

Research article

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A new species of *Acantholeberis* (Crustacea, Branchiopoda) suggests an ancient geographic distribution of the genus in South America

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Abstract. *Acantholeberis smirnovi* Paggi & Herrera-Martinez, 2020 (Cladocera: Acantholeberidae) was recently described from Andean habitats in South America. The presence of a population on the eastern coast of the continent brought the hypothesis of a new Neotropical species different from *A. smirnovi*. This hypothesis was confirmed from morphological, ecological and biogeographic evidence.

Acantholeberis accolismaris Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. differs from *A. smirnovi* in the morphology of the head, the valves and the limbs (especially the second and third limbs). Species of *Acantholeberis* are adapted to live in acid water bodies, with *A. accolismaris* sp. nov. presenting the same ecological requirements. However, the new species is adapted to live in temporary ponds near the Atlantic Ocean in Brazil in altitudes ranging between 4 and 15 meters above sea level. Considering that Acantholeberidae is an ancient taxon with a pre-Mesozoic origin, geological and environmental changes may have been relevant to speciation and to the observed biogeographic pattern of species of *Acantholeberis* in Neotropics.

Keywords. Altitude, biogeography, limbs, morphology, taxonomy.

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Introduction

The phylogenetic status of *Acantholeberis* Lilljeborg, 1853 was improved after Dumont & Silva-Briano (1998) created Radopoda – a clade formed by anomopods that bear many specializations on the limbs to scrape substrates. They observed several morphological traits on the limbs that confirm a separated evolution of *Acantholeberis* Lilljeborg, 1853 from the core-group of Macrothricidae Norman & Brady, 1867. In terms of diversity, Acantholeberidae Smirnov, 1976 sensu Dumont & Silva-Briano (1998) was considered monotypic for a long time, even populations outside their natural biogeographic zone (in the Neotropical zone) belong to *Acantholeberis curvirostris* (O.F. Muller, 1776) (Smirnov *et al.* 1995).

Curiously, no regional checklist of Cladocera from the Neotropics reports *Acantholeberis* (Elmoor-Loureiro 1997, 2000; Zoppi de Roa & López 2008; Escalante & Kotov 2015; Kotov & Fuentes-Reines 2015). Quite recently, populations of the genus in the Andean lakes of Colombia were discovered to differ from *A. curvirostris* as known in literature (Smirnov 1992; Dumont & Silva-Briano 1998; Hudec 2010). Then, Paggi & Herrera-Martinez (2020) described a new species from the Neotropics – *Acantholeberis smirnovi* Paggi & Herrera-Martinez, 2020 – based on the morphology of the valves and the proportion of some setae in the first, second and third limbs. It is possible that the population from Venezuela mentioned by Smirnov *et al.* (1995) belongs to *A. smirnovi*.

Observing the geographic distribution of *Acantholeberis*, Paggi & Herrera-Martinez (2020) indicated that the current distributional pattern observed for *A. smirnovi* might be the result of transport by birds, which may have started as early as in the Cretaceous, and that environmental changes drove the extinction and speciation processes locally. However, a new hypothesis can be raised when reports of *Acantholeberis* in other parts of the Neotropical region are studied. Here, our objective is to add new data to the biogeography of the genus with reports from eastern Brazil. At the same time, we describe a new species of *Acantholeberis* from the Neotropics.

Material and methods

The material studied here was collected in temporary ponds near the Atlantic Ocean in Brazil in altitudes ranging between 4 and 15 meters above sea level (Fig. 1). An environmental characterization of the areas where *Acantholeberis accolismairis* sp. nov. was collected can be found in Freiry *et al.* (2020, 2021) and Araújo *et al.* (2013). Specimens were selected and placed in drops of glycerin on slides with the use of a binocular stereo microscope. Afterwards, specimens were dissected using two sharp tungsten needles. The morphology of the limbs and other structures was studied with a phase contrast microscope Olympus BX41. The description style follows Van Damme (2016), separating the anatomic structures

into blocks (general habitus, carapace, cephalic, thoracic limbs, abdominal and postabdominal). The enumeration of limb setae follows the criteria of Kotov (2000a, 2000b) and Kotov *et al.* (2010). All drawings were prepared in a camera lucida coupled to an Olympus BX41 phase contrast microscope and digitally treated using the software Adobe Photoshop CS5. Photographs were taken using a USB DI-5.0 HD coupled to a Zeiss phase contrast microscope and processed with the software Micro Capture 6.9.12.

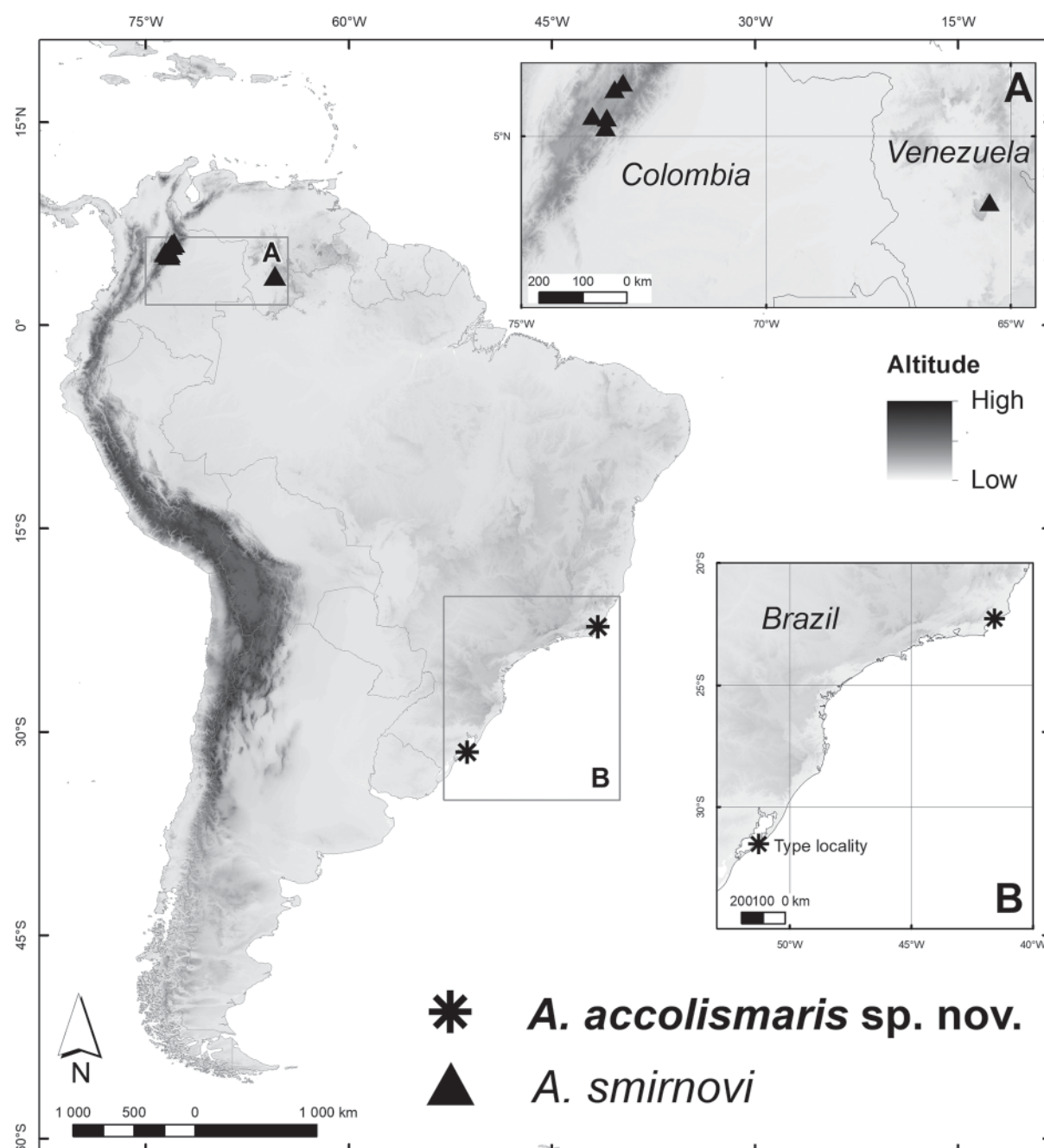


Fig. 1. Geographic distribution of species of *Acantholeberis* Lilljeborg, 1853 in South America. Black triangles represent previous records of *A. smirnovi* Paggi & Herrera-Martinez, 2020 (Paggi & Herrera-Martinez 2020). Asterisks show records of *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov.

Abbreviations of the scientific collections

EL = personal collection of Lourdes Maria A. Elmoor-Loureiro
FDRS = personal collection of Francisco Diogo Rocha Sousa
MZUSP = Museum of Zoology of the University of São Paulo

Abbreviations used in the figures and the text

as = accessory seta
as' = apical endopodite seta
as'' = apical endopodite seta
ds = distal spine of antennal base
dss = distal sensory seta of antennal base
ejh = ejector hooks
en = endite
ep = epipodite
ex = exopodite
gfp = ganthobasic filter comb
gn = gnathobase
IDL = inner distal lobe
il = inner lobe
ls1 = first lateral seta
ls2 = second lateral seta
ODL = outer distal lobe
pep = pre-epipodite
s = sensillum

Results

Taxonomy

Class Branchiopoda Latreille, 1817
Order Anomopoda Sars, 1865
Family Acantholeberidae Smirnov, 1976 sensu Dumont & Silva-Briano 1998
Genus *Acantholeberis* Lilljeborg, 1853

Acantholeberis accolismaris Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov.
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Figs 2–7

Differential diagnosis

Acantholeberis accolismaris Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. differs from the Andean *A. smirnovi* Paggi & Herrera-Martinez, 2020 in the relatively straight ventral margin of the head, with a truncated protuberance near the labrum. The differences in the limbs between the two species pertain to the proportion of several setae; however, the main differences might be found when observing the exopodite setae and the scrapers of the inner portion of the second limb (Fig. 3E, G), with scraper 6 markedly longer than scrapers 5 and 7. Seta 3 on the exopodite of the third limb in *A. accolismaris* sp. nov. is about 0.8 times as long as seta 4, whereas in *A. smirnovi* it is about 0.6 as long. The lateral and ventral surface of the postabdominal claw of *A. accolismaris* sp. nov. is completely covered by short and thin spines while these spines in *A. smirnovi* are relatively long and organized in three ventral groups. We observed that the lengths of the branches of the antenna in *A. accolismaris* sp. nov. are markedly different (the endopodite is about 0.8 times as long as the exopodite) while in *A. smirnovi* they are of similar length; however, this difference might represent a variable morphological trait. The

morphological description of *A. curvirostris* in the literature is very ambiguous; however, scrapers 5 and 6 on the second limb are of similar length. Furthermore, the length of seta 6 of the exopodite on the third limb is shorter than half the length of seta 7 (Dumont & Silva-Briano 1998; Hudec 2010). These proportions are different in *A. accolismaris* sp. nov.

Etymology

The specific name refers to geographic distribution of the new species and comes from Latin words ‘*accola*’ (‘who lives nearby’) and ‘*marae*’ (‘sea’).

Material examined

Holotype

BRAZIL • adult parthenogenetic ♀, undissected in a tube with 92% ethanol; Rio Grande do Sul, Tavares, Planície Costeira; 31°30'40" S, 51°16'02" W; wetland; MZUSP42444.

Paratypes

BRAZIL • 18 adult parthenogenetic ♀♀; same collection data as for holotype; Oct. 2016; Raquel Fontoura Freiry leg.; FDRS0696, EL03499 • 50 ♀♀, mostly juveniles; Rio de Janeiro, Macaé, Restinga de Jurubatiba; 22°14'53.1" S, 41°35'1.2" W; Feb. 2010; pond; Paloma Marinho Lopes leg.; EL02092, FDRS0697.

Type locality

A wetland in the Planície Costeira, Tavares, Rio Grande do Sul, Brazil (31°30'40" S, 51°16'02" W).

Description (adult parthenogenetic females)

GENERAL HABITUS (Figs 2A–E, 5A–B). Body elongated, sub-rectangular in lateral view, oblong in ventral and dorsal views, length 0.7–1.5 mm, height/length ratio about 0.45–0.75; posterodorsal and posteroventral angles prominent. Dorsal margin regularly arched and interrupted by a shallow cervical sinus. Ventral margin clearly arched in anterior part, posterior part slightly curved inwards giving a straight aspect in lateral view. Posterior margin concave near posterodorsal angle. In dorsal and ventral views body not laterally compressed, without lateral projection.

CARAPACE (Figs 2A–G, 6A–H). Brownish; dorsal and ventral margins smooth; ventral margin with thick rim (more evident towards anterior part of carapace). Setae on ventral margin organized in three groups: first one set with 8–10 outwardly directed setae and 40–50 plumose and ventrally directed setae (Figs 2A, D, F–G, 6G–H); second one set of short naked setae (Fig. 6C); third one armed with long setae bearing protuberances armed with spinulae at the basis (Fig. 6E). Setae on posterior margin organized in two groups: first one has up to eight long setae with slightly curved proximal part and basis armed with spinulae (Fig. 6D), second one armed with setae decreasing in length towards posterodorsal angle and bases armed with spinulae (Fig. 6F).

CEPHALIC STRUCTURES (Figs 2, 5). **Head** (Figs 2A–E, 5A–B). Elongated, triangular, about 0.4 times as long as body. Dorsal margin smooth and slightly arched, a shallow cervical sinus present. Ventral margin with two inflated parts, the first one slight and positioned near base of antennule, the second one relatively robust, sometimes folded or truncated and positioned above labrum; marginal line between inflated parts straight. Ocellus markedly smaller than compound eye. Dorsal organ shorter than compound eye, rounded, with fine ring, positioned near cervical sinus (Fig. 2I). Rostrum not developed. **Labrum** (Fig. 2A, H) with a long horn armed with five rows of minute setulae. **Antennule** (Figs 2J–K, 5C–H). Slightly shorter than head length and dilated in distal part. Ventrolateral basal sensory seta about 0.2 times length of antennular body. Antennular body armed with up to eleven transverse rows of spinules, distal part armed with several teeth surrounding insertion of aesthetascs; inner face with

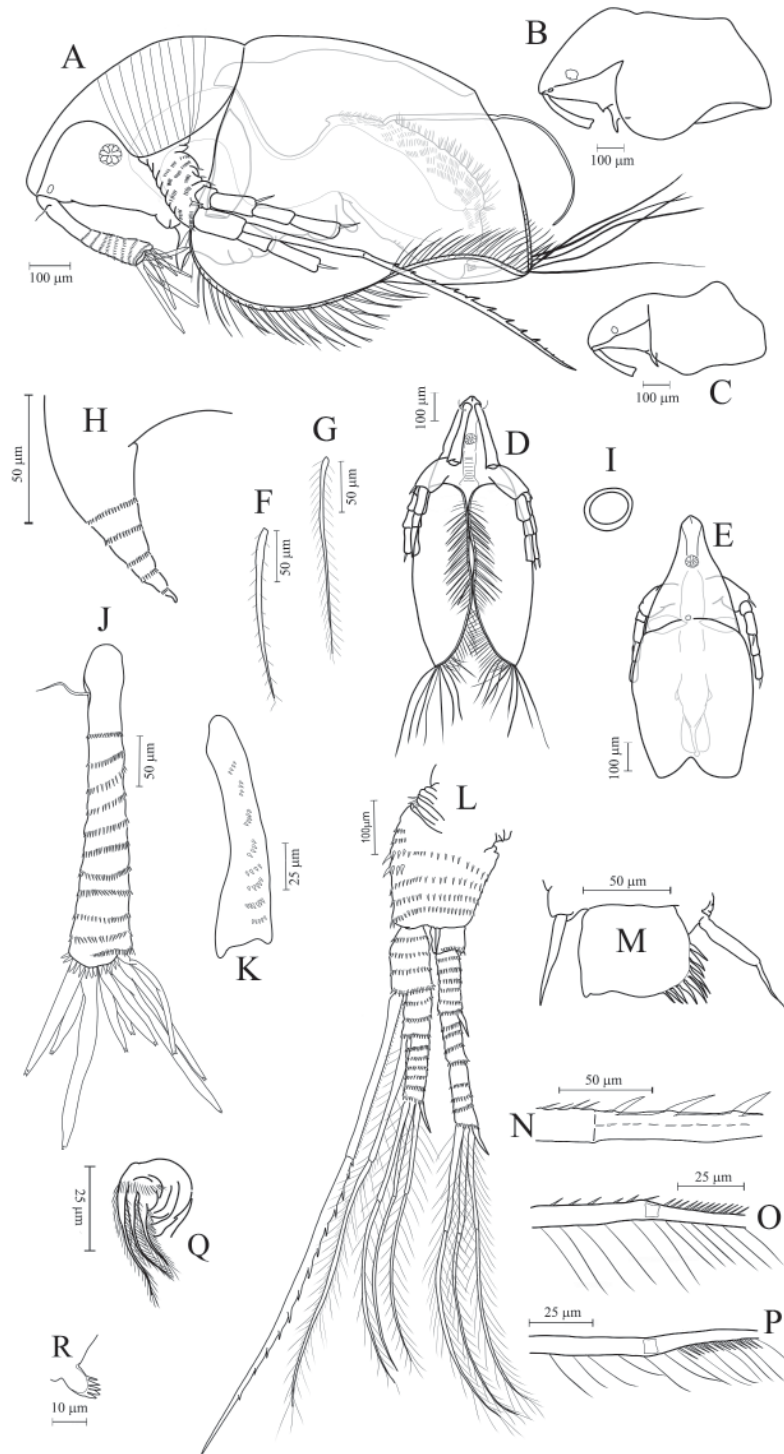


Fig. 2. *Acantholeberis accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov., parthenogenetic female from the Planície Costeira, Rio Grande do Sul (FDRS0696). **A.** Habitus, holotype. **B–C.** Lateral view, shape variation of carapace. **D.** Ventral view. **E.** Dorsal view. **F.** Outwardly directed setae. **G.** Plumose and ventrally directed setae. **H.** Horn-like outgrowth anterior to labrum. **I.** Dorsal organ. **J.** Antennule. **K.** Idem, inner surface. **L.** Antenna. **M.** Idem, first segment of exopodite, distal outer spine and inner sensory seta on basal segment. **N.** Idem, lateral seta of first segment of endopodite, armature detail. **O–P.** Morphology of apical setae of endopodite and exopodite. **Q.** Maxilla. **R.** Idem, short crown-like seta.

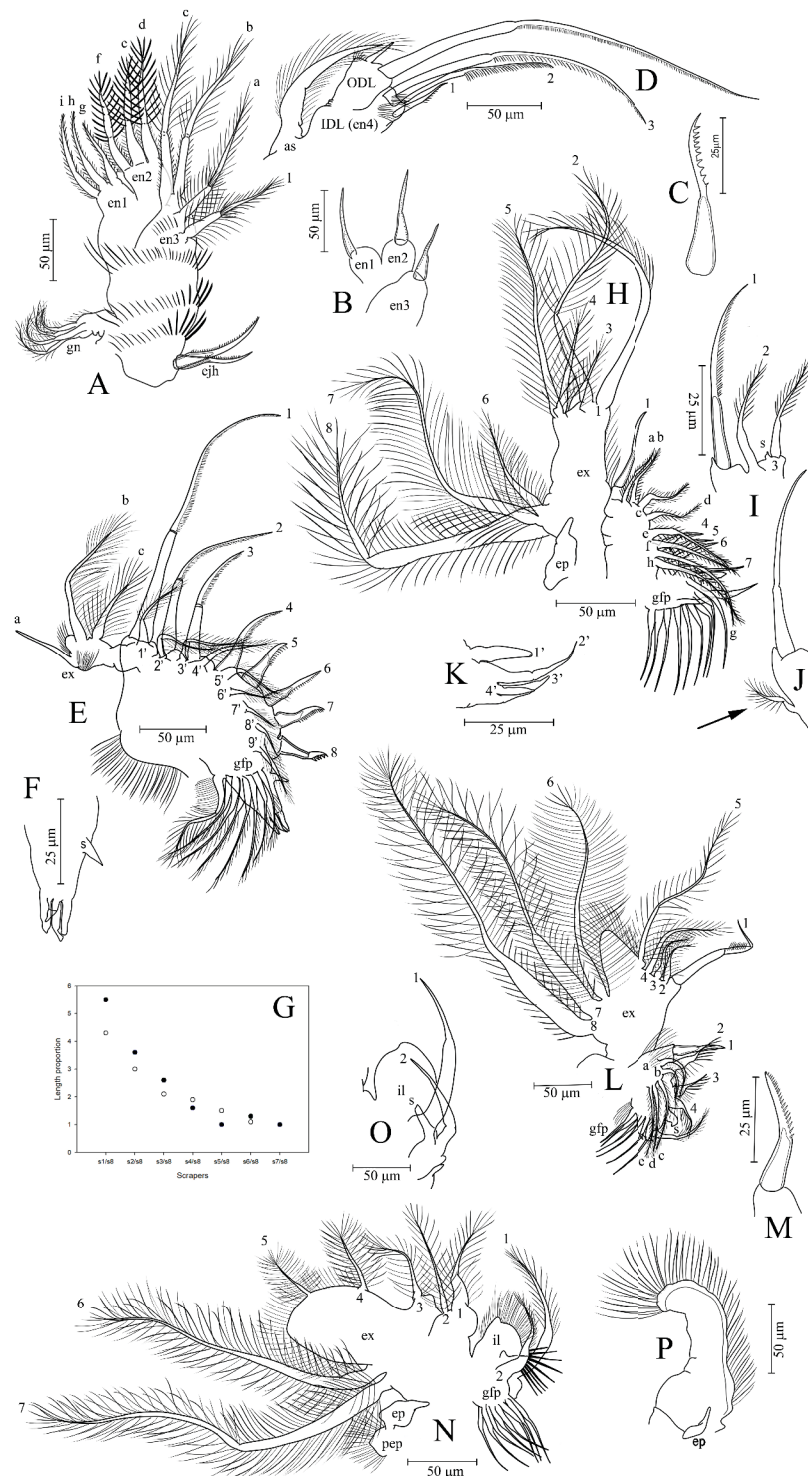


Fig. 3. *Acantholeberis accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov., parthenogenetic female from the Planície Costeira, Rio Grande do Sul (FDRS0696). **A.** First limb. **B.** Idem, endites and stiff setae. **C.** Idem, morphology of stiff setae of endites. **D.** Inner distal lobe and outer distal lobe. **E.** Second limb. **F.** Idem, gnathobase. **G.** Idem, comparative system of proportion of scrapers, white circles represent *A. smirnovi* Paggi & Herrera-Martinez, 2020 and black circles represent *A. accolismaris* sp. nov. **H.** Third limb. **I–J.** Idem, distal endite, arrow indicating the posterior seta. **K.** Idem, ganathobase. **L.** Fourth limb. **M.** Idem, seta 1 of distal endite. **N.** Fifth limb. **O.** Idem, inner lobe. **P.** Sixth limb.

teeth organized in up to eight groups (Figs 2K, 5C–F). Nine aesthetascs with forked apex, four of them markedly longer than others. *Antenna* (Figs 2L–P, 5I–O). Coxal region folded; basal segment robust, covered with transverse rows of fine spines and spinules, apical spine of similar length to spine of second segment of exopodite, sensory setae about two times as long as first segment of exopodite (Figs 2M, 5J–K). Branches markedly different in length, with cylindrical segments covered with rows of spinules; endopodite about 0.8 times as long as exopodite. Exopodite with four segments: first segment shorter than rest, armed with robust spines laterally (Figs 2M, 5J); second segment elongated, spine about middle length of segment itself; third segment without apical setae; fourth segment elongated with spine and three apical bisegmented setae armed with lateral spines and long setulae, spine about 0.5 times as long as segment itself. Endopodite with three segments: first segment armed with exceptionally long sclerotized and bisegmented seta which bears, on proximal part, thin lateral spines, distal part armed with 10–13 robust spines reducing in size distally (Figs 2N, 5N); second segment with long (but shorter than seta on first segment) and bisegmented seta which bears, on proximal part, short setulae, distal part with short and fine spines and setulae (Fig. 5O); third segment with spine and three apical bisegmented setae (Figs 2O–P, 5L–M), spine about 0.5 times as long as segment itself. Antennal formula: spines 0101/001, setae 0003/113. *Maxilla* (Figs 2Q–R, 5P–Q). Well developed, with three setulated setae and one short stout protuberance resembling crown-like seta.

THORACIC LIMBS (Figs 3, 7). Six pairs of thoracic limbs. *Limb I* (Figs 3A–D, 7A–C). Accessory seta plumose, ODL armed with two apical setae; first seta short and naked, about 0.2 times as long as second seta; second seta bisegmented, armed with short spinule on distal part, markedly long, about 2.6 times as long as accessory seta. IDL (en4) with two rows of short setulae on posterior surface and three apical setae; first seta about two times as short as second seta; second seta bisegmented and armed with short setulae on distal part about, 1.8 times as short as third seta; third seta bisegmented, markedly long and armed with short setulae on distal part. Endite 3 armed with a anterior seta (1), three posterior setae (a–c) which bears short setulae and a stiff seta, setae (a–b) similar in length and slightly longer than seta (c), stiff setae denticulated, shorter than setae (a–c). Endite 2 armed with three posterior setae (d–f) and a denticulated stiff seta, setae (d–f) bisegmented with proximal part armed with short spinulae, distal part plumose. Endite 1 with three posterior setae (g–i) and a denticulated stiff seta; setae (g–i)

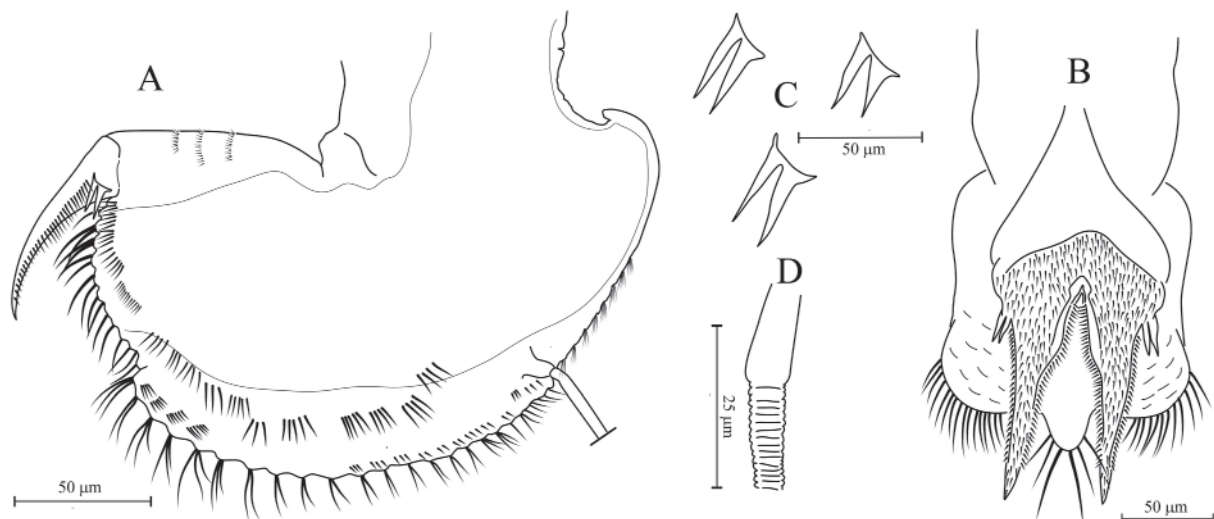


Fig. 4. *Acantholeberis accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov., parthenogenetic female from the Planície Costeira, Rio Grande do Sul. **A.** Lateral view of postabdomen. **B.** Apical view of postabdomen. **C.** Basal spines variation. **D.** Detail of postabdominal setae showing difference between proximal and distal segment.

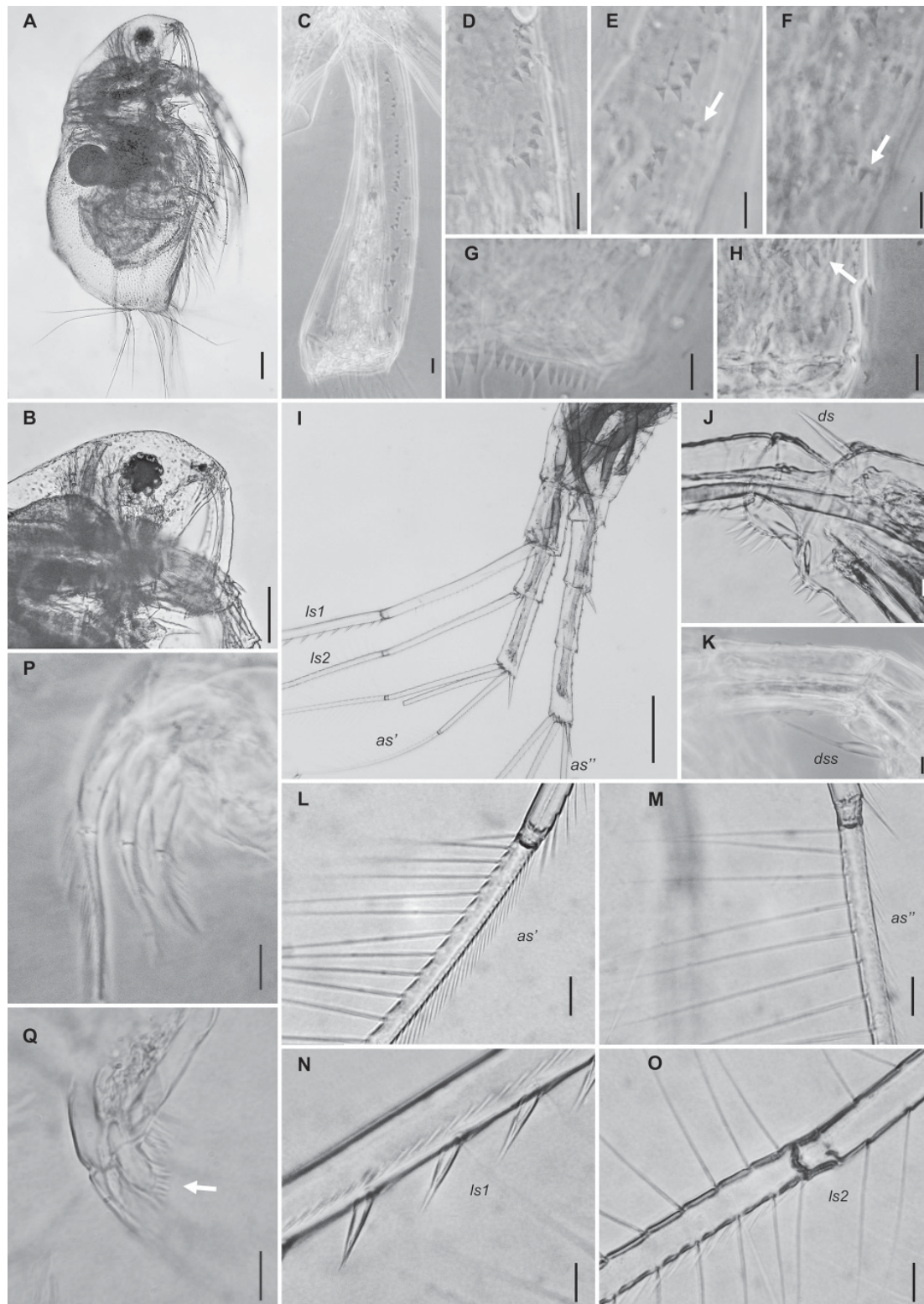


Fig. 5. *Acantholeberis accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov., parthenogenetic female from Jurubatiba National Park. **A.** Habitus. **B.** Head. **C.** Antennule. **D–H.** Antennule ornamentation (arrows show teeth organized in groups). **I.** Antenna. **J–K.** Antenna in lateral view, showing its base and first exopodite segment. **L–O.** Antennal setae. **P–Q.** Maxilla (arrow shows the short crown-like seta). Scale bars: A–B, I = 100 μ m; C–H, J–Q = 10 μ m.

bisegmented and armed with short spinulae. Two ejector hooks of different lengths armed with short spinulae on distal part. Gnathobase with four setulated setae. *Limb II* (Figs 3E–G, 7D–G). Exopodite elongated, armed with three setae (a–c); setae (a) and (c) of similar length; seta (b) about 1.8 times as long as others. Inner lobe with eight bisegmented scrapers and nine setulated soft setae; scrapers 1–4 armed with short and fine spinulae on distal part, scrapers 5 and 6 armed with thin denticles, scraper 6 armed with short spines, scraper 8 armed with up to five thick denticles; scraper 6 markedly longer than scrapers 5, 7 and 8 which are similar in length. Gnathobase with naked proximal portion, distal

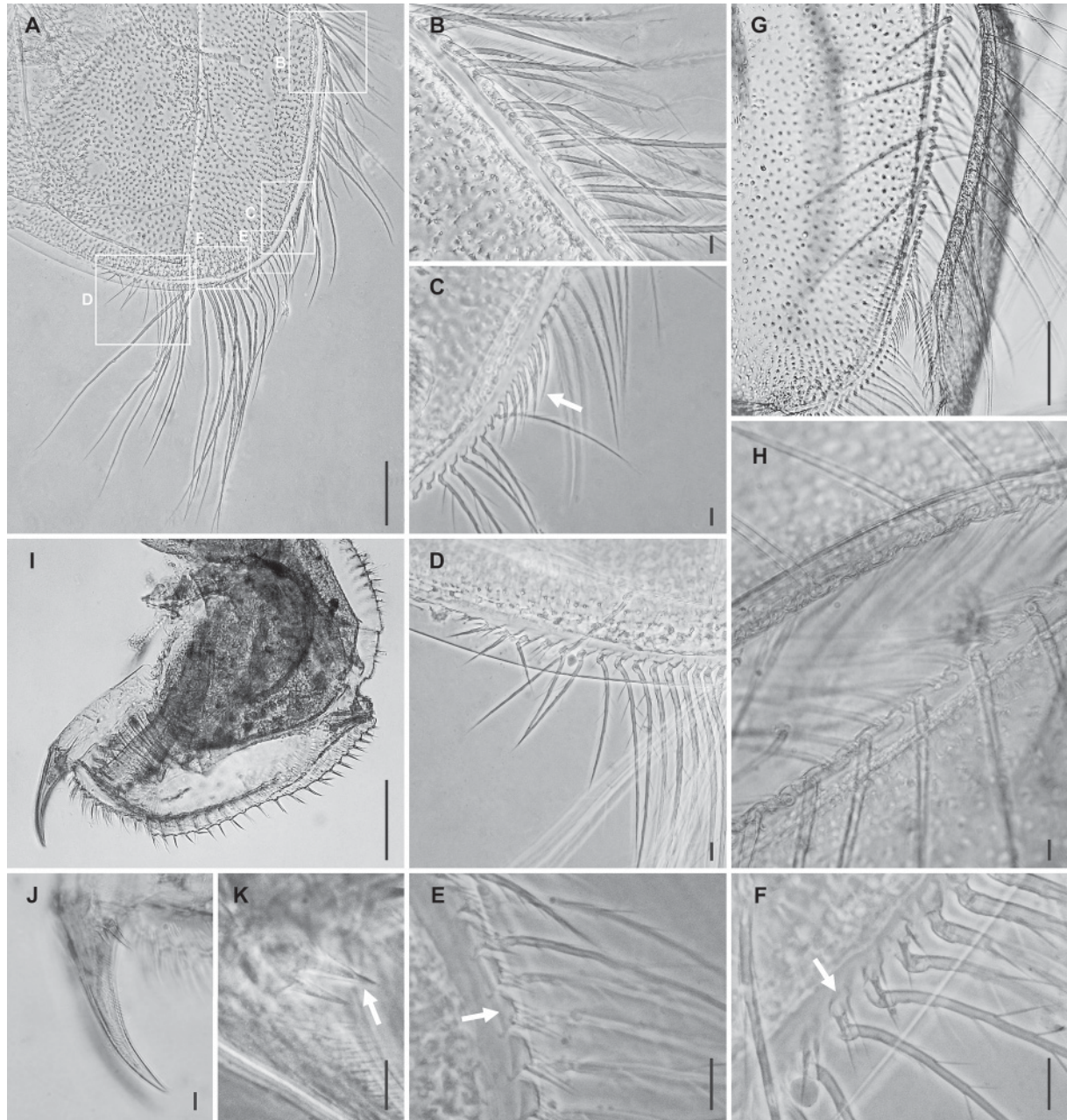


Fig. 6. *Acantholeberis accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov., parthenogenetic female from Jurubatiba National Park. **A.** Valve. **B–F.** Close view of selected regions of valve margin, as defined in subfigure A (arrows indicate some fine details). **G–H.** Carapace ventral view. **I.** Postabdomen. **J.** Postabdominal claw. **K.** Basal spines of the claw (arrow). Scale bars: A, G, I = 100 μ m; B–F, H, J–K = 10 μ m.

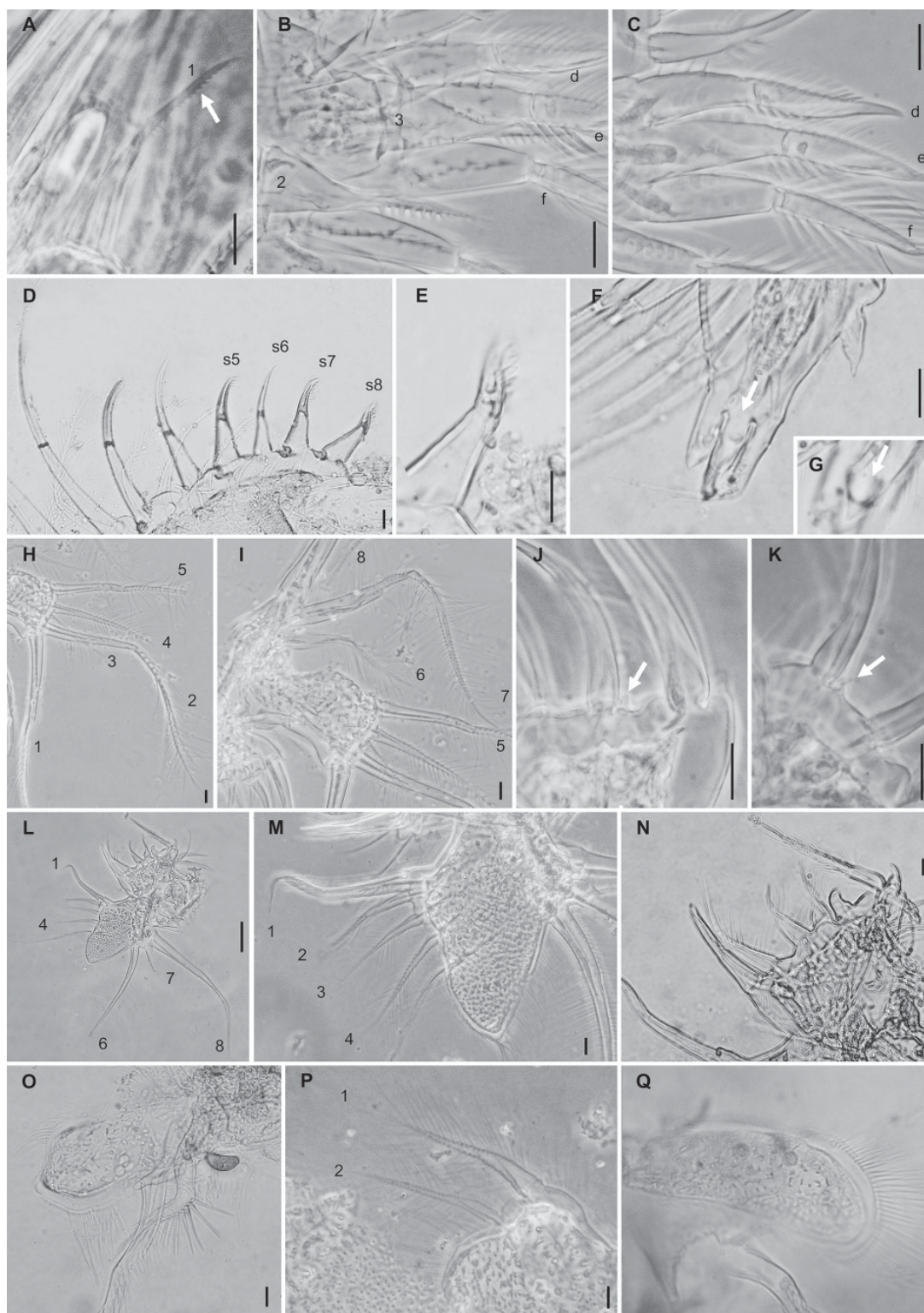


Fig. 7. *Acantholeberis accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov., parthenogenetic female from Jurubatiba National Park. **A–C.** First limb. **A.** Stiff seta on first endite. **B.** Stiff setae on second and third endites. **C.** Setae d–f on endite 2. **D–G.** Second limb. **D.** Partial view of the endite. **E.** Scraper 8. **F–G.** Gnathobase, arrow shows the position of fourth element. **H–K.** Third limb. **H–I.** Exopodite. **J–K.** Distal endite, arrows indicate the sensilla. **L–N.** Fourth limb. **L.** General view. **M.** Exopodite. **N.** Endite and gnathobase. **O–P.** Fifth limb. **Q.** Sixth limb. Scale bars: 10 µm.

portion armed with five elements; filter comb with eight setulated setae. *Limb III* (Figs 3H–K, 7H–K). Epipodite with short finger-like projection. Exopodite rectangular about 2.4 times as high as wide, with five distal (1–5) and three plumose lateral setae (6–8); seta 1 slightly shorter than seta 8; seta 3 about 0.8 times as long as seta 4; seta 4 about 0.7 times as long as exopodite; seta 6 about 0.4 times as long as seta 7; seta 8 about 0.7 times as long as exopodite. Distal endite armed with one posterior and three anterior setae (1–3) and two elements (Fig. 7H–J); setae 1 with spinulae on distal part, about 1.8 times as long as setae 2–3 which are setulated on distal part and have similar length. Basal endite with four setae (4–7) increasing in length distally, seta 7 markedly longer than seta 6. Eight long and setulated posterior setae increasing in length towards gnathobase (a–h). Gnathobase armed with four elements (1'–4'), bottle-shaped sensillum on proximal part (1'), distal part with element of sharp apex (2') and two naked elements (3'–4'). Filter comb with nine setae. *Limb IV* (Figs 3L–M, 7L–N). Exopodite subquadrangular with portion between distal and lateral setae densely setulated; five distal (1–5) and three lateral setae (6–8); seta 1 geniculated and unilaterally armed with short spinulae, about two times as long as seta 2; seta 5 about 0.6 times as long as seta 1 and about 1.8 times as long as seta 4; lateral setae plumose, seta 8 longer than setae 7 and 6. Distal endite with four setae (1–4), seta 1 scraper-like and with thin spine at distal portion, flaming-torch-like setae (3–4) armed with long setulae, seta 3 is shorter than setae 2 and 4. Basal endite armed with five setulated setae increasing in length towards gnathobase (a–e). Gnathobase thick, with three elements and curved setulated seta; filter plate with eight setae. *Limb V* (Figs 3N–O, 7O–P). Pre-epipodite rounded and densely setulated, epipodite oval with short projection. Exopodite wide and lobed, armed with five (1–5) distal and two (6–7) lateral plumose setae; setae 1–2 of similar length and longer than seta 3; seta 4 slightly longer than seta 5; setae 6 and 7 of similar length. Inner lobe wide and densely setulated, armed with two long setae (1–2) and element; seta 2 armed with up to 9 stiff setae followed by setulae, about 0.6 times as long as seta 1; setae 2 setulated. Gnathobasic filter plate with eight long setae. *Limb VI* (Figs 3P, 7Q). Epipodite with short projection. Limb as elongated lobe, unilaterally setulated, with curved apex and wide base.

POSTABDOMINAL STRUCTURES. *Abdomen* (Figs 4A, 6I). Elongated regularly arched, armed with up to ten rows of setulae of similar length, about 0.6 times as long as postabdomen. *Postabdomen* (Figs 4A–B, 6I). In frontal view clearly trilobed and massive. In lateral view wide, height/length ratio about 0.7. Ventral margin slightly straight and armed with up to five rows of short setulae. Dorsal margin separated in anal and preanal parts; preanal margin armed with up to twenty-four groups organized in 2–3 long setulae; anal margin about 0.3 of preanal margin length, armed with 10–12 groups of setulae. Postabdominal setae longer than postabdomen length, bisegmented, proximal part of distal segment folded and densely setulated (Fig. 4D). *Postabdominal claws* (Figs 4A–B, 6I). About 0.4 times as long as postabdomen, with lateral and ventral surfaces completely covered with short and thin spines, pecten armed with thin spines. *Basal spines* (Figs 4C, 6K). Cluster with two robust spines of variable length inserted laterally on postabdominal claws.

Ephippial females and males

Not studied.

Distribution and biology

So far *Acantholeberis accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. is distributed in shallow water bodies on the Brazilian coast (Fig. 1). It was found in temporary ponds in Rio de Janeiro and Rio Grande do Sul States. This is an acidophilic species living in water bodies with a pH between 4.2 and 5.1, at altitudes between 4 and 15 m a.s.l., preferentially associated with macrophytes.

Discussion

Now, the diversity in *Acantholeberis* is better understood with the description of *A. smirnovi* (Paggi & Herrera-Martinez 2020) and *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. Nevertheless, a detailed redescription of *A. curvirostris* is still necessary because the literature data are sometimes controversial when we observe specially the limbs (Fryer 1974; Dumont & Silva-Briano 1998; Hudec 2010). At least one hypothesis emerges from these observations: *A. curvirostris* might represent several sibling species in the Holarctic zone as is known for species groups in *Daphnia* O.F. Müller, 1785, *Chydorus* Leach, 1816, *Moina* Baird, 1850, and *Alonella* Sars, 1862 (Bekker *et al.* 2016, 2018; Kotov *et al.* 2016; Neretina *et al.* 2021) without assigning new species. Such findings agree with Frey's concept of non-cosmopolitanism in Cladocera, including Macrothricidae-like species (Frey 1987, 1988). Taking into account the literature data, the most consistent difference observed between *A. curvirostris* and *A. accolismaris* sp. nov. is related to the proportion of the setae on the second and third limbs (see differential diagnosis).

Looking for specific morphological traits of the Neotropical species of *Acantholeberis*, we can confirm there are several differences from the other taxa. The posteriormost part of the valves of *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. seems to be slightly folded inward (Figs 2A, D, 5A), the marginal contour of the head bears a folded or truncated protuberance positioned above the labrum (Figs 2A, 5B), teeth on the inner face of the antennules are organized in up to eight groups (Figs 2K, 5C–F) and the branches of the antennae are clearly different in length (Figs 2L, 5I).

The main differences between *A. smirnovi* and *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. can be observed when comparing the limbs. Here, we consider the first limb as the most conservative in both species; however, there are some differences in the IDL where seta 2 is markedly shorter than seta 3 (Fig. 3A). The second limb bears differences in the proportion of the setae of the exopodite and scrapers, as indicated by the graphic system of proportions (Fig. 3E, G); the most conspicuous differences are related to the length of scraper 6 when compared to scrapers 5 and 7, but, when modulating the length of scrapers 1–7 in function of the eighth scraper many minute differences may be observed (Fig. 3G).

On the third limb, there is a consistent difference related to setae 3–4 of the exopodite: in *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. their proportion is about 0.8 while in *A. smirnovi* it is about 1.5. The third limb also bears differences in length and morphology of setae 6–7 of the distal part of the endite (Fig. 3H). Seta 5 on the exopodite of the fourth limb in *A. accolismaris* sp. nov. is about two times as long as seta 4 while in *A. smirnovi* this proportion is about 1.3. On the same limb, the length of flaming-torch seta (4) in *A. accolismaris* sp. nov. is similar to that of the flaming-torch seta (2), a feature not observed in *A. smirnovi* (Paggi & Herrera-Martinez 2020). Furthermore, the seta 2 on the inner lobe of the fifth limb in *A. accolismaris* sp. nov. is armed with up to 9 stiff setulae (Fig. 3N); the same seta in *A. smirnovi* bears more than twelve stiff setulae (Paggi & Herrera-Martinez 2020).

Ecological data point out that species of *Acantholeberis* are adapted to live in acid water bodies (Fryer 1974; Paggi & Herrera-Martinez 2020), the same was observed for *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. which was found in habitats with a pH ranging between 4.2 and 5.1. Perhaps this mode of life could be regarded as ancestral for the acantholeberids. However, the geographic distribution of the species within this genus is not similar: *A. curvirostris* occurs in Holarctic water bodies at latitudes 23°–63° N with different conditions related to altitude (Fryer 1974; Smirnov 1976; Flössner 2000), a pattern very similar to that of *Ophryoxus* Sars, 1862, a former Macrothricidae (Fryer 1974). *Acantholeberis smirnovi* was collected in environments located at latitudes 3°–5° N at an altitude range from 2700 to 4030 m a.s.l. (Fig. 1).

In the case of *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov., the geographic distribution is restricted to temporary water bodies located up to 15 m a.s.l. along the Brazilian coast at latitudes 22°–41° S (Fig. 1). The narrow distribution of species seems to be well documented as several areas in Brazil were sampled and no specimens of *Acantholeberis* were mentioned (Sousa & Elmoor-Loureiro 2008, 2012; Lansac-Tôha *et al.* 2009; Santos-Winiewski *et al.* 2011; Rocha *et al.* 2011; Brito *et al.* 2020), even those studies performed on the Brazilian coast at latitudes lower than 22° S (Sousa *et al.* 2009; Van Damme & Dumont 2010; Soares & Elmoor-Loureiro 2011; Diniz *et al.* 2020). Thus, *A. accolismaris* sp. nov. or any sibling species is lacking on most part of the Brazilian territory.

Reconstructing the biogeographic puzzle involving cladoceran species requires several tools; however, the morphology of the species groups and the geological history of the planet might present some clues. For instance, Paggi & Herrera-Martinez (2020) raised the hypothesis that the presence of *Acantholeberis* in South America is relatively recent (Miocene, together with the uplift of the Andes) and was mediated through dispersion by migratory birds acting as vectors of propagules from North America to areas where *A. smirnovi* occurs currently. In our opinion, the findings of *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. contribute as a new element to this puzzle. As Cladocera represents an ancient group of Crustacea (Van Damme & Kotov 2016; Van Damme *et al.* 2021), it is possible that as yet unrecognized populations of Acantholeberidae have been widely distributed on the South American territory since the Mesozoic.

Before the uplift of the Andean region, that has occurred approximated ca 65 million years ago, South America was separated in two portions, the North Gondwana Province and the South Gondwana Province due to a marine transgression. Such a transgression had more impact on the biota of the meridional South America. In this viewpoint, many populations of Acantholeberidae could already have been isolated and submitted to several environmental changes. The Miocene brought the uplift of the Andes, a possible vicariant event to Acantholeberidae population. At the same time, South America suffered from a subsequent marine transgression event, changes in temperature and glaciations in the eastern portions of the continent that drove the process of extinction, speciation and conditioned the geographic distribution of several extant biological groups (Posadas & Ortiz-Jaureguizar 2010; Ribeiro *et al.* 2010). Although more studies are necessary, these events might be the drivers of isolation of *A. accolismaris* Sousa, Elmoor-Loureiro & Álvarez-Silva sp. nov. and *A. smirnovi* in South America.

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