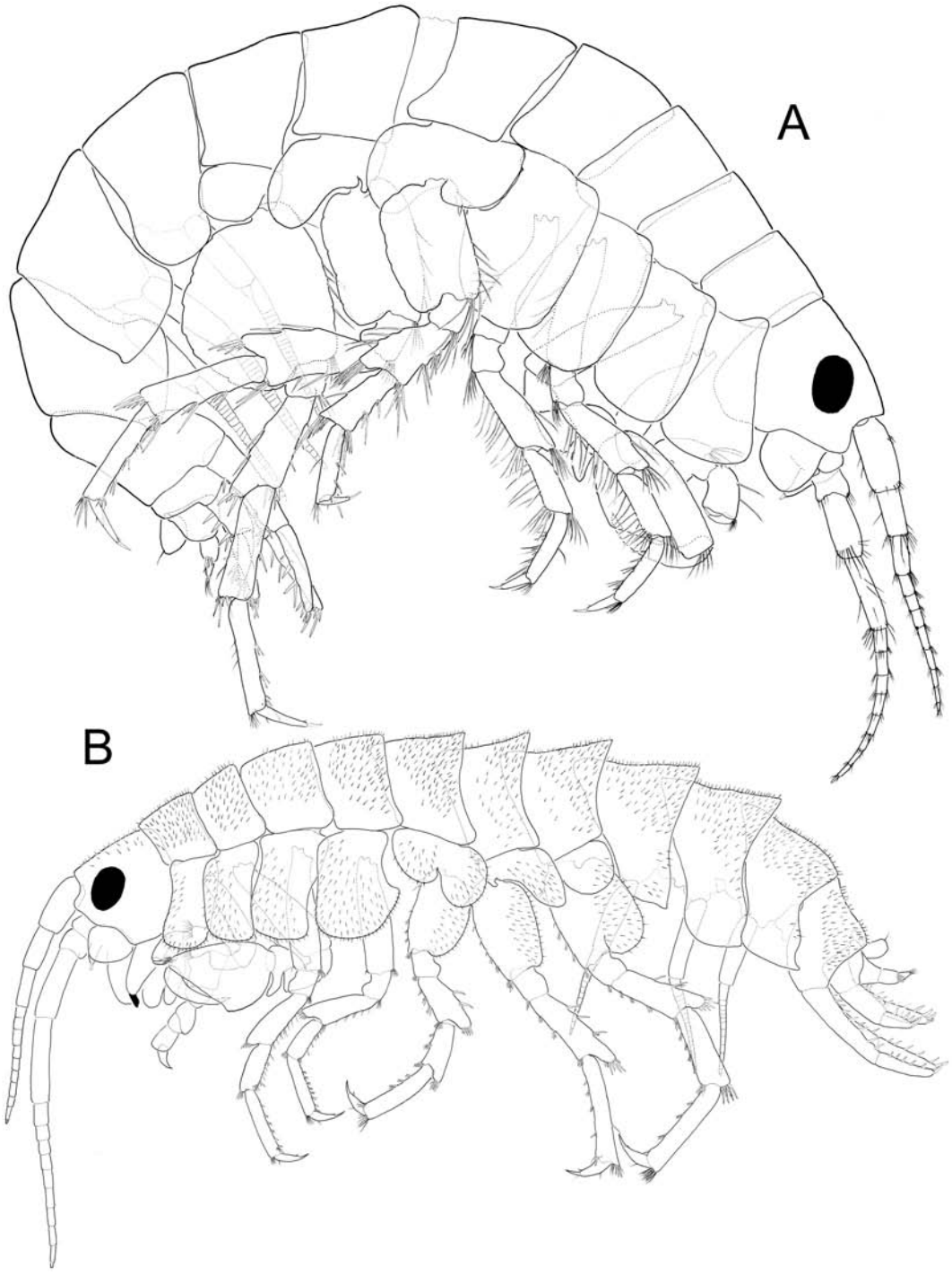

FIGURE 13 A, *Hyalella hirsuta* Jaume sp. nov., male 7.8 mm habitus, lateral; B, *Hyalella solida* Chevreux, 1907, male 10.3 mm habitus, lateral. Notice figures not at same scale.

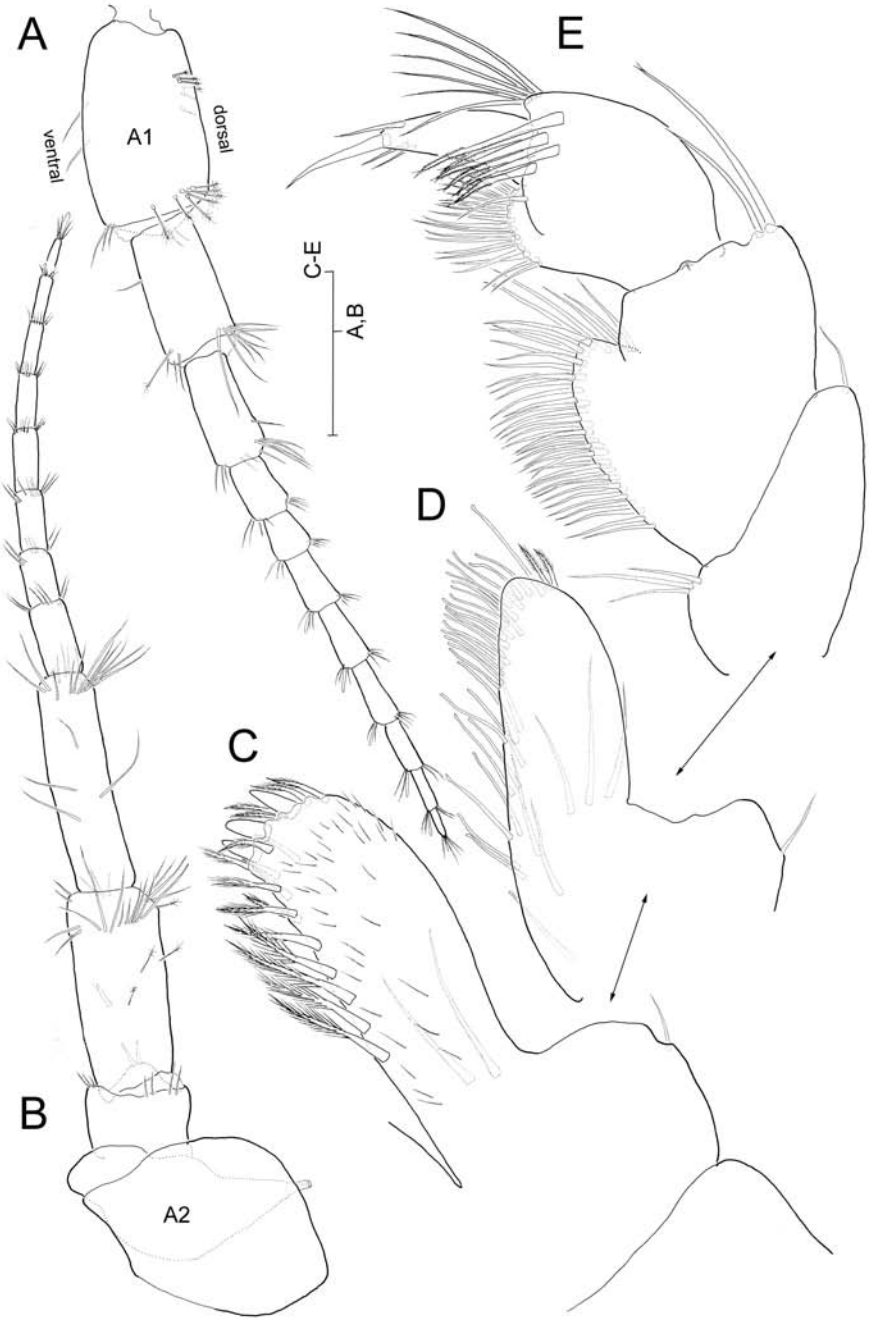

FIGURE 14 *Hyalella hirsuta* Jaume sp. nov., male. A, right antennule, lateral; B, right antenna, lateral; C, basis and basal endite of maxilliped; D, ischium and ischial endite; E, merus-dactylus. Scale bars: 0.2 mm (A, B); 0.1 mm (C-E).

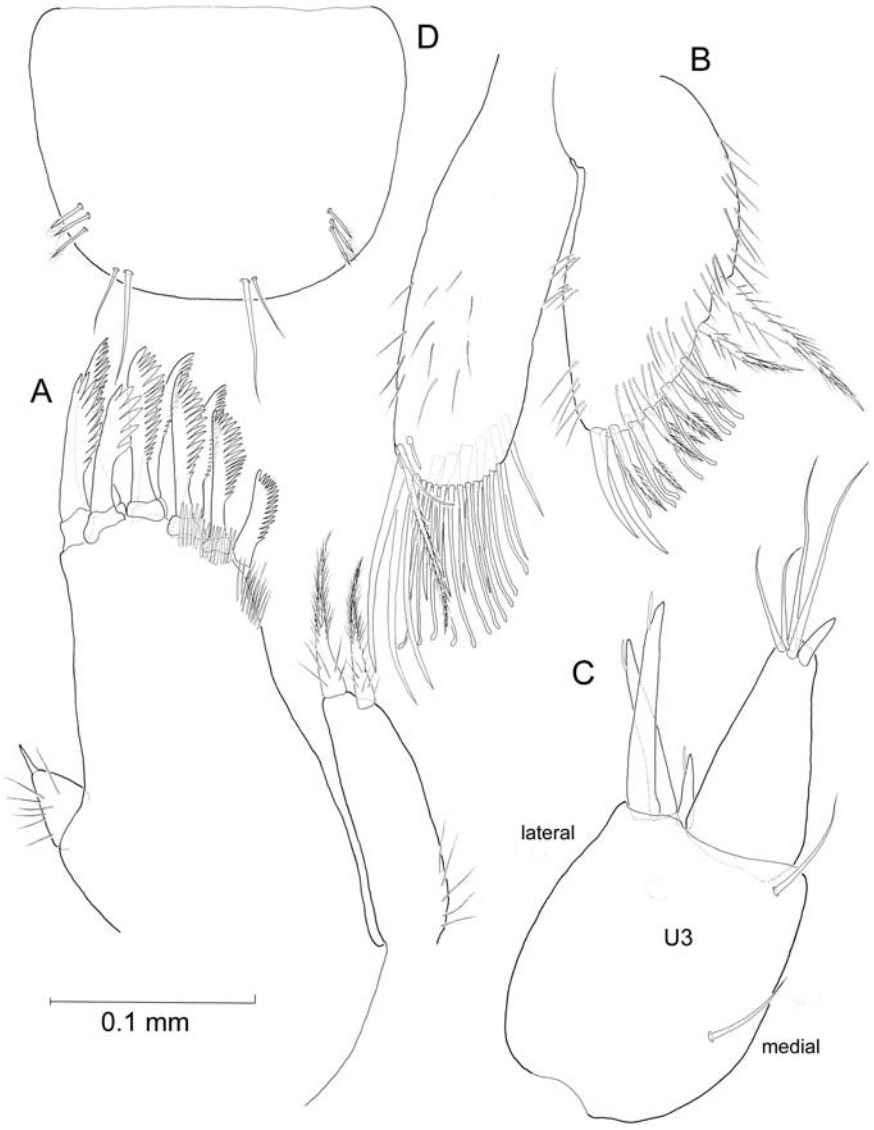

FIGURE 15 *Hyalella hirsuta* Jaume sp. nov., male. A, maxillule; B, maxilla; C, right uropod III, dorsal; D, telson, dorsal.

used to sequence *cox1* [GENBANK access. no. MW714606].

Coata Bay (in front of Capachica Peninsula) [15.733563S; 69.790862W]. On mosses, 15 m depth. Collected by M. Alonso & D. Jaume, 31/03/2019. Single male [IMEDEA/4TK7A4].

Coata Bay [15.60877S; 69.89948W]. On mud, 10 m depth. Collected by O. Kroll, 26/04/2007. Five males and five females [IMEDEA/1395].

Coata Bay (in front of Capachica Peninsula). On *Chara* bottom, 15 m depth. Collected by M. Alonso & D. Jaume, 30/03/2019. Four males and three females [IMEDEA/4TK5A2].

Perú: Mouth of Puno Bay (northern basin of Lake Titicaca) [15.753860S; 69.800983W]. Black conchiferous mud, 30 m depth. Collected by M. Alonso & D. Jaume, 31/03/2019. Five males and one female [IMEDEA/4TK8C].

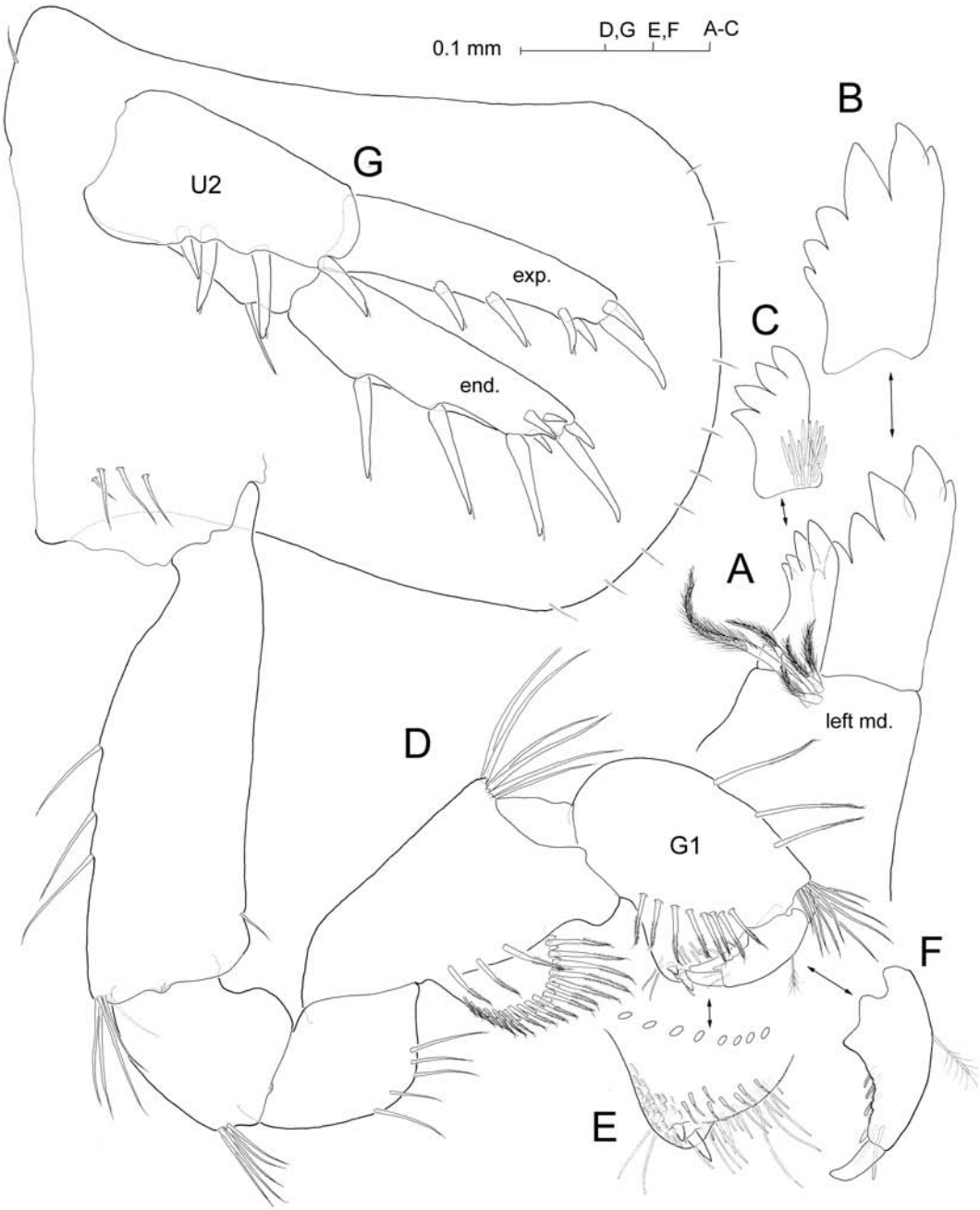

FIGURE 16 *Hyalella hirsuta* Jaume sp. nov., male. A, distal portion of left mandible; B, inset of incisor of latter; C, inset of lacinia; D, left gnathopod I, medial; E, inset of palm margin of latter, medial; F, inset of dactylus-unguis, medial; G, right uropod II, lateral.

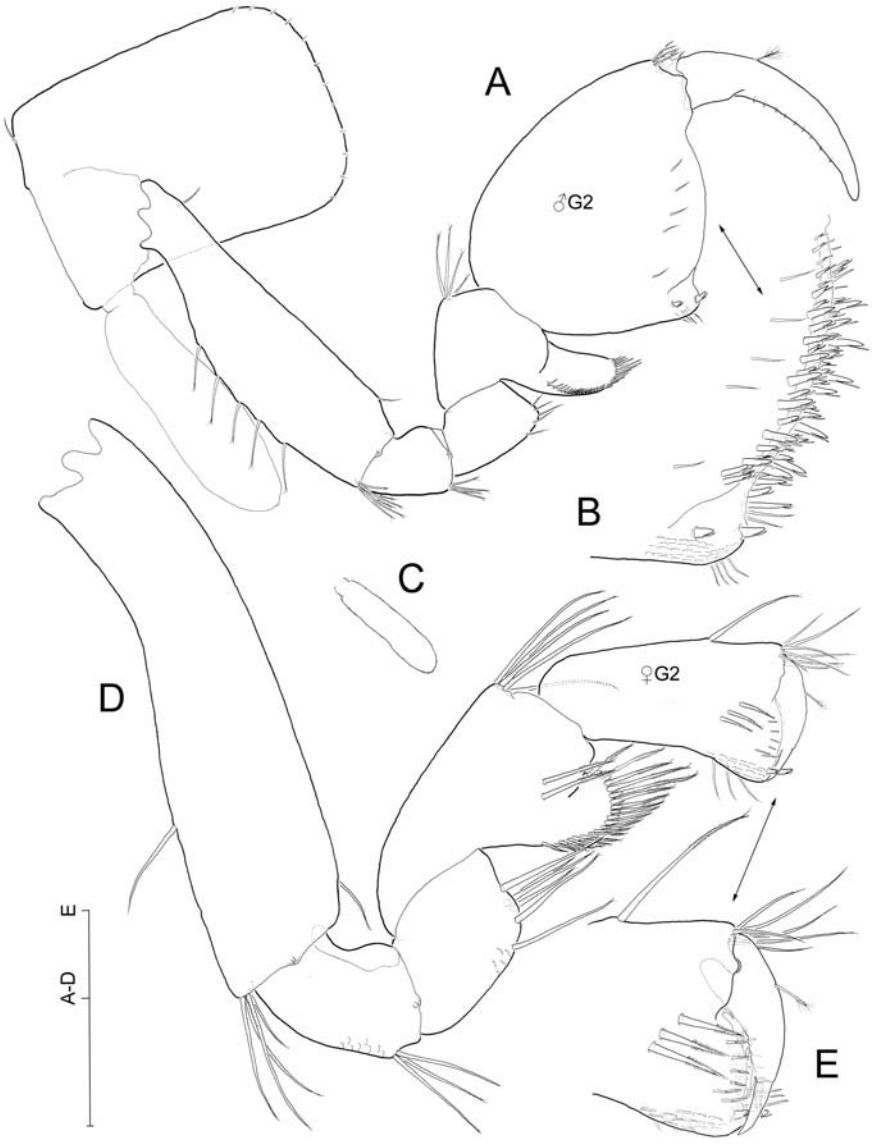

FIGURE 17 *Hyalella hirsuta* Jaume sp. nov., A, left male gnathopod II, medial; B, inset of palm margin of latter, medial; C, one of sternal gills on pereionite II; D, brooding female left gnathopod II, medial; E, inset of distal portion of latter, medial. Notice limbs not figured at same scale. Scale bars: 0.4 mm (A, C); 0.2 mm (B, D, E).

Perú: Chillora (Capachica Peninsula) [15.56018S; 69.85825W]. Black conchiferous mud with some vegetation, 15 m depth. Collected by M. Alonso & D. Jaume, 27/03/2019. Ten males and 12 females [IMEDEA/4TK2B2].

Perú: Escallani (northern shore of Capachica Peninsula; northern basin of Lake Titicaca), ~150 m offshore [15.478119S; 69.88840W]. On *Chara* bottom, ~10 m depth. Collected by M. Alonso & D. Jaume, 27/03/2019. Four males and two females [IMEDEA/4TK1A1].

Perú: Isla Ticonata (Capachica Bay; northern basin of Lake Titicaca) [15.63700S; 69.7920W]. Conchiferous bottom; 26 m

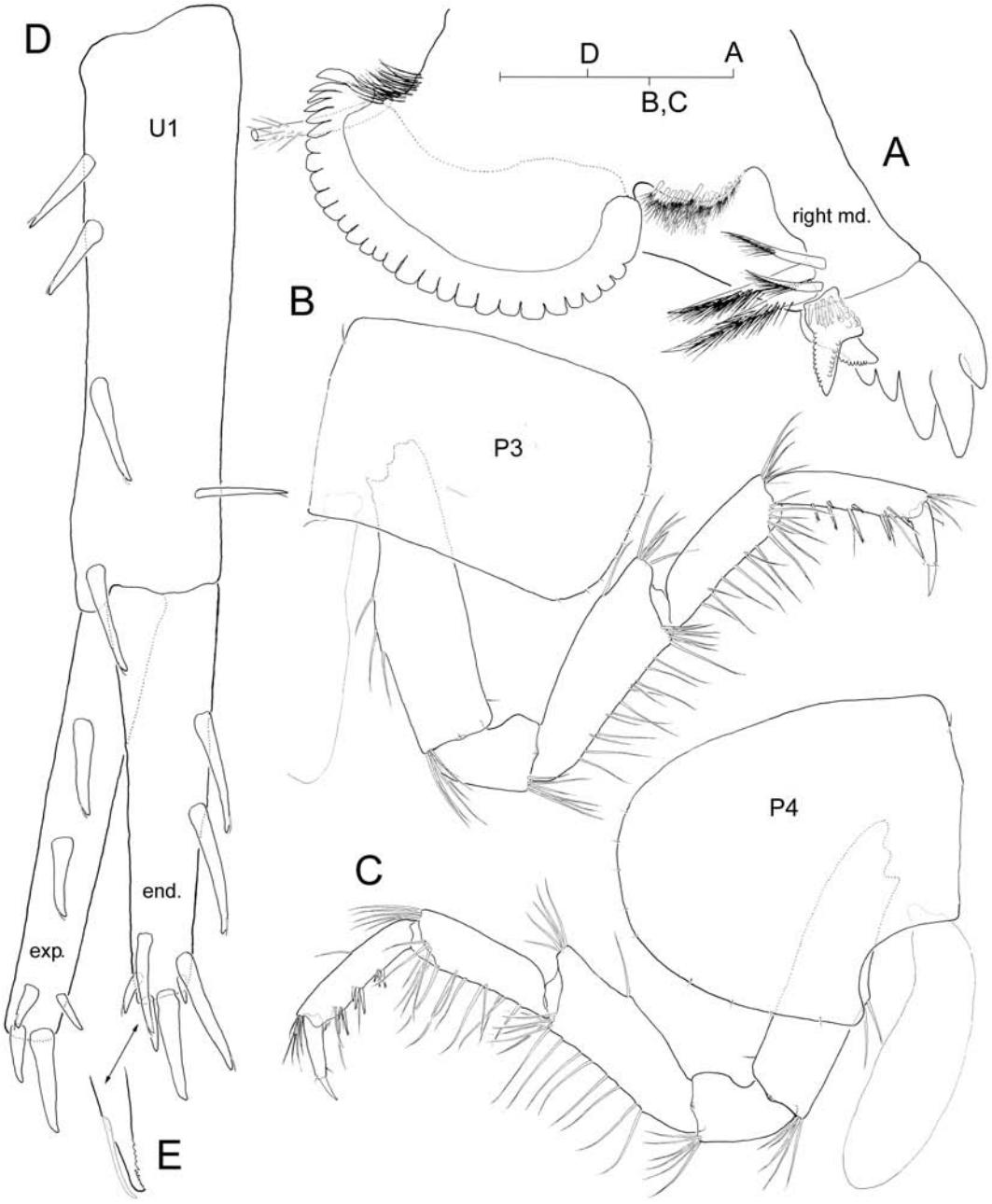

FIGURE 18 *Hyalella hirsuta* Jaume sp. nov., male. A, right mandible; B, right pereiopod III, lateral; C, left pereiopod IV, lateral; D, left uropod I, posterior; E, inset of distal portion of modified spine on endopod of latter. Scale bars: 0.1 mm (A, D); 0.4 mm (B, C).

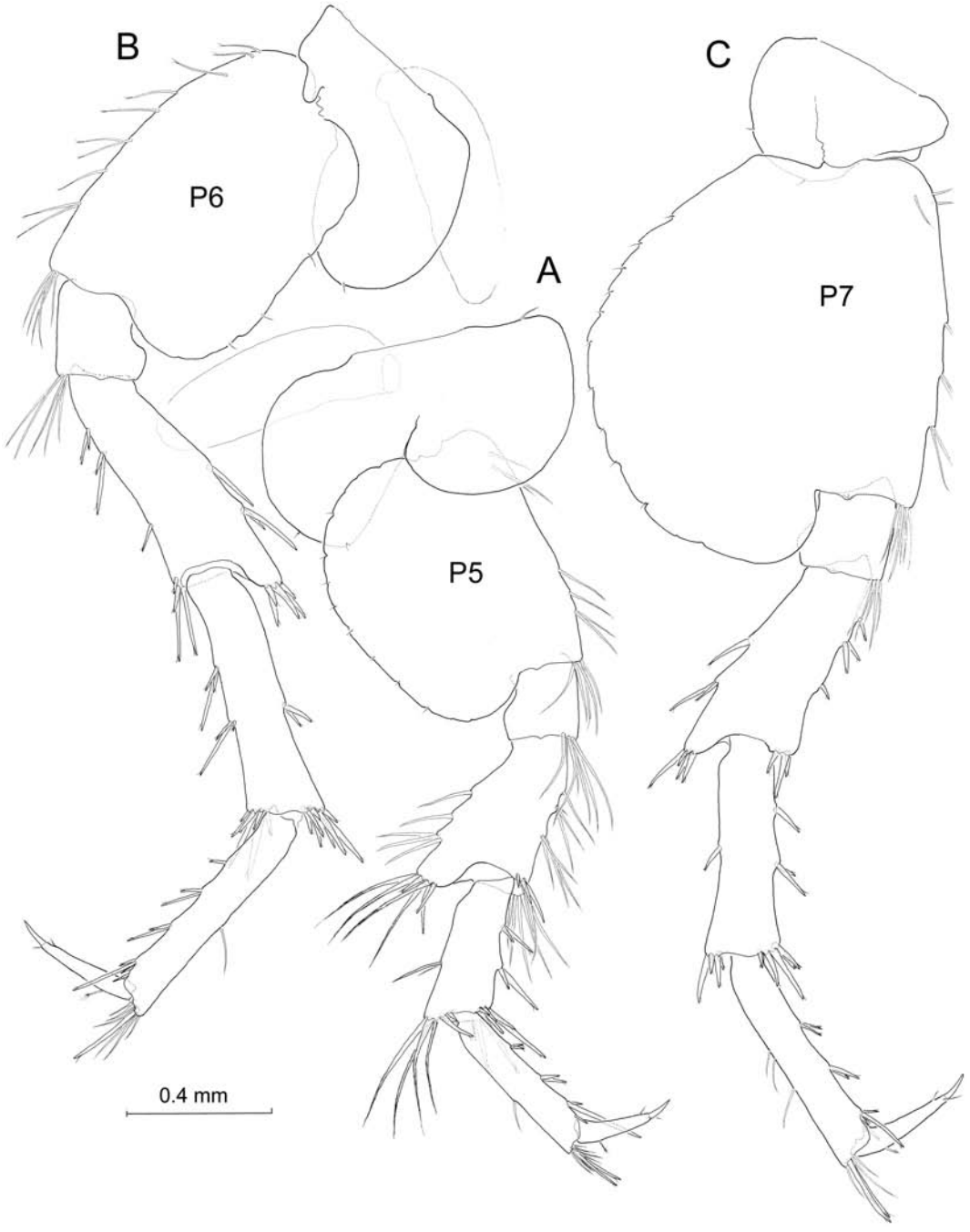

FIGURE 19 *Hyalella hirsuta* Jaume sp. nov., male. A, right pereiopod V, lateral; B, left pereiopod VI, lateral; C, left pereiopod VII, medial.

depth. Collected by O. Kroll, 11/07/2007. Two males [IMEDEA/30-5C], of which one used to sequence mitogenome [GENBANK access. no. MT672019].

Perú: Isla del Sol (northern basin of Lake Titicaca) [16.04000S; 69.15600W]. Conchiferous bottom, 21 m depth. Collected by O. Kroll, 20/07/2007. Single male and brooding female [IMEDEA/32–7(4)].

Diagnosis. Body smooth. Eye ellipsoid. Five pairs of sternal gills, on pereionites III–VII. Incisor of both mandibles toothed, left 6-cuspidate, right 5-cuspidate. Maxillule (= Maxilla 1) basal endite (= outer plate) with nine rake-like spines; endopod (= palp) short (i.e., much shorter than half of distance between base of segment and distolateral angle of basal endite), with single simple distal seta; seta reduced, less than half length of segment. Maxilla (= Maxilla 2) with only one hypertrophied pappose seta proximally on medial margin of coxal endite (= inner plate). Male gnathopod II propodus massive, triangular; palm margin as long as posterior margin. Coxal plate IV as long as broad, with deeply excavate posterior margin. Pereiopods III–V hairy. Male uropod I endopod with short, faintly modified subterminal spine. Uropod III ramus shorter than protopod. Telson about as long as broad, devoid of spines, with one pair of unequal simple setae at each side on distal margin.

Etymology. From Latin *hirsutus* (= hairy), species name refers to the series of long simple setae running along the medial margin of merus and carpus of pereiopods III–V.

Distribution. Endemic to Lake Titicaca.

Male. Body (fig. 13A) up to 9.0 mm long, smooth. Eye ellipsoid. Head about equal in length to pereionites I–II combined; head lobe evenly rounded. Five pairs of sternal gills (fig. 17C), one on each of pereionites III–VII. Epimeral plates unarmed; distal margin of plate I evenly rounded; plates II–III posterodistally acuminate but with angles hardly produced.

Antennule (= Antenna I) (figs 13A, 14A) attaining more than two-thirds length of antenna. Peduncle longer than head; relative length of peduncle segments 1–3 as 1: 0.71: 0.55. Flagellum as long as peduncle.

Antenna (= Antenna II) (figs 13A, 14B) less than half body length, peduncle longer than head; relative length of peduncle segments 4–5 as 0.94: 1. Flagellum shorter than peduncle.

Labrum (= upper lip) and paragnaths (= lower lips) not figured, ordinary, latter with inner lobes not developed.

Left mandible (fig. 16A–C) with toothed, 6-cuspidate incisor and 4-cuspidate lacinia, latter with patch of densely set short spinules on proximal portion of inner surface. Mandible setal row comprising two main pappose setae and three accessory pappose setae disposed in two parallel rows (2 + 3).

Right mandible (fig. 18A) with 5-cuspidate incisor and trifid lacinia, latter with series of round marginal denticles on each cusp distributed as figured, and with patch of densely set short spinules on proximal portion of inner surface. Mandible setal row comprising 2 + 2 pappose setae.

Maxillule (= Maxilla 1) (fig. 15A) coxal endite (= inner plate) narrow and tapering, with two pappose setae on tip. Basal endite (= outer plate) with nine stout rake-like spines. Endopod (= palp) unsegmented, tapering, short (i.e. shorter than half distance between base of segment and distolateral angle of basal endite), with simple, reduced (i.e., less than half length of segment) seta on tip.

Maxilla (= Maxilla 2) (fig. 15B) with single hypertrophied pappose seta proximally on inner margin of coxal endite (= inner plate).

Maxilliped (fig. 14C–E) ordinary; basal endite (= inner plate) with three short

cuspidate spines on distal margin; rest of limb as figured.

Pereiopodal coxae (fig. 13A) I–IV similar in length, each much longer than broad; coxa I (fig. 16D) with anteroproximal angle produced into lobe and with slightly concave anterior margin. Coxa IV (fig. 18C) as long as broad, with deeply excavate posterior margin. Coxa V (fig. 19A) bilobed, posterior lobe longest. Coxa VI with reduced anterior lobe and strongly overhanging posterior lobe (fig. 19B). Coxa VII with only posterior lobe developed (fig. 19C).

Coxal gills present on gnathopod II (fig. 17A) and pereiopods III–VI (figs 18B, C, 19A, B), sacciform.

Gnathopod I (fig. 16D–F) subchelate; propodus much shorter than carpus (attaining about 82% length of carpus), ~1.4 times longer than broad, expanded distally; up to eight bipectinate setae in a row on medial surface of segment as figured; palm angle marked by two unequal flagellate spines; palm margin convex, armed as figured.

Gnathopod II (fig. 17A) subchelate; propodus massive, triangular, 1.2 times as long as broad with posterior margin about as long as palm margin; palm angle placed at about 43% length of segment, marked with two reduced flagellate spines; palm margin strongly armoured (fig. 17B).

Pereiopods III–IV (fig. 18B, C) about similar in length; both with medial margin of merus and carpus lined with numerous long simple setae.

Pereiopods V–VII (fig. 19A-C) elongate, each with basis about as long as corresponding ischium-merus combined. Basis of pereiopod VI not as strongly expanded posteriorly nor with posterodistal angle so strongly overhanging as in pereiopods V and VII, with straight posterior margin. Pereiopod V strongly setose, especially on merus (fig. 19A).

Pleopods I–III (fig. 13A) each with protopod shorter than rami.

Uropod I (fig. 18D) rami shorter than protopod, latter armed with three spines along posterolateral margin, one spine on posterolateral distal angle, and single slender spine placed subdistally on posteromedial margin. Exopod with two spines on posterolateral margin and four on tip. Endopod, with two spines along posteromedial margin and six disposed distally, of which one (fig. 18E) slightly sexually dimorphic.

Uropod II (fig. 16G) rami longer than protopod, latter armed with three spines along posterolateral margin, one spine on posterolateral distal angle, and single slender spine placed subdistally on posteromedial margin. Exopod with two spines on posterolateral margin and four on tip. Endopod with two spines on posteromedial margin and six on tip.

Uropod III (fig. 15C) with ramus shorter than protopod. Latter with three spines on distolateral angle and two simple setae along medial margin; longest spine on distolateral angle as long as ramus. Ramus with short spine and three simple setae on tip; spine less than half ramus length.

Telson (fig. 15D) subrectangular, about 1.3 times broader than long; distal margin with pair of unequal simple setae at each side.

Brooding female. Body up to 7.8 mm long. As male in all respects except for presence of oöstegites on gnathopod II and pereiopods III–V, and structure of gnathopod II. Latter (Fig. 17D, E) with propodus slender, shorter (0.9 ×) than carpus and 1.8 times longer than broad, expanded distally; palm angle marked with two flagellate spines; palm margin slightly transverse, convex, armed as in fig. 17E; row of three bipectinate setae on medial surface of segment as figured.

Remarks. This species is characterized by the display of hairy pereiopods III–V, resembling the condition exhibited in some continental water Gammaridae (v. Pinkster, 1993); no other *Hyalella* species shows pereiopods

ornamented in such a way. Furthermore, the new species displays a faintly modified subterminal spine on the endopod of male uropod I; whereas most *Hyalella* species show either a conspicuously long and recurved modified spine or an ordinary spine at such placement, only *H. cajasi* Alonso & Jaume, 2017 from the highlands of southern Ecuador displays a spine faintly modified in the same way as the new species described herein (Alonso & Jaume, 2017).

The new taxon seems to favour relatively deep-water (up to 30 m depth), murky conchiferous bottoms mostly devoid of vegetation.

***Hyalella solida* Chevreux, 1907**
(figs 13B, 20–25)

Material examined. Perú: Chillora (Capachica Peninsula; northern basin of Lake Titicaca) [15.56018S; 69.85825W]. Black conchiferous mud with sparse vegetation, 15 m depth. Collected by M. Alonso & D. Jaume, 27/03/2019. Many specimens, both sexes [IMEDEA/4TK2C4].

Perú: Juli (northern basin of Lake Titicaca) [16.200519S; 69.461638W]. Vegetated bottom with sponges at ca. 200 m from the shore; 15 m depth. Collected by M. Alonso & D. Jaume, 28/03/2019. Many specimens, both sexes [IMEDEA/4TK4D2].

Perú: Coata Bay (to the lee of Capachica Peninsula; northern basin of Lake Titicaca) [15.726904S; 69.805414W]. On black conchiferous mud, 18 m depth. Collected by M. Alonso & D. Jaume, 30/03/2019. Single male [IMEDEA/4TK6C1].

Diagnosis. Body with five dorsal flanges, one on each pereionite V–VII and pleonite I–II; body tergites covered with densely set short setules. Eye ellipsoid. Five pairs of sternal gills, on pereionites III–VII. Incisor of both mandibles toothed, 6-cuspidate. Maxillule (= Maxilla 1) basal endite (= outer plate) with nine rake-like spines; endopod (= palp) short (i.e., much shorter than half of distance between base of segment and distolateral angle of basal endite), with single reduced (less than half length of segment) spine distally, Maxilla (= Maxilla 2) with one hypertrophied pappose seta proximally on medial margin of coxal endite (= inner plate). Male gnathopod II propodus massive, triangular; palm margin as long as posterior margin. Coxal plate IV 1.3 times longer than broad. Male uropod I endopod without modified subterminal spine. Uropod III ramus shorter than protopod. Telson broader than long, devoid of spines, with one long simple seta at each side on distal margin.

Distribution. Endemic to Lake Titicaca.

Male. Body (fig. 13B) up to 10.3 mm long, densely setulose (see also fig. 25A), with five pointed dorsal flanges, one on each of pereionites V–VII and pleonites I–II. Eye ellipsoid. Head shorter than pereionites I–II combined; head lobe evenly rounded. Five pairs of sternal gills, one on each of pereionites III–VII. Epimeral plates (fig. 25A) unarmed; plates II–III posterodistally acuminate.

Antennule (= Antenna I) (figs 13B, 20A) attaining more than two-thirds length of antenna. Peduncle longer than head; relative length of peduncle segments 1–3 as 1: 0.77: 0.71. Flagellum about as long as peduncle.

Antenna (= Antenna II) (figs 13B, 20B) less than half body length, peduncle longer than head; relative length of peduncle segments 4–5 as 0.79: 1. Flagellum shorter than peduncle.

Labrum (= upper lip) and paragnaths (= lower lips) not figured, ordinary, latter with inner lobes not developed.

Left mandible (fig. 21A) with toothed, 6-cuspidate incisor and 5-cuspidate lacinia, latter with patch of densely set short spinules on proximal portion of inner surface. Mandible setal row comprising two main pappose setae

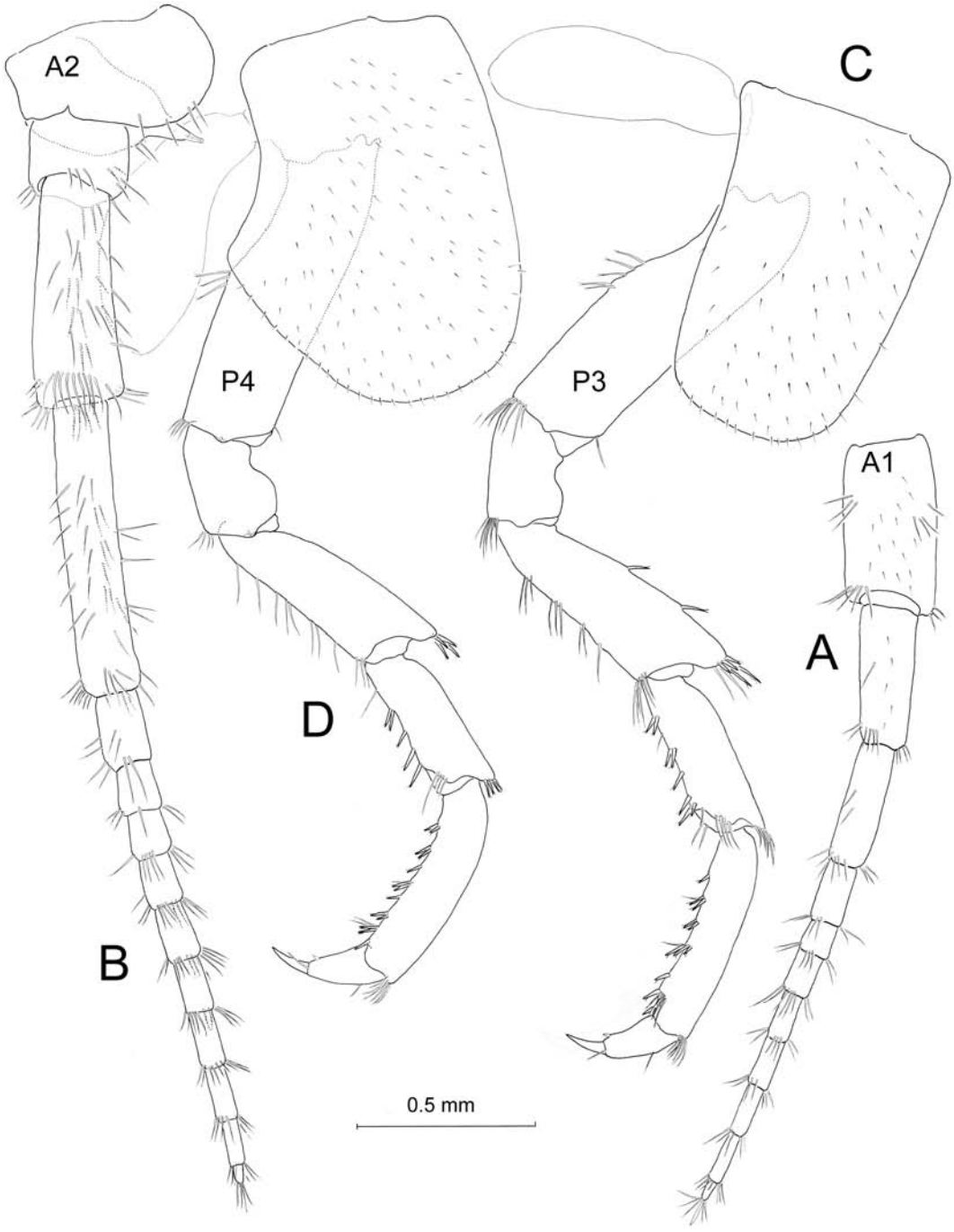

FIGURE 20 *Hyalella solida* Chevreux, 1907, male. A, right antennule, lateral; B, left antenna, lateral; C, right pereiopod III, lateral; D, right pereiopod IV, lateral.

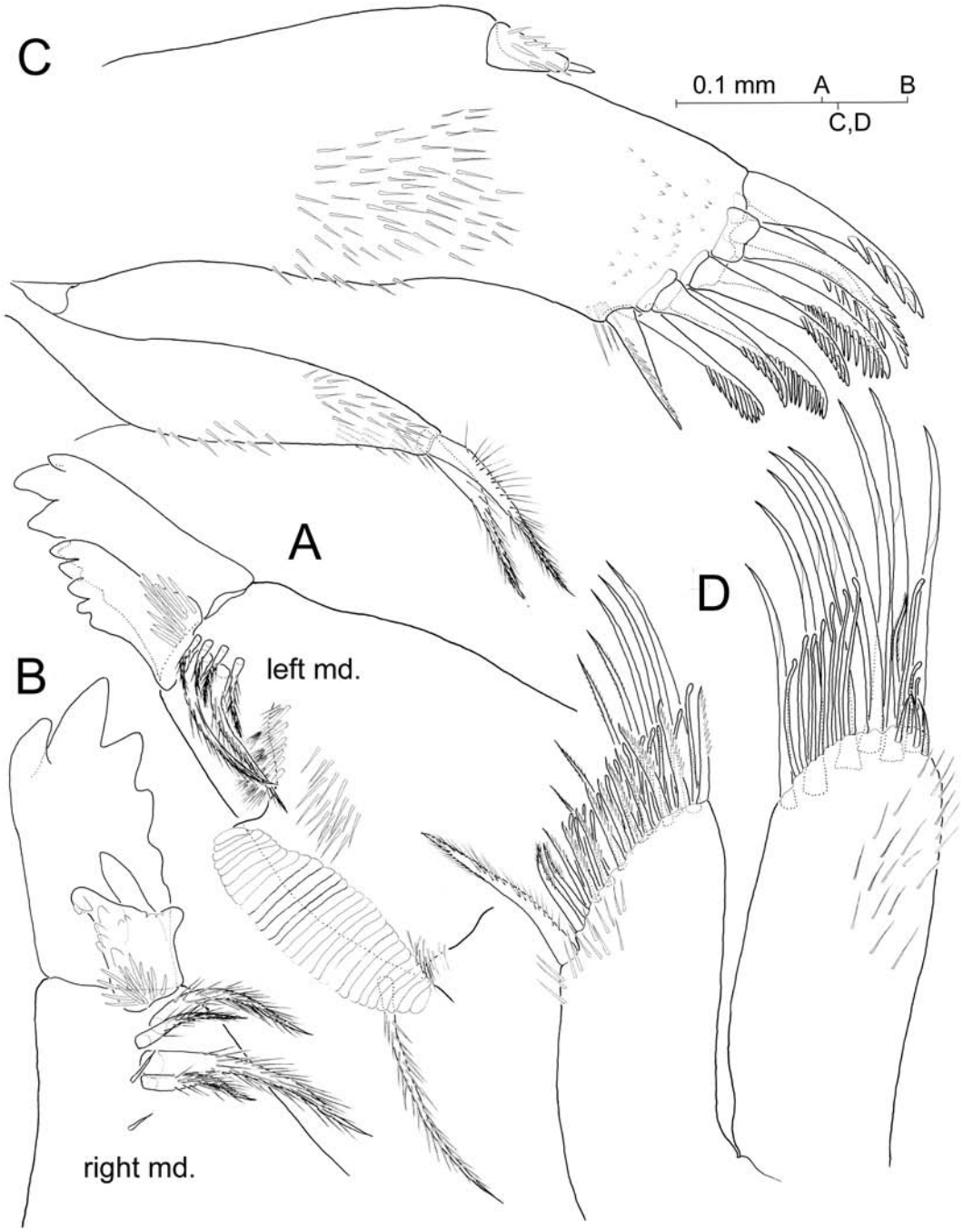

FIGURE 21 *Hyalella solida* Chevreux, 1907, male. A, left mandible; B, inset of distal portion of right mandible; C, maxillule; D, maxilla.

FIGURE 22 *Hyalella solida* Chevreux, 1907. A, male right gnathopod I, medial; B, inset of distal portion of latter, medial; C, disarticulated left maxilliped, anterior (= dorsal). Scale bars: 0.2 mm (A); 0.1 mm (B, C).

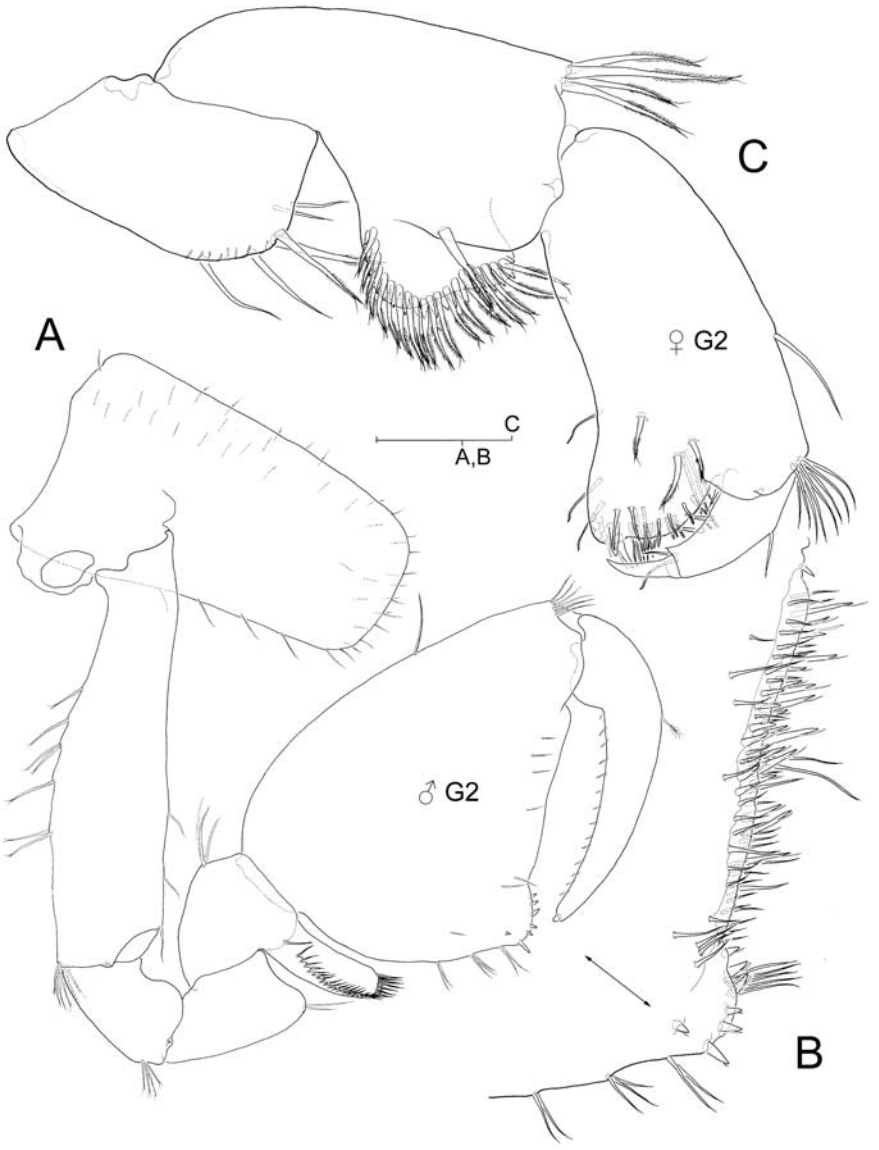

FIGURE 23 *Hyalella solida* Chevreux, 1907. A, male left gnathopod II, medial (armature along palm margin of propodus omitted); B, inset of palm margin of latter, medial; C, distal portion of left female gnathopod II, medial. Scale bars: 0.2 mm (A); 0.1 mm (B, C).

and four accessory pappose setae disposed in two parallel rows (2 + 4).

Right mandible (fig. 21B) with 6-cuspidate incisor and trifid lacinia, latter with series of round marginal denticles on each cusp distributed as figured, and with patch of densely set short spinules on proximal portion of inner surface. Mandible setal row comprising 2 + 2 pappose setae.

Maxillule (= Maxilla 1) (fig. 21C) coxal endite (= inner plate) narrow and tapering, with two pappose setae on tip. Basal endite (= outer plate) with nine slender rake-like spines. Endopod (= palp) unsegmented, tapering,

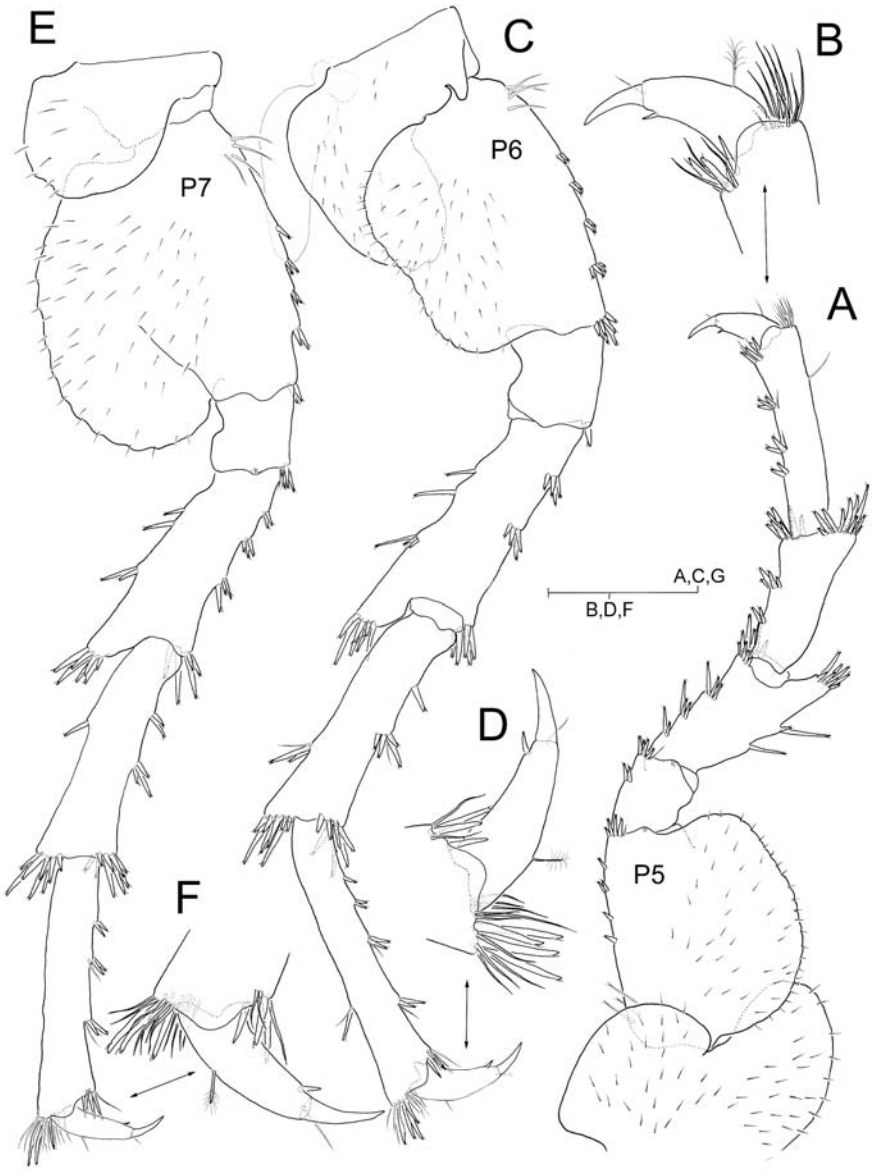

FIGURE 24 *Hyalella solida* Chevreux, 1907, male. A–B, right pereiopod V, lateral (coxal gill omitted); C–D, right pereiopod VI, lateral; E–F, right pereiopod VII, lateral. Scale bars: 0.5 mm (A, C, E); 0.1 mm (B, D, F).

short (i.e., shorter than half distance between base of segment and distolateral angle of basal endite), with simple, reduced (i.e., less than half length of segment) seta on tip.

Maxilla (= Maxilla 2) (fig. 21D) with single hypertrophied pappose seta proximally on inner margin of coxal endite (= inner plate).

Maxilliped (fig. 22A) ordinary; basal endite (= inner plate) with three short cuspidate spines on distal margin; rest of limb as figured.

Pereiopodal coxae (fig. 13B) I–IV similar in length, each much longer than broad; coxa I (fig. 22B) with anteroproximal angle produced into lobe and with slightly concave anterior

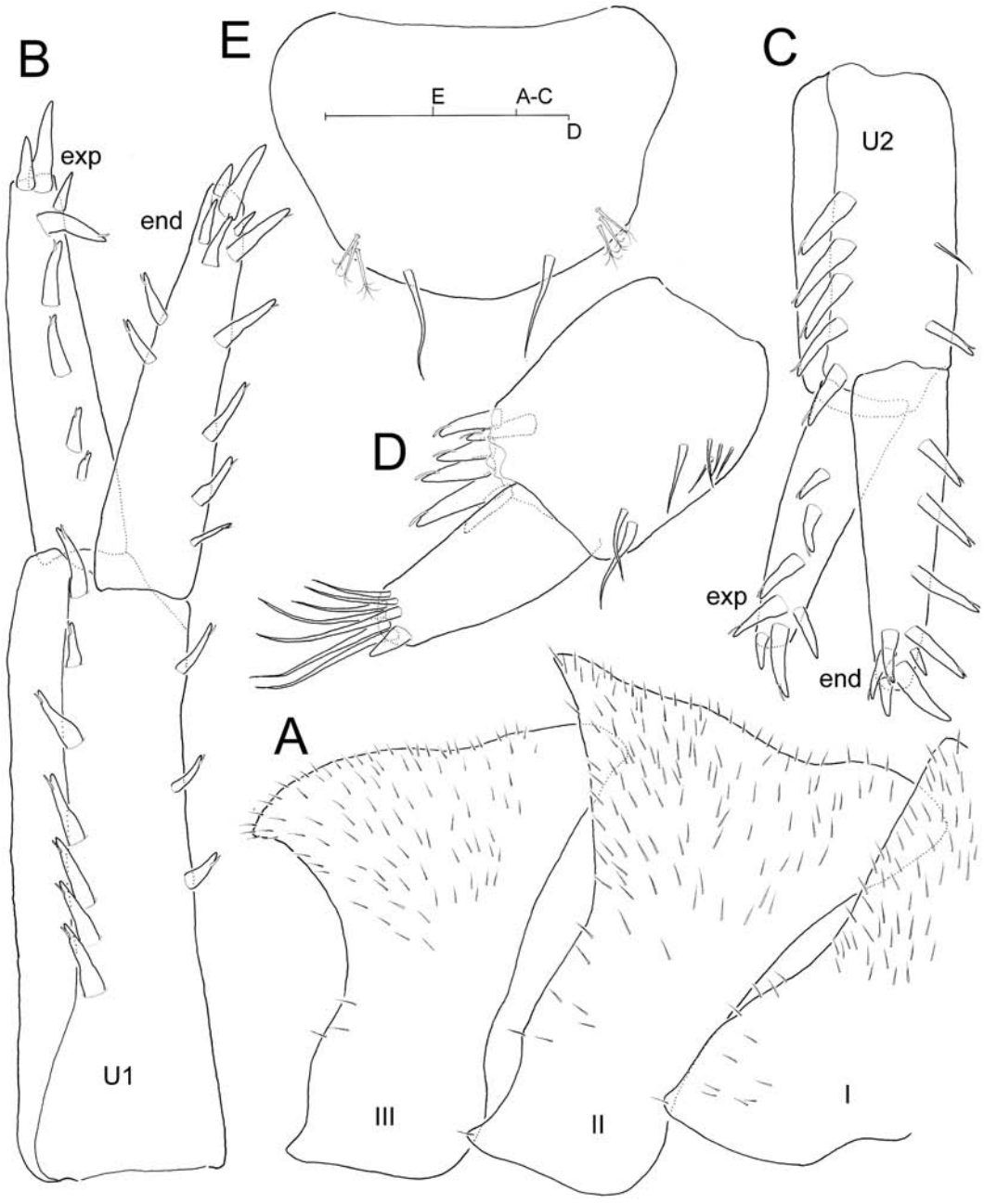

FIGURE 25 *Hyalella solida* Chevreux, 1907, male. A, right epimeral plates I–III, lateral; B, right uropod I, posterior; C, left uropod II, posterior; D, left uropod III, dorsal (= posterior); E, telson, dorsal (= posterior). Scale bars: 0.5 mm (A); 0.2 mm (B, C); 0.1 mm (D, E).

margin. Coxa IV (fig. 20D) 1.3 times longer than broad, with shallowly excavate posterior margin. Coxa V (fig. 24A) bilobed, posterior lobe longest. Coxa VI with reduced anterior lobe and strongly overhanging posterior lobe (fig. 24C). Coxa VII with only posterior lobe developed (fig. 24E).

Coxal gills present on gnathopod II (fig. 17A) and pereiopods III–VI (figs 20C, D, 24C), sacciform.

Gnathopod I (fig. 22B, C) subchelate; propodus slightly shorter than carpus (attaining about 93% length of carpus), ~1.7 times longer than broad, expanded distally; up to six bipectinate setae in a row present on medial surface of segment as figured; palm angle marked by two unequal flagellate spines; palm margin slightly convex, armed as figured.

Gnathopod II (fig. 23A, B) subchelate; propodus massive, triangular, 1.3 times as long as broad with posterior margin slightly shorter than palm margin; palm angle placed midway of segment, marked with seven short flagellate spines; palm margin armoured as in fig. 23B.

Pereiopods III–IV (fig. 20C, D) about similar in length; armature ordinary.

Pereiopods V–VII (fig. 24) elongate. Basis of pereiopods V (fig. 24A) and VII (fig. 24E) each about as long as corresponding ischium-merus combined; basis of pereiopod VI (fig. 24C) much shorter than corresponding ischium-merus combined, not as strongly expanded posteriorly nor with posterodistal angle so strongly overhanging as in pereiopods V and VII, with slightly concave posterior margin.

Pleopods I–III (fig. 13B) each with protopod shorter than rami.

Uropod I (fig. 25B) with rami shorter than protopod, latter armed with row of six spines along posterolateral margin, two spines along posteromedial margin, and one spine on each distolateral and distomedial angle of segment. Exopod with row of four spines along posterolateral margin and four spines on tip; endopod with two spines on posterolateral margin, row of four along posteromedial margin, and six spines terminally; endopod lacking modified spine.

Uropod II (fig. 25C) rami about as long as protopod, latter armed with row of four spines along posterolateral margin, one reduced simple seta on posteromedial margin, and one spine on each posterolateral and posteromedial angle of segment. Exopod with three spines along posterolateral margin and four placed distally. Endopod with three spines along posteromedial margin and six disposed distally.

Uropod III (fig. 25D) with ramus shorter than protopod, latter with transverse row of six spines on distolateral angle and about six simple setae on medial margin. Ramus with short spine and cluster of about seven simple setae on tip; spine much shorter than half ramus length.

Telson (fig. 25E) broader than long, with evenly rounded posterior margin and slightly concave lateral margins. Armature consisting on one simple seta at each side on distal margin and three short penicillate setae placed subdistally at each side.

Brooding female. Body up to 9.8 mm long. As male in all respects except for presence of oöstegites on gnathopod II and pereiopods III–V, and structure of gnathopod II. Latter (fig. 23C) with propodus slender, slightly shorter than carpus and 1.8 times longer than broad, expanded distally; palm angle marked with two long flagellate spines; palm margin slightly transverse, convex; three bipectinate setae on medial surface of segment as figured.

Remarks. This species is readily identified by its densely setulose body integument combined with the display of five pointed dorsal flanges positioned one on each of pereionites V–VII and pleonites I–II. The

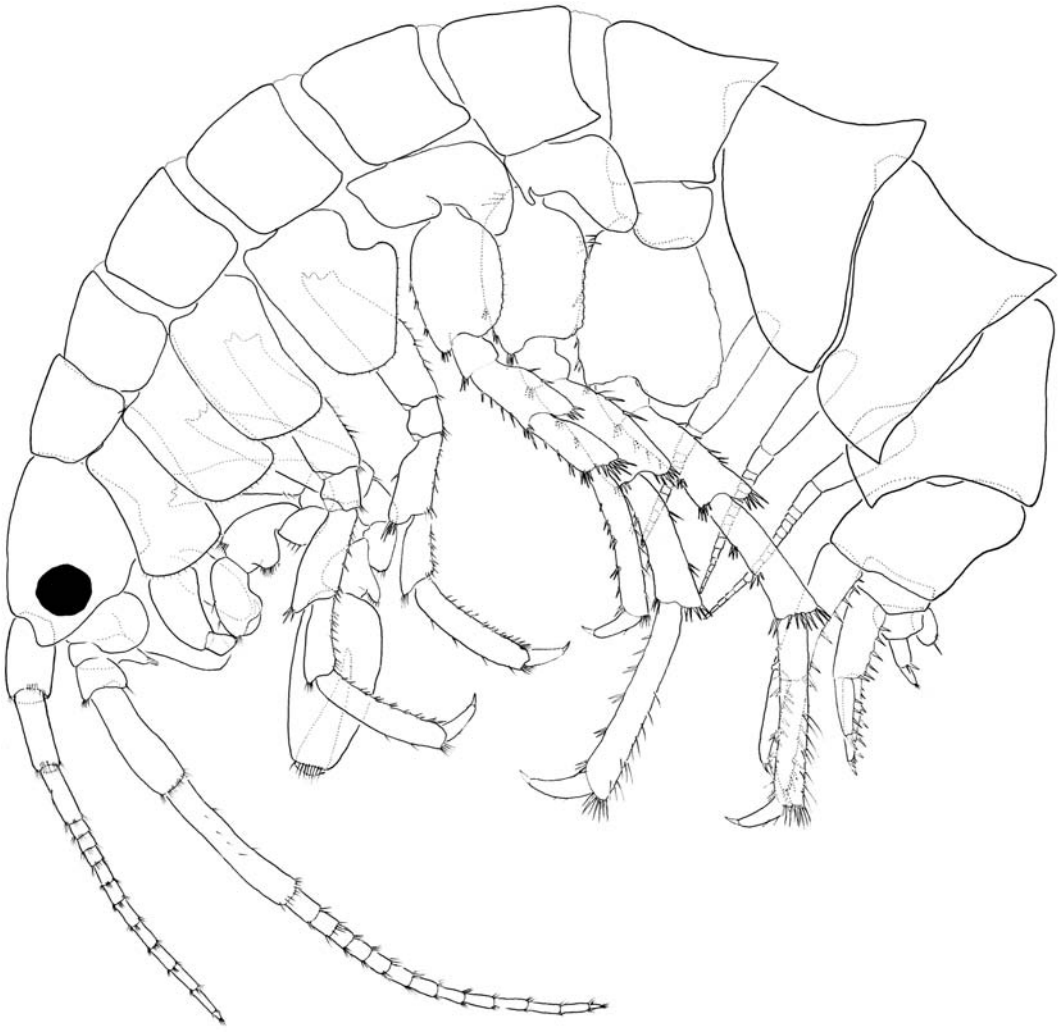

FIGURE 26 *Hyalella nefrens* González & Watling, 2003. Male 12.3 mm habitus, lateral.

strongly armoured uropods I–II are also a remarkable feature of this taxon. It was described by Chevreux (1907) based on a single male specimen collected by the expedition of de Créqui Montfort and Sénéchal de La grande to the Titicaca and never found again since its discovery, to the point that González & Watling (2003) considered it was not possible to differentiate this species and even suggested that the setulation of body somites described by Chevreux (1907) might correspond to a case of infestation with protozoans or fungi.

Hyalella nefrens González & Watling, 2003
(figs 26–33)

Material examined. Bolivia: Escoma (northern basin of lake Titicaca) [15.73621S; 69.08813W]. Sand; mud; among Totora, *Myriophyllum*, *Zannichellia*, *Isoetes*, *Nostoc*; < 1 m depth. Collected by O. Kroll, 14/12/2009. Three males and one female [IMEDEA/4798A]. One of males used to sequence mitogenome [GENBANK access. no. MT672019].

Bolivia: Huatajata (southern basin of Lake Titicaca) [16.21282S; 68.69392W]. 0.7 m

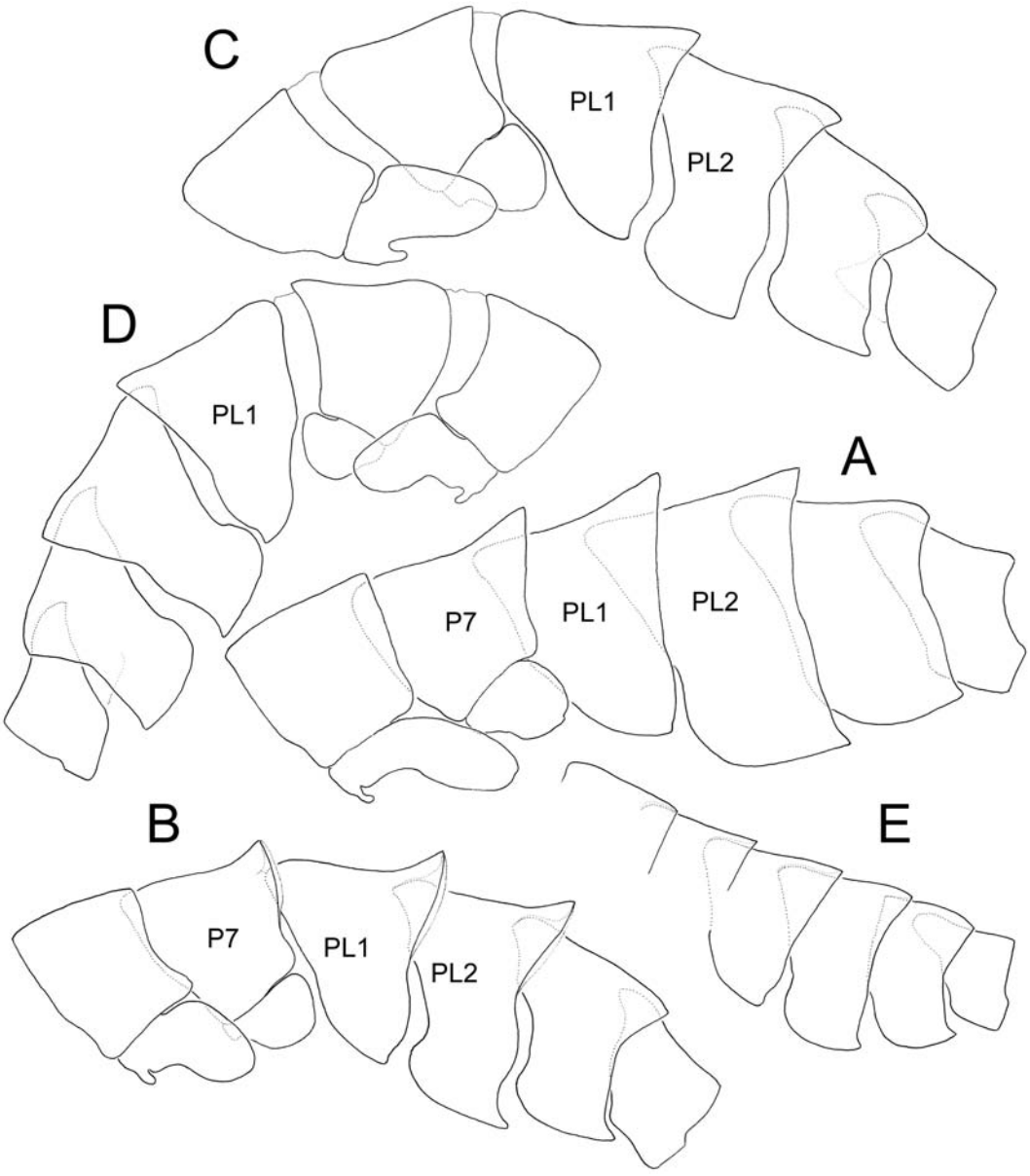

FIGURE 27 *Hyalella nefrens* González & Watling, 2003. Range of configurations of dorsal armature. A–B, specimens showing three posterodistal processes, one on each pereionite VII and pleonites I–II; C, specimen with only two processes developed, one on each pleonite I–II; D, specimen with only one process, on pleonite I; E, specimen with smooth dorsum.

depth. *Myriophyllum*. Collected by O. Kroll, 17/05/2007. Many specimens, both sexes [IMEDEA/10-5B]. Same locality and date, but between 1.5 and 2.5 m depth among *Myriophyllum*, *Elodea* and *Zannichellia* or *Ruppia*. Seven males and seven females [IMEDEA 1613C]. One of males used to sequence *cox1* [GENBANK access. no. MN582275].

Bolivia: Punta Khauani (Ajilata; northern basin of Lake Titicaca) [16.01000S; 68.81900W]. 4 m depth, among *Chara*.

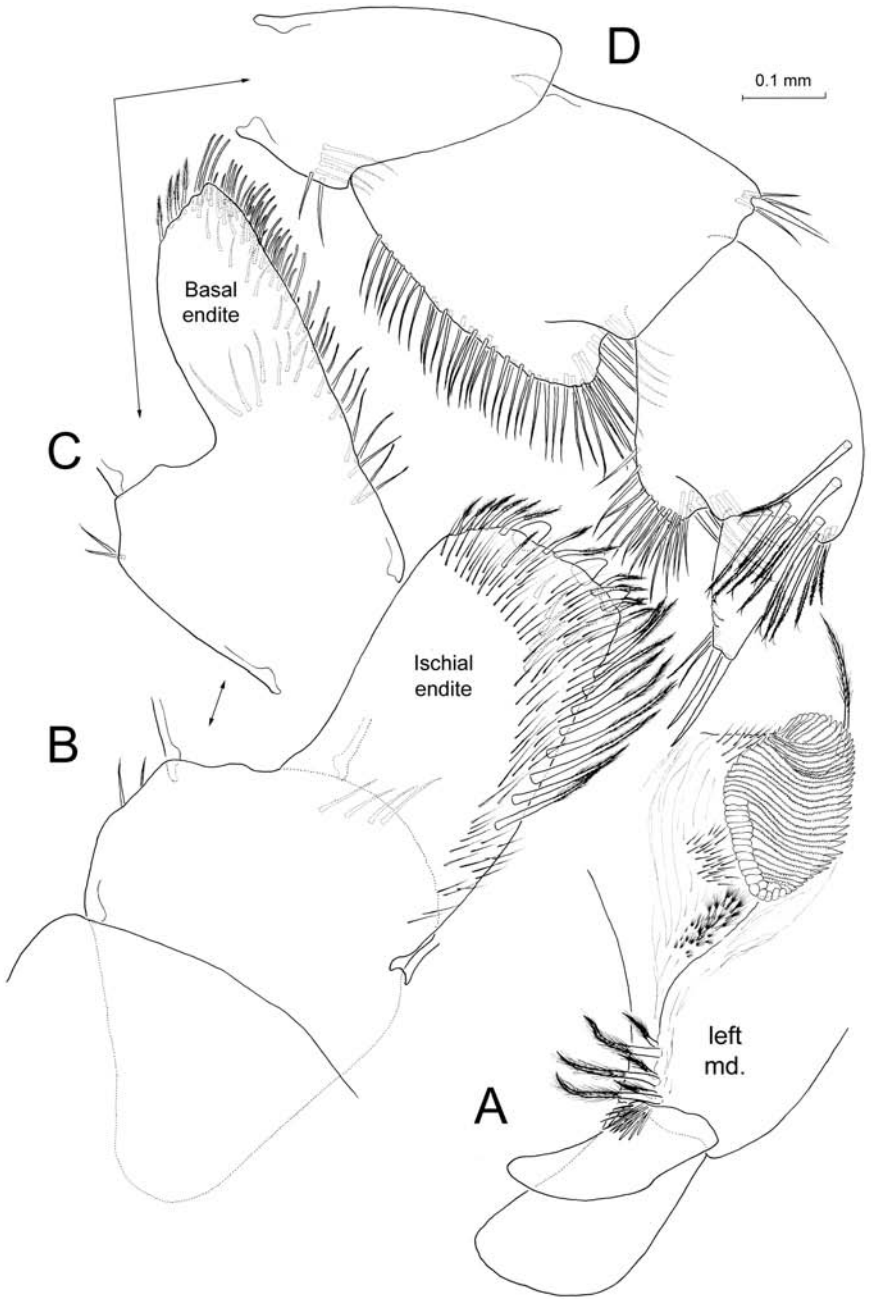

FIGURE 28 *Hyalella nefrens* González & Watling, 2003, male. A, left mandible; B–D, disarticulated left maxilliped, anterior (= dorsal).

Collected by O. Kroll, 20/07/2007. Four males and five females [IMEDEA/2314B]. One of males used to sequence *cox1* [GENBANK access. no. MN582239]. Same locality and date, but at 2 m depth among *Myriophyllum*. Two males [IMEDEA/2310F2], of which one used to sequence *cox1* [GENBANK access. no. MN582252].

Perú: Vilquechico (northern basin of Lake Titicaca) [15.23600S; 69.69400W]. Black mud

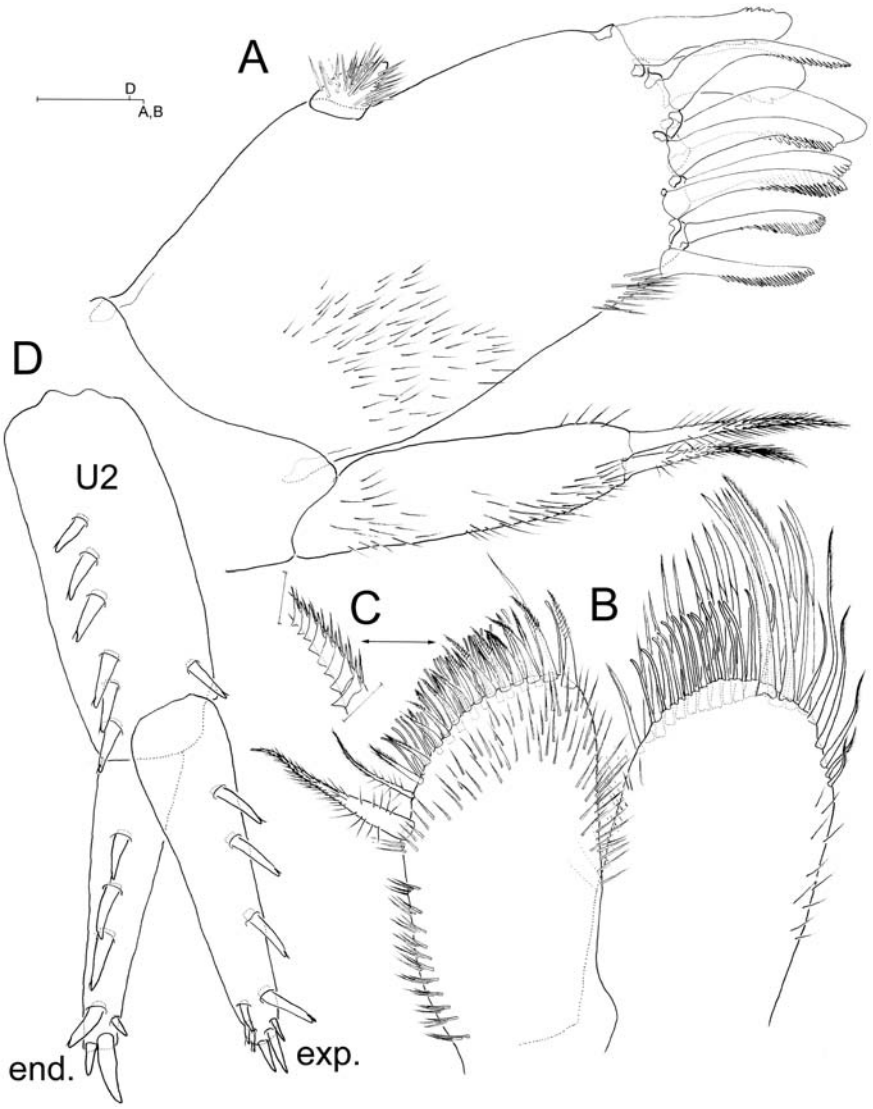

FIGURE 29 *Hyalella nefrens* González & Watling, 2003, male. A, left maxillule; B, maxilla; C, inset of one of modified spines on coxal endite of latter; D, left uropod II, posterior. Scale bars: 0.1 mm (A, B); 0.2 mm (D).

with macrophytes (*Chara*, *Myriophyllum*, *Elodea*), 3.5 m depth. Collected by O. Kroll, 06/05/2007. Two males and one female [IMEDEA/1420C]. One of males used to sequence *cox1* [GENBANK access. no. MN582249].

Perú: Isla Taquile (northern basin of Lake Titicaca). < 1 m depth. Collected by C. Juan, 07/08/2015. Single female [IMEDEA/2015-3B], used to sequence *cox1* [GENBANK access. no. MN582311].

Perú: Playa de san Juan (Juli; northern basin of Lake Titicaca) [16.202246S; 69.461093W]. <1 m depth. *Myriophyllum*. Collected by M. Alonso & D. Jaume, 19/05/2017. Single male and female [IMEDEA/3TK2B]. Male used to sequence *cox1* [GENBANK access. no. MN582299]. Same

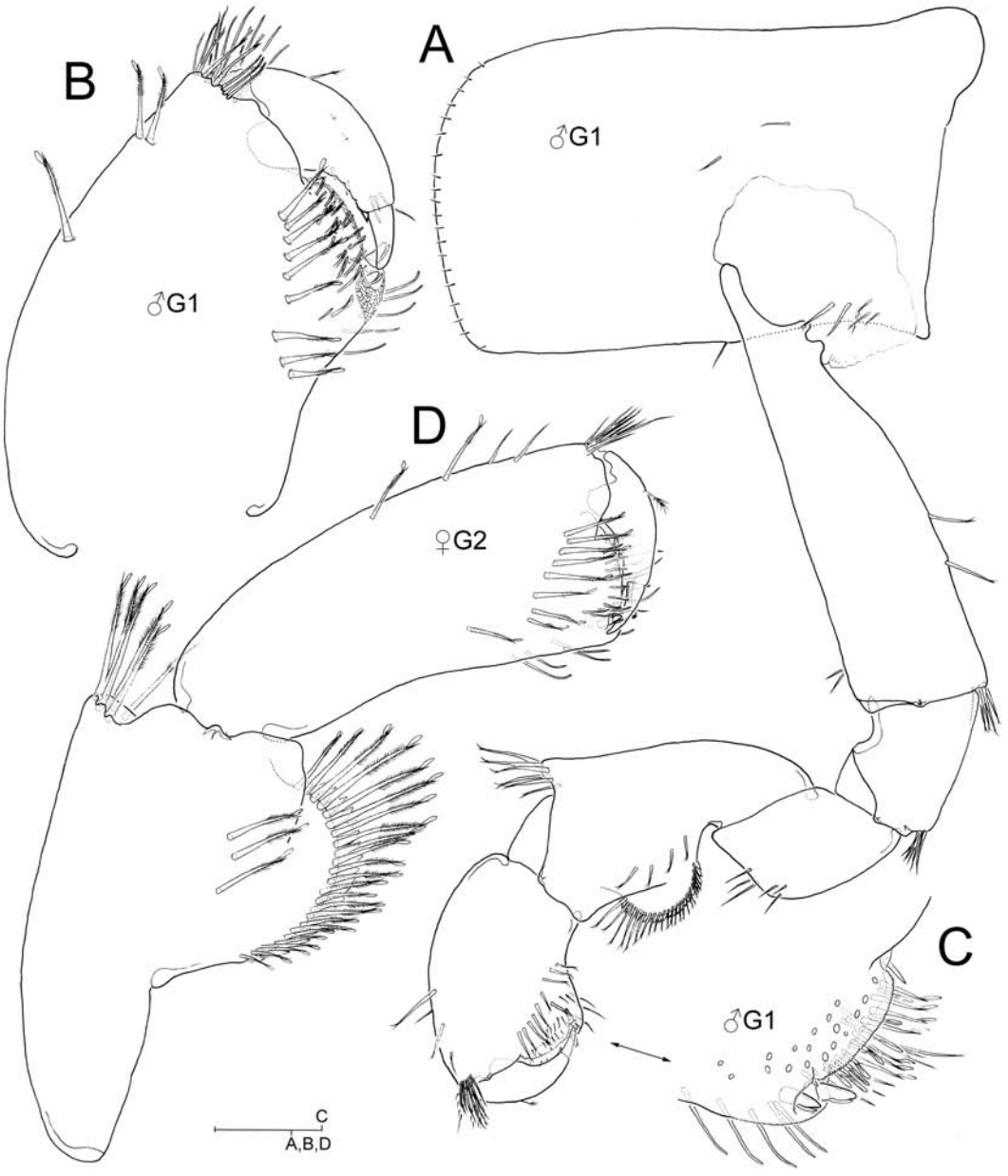

FIGURE 30 *Hyalella nefrens* González & Watling, 2003. A, male right gnathopod I, medial; B, inset of propodus+dactylus of left counterpart of latter, medial; C, inset of palm angle and armature along lateral side of palm margin of latter, medial; D, distal portion of female left gnathopod II, medial. Scale bars: 0.2 mm (A, C); 0.1 mm (B, D).

locality and date, but among *Scirpus*. Single female [IMEDEA/3TK1E], used to sequence *cox1* [GENBANK access. no. MN582298].

Perú: Juli [16.200519S; 69.461638W]. Vegetated bottom with sponges ca. 200 m from the shore; 15 m depth. Collected by M. Alonso & D. Jaume, 28/03/2019. Many specimens, both sexes [IMEDEA/ 4TK4E].

Perú: Escallani (Bahía de Capachica; northern basin of Lake Titicaca) [15.478119;

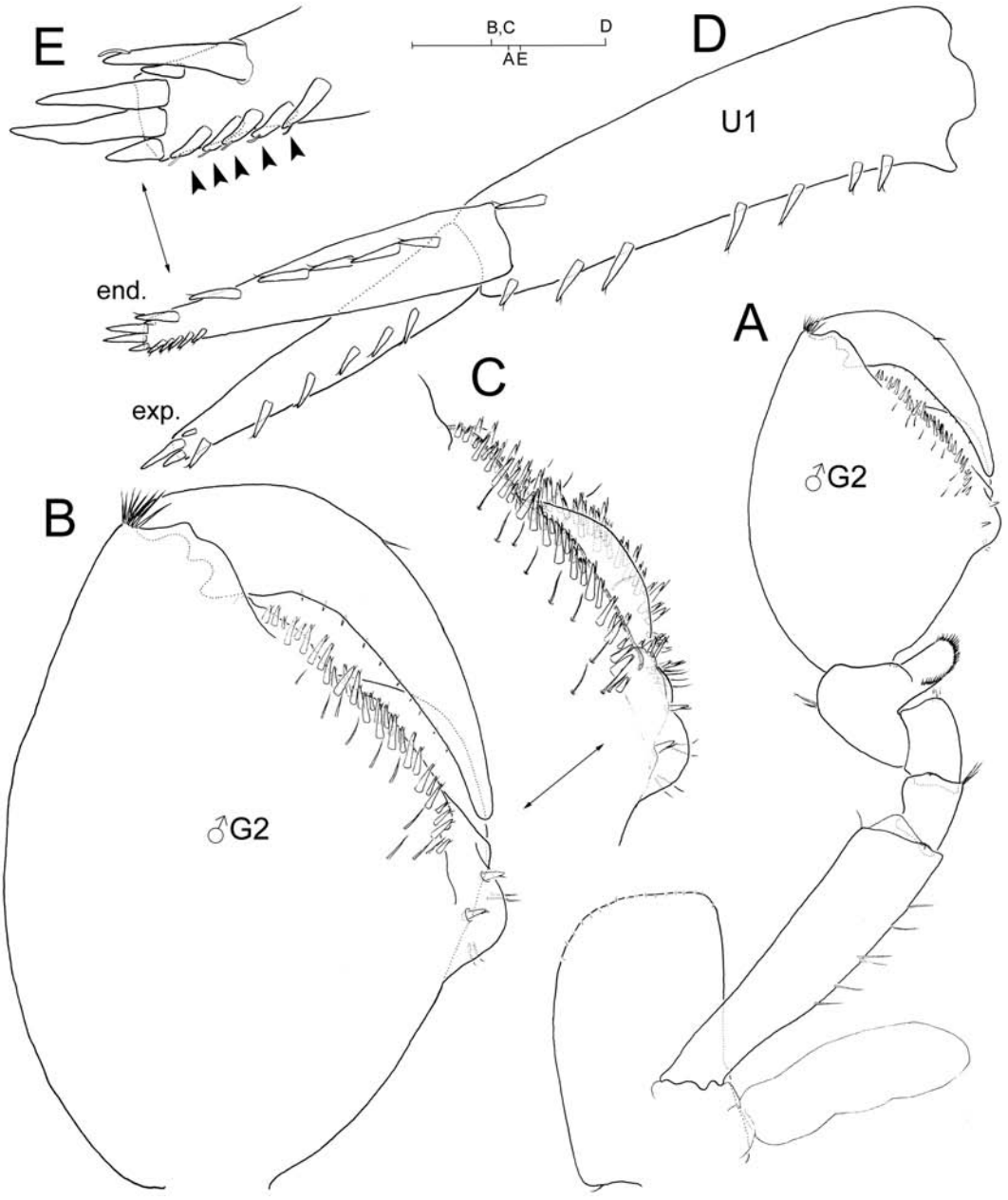

FIGURE 31 *Hyalella nefrens* González & Watling, 2003, male. A, left gnathopod II, medial; B, inset of propodus+dactylus of latter, medial (armature along outer side of palm margin omitted); C, inset of palm margin, medial; D, right uropod I, posterior; E, inset of distal portion of endopod of latter (arrowheads pointing to short spines along distolateral margin of segment, variably expressed in number among specimens). Scale bars: 0.1 mm (E); 0.2 mm (B, C); 0.5 mm (A, D).

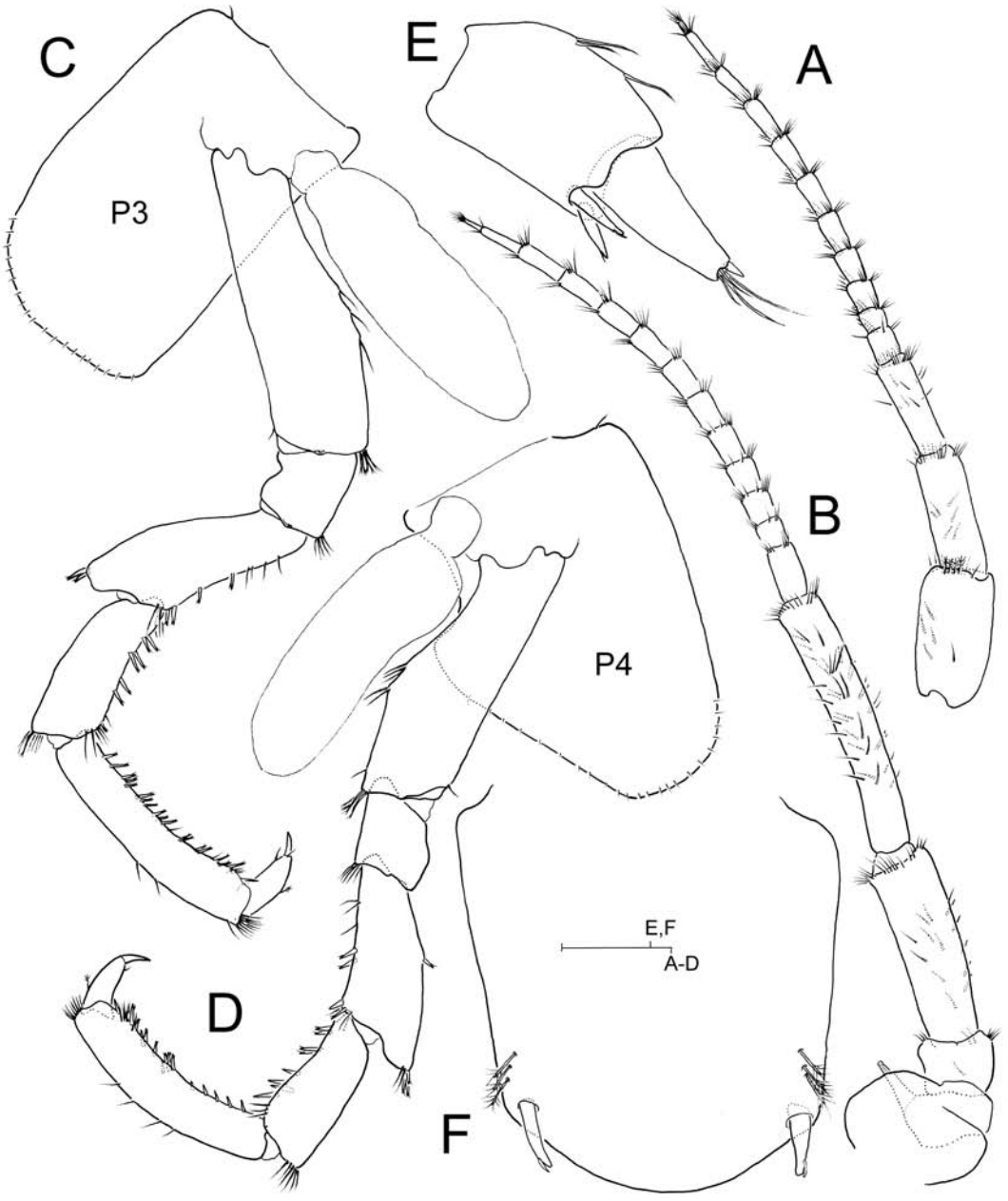

FIGURE 32 *Hyalella nefrens* González & Watling, 2003, male. A, left antennule, lateral; B, left antenna, lateral; C, right pereiopod III, medial; D, left pereiopod IV, medial; E, left uropod III, dorsal; F, telson, dorsal. Scale bars: 0.5 mm (A–D); 0.1 mm (E, F).

69.88840W]. On *Chara* bottom 10 m depth, 150 m from the shore. Collected by M. Alonso & D. Jaume, 27/03/2019. Many specimens, both sexes [IMEDEA/ 4TK1A3].

Perú: Bahía de Ramis (northern basin of Lake Titicaca) [15.32100S; 69.84600W]. 4 m depth, among *Chara*. Collected by O. Kroll, 24/06/2007. Two females [IMEDEA/24-2D], of

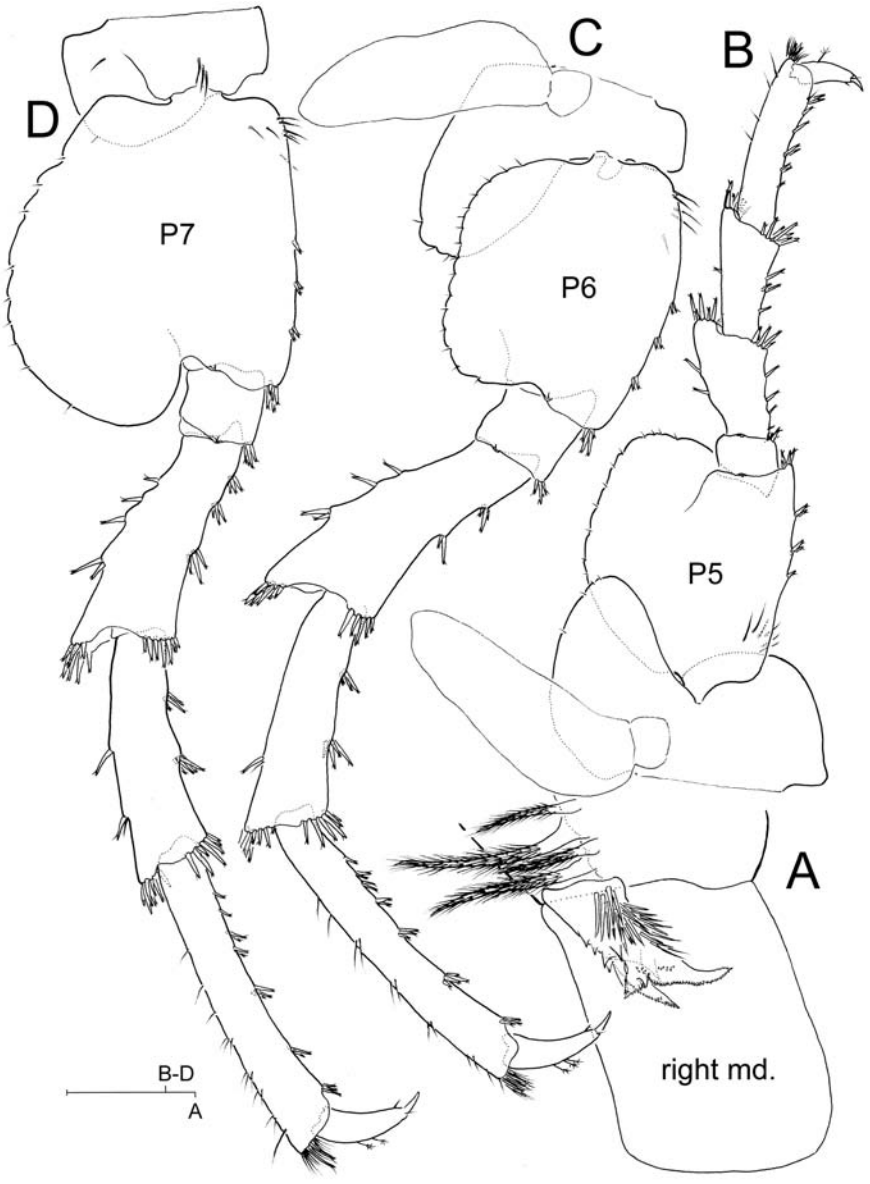

FIGURE 33 *Hyalella nefrens* González & Watling, 2003, male. A, inset of distal portion of right mandible; B, right pereiopod V, medial; C, left pereiopod VI, medial; D, left pereiopod VII, medial. Scale bars: 0.1 mm (A); 0.5 mm (B-D).

which one used to sequence *cox1* [GENBANK access. no. MN582261].

Perú: Bahía de Ramis [15.32400S; 69.83100W]. *Myriophyllum*; *Potamogeton*; 5 m depth. Collected by O. Kroll, 24/06/2007. Single specimen [IMEDEA/24-1E], used to sequence *cox1* [GENBANK access. no. MN582253].

Perú: Coata Bay, in front of Capachica Peninsula (northern basin of Lake Titicaca) [15.733563S; 69.790862W]. Among aquatic mosses, 15 m depth. Collected by M. Alonso &

D. Jaume, 31/03/2019. Many specimens, both sexes [IMEDEA/4TK7C1], of which three males used to sequence *cox1* [GENBANK access. nos. MW714607, MW714608 and MW714609].

Perú: Coata Bay [15.62553S; 69.89970W]. Mud/detritus + macrophytes (*Elodea*, some *Myriophyllum*), 2.5 m depth. Collected by O. Kroll, 26/04/2007. Single specimen [IMEDEA/1403D], used to sequence *cox1* [GENBANK access. no. MN582300].

Perú: Chocasuyu (Bahía de Copacabana; northern basin of Lake Titicaca) [16.20500S; 69.39700W]. *Potamogeton*, *Elodea*, *Myriophyllum*; 1.5 m depth. Collected by O. Kroll, 01/07/2007. Single specimen [IMEDEA/26-7C], used to sequence *cox1* [GENBANK access. no. MN582310].

Perú: Isla Ticonata (Bahía de Capachica; northern basin of Lake Titicaca; [15.64700S; 69.79100W]. Among *Chara*, 8 m depth. Collected by O. Kroll, 11/07/2007. Single specimen [IMEDEA/30-4C].

Diagnosis. Body usually with three dorsal flanges, one on each pereionite VII and pleonites I–II, rarely with two, one or none. Eye rounded. Five pairs of sternal gills, one on each pereionites III–VII. Incisor of both mandibles smooth. Maxillule (= Maxilla 1) basal endite (= outer plate) with nine spines, of which three spatulate and hardly denticulated, rest rake-like; endopod (= palp) short (i.e., much shorter than half of distance between base of segment and distolateral angle of basal endite), unarmed. Maxilla (= Maxilla 2) with only one hypertrophied pappose seta proximally on medial margin of coxal endite (= inner plate); distal margin lined with series of modified slender spines provided with transverse lamellae. Male gnathopod II propodus massive, triangular; palm margin as long as posterior margin. Coxal plate IV (1.3 times as long as broad, with deeply excavate posterior margin. Male uropod I endopod without modified subterminal spine. Uropod III ramus about as long as protopod. Telson much longer than broad, with one short stout spine at each side on distal margin.

Distribution. Endemic to Lake Titicaca.

Male. Body (fig. 26) up to 12.3 mm long, usually with three pointed dorsal flanges, one on each of pereionite VII and pleonites I–II, but occasionally with less or even none (see fig. 27). Eye rounded. Head about as long as pereionites I–II combined; head lobe roughly triangular. Five pairs of sternal gills, one on each of pereionites III–VII. Epimeral plates (figs 26–27) unarmed; plates II–III posterodistally acuminate.

Antennule (= Antenna I) (figs 26, 32A) attaining more than two-thirds length of antenna. Peduncle longer than head; relative length of peduncle segments 1–3 as 1: 1: 0.72. Flagellum longer peduncle.

Antenna (= Antenna II) (figs 26, 32B) about as long as half body length, peduncle much longer than head; relative length of peduncle segments 4–5 as 0.73: 1. Flagellum as long as peduncle.

Labrum (= upper lip) and paragnaths (= lower lips) not figured, ordinary, latter with inner lobes not developed.

Left mandible (fig. 28A) with smooth, untoothed incisor and lacinia, latter with patch of densely set short spinules on proximal portion of inner surface. Mandible setal row comprising three main pappose setae and three accessory pappose setae disposed in two parallel rows (3 + 3). Rest of mandible as figured.

Right mandible (fig. 33A) with smooth incisor and sharp trifid lacinia, latter provided with sharp marginal denticles on each cusp distributed as figured, and with patch of densely set short spinules on proximal portion of inner surface. Mandible setal row consisting of 2 + 2 pappose setae.

Maxillule (= Maxilla 1) (fig. 29A) coxal endite (= inner plate) narrow and tapering,

with two pappose setae on tip. Basal endite (= outer plate) with nine rake-like spines, of which three distolateral almost untoothed and spatulate. Endopod (= palp) unarmed, unsegmented, tapering, short (i.e,. shorter than half distance between base of segment and distolateral angle of basal endite), covered with long, densely set spinules.

Maxilla (= Maxilla 2) (fig. 29B) coxal endite (= inner plate) with single hypertrophied pappose seta proximally on inner margin and with distal margin lined with series of modified slender spines provided with series of transverse lamellae (fig. 29C) similar to those described above in *H. gonzalezi* Jaume sp. nov.

Maxilliped (fig. 28B–D) ordinary; basal endite (= inner plate) with three short cuspidate spines on distal margin; rest of limb as figured.

Pereiopodal coxae (fig. 26) I–IV similar in length, each much longer than broad; coxa I (fig. 30A) with anteroproximal angle produced into lobe and with straight anterior margin. Coxa IV (fig. 32D) 1.3 times longer than broad, with strongly excavate posterior margin. Coxa V (fig. 33B) bilobed, posterior lobe much longer than anterior lobe. Coxa VI with reduced anterior lobe and strongly overhanging posterior lobe (fig. 33C). Coxa VII with only posterior lobe developed (fig. 33D).

Coxal gills present on gnathopod II (fig. 31A) and pereiopods III–VI (figs 32C, D, 33B–D), sacciform.

Gnathopod I (fig. 30A–C) subchelate; propodus much shorter than carpus (attaining about 81% length of carpus), ~1.7 times longer than broad, expanded distally; up to nine bipectinate setae in a row on medial surface of segment as figured; palm angle marked by two unequal flagellate spines; palm margin strongly convex (fig. 30C), armed as figured.

Gnathopod II (fig. 31A–C) subchelate; propodus massive, triangular, 1.3 times as long as broad with posterior margin slightly shorter than palm margin; palm angle protruding as rounded bulge, placed at 38% length of segment, marked with two reduced flagellate spines; palm margin armoured as in fig. 31C.

Pereiopods III–IV (fig. 32C, D) about similar in length; armature ordinary.

Pereiopods V–VII elongate (fig. 33B-D), with basis much longer, shorter or equal to corresponding ischium-merus combined, respectively; basis of pereiopod VI with posterodistal angle not so strongly overhanging as in pereiopods V and VII.

Pleopods I–III (fig. 26) each with protopod shorter than rami.

Uropod I (fig. 31D) with rami shorter than protopod, latter armed with row of six spines along posterolateral margin and single spine subdistally on each posterolateral and posteromedial angle of segment. Exopod with row of five spines along posterolateral margin and four spines on tip; endopod with five spines along posteromedial margin, subterminal row of up to five short spines (number variable, some specimens even with only one spine; fig. 31E) on posterolateral margin, and five spines on tip; endopod lacking modified spine.

Uropod II (fig. 29D) rami about as long as protopod, latter armed with row of five spines along posterolateral margin and one spine subdistally on each posterolateral and posteromedial angle of segment. Exopod with three spines along posterolateral margin and seven placed distally. Endopod with three spines along posteromedial margin and four disposed distally.

Uropod III (fig. 32E) with ramus about as long as protopod, latter with two short spines (exceptionally three or four) on distolateral angle and about three simple setae on medial margin. Ramus with short spine and cluster of about four simple setae on tip; spine much shorter than half ramus length.

Telson (fig. 32F) much longer than broad, with evenly rounded posterior margin and

straight lateral margins. Armature consisting on one flagellate spine at each side on distal margin and three short penicillate setae placed subdistally at each side.

Brooding female. Body up to 10.2 mm long. As male in all respects except for presence of oöstegites on gnathopod II and pereiopods III–V, and structure of gnathopod II. Latter (fig. 30D) with propodus elongate, slightly longer (~1.1 ×) than carpus and 2.4 times longer than broad, expanded distally; palm angle marked with two long flagellate spines; palm margin slightly transverse, convex; up to nine bipectinate setae in a row on medial surface of segment as figured.

Remarks. This species, described by González & Watling (2003), is remarkable for its smooth, untoothed mandibular incisors; no other *Hyalella* shows such a feature, otherwise rarely reported to occur among continental water amphipods (e.g., Jaume & Wagner, 1998), but quite frequent in marine forms. The species is notorious also for the display of three dorsal processes, one on each pereionite VII and pleonites I–II, but availability of a large number of specimens for inspection has enabled here to fix the level of variability in the expression of both the number and development of these processes, which can vary from the usual three to display none (fig. 27). Other remarkable features of this taxon include the much longer than broad telson, the unarmed endopod of maxillule and the row of lamellate spines of the coxal endite of maxilla, the last feature shared only with *H. gonzalezi* Jaume sp. nov., described above.

Integrative taxonomy

In a previous paper (Jurado-Rivera et al., 2020), we explored the *Hyalella* species diversity of South America based on mitochondrial *cox1* sequences and implementing different molecular species delimitation criteria. We recorded the presence of at least eleven different Molecular Operational Taxonomic Units (MOTUs) in the Titicaca, and the occurrence of a remarkable disagreement between morphology and genetic data within this assemblage. We reported cases in which several MOTUs corresponded to the same morpho-species, while in others a particular MOTU was shared by a heterogeneous array of species, including some with smooth and others with heavily armoured body.

Our ongoing research on the morpho-species of the Titicaca also suggests that they cannot be diagnosed as straightforward as we tentatively proposed in a previous work (see dichotomic key to species in Jurado-Rivera et al., 2020), and that detailed redescriptions and sequence assignments are required to disclose the true magnitude of this species flock. Herein we formally describe two new species whose *cox1* sequences were already used in our previous study, whereas describe and provide *cox1* sequences of a third one not reported yet. Unfortunately, we failed in obtaining valid *cox1* sequences of *H. solida*, but provide three new sequences of *H. nefrens*. All these data were merged in our *Hyalella cox1* sequence dataset and analysed in the same way as described in Jurado-Rivera et al. (2020). Results (fig. 34) confirm the non-resolutive power of adopting a barcoding approach to distinguish among these morphologically highly divergent species: whereas *H. hirsuta* Jaume sp. nov. and *H. nefrens* share the same MOTU (= A1), *H. gonzalezi* Jaume sp. nov. and *H. krolli* Jaume sp. nov. arise in molecular phylogenetic analyses as the closest relatives and sharing the same MOTU (= C1) as *H. armata*, perhaps the most unusual of all Titicacan species, characterised by the display of an extremely elongate, laterally-directed spine on each of pereiopodal coxal plates I–IV (González & Coleman, 2002). Whereas in *H. gonzalezi* Jaume sp. nov.

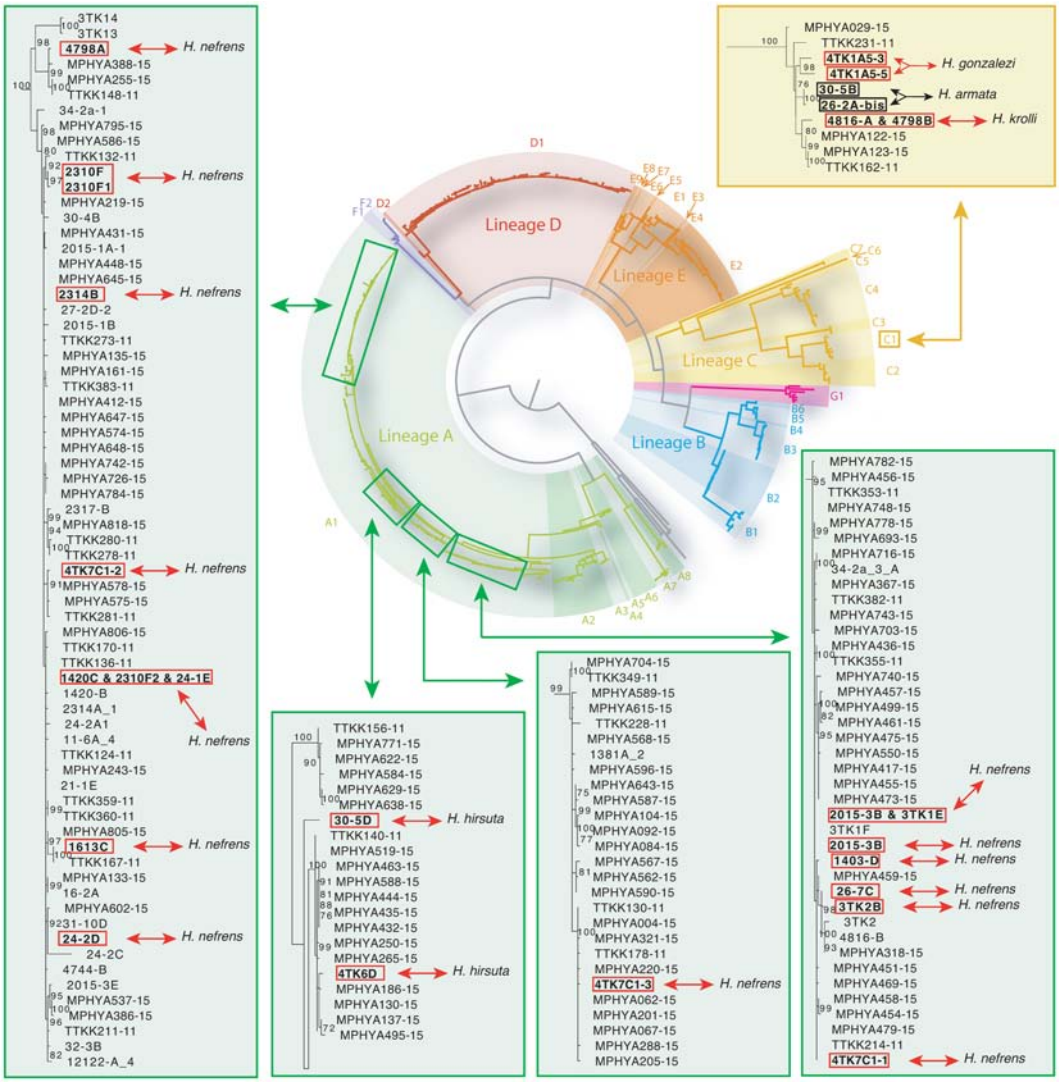

FIGURE 34 Discordance between morpho-species and Molecular Operational Taxonomic Units (MOTUs) in the *Hyalella* species flock of lake Titicaca. Bayesian phylogenetic tree depicting evolutionary relationships among South American *Hyalella cox1* haplotypes and their respective molecular species delimitations follow Jurado-Rivera et al. (2020). Only MOTUs A1, A2, A5, A6–A8, B1, C1, D1, E1 and E2 account with representatives in lake Titicaca. Notice analysis cannot discern between *H. nefrens* and *H. hirsuta* Jaume sp. nov., and same holds for species triplet *H. gonzalezi* Jaume sp. nov. / *H. krolli* Jaume sp. nov. / *H. armata*.

the close kinship with *H. armata* could explain the common display of peculiar morphological features such as the presence of body dorsal humps and the truncate endopod (= palp) of maxillule with a comparatively long distal seta, there is nothing in the morphology of *H. krolli* Jaume sp. nov. denoting its closeness to *H. armata* except for the truncate endopod of maxillule displaying a comparatively long distal seta.

The morphological diversification and the incongruence between morphological and molecular boundaries as found in the *Hyalella* of the Titicaca could be related to

the sharp changes in hydrological conditions experienced by the lake in the past, which included dramatic fluctuations in water level and salinity (see Jurado-Rivera et al., 2020, and references herein). Such environmental shifts could have triggered rapid changes in the morphology and the ecological differentiation of the species, followed by phenotypic convergence among the diverse lineages. But the intervention of other factors such as phenotypic plasticity, incomplete lineage sorting, or admixture between divergent lineages, cannot be ruled out to explain the morphological-genetic incongruence described herein, since similar patterns have been recorded also among the *Gammarus* species flock of Lake Ohrid, which had a very stable geological history compared to the Titicaca (Wysocka et al., 2013).

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References

Adamowicz, S.J., Marinone, M.C., Menu-Marque, S., Martin, J.W., Allen, D.C., Pyle, M.N., De los Ríos, P., Sobel, C.N., Ibañez, C., Pinto, J. & Witt, J.D.S. (2018) The *Hyalella* (Crustacea: Amphipoda) species cloud of the ancient Lake Titicaca originated from multiple colonizations. *Mol. Phylogenet. Evol.*, 125, 232–242.

Alonso, M. & Jaume, D. (2017) A new species of *Hyalella* from the High Andes of Ecuador (Crustacea: Amphipoda: Hyalellidae). *Zookeys*, 686, 1–13.

Archundia, D., Duwig, C., Spadini, L., Uzu, G., Guédron, S., Morel, M.C., Cortez, R., Ramos, O., Chincheros, J. & Martins, J.M.F. (2017) How Uncontrolled Urban Expansion Increases the Contamination of the Titicaca Lake Basin (El Alto, La Paz, Bolivia). *Water, Air Soil Pollut.*, 228, 44. https://doi.org/10.1007/s11270-016-3217-0

Bousfield, E.L. (1982) Amphipoda. Gammaridea. In: S.P. Parker (Ed) *Synopsis and Classification of Living Organisms*, 2: 254–285. McGraw-Hill Book Company, New-York.

Bulycheva, A. (1957) The Sea Fleas of the USSR and Adjacent Waters (Amphipoda: Talitroidea). Keys to the Fauna of the USSR. *Akademiia Nauk SSSR, Opredeliteli po Faune SSSR*, 65, 1–185 (in Russian).

Chevreux, E. (1904) Mission G. de Créqui-Monfort et Sénéchal de la Grange. Note preliminaire sur les amphipodes recueillis par M. le Dr. Neveu-Lemaire dans le Lac Titicaca (Julliet, 1903). *Bull. Soc. zool. France*, 29, 131–134.

Chevreux, E. (1907) Chapitre III. Faune et Flore des Lacs des Haut Plateaus. V. Arthropodes (Crustacés). 1. Amphipodes. In: M. Neveu-Lemaire (Ed) *Mission Scientifique G. de Créqui-Monfort et Sénéchal de la Grange, 1. Les Lacs des*

Hauts Plateuaux de l'Amerique du Sud, pp. 147–168. Imprimerie Nationale, Paris.

Chevreux, E. (1908) Études sur la faune du Turkestan basées sur les matériaux recueillis par D. D. Pedaschenko. II. Crustacés Amphipodes. *Travaux de la Soc. Imp. D. Nat. St-Pétersbourg*, 37, 91–102.

Coleman, C.O. & González, E.R. (2006) New Hyalellids (Crustacea, Amphipoda, Hyalellidae) from Lake Titicaca. *Org. Divers. Evol., 6, Electr. Suppl.*, 10, 1–28.

Copilas-Ciocianu, D. & Sidorov, D. (2021) Taxonomic, ecological and morphological diversity of Ponto-Caspian Gammaridean amphipods: A review. *Org. Divers. Evol.* (in press).

Crawford, G.I., Harrison, K., Lincoln, R.J. & Boxshall, G.A. (1993) An introduction to the species flock of amphipods (Crustacea) of Lake Titicaca. In: Workshop *Speciation in Ancient Lakes. Evolution, Biodiversity, Conservation*. Mont-Rigi, Robertsville, Belgium, Scientific Station of the Hautes-Fagnes, Abstract 18.

Cristescu, M.E., Adamowicz, S.J., Vaillant, J.J. & Haffner, D.G. (2010) Ancient lakes revisited: from the ecology to the genetics of speciation. *Mol. Ecol.*, 19, 4837–4851.

Faxon, W. (1876) Exploration of Lake Titicaca by Alexander Agassiz and S.W. Garman. IV. Crustacea. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 3, 361–375.

González, E.R. & Coleman, C.O. (2002) *Hyalella armata* (Crustacea, Amphipoda, Hyalellidae) and the description of a related new species from Lake Titicaca. *Org. Divers. Evol.*, 2, 261–262.

González, E.R. & Watling, L. (2001) Three new species of *Hyalella* from Chile (Crustacea: Amphipoda: Hyalellidae). *Hydrobiol.*, 464, 175–199.

González, E.R. & Watling, L. (2003) Two new species of *Hyalella* from Lake Titicaca, and redescriptions of four others in the genus (Crustacea: Amphipoda). *Hydrobiol.*, 497, 181–204.

Grabowski, M. (2014) *Check-List for Ponto-Caspian Amphipods* (*Brackish & Freshwater*). Caspian Sea Biodiversity Project under umbrella of Caspian Sea Environment Program. Available from: http://www.zin.ru/projects/caspdiv/caspian_amphipods.html

Grabowski, M., Wysocka, A. & Mamos. T. (2017) Molecular species delimitation methods provide new insight into taxonomy of the endemic gammarid species flock from the ancient Lake Ohrid. *Zool. J. Linn. Soc.*, 181, 272–285.

Hoffman, J. (2006) Adaptative radiation of *Hyalella* (Crustacea, Amphipoda) in Lake Titicaca. The *Hyalella longipes-lucifugax*-complex – A taxonomical case study. *International Symposium "Speciation in Ancient Lakes, SIAL IV*. Abstracts. Berlin.

Hou, Zh. & Sket, B. (2016) A review of Gammaridae (Crustacea: Amphipoda): The family extent, its evolutionary history, and taxonomic redefinition of genera. *Zool. J. Linn. Soc.*, 176, 323–348.

Jaume, D. & Wagner, H.P. (1998) New cave-dwelling amphipods (Lysianassidae, Hadziidae) from the Dominican Republic (Hispaniola). *Contrib. Zool.*, 68, 37–66.

Jurado-Rivera, J.A., Zapelloni, F., Pons, J., Juan, C. & Jaume, D. (2020) Morphological and molecular species boundaries in the *Hyalella* species flock of Lake Titicaca (Crustacea: Amphipoda). *Contrib. Zool.*, 89, 353–372.

Krapp-Schickel, T. & Sket, B. (2015) *Melita mirzajanii* n. sp. (Crustacea: Amphipoda: Melitidae), a puzzling new member of the Caspian fauna. *Zootaxa*, 3948, 248–262.

Latreille, P.A. (1816) Amphithoé. In: Biot, J.B., Bosc, L.A.G., Chaptal, J.A., Desmarest, A.G., Dutour, M., Huzard, J.B., Lamarck, J.B.P.A. de Monet de, Latreille, P.A., Lucas, J.A.H., Olivier, G.A., Palisot de Beauvois, A.M.F.J., Parmentier, A.G., Patrin, E.M.L., Richard, L.C., Sonini, C.S., Thouin, A., Tollard, C., Vieillot, L.P., Virey, J.J. & Yvart, J.A.V. (Eds) *Nouveau dictionnaire d'histoire naturelle, appliquée aux arts, à l'agriculture, à l'économie rurale et domestique, à la médecine, etc. 2nd*

edition, Vol. 1, pp. 470–471. Librairie Deterville, Imprimerie d'Abel Lanoë, Paris. https://doi.org/10.5962/bhl.title.20211

Lauzanne, L. (1992) Native species. The *Orestias*. In: C. Dejoux & A. Iltis (Eds) *Limnological Knowledge*, pp. 405–419. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Monaghan, M.T., Balke, M., Pons, J. & Vogler, A.P. (2006) Beyond barcodes: Complex DNA taxonomy of a South Pacific island radiation. *Proc. R. Soc. B.*, 273, 887–893.

Pinkster, S. (1993) A revision of the genus *Echinogammarus* Stebbing, 1899 with some notes on related genera (Crustacea, Amphipoda). *Mem. mus. civ. stor. nat. Verona*, 10, 1–185.

Sars, G.O. (1894) Crustacea Caspia. Contributions to the knowledge of the carcinological fauna of the Caspian Sea. Part III. Amphipoda. Gammaridae (part). *Bull. Acad. Imp. Sc. St.-Pétersbourg*, 1, 179–242.

Sars, G.O. (1896) Crustacea Caspia. Contributions to the knowledge of the carcinological fauna of the Caspian Sea. Amphipoda. Gammaridae (Supplement). *Bull. Acad. Imp. Sc. St.-Pétersbourg*, 4, 421–512.

Sket, B. (2000) *Fuxiana yangi* g.n., sp.n. (Crustacea: Amphipoda), a"baikaloid" amphipod from the depths of Fuxian Hu, an ancient lake in the karst of Yunnan, China. *Arch. Hydrobiol.*, 147, 241–255.

Sket, B. & Fiser, C. (2009) A new case of intralacustrine radiation in Amphipoda. A new genus and three new species of Anisogammaridae (Crustacea, Amphipoda) from the ancient lake Fuxian Hu in Yunnan, China. *J. Zool. Syst. Evol. Res.*, 47, 115–123.

Smith, S.I. (1874) The Crustacea of the freshwaters of the United States. A synopsis of the higher freshwater Crustacea of the Northern United States, Appendix F. Natural History. *Report of the Commissioner for 1872 and 1873. United States Commission of Fish and Fisheries*, 2, 637–661.

Takhteev, V.V. (2000) Trends in the evolution of Baikal amphipods and evolutionary parallels with some marine Malacostracan faunas. *Adv. Ecol. Res.*, 31, 197–220.

Takhteev, V.V. (2019) On the current state of taxonomy of the Baikal Lake amphipods (Crustacea: Amphipoda) and the typological ways of constructing their system. *Arthropoda Sel.*, 28, 374–402.

Vila, I., Pardo, R. & Scott, R. (2007) Freshwater fishes of the Altiplano. *Aquat. Ecosyst. Health Manag.*, 10, 201–211.

Weckel, A.L. (1910) Freshwater amphipods from Peru. *Proc. U. S. Nat. Mus.*, 38, 623–624.

Wysocka, A., Grabowski, M., Sworobowicz, L., Burzynski, A., Kilikowska, A., Kostoski, G. & Sell J. (2013) A tale of time and depth: intralacustrine radiation in endemic *Gammarus* species flock from the ancient Lake Ohrid. *Zool. J. Linn. Soc.*, 167, 345–359.

Zapelloni, F., Pons, J., Jurado-Rivera, J., Jaume, D. & Juan, D. (2021) Phylogenomics of the *Hyalella* amphipod species-flock of the Andean Altiplano. *Sci. Rep.*, 11(1), 366. https://doi.org/10.1038/s41598-020-79620-4.

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