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Alona kaingang (Crustacea, Cladocera, Aloninae): a new species of the *pulchella*-group, with identification key to Neotropical species

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Abstract

Background: The subfamily Aloninae has been the focus of extensive studies on the Chydoridae because it has a higher diversity of species and due to the necessity of a detailed redescription of many taxa, especially using characters related to thoracic appendages. The polyphyletic genus *Alona* was redefined recently, and many species complexes were translocated to natural groups. The *pulchella*-group is a candidate for removal from *Alona* because it forms a well-defined complex of species, besides possessing higher species diversity. At present, 50 % of the known species occur in the Neotropics.

Results: In this study, we described *Alona kaingang*, a new species of *pulchella*-group distributed in southern South America. The new species shares with other species similarities in general features of limbs but, for instance, differs from South American *Alona glabra* because it has a short postabdomen. *Alona kaingang* sp. nov. differs from Andean *Alona alti plana* because it has thick denticles on the postabdomen and longitudinal lines on the carapace are absent. Differences in the limbs were also observed.

Conclusions: *Alona kaingang* sp. nov. is part of the group that contains species with complete connection between the main head pores. Like other species of the *pulchella*-group, *A. kaingang* sp. nov. also has similarities with the genus *Ovalona*. The relationship between these two species groups still needs to be tested.

Keywords: Chydoridae; *Alona anamariae*; *Ovalona*; Head pores; Limb; Postabdomen

Background

Chydoridae Dybowski & Grochowski, 1894 *emend.* Frey, 1967 is the most speciose family of the superorder Cladocera (Crustacea: Branchiopoda). The majority of its species inhabit the littoral zones of inland waters, usually associated with aquatic vegetation or fine sediments (Smirnov 1971; Kotov 2006). The subfamily Aloninae Dybowski & Grochowski, 1894 *emend.* Frey, 1967 has been extensively studied for two main reasons: (1) the high diversity of species and (2) the need for detailed redescriptions of taxa, especially using characters related

to thoracic appendages (Smirnov 1998; Kotov 2000a, b; Sinev and Kotov 2000; Sinev 2001a; Sinev and Kotov 2001; Kotov and Elías-Gutiérrez 2002; Kotov 2003; Van Damme et al. 2003; Kotov and Sanoamuang 2004; Sinev and Elmoor-Loureiro 2010; Van Damme et al. 2010; Sinev and Atroschenko 2011).

Many of the recent morphological investigations and redescriptions of species within this subfamily were related to the genus *Alona* Baird, 1843 and resulted in the description of new genera or species translocations to correlated groups, such as *Karualona* Dumont & Silva-Briano, 2000 (Dumont and Silva-Briano 2000; Sinev and Hollwedel 2005), *Nicsmirnovius* Chiambeng & Dumont, 1999 (Kotov 2003; Van Damme et al. 2003), *Parvalona* Van Damme, Kotov & Dumont, 2005 (Van Damme et al. 2005), *Armatalona* Sinev, 2004 (Sinev 2004a), *Matralona*

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Van Damme & Dumont, 2009 (Van Damme and Dumont 2009), *Maraura* Sinev & Shiel, 2008 (Sinev and Shiel 2008), *Miralona* Sinev, 2004 (Sinev 2004b), and *Leberis* Smirnov, 1989 (Sinev et al. 2005). Together, these studies highlighted the polyphyletic nature of *Alona*, which has been supported by phylogenetic analysis (e.g., Elmoor-Loureiro 2004; Sacherová and Hebert 2003).

Recently, Van Damme and Dumont (2008a) redescribed *Alona quadrangularis* (O.F. Müller 1776) using specimens from the Palearctic region, where it was originally described. Based on the morphology of the postabdomen, terminal claws, and limb setae, these authors defined consistent diagnostic characters for the genus *Alona*. Subsequently, some species complexes that had previously been attributed to *Alona* sensu lato were studied and allocated to new genera, such as *Phreatalona*, which corresponds to the *protzi*-complex (Van Damme et al. 2009); *Coronatella*, which corresponds to the *rectangula*-complex (Van Damme and Dumont 2008b); *Brancelia* (Van Damme and Sinev, 2011) which corresponds to the *hercegovinae*-complex; and *Anthalona* Van Damme, Sinev & Dumont, 2011, which includes species of the *verrucosa*-complex (Van Damme et al. 2011). However, several groups still require removal from *Alona* sensu lato, such as the *pulchella*-group (Van Damme et al. 2010).

The *pulchella*-group is a well-defined complex of species of *Alona* sensu lato characterized by postabdomen with marginal denticles merged and moderately developed and inner distal lobe (IDL) with three setae, seven setae on the exopodite of the third limb, reduced filter comb on the fifth limb, and two lateral aesthetascs on male antennules (for more details, see Sinev et al. 2012). The *pulchella*-group has at least 16 valid species and six species waiting for revision, which have been reported for all continents, except Antarctica (Sinev 2001b, c; Sinev 2002a; Sinev 2009; Kotov et al. 2010; Van Damme et al. 2010; Sinev et al. 2012; Sinev and Silva-Briano 2012).

In the Neotropics, the *pulchella*-group is highly diverse presenting species with the entire morphological range known for the group, including the specialized species *Alona bromelicola* Smirnov, 1988 (Sinev 2002a). Specifically for South America, there are reports of three species (Sinev 2001b; Kotov et al. 2010); however, the knowledge of the *pulchella*-group remains incipient. This manuscript is part of an extensive review concerning populations of *Alona* sensu lato in Brazil, aims to evaluate the morphology, and describes a new species of the *pulchella*-group.

Methods

Selected specimens were transferred to slides containing glycerin and dissected under a stereomicroscope. The morphology of appendages and other structures was studied using a phase-contrast microscope. Some animals were dehydrated in an acetone series (50, 70, 90 and 100 %) and critical point-dried or air-dried, mounted on aluminum stubs, coated with

gold, and examined under a JEOL-JSM 7001F scanning electron microscope. Enumeration of limb setae and other structures proceeded from the epipodite to the gnathobase, without relation to homology, according to the recent literature (Van Damme and Dumont 2007; Sinev and Kobayashi 2012). Drawings were prepared using a camera lucida.

Results

Systematics

Class Branchiopoda Latreille, 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 kaingang Sousa, Elmoor-Loureiro & Santos
(Figs. 1, 2, 3, and 4)

Type material

Holotype. Undissected, adult parthenogenetic female in a tube with 90 % ethanol deposited at the Museum of Zoology of the University of São Paulo under access number MZUSP 29593. The label of the holotype is “*Alona kaingang* n. sp., 1 parth. ♀ from Mostardas—Lagoa de São Simão, RS, Brazil, Holotype.”

Paratypes. Two undissected adult parthenogenetic females in tubes with 90 % ethanol, deposited at the Museum of Zoology of the University of São Paulo under access number MZUSP 29594. One adult parthenogenetic female and one adult male, undissected in tubes with 90 % ethanol, deposited at the National Museum of Rio de Janeiro under access numbers MNRJ 23739 and MNRJ 23740. Eight specimens selected as paratypes (access number EL02477), and several unstudied individuals (access numbers EL02165 and EL02166) are deposited at the Laboratório de Biodiversidade Aquática, Universidade Católica de Brasília.

Type locality

Pond in São Simão, Mostardas, Rio Grande do Sul state, Brazil (30° 57' 06" S, 50° 42' 29" W).

Etymology

The name *kaingang* refers to indigenous people who inhabited the southern portion of South America before the European colonization. The *kaingang* people were distributed in South-eastern and Southern Brazil, where the new species occurs.

Material examined

Twenty-three adult parthenogenetic females and five males from the pond in São Simão, Mostardas, Rio Grande do Sul state, Brazil (30° 57' 06" S, 50° 42' 29" W). Material collected by Lourdes M. A. Elmoor-Loureiro on 01.i.2002. Four adult parthenogenetic females from the Limnology

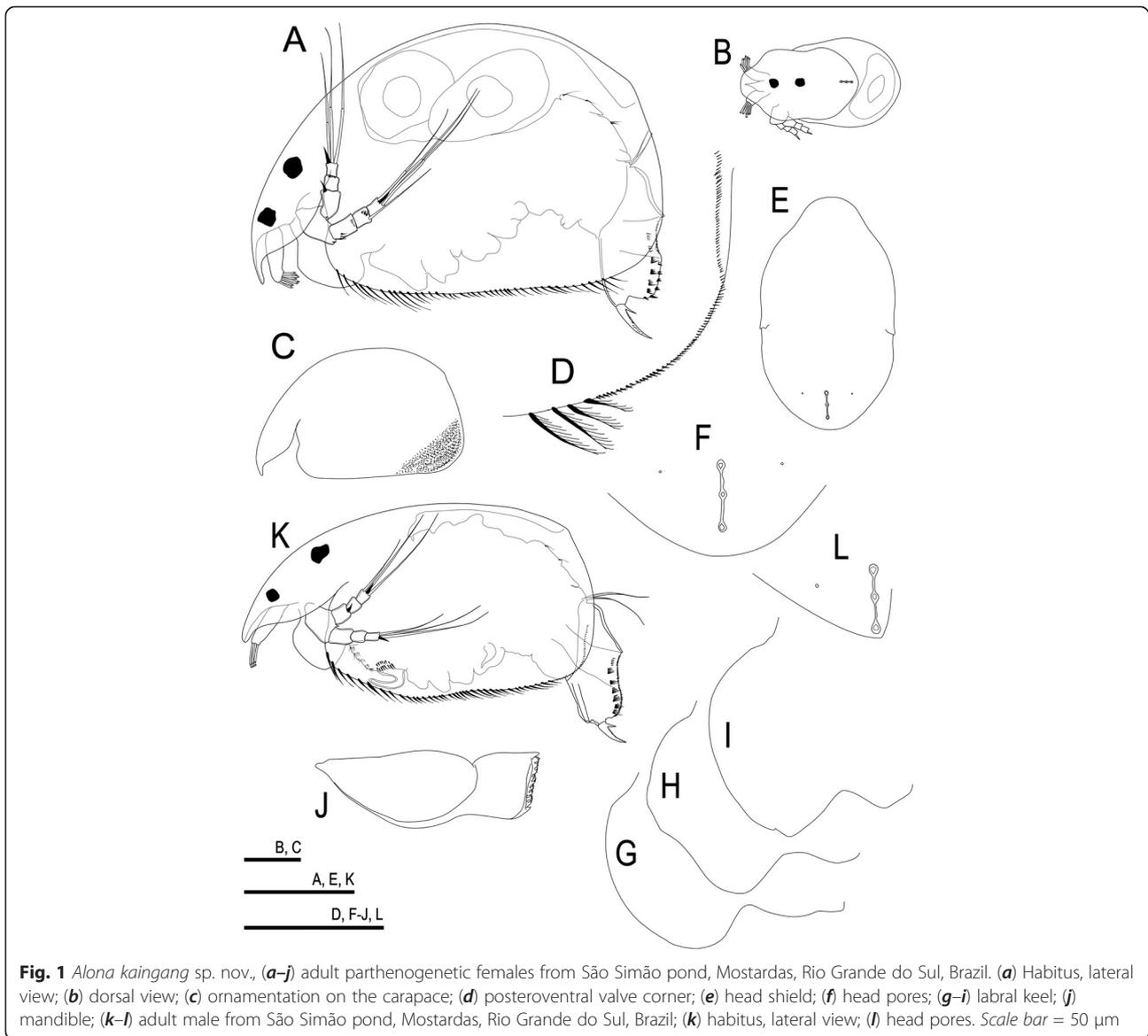


Fig. 1 *Alona kaingang* sp. nov., (a–j) adult parthenogenetic females from São Simão pond, Mostardas, Rio Grande do Sul, Brazil. (a) Habitus, lateral view; (b) dorsal view; (c) ornamentation on the carapace; (d) posteroventral valve corner; (e) head shield; (f) head pores; (g–i) labral keel; (j) mandible; (k–l) adult male from São Simão pond, Mostardas, Rio Grande do Sul, Brazil; (k) habitus, lateral view; (l) head pores. Scale bar = 50 μ m

Laboratory cultivation facility, Instituto de Ciências da Natureza, Universidade Federal de Alfenas; specimens collected at Furnas Reservoir, Minas Gerais state, Brazil (21° 03' 24" S, 46° 00' 18" W), leg. M. J. Santos-Wisniewski (EL02167). Four adult parthenogenetic females from a marginal pond in São Simão Reservoir, Minas Gerais state, Brazil (18° 48' 50" S, 50° 22' 58" W); material collected by José Roberto Debastiani-Júnior (EL02173).

Diagnosis

Female. Of small size, length 0.33–0.40 mm. Body ovoid with dorsal margin strongly arched. In dorsal view, it shows weak lateral compression, dorsal keel absent. **Head.** Head shield with posterior margin rounded, rostrum short, three main head pores subequal in size,

narrowly connected, postpore distance (PP) less than 0.2 interpore distance (IP). Lateral pores tiny, inserted midway between the main pores and the head shield margin. Labral keel wide, apex obtuse, without setae or denticles. **Carapace.** No striations or longitudinal lines. Ventral margin with 40–50 setae arranged in three groups. Posteroventral corner without denticles, with numerous spinules. **Antennules.** About 2–2.5 times as long as wide, four rows of setules on antennular body, distalmost row shorter than the others. Antennular sensory seta slender and long, about 1.5 times smaller than antennular body. Nine terminal aesthetascs of different sizes. **Antenna.** Antennal formula: spines 001/101, setae 113/003. Spine on first endopodite segment not exceeding the length of the second segment. Apical setae of endopodite and exopodite with lateral spinules. **Postabdomen.** Of moderate

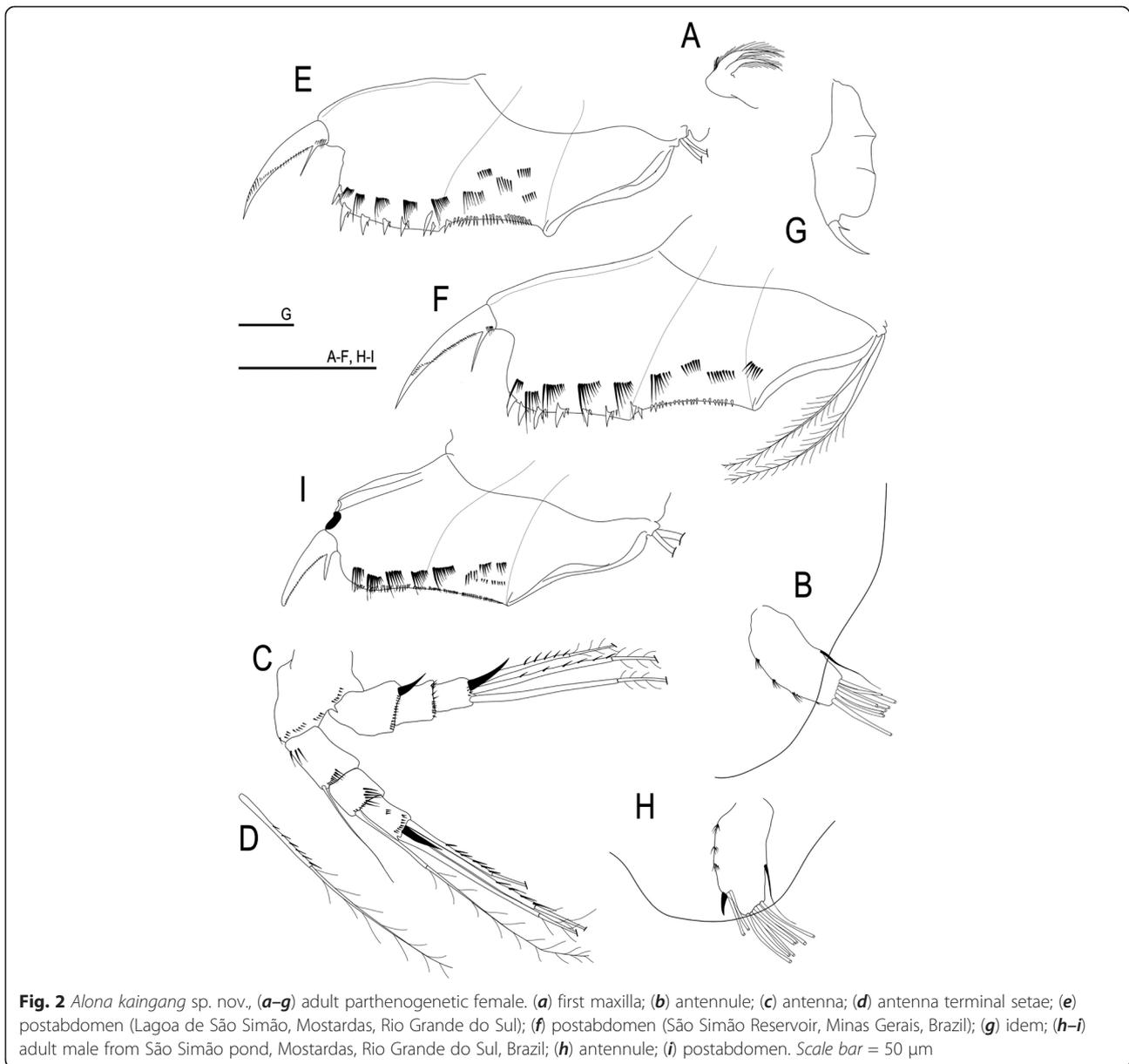


Fig. 2 *Alona kaingang* sp. nov., (a–g) adult parthenogenetic female. (a) first maxilla; (b) antennule; (c) antenna; (d) antenna terminal setae; (e) postabdomen (Lagoa de São Simão, Mostardas, Rio Grande do Sul); (f) postabdomen (São Simão Reservoir, Minas Gerais, Brazil); (g) idem; (h–i) adult male from São Simão pond, Mostardas, Rio Grande do Sul, Brazil; (h) antennule; (i) postabdomen. Scale bar = 50 μ m

size (about 2–2.4 times as long as wide). Pre-anal margin longer than anal margin and shorter than postanal margin. Anal margin inserted in a depression, with distinct angles. Lateral fascicles arranged in five to seven groups, first spines of each group thick and exceeding margin of postabdomen. Six to seven clusters of marginal denticles. Postabdominal setae with proximal portion naked, distal portion with short setules armed bilaterally. *Terminal claw*. Longer than anal margin, with one row of spinules on its base. *Basal spine*. Long, without spinules or setules. *Limb I*. First endite with two marginal setae, IDL with three setae; setae 2–3 bisegmented, long and with strong proximal spines. *Limb II*. Exopodite elongated, with setae present; scrapers 3, 5, 6, 7, and 8 with robust denticulation. *Limb III*. Exopodite with seven marginal

setae; first seta longer than the second, fifth, and seventh setae less than half length of sixth seta, fourth setae geniculated. *Limb IV*. Exopodite with six marginal plumose setae; fourth seta thick and more than half length of fifth seta; sixth seta half as long as fifth seta. *Limb V*. Exopodite with slight depression in middle portion, with four plumose setae; seta 3 smaller than setae 1 and 2; Gnathobase without elements or setae. *Male*. Body elongated, smaller than female (length 0.27–0.30 mm). Ventral margin armed with 40 setae followed by fine spinules. Posteroventral corner without denticles. Head with short rostrum. Main pores nearly connected. Lateral pore inserted between distal and median pores. *Antennules*. With three rows of short setules on the antennular body. Nine (juvenile) to 11 (adult) aesthetascs present: seven to nine terminal and

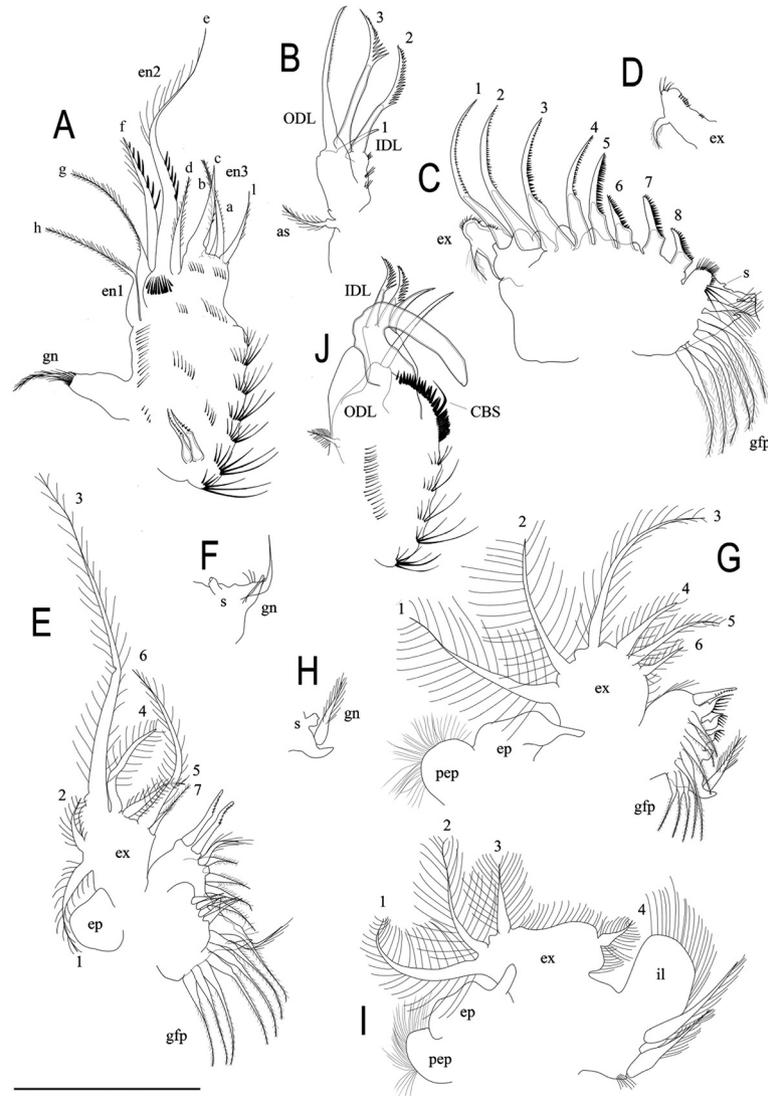


Fig. 3 *Alona kaingang* sp. nov., adult parthenogenetic females from São Simão pond, Mostardas, Rio Grande do Sul, Brazil. (a) limb I; (b) idem; (c) limb II; (d) idem; (e) limb III; (f) idem, sensillum and gnathobasic elements; (g) limb IV; (h) idem, gnathobase; (i) limb V; (j) limb I (male). Scale bar = 50 μ m

two lateral. Male seta short, thick, about 4.6 times shorter than length of antennular body. *Postabdomen*. Narrowing toward distal portion, anal angle not defined. Marginal clusters of spinules present, at least five postanal fascicles. Gonopores opening ventrally, subapically to terminal claw. *Terminal claw*. Smaller than that of female, tip blunt, with slender spinules implanted at base. Basal spine about one-third length of terminal claw. *Limb I*. With copulatory hook U-shaped; copulatory brush seta slender; IDL with three setae. Seta 1 long, thick, subequal in size to setae 2 and 3.

Description of adult parthenogenetic female

Habitus (Fig. 1(a–c)). Compared to other species of the genus, it is a small-sized animal (0.33–0.40 mm), colorless,

and transparent. Carapace ovoid in lateral view, about 1.5 times as long as high, dorsal margin strongly arched. In dorsal view, it shows weak lateral compression. Dorsal keel absent.

Head (Fig. 1(a, e, f)). Ocellus as large as eye. Head shield about 1.7 times as long as wide, with posterior margin rounded. Rostrum short, blunt, projected toward ventral margin. Three main head pores of subequal size, narrowly connected (Fig. 4(a)), PP less than 0.2 IP. Lateral pores tiny, inserted midway between main pores and head shield margin. *Labrum* (Fig. 1(g–i)). Labral keel wide in lateral view, anterior margin slightly convex, apex obtuse, lateral projections not observed, without setae or denticles. In some specimens, the margin is sub-quadrangular (Fig. 1(h)) or with a small depression near

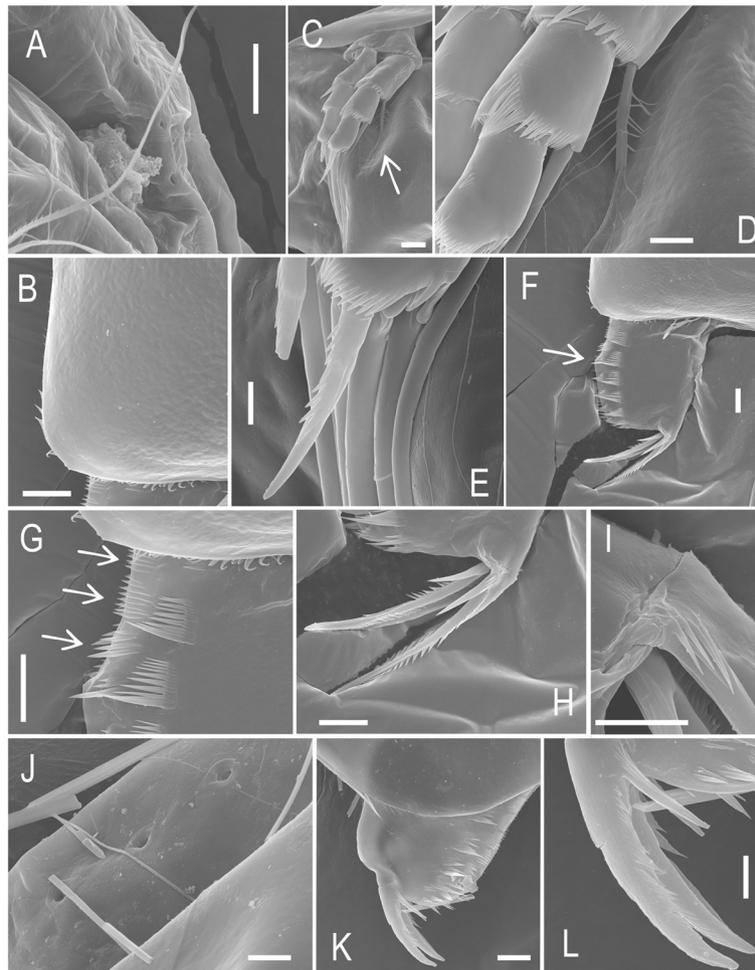


Fig. 4 *Alona kaingang* sp. nov., (**a–i**) adult parthenogenetic females from São Simão pond, Mostardas, Rio Grande do Sul, Brazil. (**a**) main head pores; (**b**) valves. (**c**) antenna—arrow showing seta on the first exopod segment; (**d**) idem. (**e**) antenna—apical spine on the first endopod segment; (**f**) postabdomen—arrow showing spinules on the anal margin; (**h**) terminal claw; (**i**) idem—base with spinules; (**j–l**) adult male from Lagoa de São Simão, Mostardas, Rio Grande do Sul, Brazil. (**j**) main head pores. (**k**) valves and postabdomen; (**l**) terminal claw. Scale bar indicates 5 μm to figures (**d**), (**e**), (**i**), (**j**), (**l**); 10 μm to figures (**a**), (**b**), (**c**), (**f**), (**g**), (**h**), (**k**)

the apex (Fig. 1(i)). *Mandible* (Fig. 1(j)). Well developed in relation to body size (Fig. 1(a)). *First maxilla* (Fig. 2(a)). Relatively well developed, with two long setulose setae.

Carapace (Figs. 1(c, d) and 4(b)). No striations or longitudinal lines (Fig. 4(b)), punctuated ornamentation observed in optical microscopy. Ventral margin straight, with 40–50 setae arranged in three groups, proximal group long, middle group short, posterior group increasing in size toward posteroventral corner. These setae are followed by spinules not arranged in groups, proximal-most spinule projecting beyond margin.

Antennules (Fig. 2(b)). Reaching the tip of the rostrum, setae sometimes exceeding it, about 2–2.5 times longer than wide, four rows of setules on antennular body, distal-most row shorter than the others. Antennular sensory seta slender and long, about 1.5 times smaller than antennular body, inserted at two thirds of antennule

length, counting from the base. Nine terminal aesthetascs of different sizes. None of these aesthetascs exceeded the length of the antennules.

Antenna (Figs. 2(c, d) and 4(e)). Coxal setae not studied. Basipodite thick, with many spinules and one long spine. First exopodite segment with long thick spinules near the base (3–4) and terminal portion. Second exopodite segment with long thick spinules on the terminal portion. Endopodite segments with short spinules. Antennal formula: spines 001/101, setae 113/003. Seta on first exopodite segment thick and plumose (Fig. 4(c, d)), not reaching mid length of terminal setae. Seta on second exopodite bisegmented, long, with many setules. Spine on first endopodite segment without denticles, reaching but not exceeding distal end of second segment. Apical spines of endopodite and exopodite of different sizes, with denticles (Fig. 4(e)). Two apical setae

of endopodite and exopodite with spinules, which are larger on the first segment.

Abdomen. About two times shorter than thorax. Two or three abdominal setae (Fig. 1(a)).

Postabdomen (Fig. 2(e–g)). Moderate size, relatively wide, about 2–2.4 times as long as wide, ventral margin slightly rounded, with at least three rows of spinules. Pre-anal margin longer than anal margin and shorter than postanal margin. Anal margin concave, clearly inserted in a depression and with two distinct angles (Fig. 4(f–g)), with many spinules forming groups (Fig. 4(g)). Postanal margin rounded, not projected. Lateral fascicles arranged in five to seven groups, first spines of each group thick and exceeding margin of postabdomen. Six to seven clusters of marginal denticles, distal ones with two to three marginal denticles (apparently fused when observed in optical microscopy but superimposed in layers when observed in SEM (Fig. 4(f)); proximal denticles may be separated; length of the marginal denticles on the postanal margin of the postabdomen about two times longer than the width of the base of the denticles. **Postabdominal seta** (Fig. 2(e)). Approximately half as long as postabdomen, proximal portion naked. Short setules armed bilaterally, inserted toward distal portion. **Terminal claw** (Fig. 2(e–g)). Implanted at projected basis from the postabdomen, longer than anal margin, uniformly curved, with one row of spinules on the base (Fig. 4(i)); pecten armed with rows of internal and external spinules; inner row composed of robust spinules; outer row with spinules increasing in size toward distal region (Fig. 4(h)). Basal spine long, about two times as long as width of claw at its base, without spinules or setules.

Five pairs of limbs

Limb I (Fig. 3(a, b)). Epipodite not studied. Outer distal lobe (ODL) with thin seta, serrulate in distal part, about same size as the longest IDL seta. Accessory seta relatively small, setulated, and implanted near base of ODL. IDL with three groups of spinules on its face, three setae present; seta 3 smaller than the others, naked. Setae 2–3 bisegmented, long, and with strong proximal spines and narrow distal portion with short denticles. Third endite with four setae, one of them thinner than the others (1); inner seta densely setulated, longer than the other two (a, b). Second endite with one row of spinules; three setae of different sizes, seta 4 (e) about 2.7 times longer than seta 3 (f) and about 4.3 times longer than seta 5 (d); setae 3–4 (d–f) with thick spinules on the lateral face, seta 4 (e) with spinules not exceeding median portion; seta 5 (d) about half length of seta 3 (f). First endite with two marginal setae (g, h), bisegmented, similar in size and slightly setulated. No specialized elements on endites. Ejector hooks similar in size. Ventral face of limb with seven rows of setules organized in clusters,

decreasing toward distal portion. Gnathobase thick, corresponding to a densely setulated seta.

Limb II (Fig. 3(c, d)). Exopodite elongated, with tiny proximal spinules, and distally setulated. Seta on exopodite present, setulated, about two to three times smaller than the exopodite itself. Endite armed with eight scrapers gradually decreasing in size toward the gnathobase, sixth and eighth scrapers smaller than seventh. Scrapers 1, 2, and 4 armed with fine denticulation, other scrapers with robust denticulation. Proximal portion of gnathobase wide, apex armed with fine spinules followed by long setules (in the populations from Minas Gerais state, these appear shorter, thinner, and more numerous), distal portion armed with sensillum and three elements, first element geniculated and setulated. Filter comb with seven setae, first seta short and densely setulated; other setae long, with setules and implanted from median portion.

Limb III (Fig. 3(e, f)). Epipodite round, without evident projections (in the populations from Minas Gerais state, long projections were observed). Exopodite subquadrangular, with seven marginal setae arranged in 2 + 5. First seta longer than second; third seta about 1.8 times longer than sixth seta; fourth seta geniculated; fifth and seventh setae less than half length of sixth seta; all setae are plumose, except the seventh. Distal endite with three setae, two scraper-like of different sizes, third seta geniculated and armed with many setules implanted bilaterally; four plumose setae, increasing in size towards the gnathobase. Basal endite with four soft setae, increasing in size towards the base. Gnathobase armed with four elements, first a cylindrical sensillum, second a strong geniculated seta, setulated on the base, third and fourth elements with acute tip and without setules. Filter comb with seven long setae.

Limb IV (Fig. 3(g, h)). Pre-epipodite round, densely setulated, epipodite oval with short projections. Exopodite round with six marginal plumose setae; setae 1 and 2 subequal in size, third seta longer than all others, fourth seta thick exceeding the half length of fifth seta; sixth seta half as long as fifth seta. Distal endite with four setae, one scraper-like and three flaming-torch-like, decreasing in size toward the base. Basal endite with three soft setae, increasing in size toward the base. Gnathobase armed with one globular sensillum and a setulated seta implanted on robust base. Filter plate with five slender setae.

Limb V (Fig. 3(i)). Pre-epipodite round and densely setulated, epipodite with short projections. Exopodite rectangular, not divided in lobes, about twice as long as wide, slight depression in the middle portion; four plumose setae, decreasing in size toward internal lobe; setae 1 and 2 subequal in size, seta 3 markedly smaller than setae 1 and 2; seta 4 about half length of seta 3. Internal lobe wide, oval and with long setules; two setulated setae

of similar size on the inner face of the lobe, no elements between these setae. Gnathobase reduced, without elements or setae.

Ephippial female: Unknown.

Description of male

Habitus (Fig. 1(k)). Smaller than female, length 0.27–0.30 mm, about 1.6 times as long as high. Body elongated, weak lateral compression, body less arched than in female. *Head* with short rostrum, ocellus smaller than eye. Main pores close to each other, nearly connected, lateral pores tiny and arranged in different positions than in females (between distal and median pores; Fig. 1(l)). *Carapace* without punctuated ornamentations or longitudinal lines (Fig. 4(k)), ventral margin armed with 40 setae followed by fine spinules, the most proximal exceeding the line of posterior margin.

Antennules (Fig. 2(h)). Slightly exceeding the tip of the rostrum, about two times as long as wide, with three rows of short setules on antennular body; seven (juvenile) to nine (adult) aesthetascs of different sizes (none of these aesthetascs exceeds the length of antennules). Two lateral aesthetascs of different sizes, about half as long as antennular body; sensory seta long, about 1.4 times shorter than antennular body. Male seta short, thick, about 4.6 times shorter than length of antennular body, inserted at three thirds of antennular length, counting from base.

Antenna as described for females.

Postabdomen (Fig. 2(i)). Smaller than in female, narrowing distally; anal margin longer than postanal margin, angles not defined; marginal clusters of spinules present (Fig. 4(k)); at least five postanal fascicles, distal-most fascicle exceeding the margin line; anal margin with spinules differentiated in three groups; gonopores opening ventrally, subapically to terminal claw. *Terminal claw* smaller and thicker than in female, tip not acute (Fig. 4(l)). Slender spinules implanted at the base of terminal claw (as in the female). Basal spine about one-third length of terminal claw.

Limb I (Fig. 3(j)). Smaller than in female, copulatory hook U-shaped. Copulatory brush present, copulatory brush seta slender; IDL with three setae, smaller than corresponding setae of female, seta 1 long, thick, subequal in size to the other two setae, setae 2 and 3 bisegmented and with strong proximal spines (as in female).

Differential diagnosis

Alona kaingang sp. nov. is a member of the *pulchella*-group because its posteroventral corner of the carapace is armed with many short and thin spinules not arranged in groups, three main head pores, marginal denticles of postabdomen merged and developed, lateral fascicles developed, IDL with three setae, seven setae on the exopodite of limb III, fourth setae of limb III well-developed,

gnathobase of limb V reduced, and limb VI absent. *Alona kaingang* sp. nov. also has the fourth seta on the exopodite of limb III geniculated, like many species of the *pulchella*-group (except in *Alona archeri*). As an alternative to identification, the *pulchella*-group may be separated into two groups based on the morphology of the main head pores: one group contains species with interrupted connection (including those species with main head pore isolated) and the other contains species with complete connection. Thus, *A. kaingang* sp. nov., having a complete connection between the main head pores, could be differentiated from species belonging to do the first group: *Alona capensis* (Rüher, 1914), *Alona setulosa* (Megard, 1967), *Alona cambouei* (Guerne & Richard, 1893), *Alona aguascalientensis* Sinev & Silva-Briano, 2013, *Alona nigra* Smirnov, 1996, *Alona setuloides* Smirnov & Timms, 1983, *Alona azorica* Frenzel & Alonso 1989, *Alona anastasia* Sinev Alonso, Miracle & Suhaquillo, 2012, and *Alona nuragica* Margaritora, 1971. Regarding species with complete connection between main head pores, *A. kaingang* sp. nov. is distinguished by the presence of spinules on apical setae of antenna and proximal spines on IDL setae. Besides, *A. kaingang* sp. nov. has single morphological traits such as the slight depression in the middle portion of the exopodite of the limb V.

Ecology and distribution

Alona kaingang sp. nov. has populations occurring in water bodies of the subtropical region of South America. Until now, *A. kaingang* sp. nov. has been found only in Minas Gerais state (São Simão and Furnas Reservoirs) and Rio Grande do Sul state (São Simão pond), Brazil. However, we expect that its geographical distribution is broader in the subtropical zone, because *A. kaingang* sp. nov. might be confused with *Coronatella rectangula* (Sars, 1861). This latter species likely does not occur in the Neotropics (Van Damme and Dumont 2008b), but there are many records in different Brazilian hydrographic regions (see Green 1972; Smirnov and Santos-Silva 1995; Eskinazi-Sant'Anna et al. 2005; Sterza and Fernandes 2006; Rocha et al. 2011), including the Furnas Reservoir (Santos et al. 1994; Viti et al. 2013). The review of *Coronatella* in Brazil confirmed that *C. rectangula* does not occur in Brazil, and probably not in the Neotropics (Sousa et al. 2015).

São Simão pond is located on the coastal plain of Rio Grande do Sul state and is part of the coastal wetland complex between the Atlantic Ocean and Patos Lagoon. São Simão pond does not connect with the ocean. The abiotic parameters of the pond water were as follows: pH 6.8–7.1, electrical conductivity 97.3–110 $\mu\text{S}\cdot\text{cm}^{-1}$, total phosphorus 0.104–0.139 $\text{mg}\cdot\text{L}^{-1}$, ammoniacal nitrogen 0.051–0.11 $\text{mg}\cdot\text{L}^{-1}$, dissolved oxygen 7.2–9.1 $\text{mg}\cdot\text{L}^{-1}$,

and temperature 17–17.5 °C (data available from State Foundation of Environmental Protection Henrique Luiz Roessler—FEPAM, referring to the year when the type material was collected).

Furnas Reservoir, located in Minas Gerais state, is the largest reservoir in southeastern Brazil, with a total area of approximately 1440 km². Recent data indicate that water temperature ranges between 24 and 28 °C, pH is approximately 7, electrical conductivity 19–22 μS.cm⁻¹, total nitrogen 210–318 μg.L⁻¹, total phosphorus 13–29 μg.L⁻¹, and dissolved oxygen 8–9 mg.L⁻¹ (Santos et al. 2010).

São Simão Reservoir is located on the boundary between Goiás and Minas Gerais states. Currently, it is being impacted by the uncontrolled increase in urban areas and farmland in its surroundings. This has led to eutrophication of the reservoir (Fonseca 2010).

The biology of *A. kaingang* sp. nov. is not well known. It inhabits the littoral zones of water bodies, probably among aquatic vegetation. Based on the present records, it should be found in different kinds of ecosystems: reservoirs, wetlands, and coastal lagoons. We believe that this species may occur in habitats with varying degrees of salinity. *Alona kaingang* sp. nov. is presumed to be an active scraper of substrates, based on the strong scrapers found in the limbs I and II (Fryer 1968; Van Damme et al. 2011).

Identification key for species from the pulchella-group with occurrence in the Neotropics (adult parthenogenetic females)

1. Basal spines very short, less 0.1 of the length claw, aesthetascs length about 2.6 times shorter than antennular body.....*A. bromelicola*-Central America (Sinev 2002b)
 - Basal spine longer than 0.1 of the length of the claw, aesthetascs length about 1.6–2 times shorter than antennular body..... 2
2. Labral keel with two rows of short setules.....3
 - Labral keel naked..... 4
3. Postabdomen narrowing distally, basal segment of the apical setae of antenna without spines, one seta markedly longer than the others on the anterior group on the ventral margin of the carapace,.....*Alona anamariae*—Endemic from the Central Plateau of Mexico (Sinev and Silva-Briano 2012)
 - Postabdomen wide and truncated, basal segment of the apical setae of antenna with spines, setae of the

anterior group on the ventral margin of the carapace not differentiated in length.....*A. setulosa*—From North Canada to North Mexico (Sinev, 2009; Sinev and Silva-Briano 2012)

4. Abdomen armed with two to three rows of setules.....5
 - Abdomen armed with five to six rows of long setules.....*A. nigra*—Bolivia and North Chile on the Andes (Kotov et al. 2010)
5. Main head pores connected.....6
 - Main head pores disconnected..... *A. aguascalientensis*—Endemic of the Central Plateau of Mexico (Sinev and Silva-Briano 2012)
6. Antenna, spine on the first segment of the endopodite shorter than second segment.....7
 - Antenna, spine on the first segment of the endopodite of the same length of the second segment.....*A. kaingang* sp. nov.—Southern South America
7. Carapace ornamented with longitudinal lines, length of the postabdomen about 2.5 height, postanal margin armed with 5–6 denticles.....*Alona altiplana*—Endemic from Andes (Kotov et al. 2010)
 - Carapace without longitudinal lines, length of the postabdomen about 2.8–3 height, postanal margin armed with seven to eight denticles.....*Alona glabra*—Common in the Neotropics (Sinev 2001b; Sinev and Silva-Briano 2012)

Discussion

The presence of main head pores with complete connection may be taken as the main morphological trait uniting *A. kaingang* sp. nov. with valid species *Alona pulchella* (Herrick, 1884), *A. archeri* (Sars, 1888), *A. altiplana* Kotov, Sinev & Berrios, 2010, *A. glabra* (Sars, 1901), *A. bromelicola* (Smirnov, 1988), *A. karelica* Stenroos, 1897, and *A. anamariae* Sinev & Silva-Briano, 2012. Among these species, the postabdomen of *A. kaingang* sp. nov. is quite similar to other species that present short postabdomen: the Chilean *A. altiplana* and the Mexican *A. anamariae*. All other species aforementioned have an elongated postabdomen (Sinev and Silva-Briano 2012).

Alona kaingang sp. nov. (length 0.33–0.40 mm) is smaller than *A. altiplana* (0.35–0.50 mm). Besides, *A. kaingang* sp. nov. differs from *A. altiplana* because its carapace does not have longitudinal lines (Fig. 4(b)). In

A. kaingang sp. nov., the length of the marginal denticles on the postanal margin of the postabdomen is about two times longer than the width of the base of the denticles (Fig. 2(e–f)) while in *A. altiplana*, this proportion is about 2.5–3 times longer (Kotov et al. 2010). The following morphological traits of the limbs are observed in *A. kaingang* sp. nov. but not in *A. altiplana*: (1) seta e on the endite 2 of the limb I about two times longer than seta f, (2) setae 2–3 of the IDL markedly different in length, (3) setae 2–3 of the IDL armed with proximal spines, (4) scrapers 5–8 armed with strong denticles, (5) absence of specialized structure on the endite of the limb III, (6) seta 5 on the exopodite of the limb IV longer than setae 5–6, and (7) seta 3 on the exopodite of the limb V shorter than setae 1–2.

Regarding the size, *A. kaingang* sp. nov. (length 0.33–0.40 mm) is longer than *A. anamariae* (0.28–0.30 mm) (Sinev and Silva-Briano 2012). In *A. anamariae*, the labral keel has two rows of setules, while in *A. kaingang* sp. nov., the labral keel is naked (Fig. 1(g–i)). In *A. kaingang* sp. nov., PP is less than 0.2 IP while in *A. anamariae*, PP is about 0.2–0.3 IP. Another important morphological trait observed in *A. anamariae* and that is absent in *A. kaingang* sp. nov. is the longest setae on the anterior group of margin of the carapace (Sinev and Silva-Briano 2012). The postabdomen of *A. anamariae* does not have postanal angle as evident as in *A. kaingang* sp. nov. (Fig. 2(e–f)). On the limbs, *A. kaingang* sp. nov. differs from *A. anamariae* because it has (1) setae 2–3 of the IDL markedly of different length, (2) setae 2–3 of the IDL armed with proximal spines, (3) scrapers 5–8 armed with strong denticles, (4) absence of a specialized structure on the endite of the limb III, (5) first flaming torch relatively robust, and (6) seta 5 on the exopodite of the limb III shorter than seta 7.

Recently, Sinev et al. (2012) described the Mediterranean *A. anastasia*, which has a distinct short postabdomen; however, this species may be easily separated from *A. kaingang* sp. nov. by the interrupted connection of the main head pores and absence of seta on the exopodite of the limb II. *Alona setulosa* is another species of the *pulchella*-group with a short postabdomen (Sinev 2009), and the shape of this structure is quite similar to that observed in *A. kaingang* sp. nov. Besides that, *A. setulosa* and *A. kaingang* sp. nov. share similar armature of the apical setae of the antenna and denticulation of scrapers 6–8. The armature of the apical setae of the antenna observed in *A. setulosa* and *A. kaingang* sp. nov. was also found in phylogenetically distant groups such as *Armatalona* (Sinev 2004a) and *Acanthalona* (Sinev and Kobayashi, 2012), which suggests that this morphological similarity may be the result of convergence. *Alona setulosa* differs from *A. kaingang* sp. nov. because it has a labral keel with two rows of setules and incomplete connection between

main head pores. They differ also in fine features of the limbs (see Sinev 2009).

The differences between the other species that possess main head pores with complete connection and *A. kaingang* sp. nov. go beyond the shape of the postabdomen. The South American *A. glabra* has the third seta on the exopodite of the limb V longer than the second seta, and the first flaming torch on the limb IV is relatively thinner (Sinev 2001b). Besides that, the geographical distribution of *A. glabra* extends from Argentina to the extreme North of the Neotropics, while *A. kaingang* sp. nov. is restricted to the southern regions. *Alona archeri* has a PP less than 0.5 IP, well-developed distal denticles on the postabdomen transformed in setules toward the anal margin and seta 4 on exopodite of the limb III relatively straight and markedly long (Sinev 2002b). The paleotropical *A. pulchella* has thin setules on the scrapers of the limb II and relatively long second setae on the exopodite of the limb III (Sinev 2001c). The specialized *A. bromelicola* has a markedly short seta on the exopodite of the limb II, setae 6–4 on the exopodite of the limb IV of similar length, seta 3 is of similar length to setae 1–2 and seta 5 is markedly long, and the basal spine is very short (Sinev 2002a). Some other species that are presumed to be members of the *pulchella*-group still await revision (Van Damme et al. 2010), and their affinities and differences with *A. kaingang* sp. nov. cannot be addressed yet.

Although there are several morphological differences between species in the *pulchella*-group and those related to species groups, Van Damme et al. (2013) indicated the possibility of identification mistakes. For example, some species of the *pulchella*-group has a postabdomen of a similar shape to the one observed in the *Alona gutatta*-group (e.g., *A. archeri*, *A. glabra*, and *A. bromelicola*). However, the *Alona gutatta*-group is a member of the *Hexalona*-branch, and the observation of the limbs is necessary (or at least the use of high magnification) for correct identification. Under low magnification, the postabdomen of *A. kaingang* sp. nov. resembles *C. rectangula* (= *Alona rectangula*), and many records of this species in Brazil (see Green 1972; Santos et al. 1994; Smirnov and Santos-Silva 1995; Eskinazi-Sant'Anna et al. 2005; Sterza and Fernandes 2006; Rocha et al. 2011; Viti et al. 2013) perhaps may be attributed to new species of the *pulchella*-group described here; this, however, needs to be checked.

From the evolutionary point of view, the *pulchella*-group presents an interesting duality because its species do not have many specializations on the limbs (their morphology is almost constant) but differ in habitus and, mainly, in the morphology of the postabdomen. An extreme case was observed in *A. capensis*, which is similar in its limb morphology to other species of the group, but has an elongated body, a bulge on the rostrum, and

a very peculiar postabdomen (Van Damme et al. 2013). Besides, the variability of shapes of the postabdomen in other species of *pulchella*-group (elongated, short and narrowing, short and massive, short and rounded; marginal denticles thick or thin; lateral fascicles short or long) suggests speciation mediated by evolutionary pressure on the movement, once postabdomen is the main morphological trait involved in such purpose, being also related to food collecting and shelter searching. The phylogenetic interpretation of the diversity of shapes of postabdomen in the *pulchella*-group still remains unclear, but elongated and short forms in different species may be arisen from adaptive convergence associated to similar environmental pressure.

As in other species group (e.g., *Anthalona*, *Nicsmirnovius*, *Euryalona*, *Monospilus*), the morphology of head pores may be important phylogenetically for the *pulchella*-group because of the absence of reductions or specializations in the limbs, that make difficult to do suggestions of affinities between species. However, in species with both morphologies of main head pores, a considerable level of affinities related to general shape of the postabdomen and fine structure on the limbs are observed: *A. cambouei*, a species with broken connection, has armatures of postabdomen and seta 5 of the limb III similar to species with complete connection, such as, *A. pulchella* and *A. glabra* (Sinev 2001b, c). When compared with the broken connection species *A. nigra*, *A. aguascalientensis*, and *A. setulosa*, *A. cambouei* presents differences in the armature of antenna, postabdomen, and proportions of setae on limbs I and III (Sinev 2001c, 2009; Sinev and Silva-Briano 2012). Therefore, morphology of main head pores could also be the result of convergence and to test relationship in *pulchella*-group may be useful to elucidate these issues.

The morphological boundaries of the *pulchella*-group indicate that it does not belong to the *Alona sensu stricto* (Van Damme and Dumont 2008a). It is probable that in the future, a new genus will be created and that will include *A. kaingang* sp. nov. (Van Damme et al. 2010). Moreover, the *pulchella*-group has many affinities with *Ovalona* Van Damme & Dumont, 2008, a group recently removed from *Alona*. These two groups present affinities in habitus and limbs (mainly with *Ovalona weincki*), and the only feature that is truly different is the presence of the merged (*pulchella*-group) and not-merged (*Ovalona*) denticles on the postanal margin of the postabdomen (Van Damme and Dumont 2008b). The use of molecular tools can be valuable to define the relationship between the *pulchella*-group and *Ovalona*.

Conclusions

The *pulchella*-group is now composed of at least 15 valid species. The Neotropical fauna of the *pulchella*-

group is extremely diverse and contains 50 % of the known species. *Alona kaingang* sp. nov. has connection between main head pores complete; therefore, it is separated from *A. setulosa*, *A. cambouei*, *A. aguascalientensis*, *A. nigra*, *A. setuloides*, *A. azorica*, *A. nuragica*, *A. capensis*, and *A. anastasia*. *Alona kaingang* sp. nov. resembles *A. altiplana* but differs from it mainly in the morphology of the marginal denticles of the postabdomen and fine features on the limbs. The relationship between *pulchella*-group and *Ovalona* needs to be better studied.

Abbreviations

As: accessory seta; CBS: copulatory brush seta; EL: Elmoor-Loureiro collection, at Universidade Católica de Brasília, Brazil; en: endite; ep: epipodite; ex: exopodite; fc: filtercomb; gfp: gnathobasic filter plate; gn: gnathobase; IDL: inner distal lobe; il: inner lobe; IP: interpore distance (distance between anterior and posterior major head pores); MNJR: National Museum of Rio de Janeiro, Brazil; MZUSP: Museum of Zoology of the University of São Paulo, Brazil; ODL: outer distal lobe; pep: pre-epipodite; PP: postpore distance (distance between posterior major head pore and posterior border of head shield); s: sensillum; SEM: scanning electron microscopy.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FDRS, LMAEL, and SS wrote the manuscript. FDRS and LMAEL produced the drawings, photographs on the SEM, and taxonomic discussion. SS discussed the taxonomic parts, made the intellectual contributions, and reviewed the manuscript. All authors read and approved the final manuscript.

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