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Description of a new species of the *costata*-group (Cladocera, Chydoridae, Aloninae) from Brazil

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Abstract

The aim of this study is to describe a new species of the *costata*-group from Brazil. *Alona margipluma* **sp. nov.** shares morphological traits with *A. costata* Sars, 1862, *A. natalensis* Sinev, 2008, and *A. cheni* Sinev, 1999, but differs from them in: (i) thin setulae between the marginal setae on the valves, (ii) setae 4–5 on the exopodite of limb III long and different in length, (iii) bottle-shaped sensillum on the basal endite of limb IV. For identification of *Alona margipluma* **sp. nov.** it is necessary to check carefully the main head pores and postabdomen characters since the former superficially resemble *A. iheringula*, *A. setigera* and *Alona guttata*.

Key words: *Alona costata-group*, head pores, morphology, taxonomy, Neotropics, South America

Introduction

The *costata*-group is a well-defined species complex of the subfamily Aloninae Dybowski & Grochowski, 1894 emend. Frey, 1967 belonging to the Hexalona-branch (Van Damme *et al.* 2010). Its representatives have a number of conservative morphological features, such as transverse lateral head pores, each with a pocket-like cavity, inner distal lobe of limb I with three setae, limb I with one flat plumose seta on endite 1, exopodite of limb III with seven setae, presence of filter comb on limb V, and limb VI. However, structures such as the postabdomen, main head pores and labral keel show important differences among different species (Sinev 1999; Sinev 2001; Sinev 2008; Sinev 2009a; Van Damme & Eggermont 2011; Van Damme *et al.* 2011). The *costata*-group is expected to be transferred to a new genus because it differs from the "true *Alona"* core group, which includes species with morphology similar to *Alona quadrangularis* (O.F. Müller, 1776) (Van Damme & Dumont 2008a; 2008b).

Until now, the Brazilian fauna of the *costata*-group has been represented by two species, *A. iheringula* Sars, 1901 and *A.* cf. *setigera* Brehm, 1931. *Alona iheringula* was, for a long time, considered a junior synonym of *A. rustica* Scott, 1895 (cf. Smirnov 1971). Sinev (2001) revalidated the species status of *A. iheringi* after its redescription based on Sars' material. Kotov & Sinev (2004) created a *nomem novum*, *Alona iheringula*. *Alona setigera* was initially described from New Zealand as a variation of *A. guttata* Sars, 1862 and later transferred to the genus *Biapertura* Smirnov, 1971 because it has two main head pores (Smirnov & Timms 1983). Sinev (1999) observed that the morphology of its limbs was related to the *Alona costata*-group, and it was considered as a valid species (Van Damme *et al.* 2010). In Brazil, this species was reported for the first time in São Paulo state (Santos-Wisniewski *et al.* 2001) and new records have been added for other states (Elmoor-Loureiro 2010).

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In samples from different localities in Brazil, a new species of the *costata*-group was found occurring together with *A. iheringula*, *A.* cf. *setigera,* and *A.* cf. *guttata*. The aim of this study is to describe this species.

Material and methods

Morphological analyses. The selected specimens were transferred to drops of glycerol on slides and dissected under a stereomicroscope. The morphology of the appendages and other structures was studied using a phase contrast microscopy. Enumeration of limbs setae and other structures proceeds from the epipodite to the gnathobase, without relation to homology, according to recent literature (Van Damme & Dumont 2007; Sinev & Kobayashi 2012) and to facilitate comparisons with the description of *Alona natalensis* (Sinev 2008). Drawings were prepared using a camera lucida. Two animals were dehydrated in a graded alcohol series (50%, 70%, 90%, 95% and 100%) and dried using HMDS (Hexamethyldisilazane), mounted on aluminum stubs, coated with platinum and examined under a JEOL-JSM 7001F scanning electron microscope.

Abbreviations in figures and tables. as: accessory seta; en: endite; ep: epipodite; ex: exopodite; fc: filter comb; gfp: gnathobasic filter plate; IP: interpore distance (distance between anterior and posterior major head pores); IDL: inner distal lobe; il; inner lobe; ODL: outer distal lobe; pep: pre-epipodite; s: sensillum; PA: postabdomen; PP: postpore distance (distance between the posterior major head pore and the posterior border of the head shield); P2: limb II; P3: limb III; P4: limb IV; P5: limb V; P6: limb VI; GEEA: Research Group on Aquatic Environments, Universidade Católica de Brasilia, Brazil.

Abbreviations of the collections. FDRS: Personal collection of Francisco Diogo Rocha Sousa. CLLA: Slides collection of the GEEA, at Universidade Católica de Brasília, Brazil. MZUSP: Museu de Zoologia da Universidade de São Paulo, Brazil.

Results

Taxonomy

Class Branchiopoda Letreille, 1817

Order Anomopoda Sars, 1865

Family Chydoridae Dybowski & Grochowski, 1894 emend. Frey, 1967

Subfamily Aloninae Dybowski & Grochowski, 1894 emend. Frey, 1967

Genus *Alona* Baird, 1843

***Alona margipluma* sp. nov.**
(Figs. 1–4)

Etymology. the name "*margipluma*" comes from two Latin words, *margo* (= margin) and *pluma*, which refers to thin setules between ventral setae of the carapace.

Type locality. Criminosa Lake (21°40'28.8"S, 57°53'28.5"W), Porto Murtinho, Pantanal, Mato Grosso do Sul, Brazil.

Type material. Holotype: undissected, adult parthenogenetic female in a tube with 92% ethanol deposited at the Museum of Zoology of the University of São Paulo under access number MZUSP 33196. The label of the holotype is: "*Alona margipluma* sp. n., 1 parth. ♀ from to Criminosa Lake, Porto Murtinho, Pantanal, Mato Grosso do Sul, Brazil. Holotype".

Material Studied. Paratypes. Eight adult parthenogenetic females and two juveniles from Criminosa Lake (21°40'28.8"S, 57°53'28.5"W), Porto Murtinho, Pantanal, Mato Grosso do Sul, Brazil. Material collected on 10.i.2010 and 19.i.2010, leg. Adriana Maria Güntzel (FDRS0275). Two adult parthenogenetic females from the

Amonguijá River (21º41'11.3" S, 57º52'54.8" W), Porto Murtinho, Pantanal, Mato Grosso do Sul, Brazil. Material collected on 18.xi.2009, leg. Adriana Maria Güntzel (FDRS0274). Four adult parthenogenetic females and one juvenile from Baía da Célia, Fazenda Nhumirim (18°59'27.5"S, 56°39'41.0"W), Pantanal, Mato Grosso do Sul, Brazil. Material collected on 07.ix.2000 by Valéria Barros (FDRS0276). Two adult parthenogenetic females from the Cachoeira II reservoir (07°58.338'S, 38°19.628'W), Serra Talhada, Caatinga, Pernambuco, Brazil. Material collected on 18.vi.2011, leg. Leidiane Pereira Diniz (FDRS0378). One adult parthenogenetic female from Capetinga Stream (15°57'40.6" S, 47°56'36.7"W), Água Limpa Farm, Cerrado, Distrito Federal, Brazil. Material collected on 01.ix.2006 by GEEA. Two adult parthenogenetic females from Cabocla I pond (15º48'16.6"S, 47º14'58.8'W"), Campo de Instrução de Formosa, Cerrado, Distrito Federal, Brazil. Material collected on 07.viii.2009 by GEEA (FDRS0273). Six parthenogenetic females from Paranoá Lake (15°43'47" S, 47°52'58"W), Cerrado, Distrito Federal. Material collected on viii.2014 by Ciro Joko, Mariana Lessa and Elisângela Rangel (FDRS0383). Slides containing dissected individuals deposited at the Laboratório de Biodiversidade Aquática, Universidade Católica de Brasília (CLLA001 to CLLA018).

Diagnosis. *Female.* Maximum height at middle of body, body about 1.5 times as long as high, weakly compressed laterally. Head with ocellus and eye of different size. Three main pores connected; anterior pore larger than others; a wide connection between the posterior and middle pores, connection between the middle pore and anterior pore wide or relatively narrow; transverse lateral head pores about 0.7 – 0.85 IP; deep and rounded pockets, about 1.5 times longer than lateral head pores. Carapace covered by dense longitudinal lines; ventral margin almost straight, with 45–49 plumose setae per valve; thin setulae between marginal setae. Labral keel without a notch. Antennule about two times longer than wide. Antenna, antennal formula: spines 001/101, setae 113/003. Apical spine of endopodite and exopodite long, of similar size, with visible denticles. Apical setae bisegmented and setulated. Postabdomen slightly narrowing distally, about three times as long as wide. Pre-anal margin of similar size to anal margin, and shorter than postanal margin. Anal margin slightly concave, with 3–4 denticle groups. Postanal portion of postabdomen with acute distal portion. Ten-twelve well developed marginal denticles, each with several spinules on its anterior margin. Terminal claw longer than anal margin, with one group of short spinules on its base; pecten armed with a row of outer spinulae decreasing in size towards distal portion. Basal spine relatively short, shorter than width of claw base, without spinulae or setulae. Limb I with ejector hooks different in size, accessory seta implanted near the base; ODL with a thin seta, serrulated in the distal part; IDL with three setae; seta 1 small and thin; setae 2 and 3 bisegmented and armed with setulae, similar in size to ODL setae. Limb II with elongated exopodite bearing a non-setulated seta; scrapers 3 and 7 armed with robust denticles as compared to other scrapers. Limb III, fourth and fifth setae of exopodite relatively long. Limb IV, exopodite subquadrangular with setae 1–4 plumose; gnathobase armed with a bottle-shaped sensillum. Limb V, exopodite with four setae, divided into two lobes; gnathobase as a rounded lobe; filter comb with three long setae. Limb VI present. *Male* and *ephipial female* unknown.

Description. Parthenogenetic female. *Habitus* (Figs. 1A–B; 4A). Animal of a relatively large size 0.32–0.43 mm; in a lateral view, carapace oval, maximum height at middle of body, which is about 1.5 times as long as high. Dorsal margin convex. From a ventral view, body weakly compressed laterally. Dorsal keel absent.

Head (Figs. 1A; 2A–D; 4A, C). Ocellus and eye of different size. Headshield with maximum width behind the mandibular articulation, posterior margin wavy. Rostrum short, rounded, projected towards ventral margin of carapace. Three main pores connected; anterior pore larger than others; a wide connection between posterior and middle pore, connection between middle and anterior pore wide or relatively narrow. PP about 0.7–0.95 IP. Transverse lateral head pores about 0.7 – 0.85 IP, located at the level of middle main head pores. Deep and rounded pockets, about 1.5 times longer than lateral head pores.

Labrum (Fig. 2E) of moderate size. Labral keel without a notch; anterior margin convex, posterior margin with two clusters of setules.

Carapace (Fig. 1C–D; 4B) covered by longitudinal lines. Ventral margin almost straight, with 45–49 plumose setae per valve; thin setulae between marginal setae; middle group consists of short marginal setae; posterior group of long setae decreasing in size towards the posteroventral corner. Posterior margin armed with spinulae not arranged in groups and not projected beyond the margin.

Mandibles large relatively to body size.

First maxilla (Fig. 2H) well developed, with two long setulated setae.

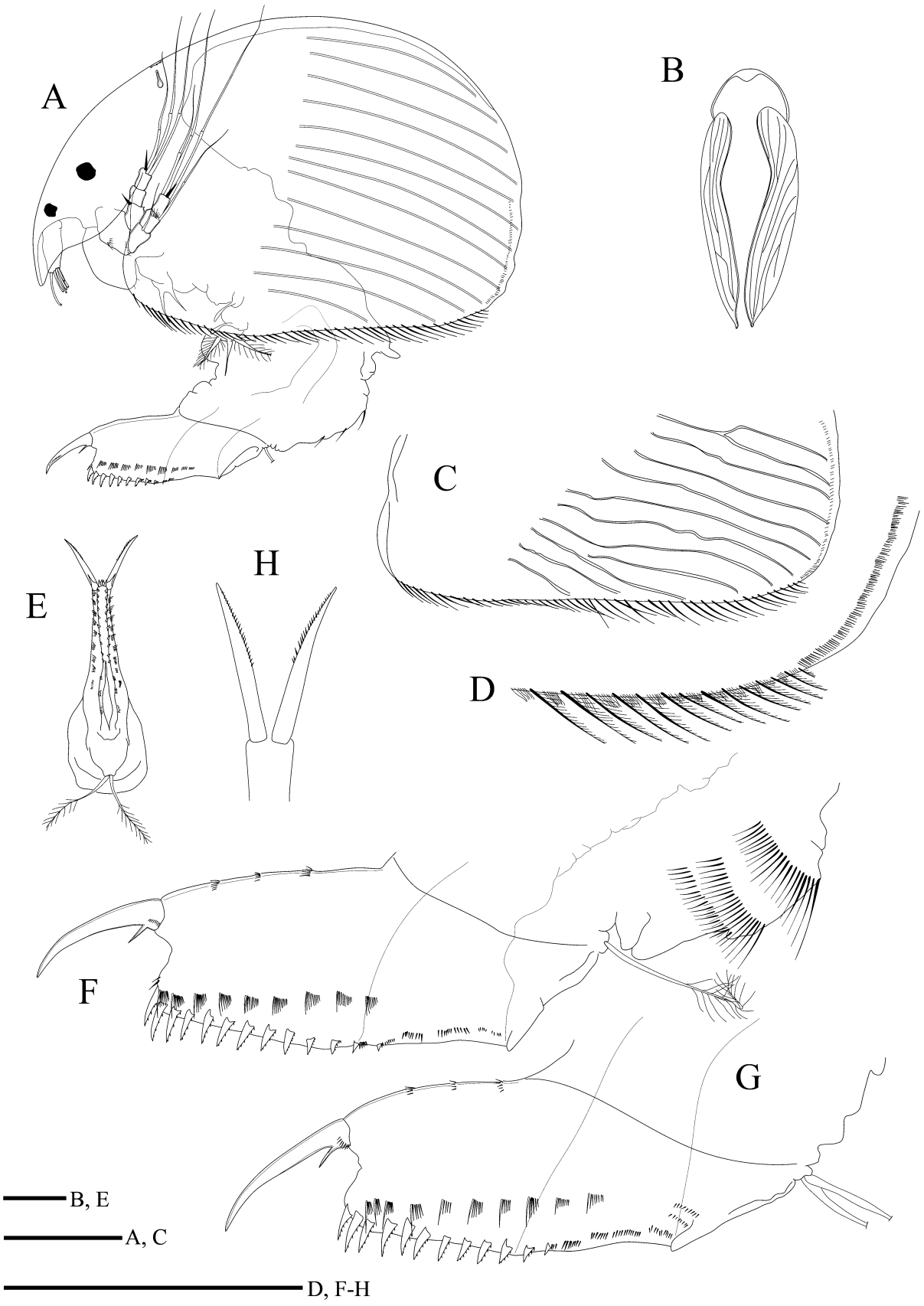

FIGURE 1. *Alona margipluma* **sp. nov.**, parthenogenetic females. A, habitus, lateral view. B, habitus, ventral view. C, carapace. D, posteroventral corner of the valves. E, postabdomen, ventral view, F-G, postabdomen, lateral view (Pernambuco state). Scale bars = 100μm.

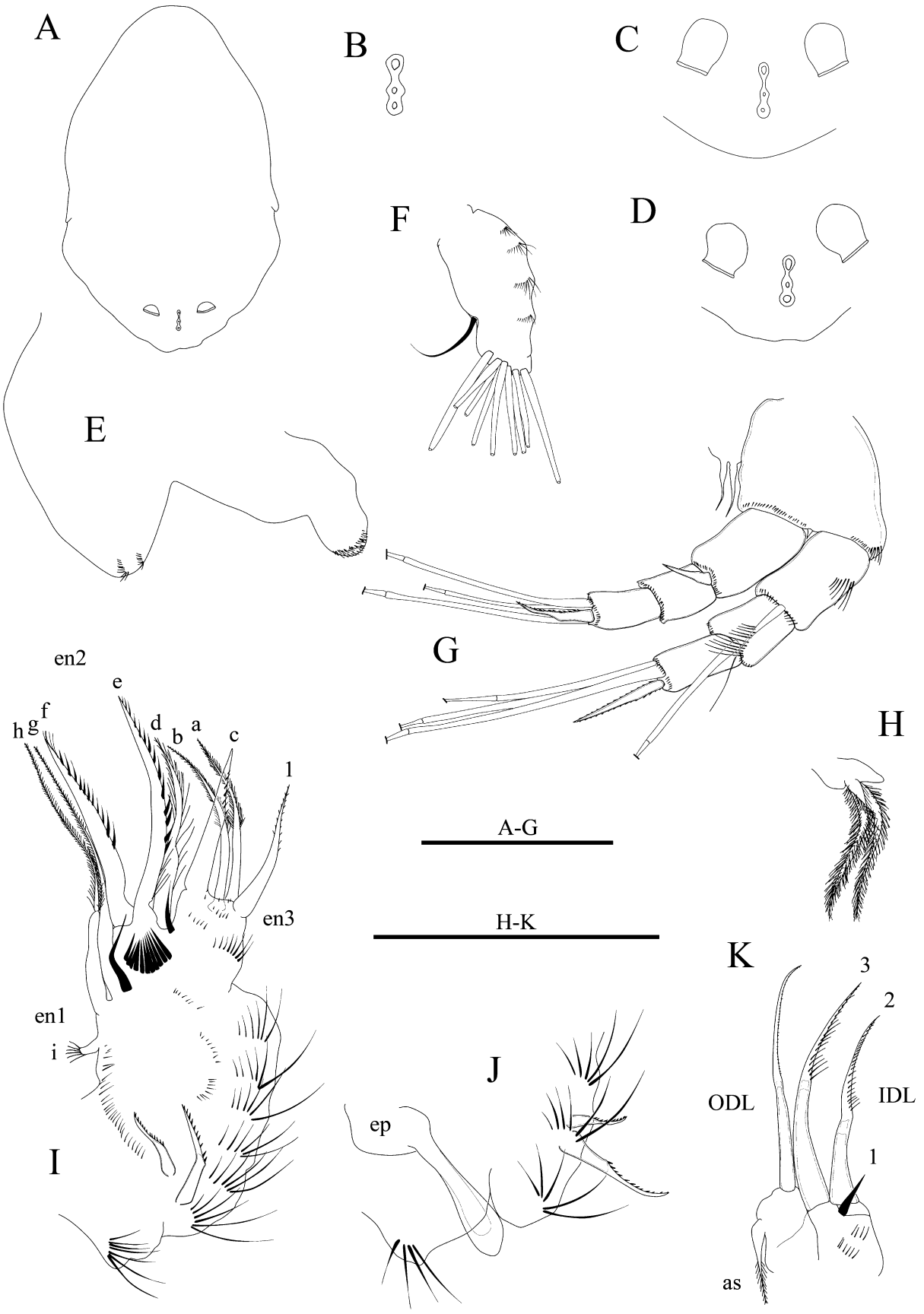

FIGURE 2. *Alona margipluma* **sp. nov.**, parthenogenetic females. A, headshield. B–D, head pores. E, labral keel. F, antenule. G, antenna. H, maxila. I, limb I. J, limb I, ejector hooks and epipodite. K, limb I, IDL and ODL. Scale bars = 50μm.

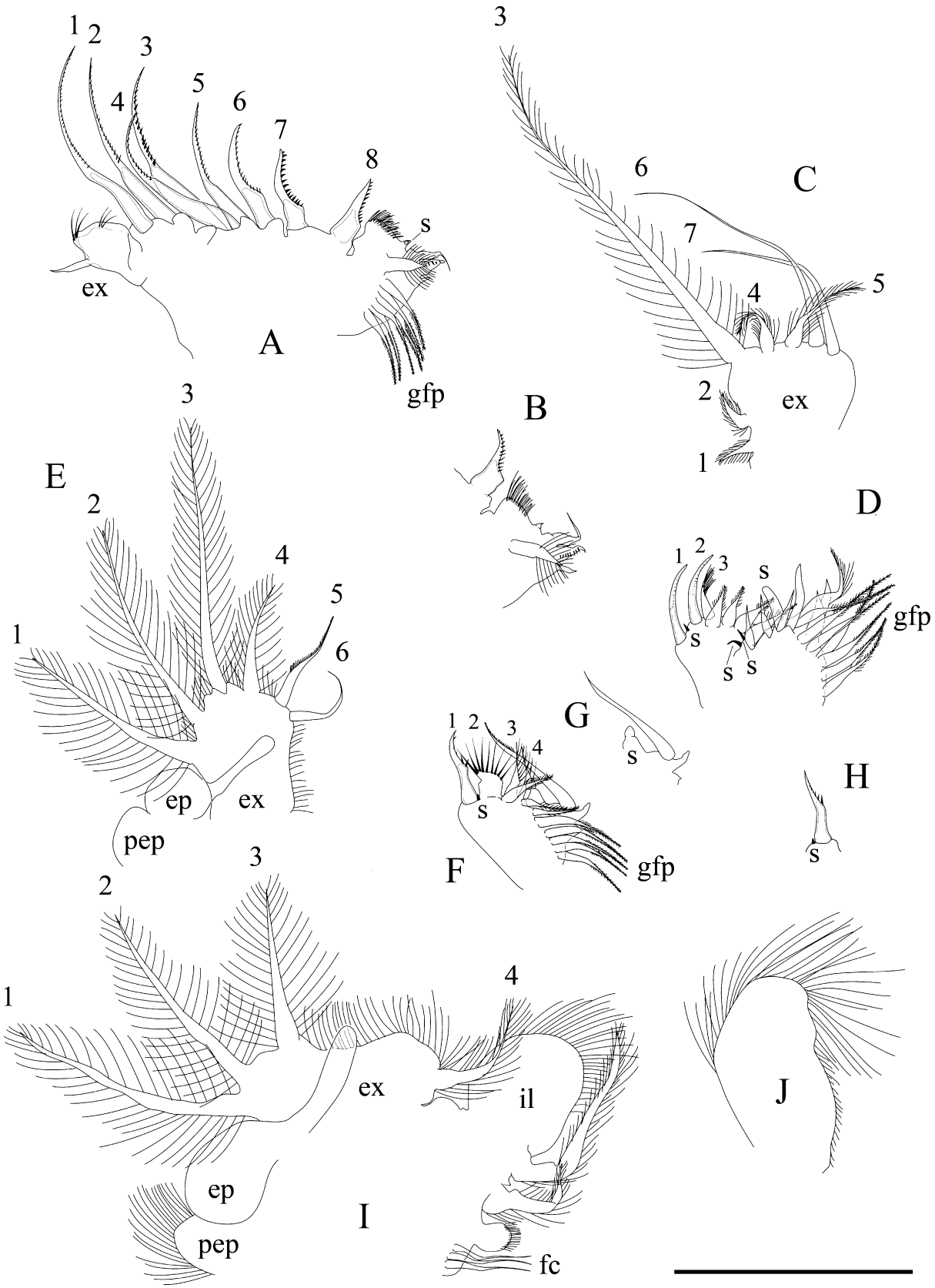

FIGURE 3. *Alona margipluma* **sp. nov.**, parthenogenetic female. A, limb II. B, limb II, gnathobase. C, limb III, exopodite. D, limb III, endites. E, limb IV, exopodite. F, limb IV, endites. G, limb IV, sensillum. H, limb IV, distal endite seta. I, limb V. J, limb VI. Scale bar = 50µm.

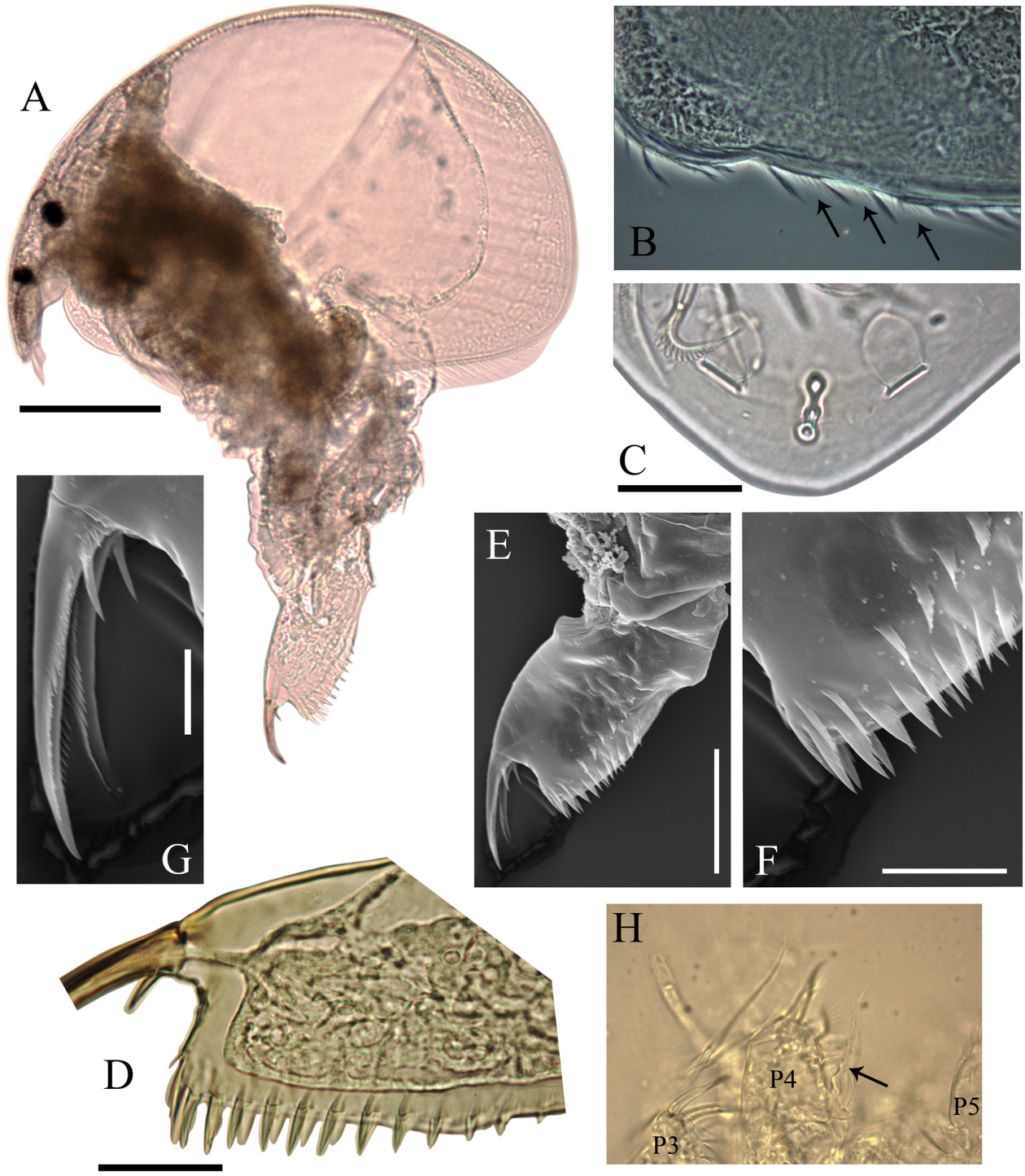

FIGURE 4. *Alona margipluma* **sp. nov.**, parthenogenetic female. A, habitus. B, ventral margin, arrows showing the setulae between setae of valves. C, main and lateral head pores. D-F, postabdomen. G, terminal claw of postabdomen. H, limb IV endite, arrow showing the bottle-shaped sensillum. Scale bars: A, E = 100µm; C, D, F = 50µm; G = 10µm.

Antennule (Fig. 2F) with length two times the width; four rows of setules on antennular body. Antennular sensory seta slender and short, about 2–2.5 times smaller than antennular body, inserted at two-thirds of antennule length from its base. Nine aesthetascs of different length, all projecting beyond the tip of rostrum. None of them exceeds the length of the antennules.

Antenna (Fig. 2G). Two coxal setae of equal length. Basal segment thick, with many spinulae and a short spine distally. First segment of exopodite with long and slender spinulae near its base and short spinulae at its terminal

portion. Second segment with long and slender setulae located at median portion and short spinulae in its terminal portion. Endopodite segments with short spinulae at its terminal portion. Antennal formula: spines 001/101; setae 113/003. Setae on first segment of exopodite thin, not reaching mid length of terminal setae. Seta on second segment of exopodite bisegmented. Spine on first segment of endopodite short, not exceeding distal end of second segment. Apical spines of similar length, longer than apical segments and with visible denticles. Apical setae bisegmented and setulated.

Abdomen about two times shorter than thorax, armed with three rows of long abdominal setae.

Postabdomen (Figs. 1E–G; 4D–G). Slightly narrowing distally, about three times as long as wide, ventral margin slightly convex with at least three rows of spinulae. Pre-anal margin of similar size to anal margin and shorter than postanal margin. Anal margin slightly concave, with 3–4 groups of spinulae. Postanal margin almost straight, distal angle acute, slightly projected. Lateral fascicles arranged in 8–9 groups; first spinule of each fascicle longer and thicker than others; first three fascicles exceeding postabdomen margin. Ten-twelve well developed marginal denticles, each one with several spinulae on its anterior margin; most proximal denticles grouped. Postabdominal seta (Fig. 1E). About two times shorter than postabdomen; proximal portion naked; long setules armed bilaterally towards its distal portion. Terminal claw (Figs. 1E–H; 4G). Implanted in a projected basis of postabdomen, slightly longer than anal margin, uniformly curved, with a group of short spinules at its base; external pecten armed with a row of outer spinules. Basal spine relatively small, shorter than width of claw at its base, without spinulae or setulae.

Six pair of limbs.

Limb I (Fig. 2J–K). Epipodite oval with a long projection. Accessory seta implanted near base of ODL, which has a thin seta, serrulated in its distal part, with length about the same as the longest IDL seta; IDL (en 4) with three groups of spinules on its face and three setae: seta 1 about three times shorter than setae 2; setae 2 and 3 bisegmented and armed with setulae, seta 3 slightly longer than seta 2. Endite III with four setae; posterior setae (a–b) setulated, longer than the other two setae (posterior seta c and anterior seta 1). Endite II with one row of spinules; three posterior setae present (d–f), setae (f) and (e) similar in length and with thick spinules on the lateral face; setae (d) exceeding the midlength of seta (e); endite armed a single anterior stiff seta. Endite I with three posterior setae (g–i), two being bisegmented and setulated in distal part (g–h) and a short flat plumose seta (i), and a thin anterior stiff seta. Ejector hooks of different length. Ventral face of the limb with six groups of setulae organized in clusters, decreasing in size towards the distal portion. Gnathobase not studied.

Limb II (Fig. 3A–B). Exopodite elongated, with two rows of setulae and a naked seta. Inner limb portion armed with eight scrapers gradually decreasing in length towards gnathobase. Scrapers 3 and 7 armed with robust denticulation. Distal armature of gnathobase armed with four elements: first a sensillum, second an element with distal portion geniculated, third element armed with denticles, fourth element relatively short, not sharp. Filter comb with seven setae; first seta short, robust, with dense and long setulae; other setae long and with short setulae.

Limb III (Fig. 3C–D). Epipodite not studied. Exopodite relatively large, subquadrangular, with five distal and two lateral setae. Setae 1–2 setulated, similar in length. Third seta long, setulated, about 1.5 times longer than the sixth seta. Fourth and fifth setae shorter than setae 6–7, but longer than setae 1–2. Sixth seta about 1.3 times longer than the seventh seta, both naked. Distal endite with one sensillum and three setae (1–3), two scraper-like and similar in length (1–2), and third seta slightly curved and armed with many setules bilaterally implanted (3); four plumose posterior setae similar in length. Basal endite with four soft anterior setae, distalmost seta very long and robust; two sensillum near to first soft seta present. Gnathobase armed with four elements, the first being a cylindrical sensillum, the second a strong geniculated seta, third and fourth elements with tip acute, naked. Filter comb with seven setae.

Limb IV (Fig. 3E–H; 4H). Pre-epipodite round. Epipodite rounded, with a long projection. Exopodite subquadrangular with six marginal setae: setae 1–4 plumose; seta 1 slightly shorter than seta 2; third seta longer than all others; fourth seta subequal in length to the fifth seta; fifth seta slightly setulated unilaterally; sixth seta naked. Distal endite with four setae (1–4), one scraper-like and armed with one sensillum at the base (1), three flaming-torch-like (3–4); the first flaming-torch robust, with long setules (2) and three soft setae increasing in size proximally. Gnathobase armed with one bottle-shaped sensillum and one setulated seta implanted on a robust base. Filter plate with five slender setae.

Limb V (Fig. 3I). Pre-epipodite oval and densely setulated; epipodite with long projection. Exopodite unclearly divided into two lobes, with four plumose setae decreasing in size towards the internal lobe; setae 1–2 similar in

length; fourth seta about 1.3–1.5 times shorter than the third seta. Internal lobe very wide, oval and with long setulae; two setulated setae on the inner face of the lobe, first seta about two times longer than the other. Gnathobase as a rounded lobe; filter comb with three long setae.

Limb VI (Fig. 3J). An elongated lobe, about two times longer than wide; apical margin of the lobe with long setulae; proximal margin armed with short setules.

Ephippial female. Unknown.

Male. Unknown.

Differential diagnosis. *Alona margipluma* **sp. nov.** clearly differs from species of the *rustica*-branch (see Hudec 1998; Sinev 1999; Sinev 2009a; Van Damme & Eggermont 2011), such as from Neotropical *A. iheringula,* in the morphology of the postabdomen, and main and lateral head pores. Differences in aforementioned structures are also observed as compared with *A. weltneri* Keilhack, 1905 (see Van Damme *et al.* 2011). Specifically, *Alona margipluma* **sp. nov.** differs from *A. setigera* because it has three main head pores. Because its large lateral head pores and distal postabdominal angle acute, *Alona margipluma* **sp. nov.** resembles *A. costata*, *A. cheni* and *A. natalensis*, but the former has different morphology of the exopodite of the limb V and the lateral head pores. Such as, in *A. cheni* and *A. natalensis* the pockets are shallow, about 2 and 3 times smaller than length of lateral head pores, respectively. In *A. costata* the pockets are deep, about two times longer than lateral head pores, and the basal spine on the postabdomen is longer than the width of terminal claw base. *Alona margipluma* **sp. nov.** has deep pockets, about 1.5 times longer lateral head pores, and basal spine is shorter than the width of terminal claw base. Furthermore, *Alona margipluma* **sp. nov.** can be recognized by the presence of thin setulae between setae on the valves, absent in another species (see Sinev 1999, 2008); long setae 4–5 on exopodite of the limb III; and bottle-shaped sensillum on the basal endite of the limb IV. Other differences among *A. costata*, *A. setigera*, *A. cheni*, and *A. natalensis* are summarized in Table 1.

Distribution and ecology. So far, *Alona margipluma* **sp. nov.** has been found only in Brazil, in five localities. However, as the records of *A. margipluma* **sp. nov.** observed in this study are very distant from each other, it is possible that distribution of this species covers a considerable portion of the country. *Alona margipluma* **sp. nov.** is a species with preference for lentic waters; however, it was also found in lotic environments, associated with leaves in backwater zones (Capetinga Stream and Amonguijá River). Water bodies inhabited by *A. margipluma* **sp. nov.** (except Baía da Célia and Paranoá Lake) have temperatures ranging between 19.7 and 33.5 ºC, electric conductivity < 300 µS/cm, pH 4.63–7.3, turbidity 31.3–100 NTU, total dissolved solids 0.071–0.187 g/L and dissolved oxygen 4.11–7.3 mg/L. In some localities, *Alona margipluma* **sp. nov.** was found together with *A.* cf. *setigera*, *A. ossiani*, *A. iheringula,* and *A. guttata*. *Alona margipluma* **sp. nov.** was collected associated exclusively with the spongy air-filled roots of *Ludwigia helminthorrhiza* Mart. in the Cachoeira II reservoir (Caatinga) or with a multispecific bank of macrophytes in the Cabocla I pond (Cerrado).

Key to the identification of American species of the *costata*-group

1	Distal angle of postabdomen projected and acute, slightly narrowed distally. Lateral head pores long, over 1/2 IP	2 (*costata*-branch)
-	Distal angle of postabdomen rounded and projected, strongly narrowed distally. Lateral head pores short, about 1/3 IP	3 (*rustica*-branch)
2	Three main head pores	*Alona margipluma* **sp. nov.**
-	Two main head pores	*Alona* cf. *setigera* Brehm, 1931
3	Rostrum elongated, labral keel with projections on its upper half	*Alona bicolor* (Frey, 1965)
-	Rostrum not elongated, labral keel without projections	4
4	Middle main head pore at similar distance of anterior and posterior head pore	*Alona rustica* Scott, 1895
-	Middle main head pore near to posterior head pore	5
5	Second seta on the exopodite of limb V slightly longer than first seta	*Alona iheringula* Kotov & Sinev, 2004
-	Second seta on the exopodite of limb V about two times shorter than first seta	*Alona hudeci* Sinev, 1999

Discussion

Fauna of the Chydoridae in Brazil are represented by about 65 valid species. Recently, a widening of the studied area and the revision of some species contributed to demonstrating the peculiar composition of the fauna, with few

species that are endemic to habitats or specific regions (see Sinev & Elmoor-Loureiro 2010; Elmoor-Loureiro *et al.* 2013; Elmoor-Loureiro 2014). However, the country has a large number of species with wide distribution, as this is the case of *A. iheringula*, *Alona* cf. *setigera* and *A. margipluma* **sp. nov.**. These three species can coexist, but are different morphologically in the features of the head pores, postabdomen and limbs. For instance, the following morphological traits are present in *A. iheringula,* but absent in *A. margipluma* **sp. nov.**: main head pores with narrow connection and short transverse lateral head pores; postabdomen narrowing distally and with distal projection slightly rounded; setae 4–5 on the exopodite of limb III of similar length; seta 4 on the exopodite of limb IV shorter than seta 5; and exopodite of the limb V clearly bilobed (see Sinev 2001).

The main difference between *A. setigera* and *A. margipluma* **sp. nov.** is that the first species has two main head pores. However, consistent differences are also observed in the limbs; *A. setigera* has seta (f) on limb I markedly longer than seta (e); setae 4–5 on the exopodite of limb III short and of similar length; exopodite of limb V clearly bilobed; and limb VI armed with setulae on the distal part (see Sinev 1999). These morphological traits are not found in *A. margipluma* **sp. nov.**

According to Sinev (2008), *Alona cheni*, *A. natalensis, A. setigera* and *A. costata* form a special lineage (*costata*-branch) within the *costata*-group because they share large lateral pores and an acute distal angle of the postabomen (for differences and similarities between these species, see Table 1). These characteristics are found also in *A. margipluma* **sp. nov.** and make it a new member of this subgroup. *Alona margipluma* **sp. nov.** seems to be closer to *A. natalensis* because of the affinities in the morphology of the main and lateral head pores, unclear division of the exopodite of limb V, and morphology of labral keel (Table 1). However, *A. margipluma* **sp. nov.** has some exclusive characteristics, such as setae 4–5 on the exopodite of limb III different in length, a bottle-shape sensillum on the distal endite of limb IV and thin setules between the valves of the marginal setae. This latter characteristic is uncommon for species of Aloninae, which generally present short spinules.

Biogeographic analyses and descriptions of species distributed in different continents have repeatedly displayed affinities among fauna from areas that were connected before continental drift. This includes some species of *Nicsmirnovius* Chiambeng & Dumont, 1999 (Van Damme *et al*. 2003), the *Alona affinis*-group (Sinev 1997; 2009b), *Ovalona* Van Damme & Dumont, 2008 (Sinev 2006; Van Damme & Dumont 2008a), *Ilyocryptus* Smirnov, 1992 (Kotov & Elias-Gutierrez 2009), *Leydigia* Kurz, 1875 (Kotov 2009; Kotov & Fuentes-Reines 2014), and *Macrothrix* Norman & Brady, 1867 (Kotov 2007). This is also evident for the *costata*-branch, as shown by the affinities between the African *A. natalensis* and South American *A. margipluma* **sp. nov.**. *Alona margipluma* **sp. nov.** and *A. natalensis* also have many morphological affinities with *A. cheni,* but the phylogenetic and biogeographic significance of these affinities needs to be further investigated. It is clear that the *costata*-branch represents a good example of the continental endemism in Chydoridae; however, analyses of molecular data may be helpful to reconstruct the evolutionary history of this linage from *costata*-group.

On the other hand, the deep pockets of lateral head pores observed in *A. margipluma* **sp. nov.** resemble those found in *A. costata*, but many differences are observed between these species. For instance, *A. costata* has a short lateral head pore when compared with its pockets, and it has a narrow connection between middle and posterior main head pores. Besides that, other differences are observed in features from limbs (see Table 1). *Alona costata* has been reported in North America in some studies (Frey 1965, 1986; Flössner & Frey 1970), but, the morphology of the limbs in these populations has not yet been completely studied. According to Frey (1965, 1986) and Sinev (1999), the status of *A. costata* in North America is unclear and it may represent a cryptic species, not allowing more comparisons with *A. margipluma* **sp. nov.**. More consistent studies on the North America populations of *A. costata* are needed.

Recently, Silva *et al.* (2014) presented good photographs of an individual cultured from material collected at Furnas Reservoir, Minas Gerais state, which they labeled as *A. iherinhula* and indicated the COI sequence. However, the photographs show main head pores with wide connection, lateral head pores large and with deep pockets, longitudinal lines on the carapace and distal region of the postanal margin of the postabdomen clearly acute, which are not morphological traits observed in the typical *A. iheringula*, but belong to *A. margipluma* **sp. nov**. Therefore, we are convinced that the specimen photographed belongs to the new species instead of to *A. iheringula.* Likewise, the COI sequence deposited at GenBank (access number KF383284) should be considered as belonging to *A. margipluma* **sp. nov.**. and the considerations based on the genetic divergence performed by Silva *et al.* (2104) might not be considered valid, so far. Keeping in mind that the specimens that led to the culture were collected from a location within the range of the new species, this idea is reinforced.

TABLE 1. Main morphological differences between species from *costata*-branch: *Alona costata* (Sinev 1999), *Alona setigera* (Sinev 1999), *Alona cheni* (Sinev 1999) and *Alona natalensis* (Sinev 2008) and *Alona margipluma* **sp. nov.**. * Morphological trait absent.

	Alona costata	*Alona setigera*	*Alona cheni*	*Alona natalensis*	*Alona margipluma* **sp. nov.**
Size (mm)	0.38–0.42	0.38–0.42	0.47	0.54	0.32–0.43
Number of main head pores	3	2	3	3	3
Connection between middle and posterior main head pores	narrow	*	wide	wide	wide
Connection between middle and anterior main head pores	narrow/wide	*	narrow	narrow	narrow/wide
Main head pores PP/IP	0.5–0.8	1.1–1.3	0.7–0.9	1–1.2	0.7–0.95
Length of lateral main head pore	about 0.75 IP	about 0.9–1 IP	about 0.9–1 IP	about 1–1.2 IP	about 0.7–0.85 IP
Depth of pockets/ length lateral head pores and shape of pockets	about 2, rounded	about 1.5, rounded	about 2, semicircular	about 3 shallow	about 1.5, rounded
P3 setae 3–4	short, different length	short, subequal length	short, subequal length	short, subequal length	long, different length
P4 endite distal sensillum	reduced	reduced	reduced	rounded	bottle-shaped
P5 exopodite, lobe division	pronounced	pronounced	pronounced	unclear	unclear
P6 setules position	distal	distal	distal and lateral	distal	distal and lateral

In conclusion, the Brazilian fauna of the *costata*-group is now composed of three species: the previously reported *A. iheringula* and *A. setigera,* and the new species, *Alona margipluma* **sp. nov.**. We found *A. margipluma* **sp. nov.** in samples containing *A. guttata*, *A.* cf. *setigera* and *A. iheringula*, and it is very difficult to distinguish among these species at a low magnification or under the stereomicroscope. Because of their co-occurrence and high similarity in general view, we suggested careful observation of the main and lateral head pores and postabdomen in order to reduce the likelihood of mistakes in identifying these species.

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