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ORIGINAL ARTICLE

Description of the male of *Coronatella paulinae* (Crustacea, Branchiopoda, Chydoridae) with an identification key for the genus based on the male morphology

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ABSTRACT

Cladoceran males are not very frequent in natural populations, since they are only produced in stress situations. Thus, only a few species have had the male morphology described. Nevertheless, whenever data concerning the morphology of males is available, they are used as a tool to resolve taxonomic problems. In this study, the morphology of *Coronatella paulinae* Sousa, Elmoor-Loureiro and Santos, 2015 (Cladocera: Chydoridae) was described and compared to other species within the genus. *Coronatella paulinae* shares the diagnostic morphological traits typically attributed to the genus: (1) gonopores opening ventrally, subapically to the postabdominal claw; (2) marginal setulae arranged in groups on the postanal margin; (3) Inner Distal Lobe (IDL) armed with three setae, of which one is the male seta. The absence of lateral aesthetascs on male antennules might also be an important diagnostic character for the genus, since they are present in other Aloninae groups. *Coronatella paulinae* males present a unique combination of morphological traits on the postabdomen, which distinguish them from

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other *Coronatella* Dybowsky and Grochowski, 1894 species, such as a marked postanal angle and an almost straight basal spine, longer than the mid-length of the postabdominal claw.

Key words
Antennules, basal spine, limb I, postabdomen, postabdominal claw.

Introduction

Cladoceran species usually exhibit cyclical parthenogenesis, where asexual reproduction is occasionally interrupted by environmental induction (*e.g.* resource quality, temperature, luminosity) and the same females might produce gamogenetic individuals able to reproduce sexually (Smirnov, 2014). This alternation between asexual and sexual reproductive cycles has an important role in the dispersion, colonization and increase of genetic variability in the novel aquatic system due to the production of resting eggs by ephipial females. Resting eggs may persist in the environment for many years and initiate new populations once stable conditions return (Grebelnyi, 1996; Cáceres and Soluk, 2002; Brock *et al.*, 2003; Santangelo, 2009; Araújo *et al.*, 2013).

Because sexual reproduction happens only occasionally, the occurrence of males in populations is rare. Notwithstanding this, adult males can be recognized by a high degree of dimorphism of the structures associated with reproduction, such as the modifications on the first limb, antennules and postabdomen (Smirnov, 1992; 1996; Kotov, 1997; Benzie, 2005). This is especially true for members of the order Anomopoda Sars, 1865, although in Ilyocryptidae Smirnov, 1971 *emend*. Smirnov, 1992, this dimorphism is less pronounced (Goulden, 1968; Kotov and Štifter, 2006; Belyaeva and Taylor, 2009; Kotov *et al.*, 2009).

Within Anomopoda, the adult males of Chydoridae Dybowski and Grochowski, 1894 *emend.* Dumont and Silva-Briano, 1998 have a wide range of morphological variability (see Smirnov, 1967; 1971). For example, in *Leydigia* Kurz, 1875 it is common to observe the presence of distal penis on the postabdomen to the opening of the gonopore (although this morphological character is absent in some species) and thirteen apical aesthetascs on the antennules (Kotov, 2009; Sinev and Sanoamuang, 2011). The males of *Leydigiopsis* Sars, 1901 have a gonopore inserted laterally in relation to the postabdomen and at least four lateral aesthetascs on the antennules (Sinev, 2004). However, the most common morphological pattern is observed in the males of *Alona* Baird, 1843 and correlated genera, which have a ventrally opening gonopore on the postabdomen and 11–15 aesthetascs on the antennule, some of which may be laterally inserted (Kotov and Sanoamuang, 2004; Sinev *et al.*, 2005; 2009; Van Damme and Dumont, 2008a; 2008b; Van Damme *et al.*, 2009; 2011; Sinev, 2012; Sinev and Shiel, 2012; Sinev and Silva-Briano, 2012).

Regarding tropical aquatic environments, Sinev and Sanoamuang (2011) stated that the stability of water bodies and the low population density associated with predation are factors that difficult the study of biological aspects of cladocerans males. However, in recent times, the male morphology has received increased attention because of its potential to allow accurate taxonomic identification when the parthenogenetic females of certain species are too similar in morphology (Sinev, 1999; 2013; Kotov, 2008; 2015; Sinev and Shiel, 2012; Kotov and Fuentes-Reines, 2015). Although the recent literature points to the importance of male morphology in cladoceran systematics, many species still lack or require improvements in the descriptions of male morphology.

Recently, new species of the genus *Coronatella* Dybowsky and Grochowski, 1894 were described from South America (Sousa *et al.*, 2015). For one of them, *Coronatella paulinae* Sousa, Elmoor-Loureiro and Santos, 2015, the description was based solely on parthenogenetic females. The present study aims to expand the characterization of this species by providing a description of the male. Additionally, we provide an identification key to separate the species of *Coronatella* based on the male morphology.

Material and methods

We transferred the selected specimens to slides containing glycerin and dissected them under an Olympus SZ61 stereomicroscope. We analyzed appendage morphology and other structures using an Olympus BX41 phase contrast microscopy. All drawings were made with the aid of a camera lucida. The enumeration and nomenclature structures of the setae of limb I followed the recent literature on the topic (Sinev and Sanoamuang, 2011). The description of the male of *C. paulinae* was made using 15 adult males taken from samples collected in the littoral region of Gambazinho Pond (19°47'7"S 42°34' 45.5"W), a small and shallow lake (11.1 ha; 10.3 m maximum depth) at the Rio Doce State Park, Minas Gerais, Brazil, collected on 18 June 2011. Rio Doce State Park (19°47'10.6"S 42°34'48.3"W) is site 4 within the Brazilian network of LTER (Long Term Ecological Research – PELD, in the Portuguese abbreviation) sites.

The following abbreviations were used in the text and figures: en = endite; ep = epipod; cbs = copulatory brush seta; IDL = inner distal lobe; IP = interpore distance (distance between anterior and posterior main head pores); PP = postpore distance (distance between posterior main head pore and posterior border of head shield); ODL = outer distal lobe; ms = male seta.

Systematics

Class Branchiopoda Latreille, 1817

Order Anomopoda Sars, 1865

Family Chydoridae Dybowsky and Grochowski, 1894 *emend.* Frey, 1967

Subfamily Aloninae Dybowsky and Grochowski, 1894 *emend.* Frey, 1967

Genus *Coronatella* Dybowsky and Grochowski, 1894

***Coronatella paulinae* Sousa, Elmoor-Loureiro and Santos, 2015**

Description. Parthenogenetic female. For description and diagnosis, see Sousa *et al.* (2015).

Adult Male. Habitus (Fig. 1). Smaller than the female, length 0.21–0.24 mm, about 1.6 times as long as it is height; maximum height close to intermediate point of the body; without dorsal keel.

Head (Fig. 1). Rostrum relatively short, rounded, not projected; ocellus and eye of similar sizes; three main head pores connected by a narrow connection, median pore smaller than proximal and distal ones, IP about three times longer than the PP, lateral head pores tiny and inserted at same level as the median main head pores (Fig. 4). Labral keel wide in a lateral view, relatively short, naked; anterior portion convex with a slight projection, apex rounded (Fig. 1).

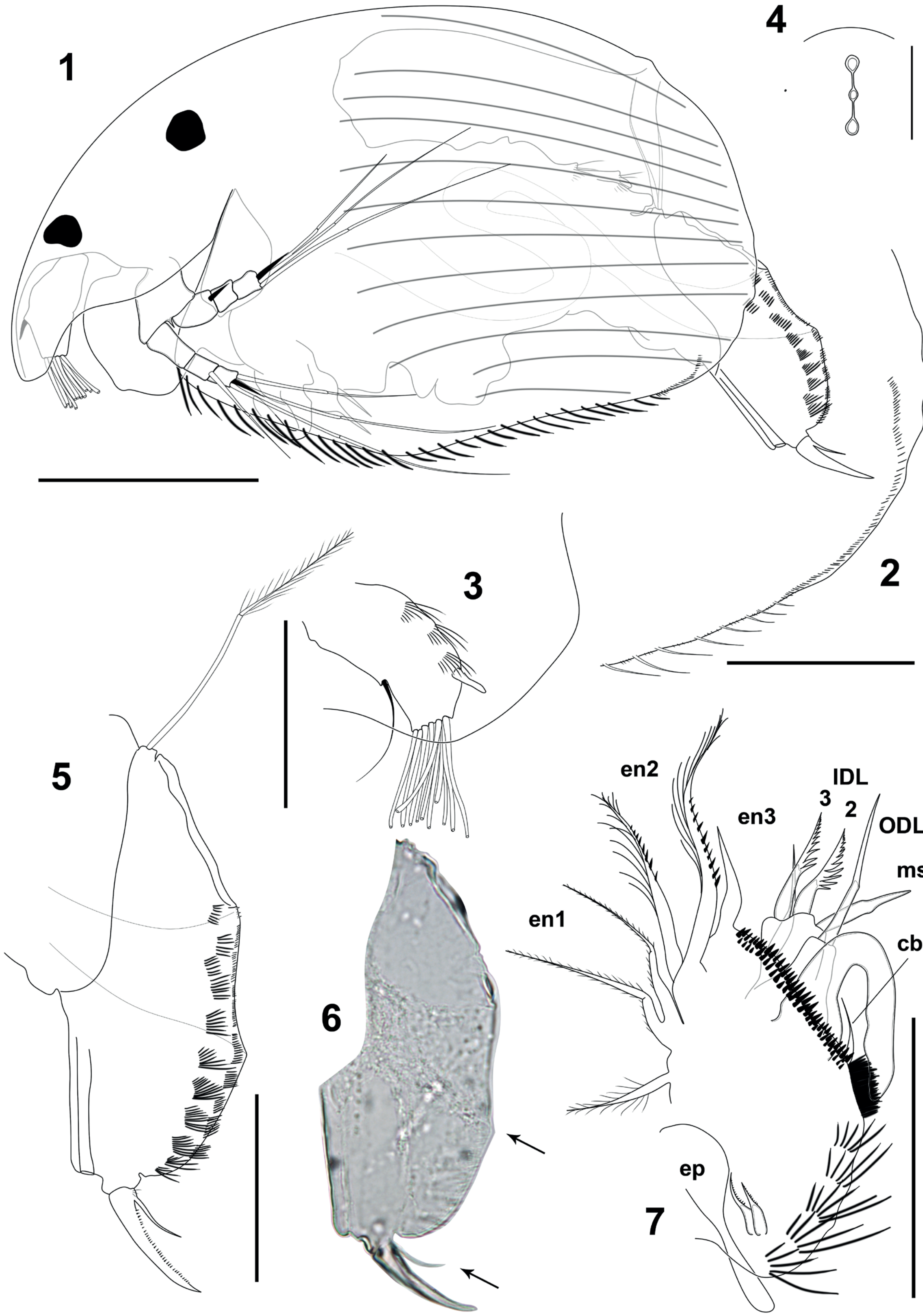
Carapace (Figs. 1, 2) relatively elongated, longitudinal lines present; ventral margin armed with 22–24 slightly plumose setae not differentiated in groups, followed by fine spinulae, the most proximal spinulae not exceeding the line of the posterior margin; fine and short spinulae between ventral setae present. Posteroventral corner without denticles.

Antennules (Fig. 3). Not exceeding the tip of rostrum, about two times as long as wide: three rows of setulae similar in length on the antennular body. Eleven apical aesthetascs of different lengths, shorter than the length of the antennular body. Sensory seta about 1.5 times shorter than the length of the antennular body. Male seta short and robust, not sharp, about 4.3 times shorter than the length of the antennular body, inserted at the distal third of the antenular body.

Thorax (Fig. 1) two times longer than the abdomen; one row of abdominal setae present.

Postabdomen (Figs. 5, 6). Moderately rectangular, smaller than the female's, about 2.3–2.6 times longer than its height, narrowing towards the distal portion; anal and postanal margin similar in length; postanal angle relatively well defined; postanal margin armed with 4–5 groups of unmerged setulae, the distalmost being longer; 7–10 lateral fascicles, distalmost fascicles exceeding the marginal line; anal margin with spinulae arranged in three groups; gonopores opening ventrally, subapically to the postabdominal claw. Postabdominal setae about two times longer than the postabdominal length, bisegmented, distal segment setulated. Postabdominal claw smaller and more robust than in the female, tip acute; pecten formed by thick and short spinulae. Basal spines slender, longer than the mid-length of the postabdominal claw, straight or slightly curved near the tip; with a row of fine spinulae inserted on its base.

Limb I (Fig. 7). Smaller than the female's, copulatory hook U-shaped, arms relatively similar in length. Copulatory brush present; seta of the copulatory brush



Figures 1–7. *Coronatella paulinae* Sousa, Elmoor-Loureiro and Santos, 2015. Male from Gambazinho Pond, Minas Gerais state, Brazil: 1, Habitus, lateral view, adult; 2, Posteroventral corner of the carapace; 3, Antennule; 4, Head pores; 5, Postabdomen; 6, Idem, outline, arrows showing the postanal angle and the variability of basal spine shape; 7, Limb I. Scale bars: 1 = 50 μm; 2, 3, 5, 7 = 25 μm; 4 = 12 μm. Abbreviations: cbs = copulatory brush seta; en = endite; ep = epipod; IDL = inner distal lobe; ms = male seta; ODL = outer distal lobe.

with about 0.7 of the length of the male seta on the IDL; five clusters of long setulae inserted on the body of the limb. IDL with two setae (2–3) of different lengths and armed with short proximal spines; seta of the ODL about 1.7 times longer than the smallest IDL seta (2), slightly serrated. Male seta relatively long (ms), similar in length to the smallest IDL seta (2).

Other limbs similar to those of the parthenogenetic female (see Sousa *et al.*, 2015).

Remarks. The morphological traits presented in the description of *Coronatella paulinae* males are widely used to discriminate males from females in Chydoridae. The shape of the male postabdomen of *C. paulinae* resembles the one in *Coronatella poppei* (Richard, 1897), *Coronatella rectangula* (Sars, 1862), and *Coronatella circumfimbriata* (Megard, 1967). However, it can be distinguished from these other species by the armature of the basal spine, which is long and almost straight in the present species (Fig. 5). Compared to *Coronatella anemae* Van Damme and Dumont, 2008, *C. paulinae* differs in the habitus and shape of the postabdomen (see Van Damme and Dumont, 2008b). As for the males of *Coronatella holdeni* Van Damme and Dumont, 2008, *Coronatella undata* Sousa, Elmoor-Loureiro and Santos, 2015, *Coronatella monacantha* (Sars, 1901), and *Coronatella serratalhadensis* Sousa, Elmoor-Loureiro and Santos, 2015, these are unknown, so far.

In the paper where the genus *Coronatella* was defined, Van Damme and Dumont (2008b) showed illustrations of the postabdomen of *Coronatella eucostata* (Sars, 1894), which is similar, in terms of general morphology, to *C. paulinae*. The validity of *C. eucostata* is still questioned because of a possible synonymy with *Alona novaezealandiae* Sars, 1904. Despite this, *C. eucostata* has a short basal spine, which differentiates it from *C. paulinae*.

Identification key for males of *Coronatella* species

1a Postabdomen elongated, about 3.3 times longer than its height ....
.... *Coronatella anemae* [Afrotropical region (Van Damme and Dumont, 2008b]
1b Postabdomen short, about 2.3–2.6 times longer than its height 2
2a Basal spine longer than the mid-length of the postabdominal claw 3
2b Basal spine shorter than the mid-length of the postadominal claw ....
.... *Coronatella rectangula* [Palaearctic region (Van Damme and Dumont, 2008b)]
3a Basal spine sinuous 4
3b Basal spine almost straight .... *Coronatella paulinae* [Neotropical region: Brazil (Sousa *et al.*, 2015)]
4a Postanal angle evident, sinuosity located at the mid-length of the basal spine ....
.... *Coronatella poppei* [Neotropical region: Brazil, Chile (Sousa *et al.*, 2015)]
4b Postanal angle not expressed, sinuosity located at the distal portion of the basal spine ....
.... *Coronatella circumfimbriata* [Nearctic region (Sinev, 2009; Sinev and Silva-Briano, 2012)]

Discussion

Males of *Coronatella paulinae* has the typical morphological traits associated with the genus (Van Damme and Dumont, 2008b), such as: (1) ventrally opening gonopores, subapical to postabdominal claw; (2) marginal setulae on the postabdomen arranged in groups and (3) IDL armed with three setae, of which one is the male seta. The morphology of the antennule also seems to be consistent within the genus, because available descriptions indicate the presence of 11 apical aesthetascs similar in length and the absence of lateral aesthetascs (Alonso, 1996; Flössner, 2000; Sousa *et al.*, 2015).

In genera from the *Coronatella*-branch (Van Damme and Dumont, 2008b), the male antennule has a variable morphology. For example, males from *Anthalona* Van Damme, Sinev and Dumont, 2011 do not bear lateral aesthetascs on antennules, but have up to 12 apical aesthetascs (Sinev and Hollwedel, 2002; Van Damme *et al.*, 2011). *Karualona* Dumont and Silva-Briano, 2000 males bear one lateral and 10 apical aesthetascs on the antennules (see Alonso and Pretus, 1989). Males from *Extremalona* Sinev and Shiel, 2012 have antennules with six lateral aesthetascs (Sinev and Shiel, 2012). *Celsinotum* Frey, 1991 males have two–six lateral aesthetascs, and up to 12 apical ones (Frey, 1991; Sinev and Kotov, 2012), while *Leberis* Smirnov, 1989 has two lateral aesthetascs plus seven (Kotov and Fuentes-Reines, 2015) or nine apical ones (Sinev *et al.*, 2005). The same armature present in *Leberis* is observed in *Ovalona* Van Damme and Dumont, 2008 (Sinev, 2015), in the *elegans*-group of *Alona sensu lato* (Sinev *et al.*, 2009) and *Magnospina dentifera* (Sars, 1901) (Sousa *et al.*, 2016). So far, the male of *Bergamina* Elmoor-Loureiro, Santos-Wisniewski and Rocha, 2013 is unknown (Elmoor-Loureiro *et al.*, 2013).

The postabdomen is the most evident morphological trait related to dimorphism between females and males of Chydoridae. The potential of this structure to separate species was mentioned by Sinev (1999) when comparing males of *Alona werestchagini* Sinev, 1999, *Alona guttata* Sars, 1862, and *Alona barbulata* Megard, 1967. Females of the aforementioned species are very similar in morphology; however, consistent differences were observed in the shape of male postabdomen and, especially, at the postabdominal claw armature. Sinev (2013) highlighted that the differential morphology of *Alona ossiani harricki* Sinev, 2013 is the presence of three marginal denticles on the male postabdomen. The recently described *Leberis colombiensis* Kotov and Fuentes-Reines, 2015 can also be distinguished from its congeners through the morphology of the male postabdomen (Kotov and Fuentes-Reines, 2015). In male *C. paulinae*, the postabdomen also seems to be a key structure to differentiate it from other species of the genus. *Coronatella paulinae* males have a unique combination of characters: short postabdomen, evident postanal angle, groups of long setulae on the postanal margin, and basal spines almost straight and longer than the mid-length of the postabdominal claw.

Looking at the limb I, some differences may be observed between the males of *Coronatella paulinae* and those of other species of the genus. For instance, *C. paulinae* has relatively shorter ODL setae and the male seta is clearly more robust when compared with those of *C. poppei* (Sousa *et al.*, 2015). *Coronatella paulinae* has a relatively long male seta, similar in length to the smallest IDL seta, while in *C. circumfimbriata* the male seta is slender and longer than the IDL setae; the copulatory brush seta is almost three times shorter than the male setae (Sinev, 2009). Although the habitus, postabdomen, and antennules of *C. paulinae* are similar to those of *C. rectangula*, the two studies that present complete descriptions of limb I from males of *C. rectangula* show a different arrangement of IDL setae and male seta. Frey (1988) illustrated the male setae longer than the IDL setae, while Alonso (1996) described the male setae of *C. rectangula* with similar length to the IDL setae. It is possible that specimens observed for these authors were in different phases of development, but alternatively they could represent different species, since the Iberian Peninsula has a distinctive fauna of Chydoridae when compared with other regions of Europe (*e.g.* Sinev *et al.*, 2009). Thus, we believe that a comparison of the limb I of *C. paulinae* with *C. rectangula* with the available data is still premature.

The presence of males is fundamental to the formation of resting eggs of the Anomopoda, which impacts the dispersion, colonization and genetic variability of cladocerans in continental aquatic systems. Data from the literature have showed that the male morphology is important to Chydoridae systematics (*e.g.* Sinev, 1999; 2013; Sinev and Shiel, 2012; Kotov and Fuentes-Reines, 2015). With regards to *Coronatella*, the males can also be used to differentiate some taxa. The male of *C. paulinae* has a unique armature of the postabdomen, which distinguishes it from other species of the genus.

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