Zootaxa 5424 (3): 308–322
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5424.3.2
http://zoobank.org/urn:lsid:zoobank.org:pub:57A6932E-3AB3-4881-A534-13FA47620E1B

One more step in solving the *Chydorus* puzzle—a morphological comparison between *Chydorus tilhoi* Rey & Saint-Jeans, 1969 and *Chydorus sphaericus* (O.F. Müller, 1776) (Crustacea: Cladocera)

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Abstract

A number of species of *Chydorus* Leach, 1816 (Crustacea: Cladocera) need improvements in their taxonomy much more than any other genus within the family Chydoridae Dybowsky & Grochowski, 1894 *emend*. Frey, 1967, which makes the systematics of the genus still a puzzle that lacks several pieces. Here, we redescribe the African species *Chydorus tilhoi* Rey & Saint-Jeans, 1969 and compare its morphology with that of *Chydorus sphaericus* (O.F. Müller, 1776). The two taxa might be easily differentiated because *C. tilhoi* has a single and relatively large major head pore with a wide rim, labral keel elongated with a large spine, and postabdomen with postanal part elongated, narrowing distally and with denticles near its anal margin, organized in groups. These morphological traits are absent in *C. sphaericus*. *Chydorus tilhoi* and *C. sphaericus* also differ in the morphology of the first (Inner Distal Lobe setae), third (exopodite proportion), and fifth (exopodite shape) limbs. Based on the literature and our observations, the limb morphology of *C. tilhoi* has important similarities with that of *C. breviceps*, *C. nitidulus* and *C. dentifer*, and their translocation to a new genus seems to be a fundamental piece in the puzzle of *Chydorus*.

Key words: Africa, Congo River Basin, morphometry, redescription, taxonomy

Introduction

Intrigued by the wide distribution of *Chydorus sphaericus* (O.F. Müller, 1776) (Crustacea: Cladocera) around the world, David G. Frey (1980) wrote that "Besides death and taxes, one the accepted certainties of the modern world has been that nearly all small sub-globular individuals of *Chydorus* belong to the species *sphaericus*". Keeping in mind his initial ideas about the non-cosmopolitanism in Cladocera, he (Frey, 1980, 1985) published a series of papers

 Accepted by M. Alonso: 16 Feb. 2024; published: 14 Mar. 2024

on challenges related to *Chydorus* Leach, 1816. First, the revision of *Chydorus sphaericus* led to the description of *Chydorus brevilabris* Frey, 1980 and *Chydorus biovatus* Frey, 1985. Besides that, Frey (1982a, 1982b, 1987) also studied *Chydorus* species with honeycombed ornamentation on the carapace and translocated the species related to *Chydorus barroisi* Richard, 1894 to the new genus *Ephemeroporus* Frey, 1982 (see Frey 1982c).

In the last two decades, the systematic and taxonomy of *Chydorus* have received less attention compared to other cladoceran groups, especially those within Aloninae (e.g. Van Damme *et al.* 2003; Van Damme & Dumont 2008, Van Damme *et al.* 2011, Sousa *et al.* 2016; Sinev & Dumont 2016; Sinev 2015, 2020; Sinev *et al.* 2023). As a result, improvements are needed in our knowledge on the taxonomy, morphology, geographical distribution, and ecological features of a proportionally larger number of species in *Chydorus* than in any other genus of Chydoridae Dybowsky & Grochowski, 1894 *emend.* Frey, 1967. The only exception is *Chydorus sphaericus* (Balyaeva & Taylor 2009; Kotov *et al.* 2016; Klimovsky & Kotov, 2015; Karabanov *et al.* 2022), which was revised recently.

Thus, the taxonomy and systematics of *Chydorus* are still a puzzle lacking several pieces. Using only taxonomic features, an overview based on recent literature (Smirnov & Sheveleva 2010; Kotov *et al.* 2013, Sinev 2014; Klimovsky & Kotov, 2015; Sinev *et al.* 2022) suggests that more than 50% of the 68 names available at the species level should be considered as *species inquerenda* or lacking improvements in their taxonomic resolution. Furthermore, there are several taxa whose primary types have not been designated, making the resolution of taxonomic problems more difficult. Besides that, the inventory of megadiverse continents, initially the Neotropical and Afrotropical zones, is far from satisfactory, as the number of species reported there is lower than in the Oriental and Palearctic zones.

During the 21st century, only five *Chydorus* species have been described or redescribed (Smirnov & Sheveleva 2010; Sinev 2014; Klimovsky & Kotov, 2015; Sinev *et al.* 2022). Besides that, populations of *Chydorus* cf. *biovatus* from the Central Yakutia in Russia, were completely studied (Klimovsky & Kotov, 2015). Even species with shapes that are very different from the *sphaericus*-like sub-globular body, such as *Chydorus nitidulus* (Sars, 1901), *Chydorus dentifer* Daday, 1905, and *Chydorus tilhoi* Rey & Saint-Jeans, 1969 have not been completely studied. Both species bear denticles on the posteroventral corner of the carapace and an elongated postabdomen, as seen in *C. breviceps* (Stingelin, 1905).

The South American species, *Chydorus nitidulus* and *C. dentifer*, were described using only features of habitus and postabdomen. Later, Paggi (1972) indicated the absence of major head pores in adult females of *C. nitidulus,* and Smirnov (1996) illustrated for the first time the Inner Distal Lobe on the first limb of *C. dentifer*. The revision of Brazilian *Chydorus* species is in progress, and it will fill the gaps about limb morphology in *C. nitidulus* and *C. dentifer*. Regarding Afrotropical *C. tilhoi*, described from material in Lake Chad, only habitus, labral keel and postabdomen were used to support the description of new species (Rey & Saint-Jeans, 1969; Smirnov, 1971). Until now, the morphology of limbs remains unstudied. Here, we investigate the morphology of *C. tilhoi* in comparison to *C. sphaericus* and discuss the taxonomic status of the former and related species.

Material and Methods

Morphological analyses. The specimens used for this study were examined under a binocular stereomicroscope; they were placed in drops of glycerin on slides and studied under an Olympus BX41 phase contrast microscope. We dissected several individuals to investigate the appendages. Our interpretation of the morphological structures follows the suggestions of Van Damme (2016). To enumerate the limb setae, we adopted the homology criteria of Kotov (2000a; 2000b), which exhibited stability when tested in different groups of the cladocerans (Kotov *et al.* 2010). All drawings were made using a *camera lucida* and digitally covered using a graphic tablet.

SEM processing. The samples were first fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer with pH=7.3 for 4 hours. Then the samples were washed three times for 5 minutes each in distilled water, immersed in 0.5% osmium tetroxide in distilled water for 30 to 40 minutes, then were washed three times in distilled water for 10 minutes each time, dehydrated in an increasing series of alcohol concentrations, starting at 7.5% and gradually increasing to a maximum concentration of 100%. Finally, the samples were taken to the critical drying point and then placed in stubs and sputtered with gold, making them conductive and ready for electron microscopy analysis.

Abbreviations of the scientific collections. FDRS: Personal collection of Francisco Diogo Rocha Sousa.

Abbreviations used in the figures and the text. en = endite; ep = epipodite; ex = exopodite; fc = filter comb; gfp = gnathobasic filter plate; gn = gnathobase; IDL= inner distal lobe; il = inner lobe; ODL = outer distal lobe; pep = pre-epdipodite; s = sensillum.

Results

Taxonomy

Class Branchiopoda Latreille, 1817

Order Anomopoda Sars, 1865

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

Subfamily Chydorinae Dybowsky & Grochowski, 1894 *emend.* Frey, 1967

Genus *Chydorus* Leach, 1816

Chydorus sphaericus (O.F. Müller, 1776)

(Figs. 1–2)

Lynceus sphaericus O.F. Müller, 1785: p. 71–72, figs. 7–9; *Chydorus arcticus* Røen, 1987: p. 125–130, figs. 1–5; *Chydorus caelatus* Schoedler, 1862: p. 15, fig 44; *Chydorus lynceus* Langhans, 1911 in Smirnov (1971): p. 345; *Chydorus mutilus* Kreis, 1921: p. 272, figs. 42–45; *Monoculus infusorius* Schrank, 1781: p. 536.

Material Examined. Fifteen parthenogenetic females from Bjørndalsvatn Lake Sveindal-Hornnes, Norway (58°30'32.9"N, 7°33'27.1"E), material collected by Lourdes M. A. Elmoor-Loureiro on 11.viii.2018 (FDRS0698).

Description. Parthenogenetic female. *General***.** (Fig. 1A–C). In lateral view body sub-globular or rounded; dorsal margin arched, without a keel or projections; without lateral compression, lateral projections absence.

Head. Ocellus smaller than the eye (Fig. 1A–B). Rostrum short, with a sharp tip, about 1.2 times longer than the antennular body. Head shield not completely studied. Two major head pores of similar diameter, and two very small, closely-set pores between them, these tiny pores located approximately at half the distance between the major head pores (Fig. 1F).

Labrum (Fig. 1G–H). Keel not prominent, without notch, naked, distal portion elongated and triangular, with short lateral projections in frontal view.

Carapace (Fig. 1A–E). Covered by slight tubercles or hexagons; anteroventral margin with an evident flange; anterior part of ventral margin with 6–8 slender setae which are not plumose; posterior part of ventral margin naked; each valve bear 34–35 plumose setae inserted at a distance from valve ventral margin, posterior and anterior setae shorter than middle setae. Posteroventral margin without spines or denticles. Posterior margin with an acute or obtuse projection formed by the junction of dorsal and ventral margins.

Abdomen (Fig. 1A). About 3.6 times shorter than the thorax, three transverse rows of setulae at its dorsal surface.

Postabdomen (Fig. 1M). About 3.5 times longer than wide, ventral margin relatively straight; preanal margin about 1.4 times longer than the anal margin, angle clearly prominent; anal margin somewhat concave, with similar length to postanal margin, armed with several spinulae; postanal margin with distalmost portion slightly narrow, armed with 7–8 denticles which have the base about 2.5–4.5 times shorter than the length itself; lateral fascicles formed by thin and short spinulae not organized in groups. *Postabdominal setae* about 1.8 of postabdomen length, bisegmented, provided with setulae in the distal segment. *Postabdominal claw*. Bearing two basal spines and a subterminal flagellum, about 0.28 of postabdomen length; pecten organized in two groups, proximal group with short spines, distal group with long spines. *Basal spines.* Naked, proximal spine about 1.8 times shorter than the distal; distal spine about 0.15 of postabdominal claw length.

Antenna I (Fig. 1I). Approximately two times longer than wide, never reaching tip of the rostrum; antennular sensory seta about 3–3.2 times shorter than antennular body, inserted near middle of antennular body; nine aesthetascs do not extend middle of antennular body.

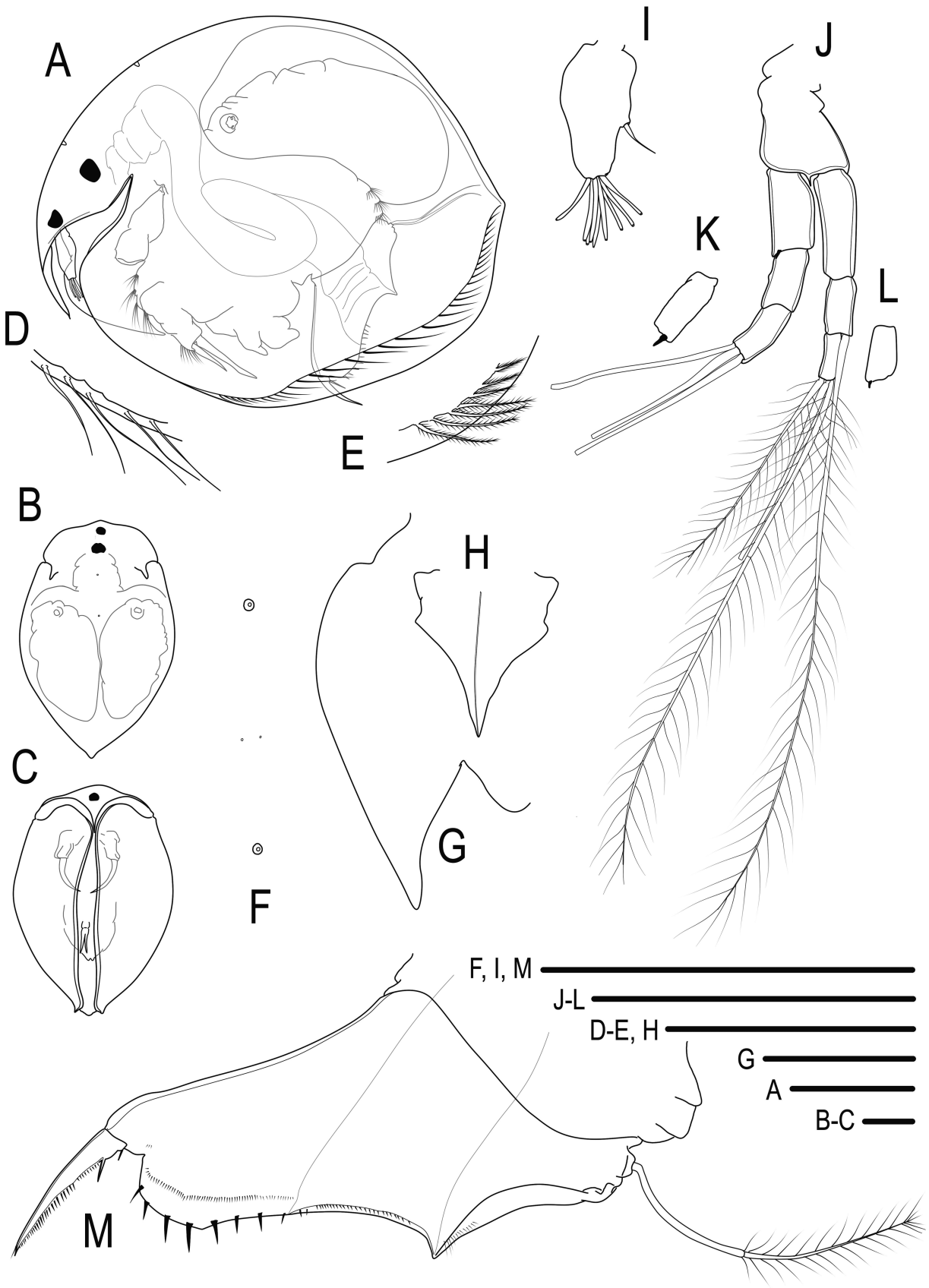

FIGURE 1. *Chydorus sphaericus* (O.F. Müller, 1776) from Bjørndalsvatn Lake Sveindal-Hornnes, Norway, parthenogenetic female. A, habitus; B, dorsal view; C, ventral view; D, ventral margin of carapace, anterior portion; E, ventral margin of carapace, posterior portion; F, head pores; G, labral keel; H, *idem*, frontal view; I, antenna I; J, antenna II; K, *idem*, distal segment of endopodite; L, *idem*, distal segment of exopodite; M, postabdomen. Scale bars = 0.1 mm.

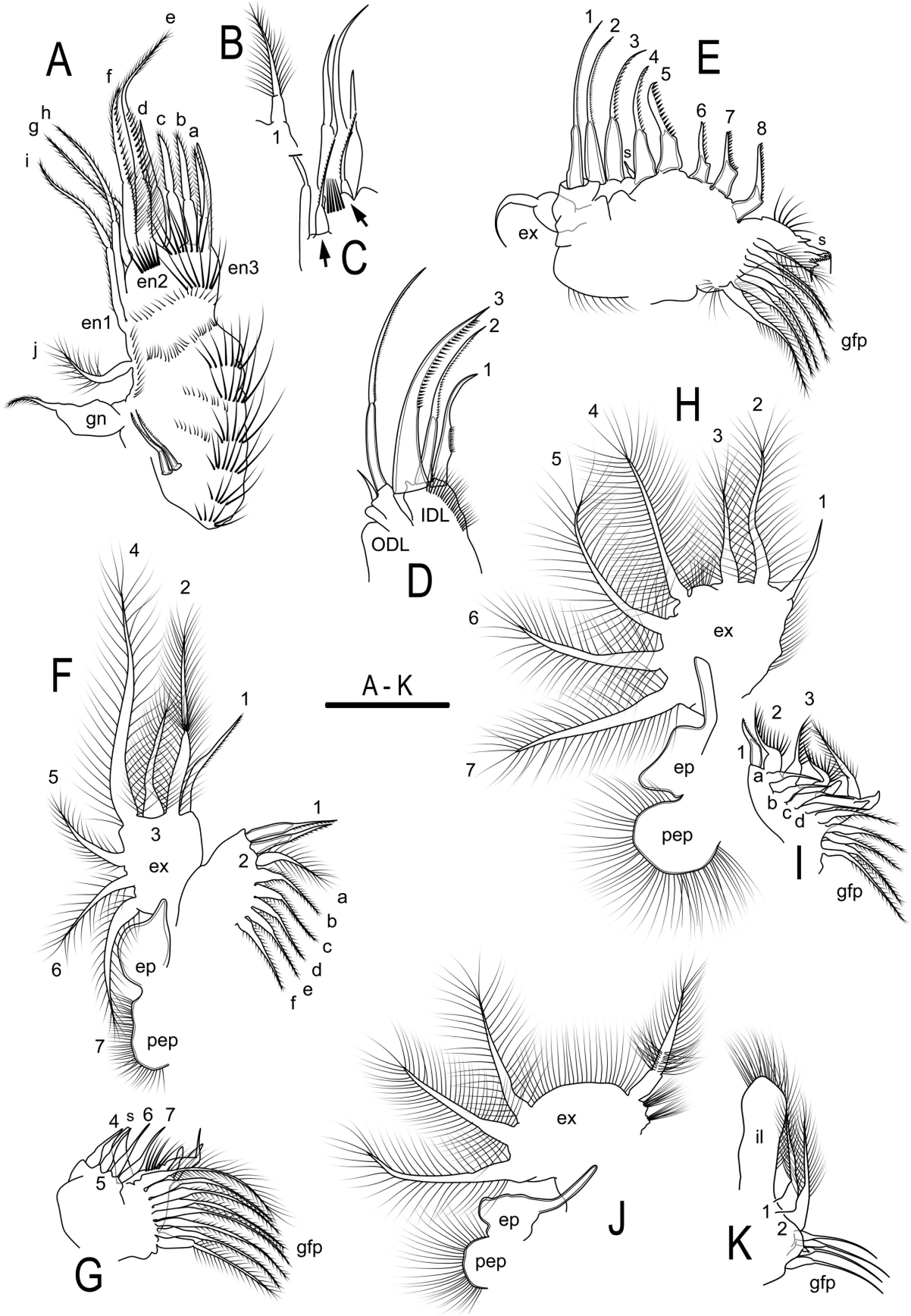

FIGURE 2. *Chydorus sphaericus* (O.F. Müller, 1776) from the Bjørndalsvatn Lake Sveindal-Hornnes, Norway, parthenogenetic female. A, limb I; B, *idem*, anterior seta 1; C, *idem*, arrow showing stiff setae; D, *idem*, ODL and IDL. E, limb II; F, limb III; G, *idem*, basal endite; H, limb IV; I, *idem*, distal and basal endites; J, limb V; K, *idem*, inner lobe. Scale bars = 0.05 mm.

Antenna II (Fig. 1J–L). Basal segment with a short and thin spine. First exopodite segment longer than first endopodite segment; second exopodite segment with a long seta, about 2.8 times longer than exopodite length itself; third exopodite segment with three apical setae, plumose, two setae about 1.9 times longer than the branch length itself, one setae of similar length to length of branch itself; apical spine of the exopodite about 2 times shorter than endopodite apical spine. First endopodite segment armed with a spine similar in length to apical spine of exopodite; third endopodite segment with tree setae, plumose, about 1.9 times longer than the branch length itself. Antennal formula (exo/endo): spines 001/101, setae 013/003.

Limb I (Fig. 2A–D). Epipodite not studied. ODL armed with a short seta and a thin serrated seta, longer than IDL third seta. IDL (en 4) with a single group of setulae on the corm, three setae present; seta 1 hook-like, about 1.5 times shorter than the second seta; seta 2 bisegmented, about 1.3 times shorter than the third seta; seta 3 long, hook-like; all setae armed with spines. Endite 3 with four setae, anterior seta 1 (Fig. 2B) similar in length to posterior seta (b), posterior seta (a) about 1.1 times shorter than seta (b), posterior seta (c) about 0.9 times longer than the seta (b). Endite 2 with three posterior setae present (d–f) and a single stiff setae (Fig. 2C); seta (d) plumose about 1.7 times shorter than seta (e); seta (f) armed laterally with short and thick setulae, about 1.3 times shorter than seta (e); seta (e) bears thick setulae on lateral face; stiff seta armed with short denticles, about 1.6 shorter than posterior seta (d). Endite 1 with three posterior setae of similar length (g–i), which are bisegmented and densely setulated on the distal part, seta (j) plumose and approximately 2.3 times shorter than seta (i); ejector hooks of similar length among themselves and armed with spines; ventral face of the limb with 7 cluster of setulae. Gnathobase with a setulated setae.

Limb II (Fig. 2E). Exopodite armed with a seta about 1.3 times longer than e exopodite itself; inner limb portion armed with eight scrapers and a single element (sensillum); scraper 1 markedly longer than the others, length of scraper 2 about 0.89 of scraper 1 length; scraper 3 about 0.9 of scraper 2 length; scraper 4 about 0.78 of scraper 3 length; scraper 5 armed with thick denticles, about 0.8 of scraper 4 length; scrapers 6–7 of similar length; scraper 8 about 1.2 times longer than scrapers 6–7. Proximal portion of the gnathobase flattened, armed with long setulae and one element; distal portion armed with three elongated elements; filter comb with seven setulated setae.

Limb III (Fig. 2F–G). Pre-epipodite densely setulated. Epipodite oval, with a short projection; exopodite with four distal (1–4) and three lateral setae (5–7); seventh seta setulated, about 1.3 times longer than the sixth and fifth setae; fourth seta setulated, about two times longer than the third seta; third seta setulated and about 1.5 times shorter than the second seta; second seta setulated, longer than the first seta, about 0.7 of fourth seta length. Distal endite with tree setae (1–3), setae 1–2 slender, armed with spines and with similar length; seta 3 about 2.6 times shorter than the setae 1–2 (not represented in Fig. 2F); six setulated posterior setae decreasing in length towards the gnathobase (a–f). Basal endite with four setae (4–7) increasing in length towards the gnathobase. Gnathobase armed with a long and cylindrical sensillum (s) and three elements; filter comb with eight setulated setae.

Limb IV (Fig. 2H–I). Pre-epipodite oval and densely setulated. Epipodite with a projection relatively long. Exopodite with seven marginal setae; setae 2–7 plumose; setae 5 and 7 similar in length; sixth seta about 1.2 times shorter than the setae 5 and 7; fourth seta about 1.6 times longer than the third seta; third seta about 1.4 times shorter than the second seta; first seta about 0.63 of second seta length. Distal endite with four setae (1–4), seta 1 scraper-like, armed with thin spines at its middle-length; flaming-torch-like setae (2–4) longer than seta 1, armed with long setulae. Basal endite armed with four setae (a–d) increasing in length towards the gnathobase. Gnathobase with a single sensillum (s) and two elements, armed with one curved setulated seta, which is shorter than the width of the endite itself; filter plate with six setae.

Limb V (Fig. 1J–K). Pre-epipodite densely setulated. Epipodite oval with, a relatively long projection; exopodite wide, rounded, and armed with four plumose setae and two setulated hillocks implanted near the first seta; fourth seta about 1.3 times longer than first seta; setae 2–3 of similar length. Internal lobe elongated, with rounded apex armed with many setulae; setae 1–2 setulated and similar in length; filter comb with four setae.

Ephippial female. Ephippium occupying posterior part of carapace, covered by lines and regular polygons, slightly pigmented on the central part. The head shield is strongly tapered posteriorly and the postpore distance is longer than in parthenogenetic female (Frey, 1980; Alonso, 1996).

Adult male. According to Frey (1980), Smirnov (1996) and Alonso (1996), the males of *C. sphaericus* are smaller than the mature females. The postabdomen has a postanal margin strongly contracted, finger-like. The postabdominal claws do not bear basal spines.

Size. Parthenogenetic female. Length 0.31–0.49 mm, height/length ratio 0.86–0.

Differential diagnosis. *Chydorus sphaericus* should be considered a complex with several sibling species worldwide still waiting for description. Members of the *sphaericus*-complex have a globular body, labral keel with distal portion elongated and triangular, postabdomen with preanal angle clearly prominent, postanal margin with distalmost portion slightly narrow armed with 7–8 denticles which have the base about 2.5–4.5 times shorter than the length itself. Members of the *sphaericus*-complex bear ODL with two setae and IDL of the first limb, with setae 1 and 3 hook-like. *Chydorus sphaericus* s.str. might be differentiated from other species within the group because the basal spines on the postabdominal claws are absent in males (Frey, 1980; Alonso, 1996; Klimovsky & Kotov, 2015).

Distribution and biology. The species was described from Denmark; however, it has been reported worldwide (Rey & Saint-Jean 1969; Frey 1980; Smirnov 1996; Elmoor-Loureiro 1997; Kotov *et al* 2010; Makino *et al*., 2023). In Eurasia, there are some populations with clear geographic and genetic separation (Kotov *et al*. 2016; Karabanov *et al.* 2022). At the same time, zoochory and anthropogenic dispersion explain several events of colonization of *Chydorus sphaericus* s.str. in Australia (Karabanov *et al.* 2022).

Chydorus tilhoi Rey & Saint-Jeans, 1969
(Figs. 3–6)

Rey & Saint-Jean (1969): p.39–40, figs 20A–D.
Chydorus brevidentatus in Smirnov (1971): p. 385–386, figs. 353–354.

Material Examined. Three adult parthenogenetic females from the Congo mainstem, Congo River Basin (-2.1329N / -4.1928N / 1.8104N, 16.2017N / 15.5258E / 23.0660E), collected on 16.12.2013, 19.12.2013 and 12.6.2014 (FDRS701). Two adult parthenogenetic females from the Kasai River, Congo River Basin (-3.6250N / -3.1764N, 18.6586E / 17.3230E), collected between 25.4.2015 (FDRS709) and 17.4.2015. Two adult parthenogenetic females from the Sankuru River, Congo River Basin (-4.2753N / 20.4415E), material collected on 28.4.2015 (FDRS710). Eleven parthenogenetic females from the Congo Basin (FDRS711), material collected between the Congo River and its tributaries in 2010.

Redescription. Parthenogenetic female. *General* (Figs. 3A–B, 6A–B). Parthenogenetic female. General (Figs. 3A–B, 6A–B). In lateral view body sub-oval, dorsal margin arched, without keel or projections; no lateral compression, absence of lateral projections.

Head. (Figs. 3E–I, 6C–G). Ocellus smaller than the eye. Rostrum short with sharp tip, similar in length to the antennular body (Fig. 3A). Head shield (Figs. 3E–G, 6F–G) with posterior part elongated, about 1.3 times longer than the wide, margin posterior rounded. Single major head pore relatively large with a wide rim.

Labrum (Figs. 3H–I, 6E). Keel not prominent, with a large denticle, distal portion elongated with rounded or obtuse apex; lateral horns present in frontal view.

Carapace (Figs. 3A–D, 6A–B, H–J). Covered by hexagons; the anteroventral margin has an evident flange; margins with thick border in ventral view; anterior part of ventral margin with 5-7 slender setae not plumose; posterior part of ventral margin naked; each valve bear 49-52 plumose setae laterally inserted, posterior and anterior setae shorter than the middle setae. Posteroventral margin with 1 or 2 denticles with sharp apex. Posterior margin clearly low, armed with fine spinules that decrease in length towards the dorsal margin.

Abdomen (Figs. 3A, 6A). About 2 times shorter than the thorax, setulae on the dorsal surface not studied.

Postabdomen (Figs. 5C–D, 6K). Tapering distally, about 4.1 times longer than wide, ventral margin straight; preanal margin concave, relatively longer than the anal margin, angle clearly prominent; anal margin relatively concave, about 1.8–2.3 times shorter than the postanal margin, armed with 1–4 groups of short spines; postanal margin elongated, straight, marginal denticles organized in two groups, distal group formed by isolated thin denticles , proximal group formed by 3–5 groups of short denticles; lateral fascicles with inconspicuous spinulae. *Postabdominal setae* about 1.7 of postabdomen length, provided with setulae in the distal segment. *Postabdominal claw*. Bearing two basal spines and a subterminal flagellum, about 0.17–0.19 of postabdomen length; pecten organized in two groups, proximal group with short and thick spines, distal group with thin and relatively long spines. *Basal spines.* Naked, proximal spine about 2.8–3.4 times shorter than the distal; distal spine about 0.3 of postabdominal claw length.

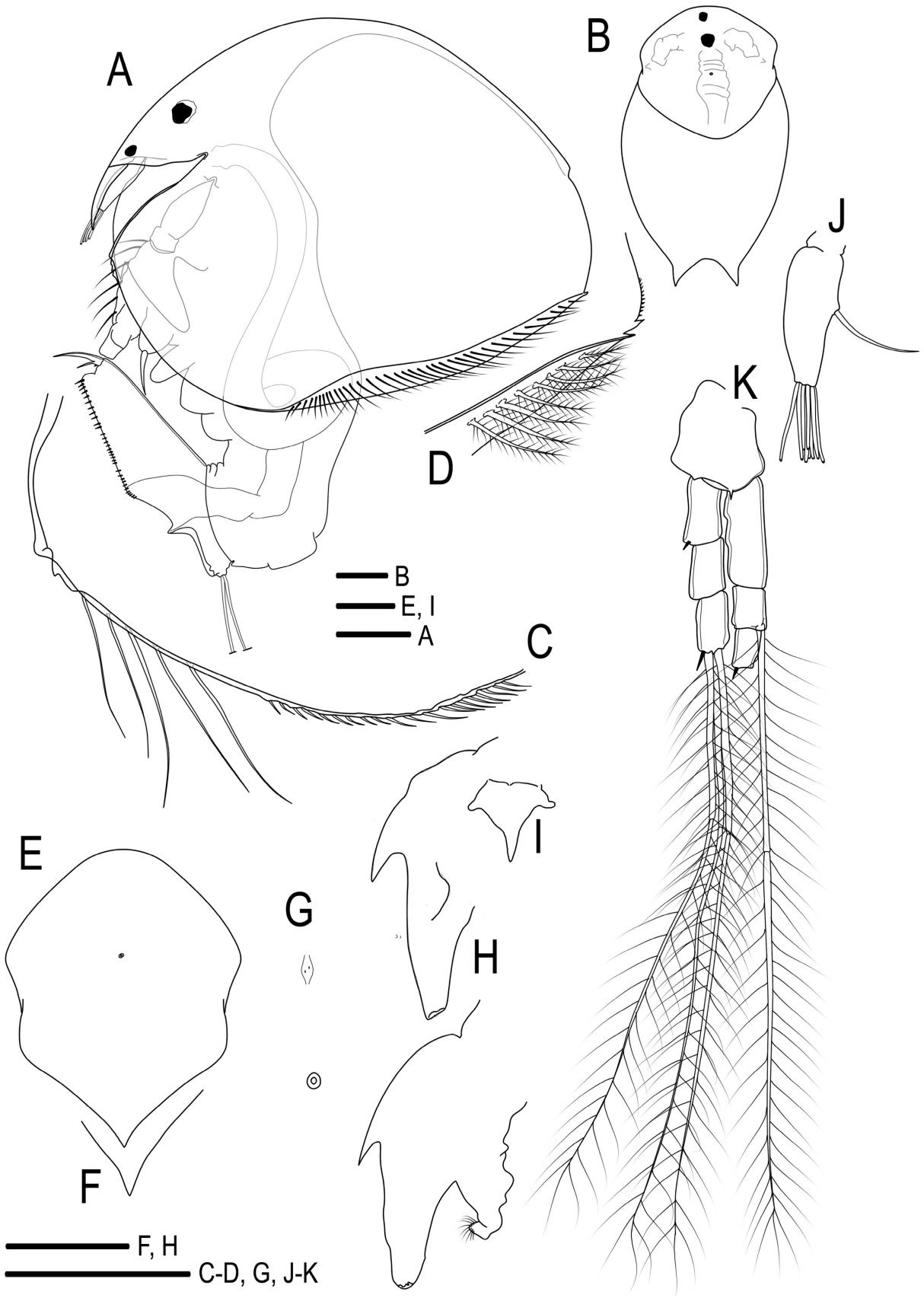

FIGURE 3. *Chydorus tilhoi* Rey & Saint-Jeans, 1969 from the Congo River Basin, Africa, parthenogenetic female. A, habitus; B, dorsal view; C, ventral margin of carapace, anterior portion; D, ventral margin of carapace, posterior portion with denticles; E, head shield; F, *idem*, rostrum; G, head pores; H, labral keel; I, *idem*, frontal view; J, antenna I; K, antenna II. The authors thank to Dr. Alexey A. Kotov and an anonymous reviewer for the valuable suggestions and criticism. Scale bars = 0.05 mm.

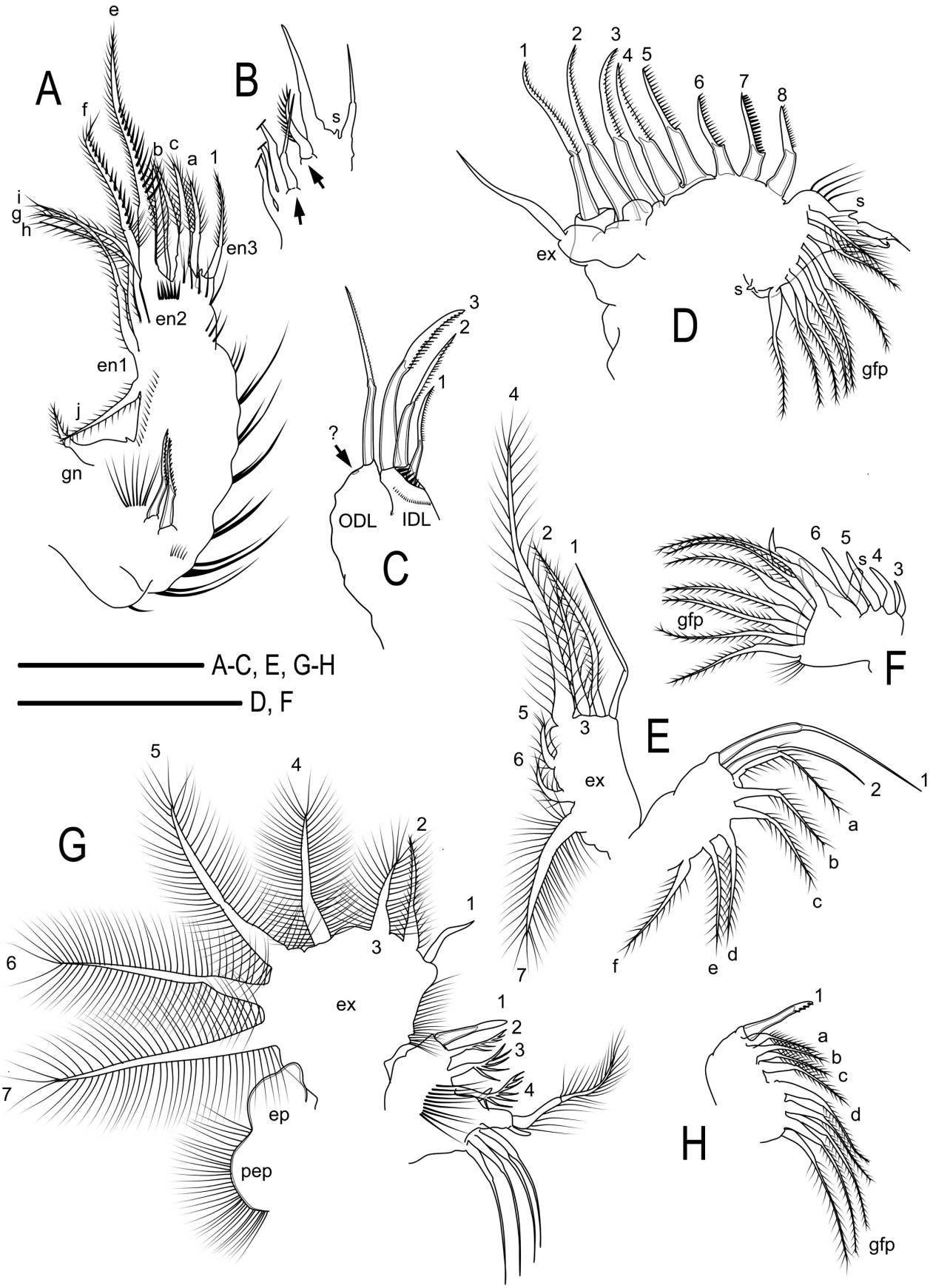

FIGURE 4. *Chydorus tilhoi* Rey & Saint-Jeans, 1969 from the Congo River Basin, Africa, parthenogenetic female. A, limb I; B, *idem*, arrows showing stiff setae; C, *idem*, ODL and IDL; D, Limb II; E, limb III; F, *idem*, basal endite; G, limb IV; H, *idem*, basal endite and gnathobasic filter plate. Scale bars = 0.05 mm.

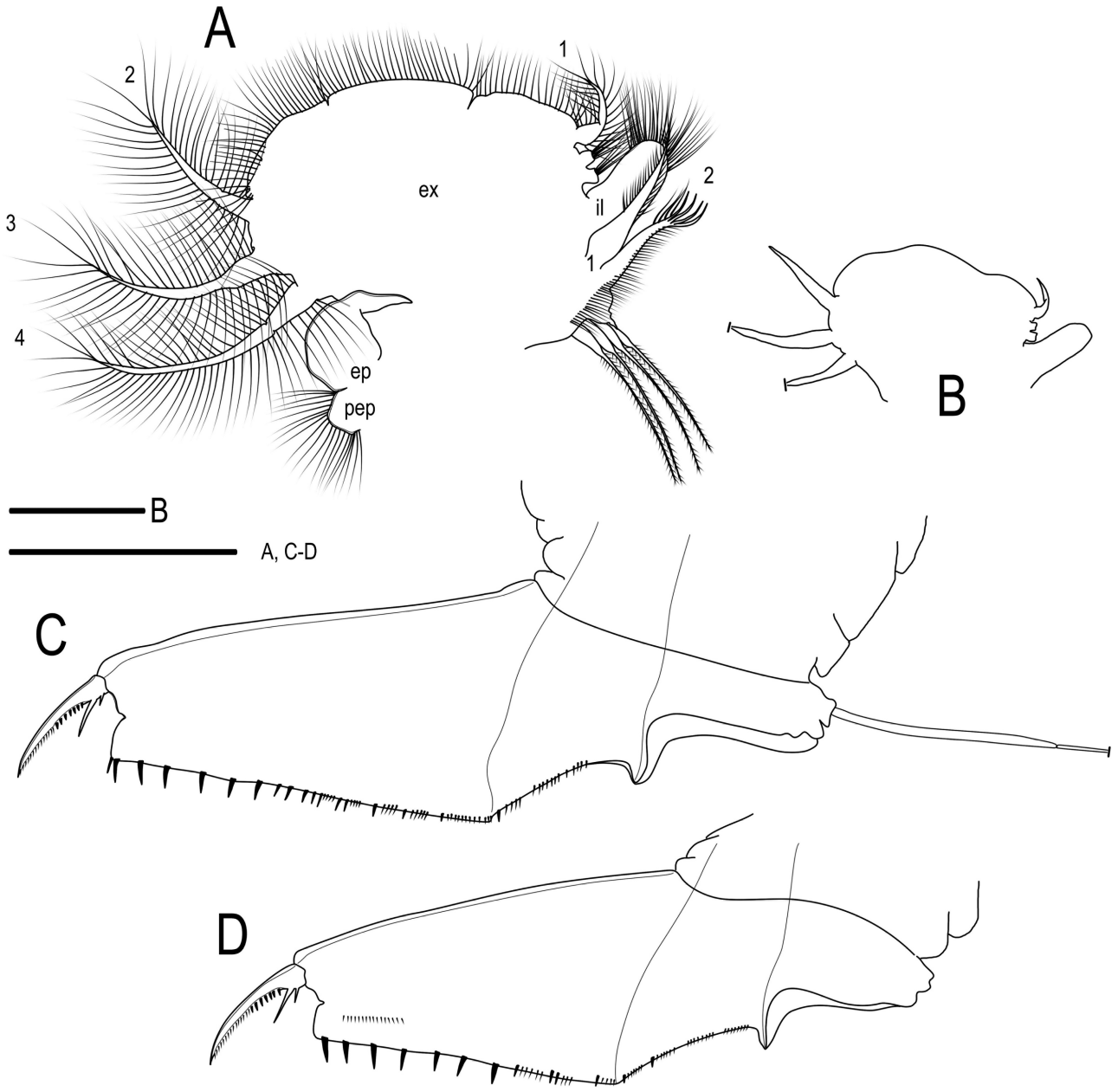

FIGURE 5. *Chydorus tilhoi* Rey & Saint-Jeans, 1969 from the Congo River Basin, Africa, parthenogenetic female. A, limb V; B, idem, outline showing variation on the exopodite shape; C-D, postabdomen. Scale bars = 0.05 mm.

Antenna I (Figs. 3J, 6D). About 2.5 times longer than wide, never reaching the tip of the rostrum; antennular sensory seta about 1.5 times shorter than the length of the antennular body, inserted near to middle length of antennular body; nine aesthetascs which extend beyond the tip of the rostrum, with half the length of the antennular body.

Antenna II (Fig. 3K). Basal segment with short, thin spine. First exopodite segment longer than first endopodite segment; second exopodite segment with a long plumose seta, about 3.1 times longer than the length of the branch itself; third exopodite segment with three apical setae similar in length, plumose, about 3 times longer than the length of the branch itself; apical spine of the exopodite approximately 2 times shorter than the apical spine of the endopodite. First endopodite segment armed with a spine similar in length to apical spine of exopodite; third endopodite segment with three setae of similar length, plumose, about 3 times longer than the length of the branch itself. Antennal formula (exo/endo): spines 001/101, setae 013/003.

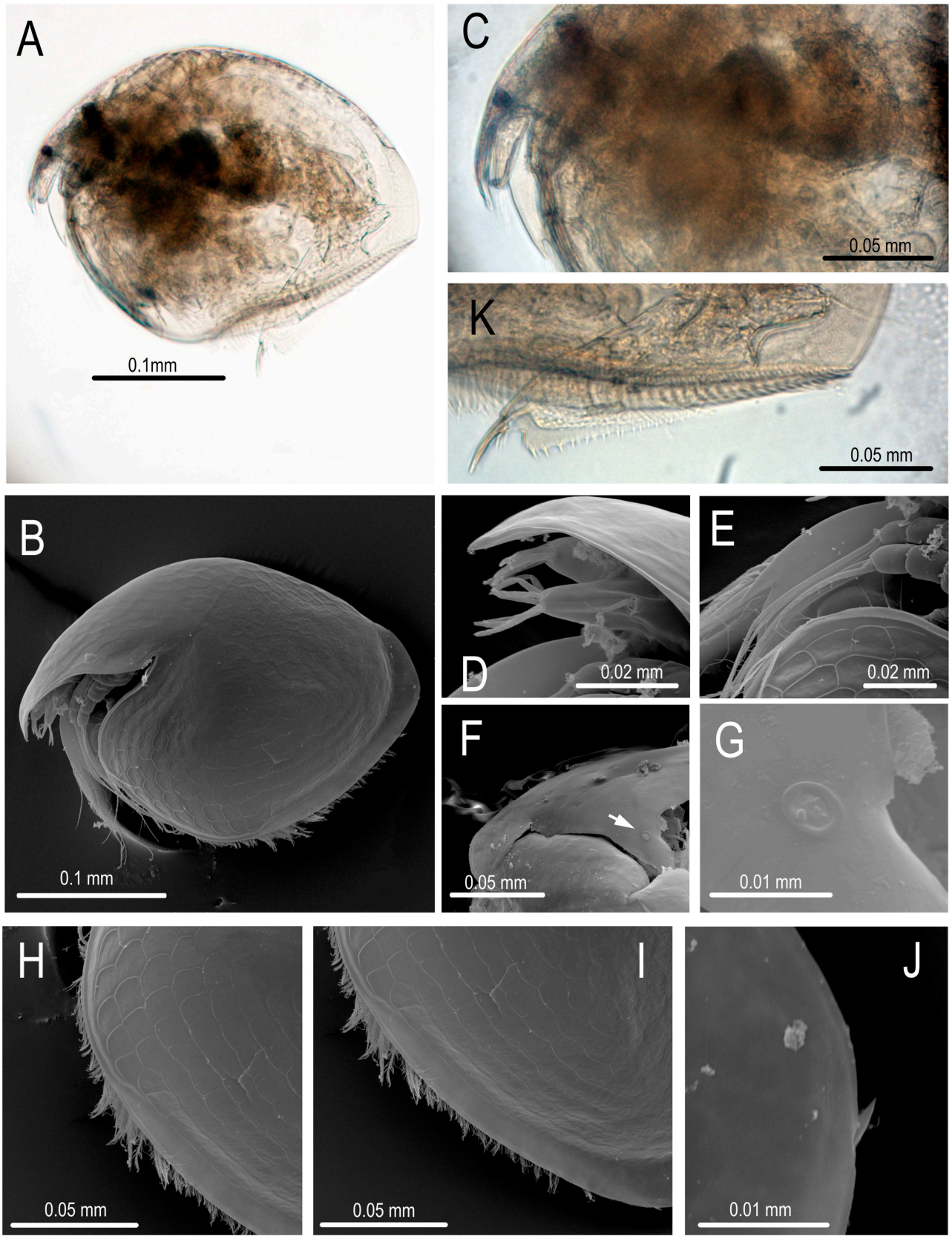

FIGURE 6. *Chydorus tilhoi* Rey & Saint-Jeans, 1969 from the Congo River Basin, Africa, parthenogenetic female. A-B, habitus; C, rostrum; D, rostrum and antennule; E, denticle on the labral keel; F, arrow showing the position of major head pore; G, major head pore with a thick rim; H, ventral margin of carapace, anterior portion; I, ventral margin of carapace, median and posterior portions; J, denticle on the posteroventral margin of carapace.

Limb I (Fig. 4A–C). Epipodite not studied. ODL armed with a thin serrated seta, similar in length to IDL third seta. IDL (en 4) with one group of short spinulae and one group of denticles on the corm, three setae present; seta 1 slender, armed with fine spinulae, about 1.4 times shorter than the second seta; seta 2 bisegmented, armed with short spines, about 1.2 times shorter than the third seta; seta 3 bisegmented, long, chitinized, armed with spines. Endite 3 with four setae, anterior seta 1 similar in length to posterior seta (b), posterior seta (a) about 1.1 times shorter than the seta (b), posterior seta (c) about 0.9 times longer than the seta (b). Endite 2 with three posterior setae present (d–f) and one stiff seta Fig. 4B); seta (d) plumose, about 2.3 times shorter than seta (e); seta (f) armed laterally with short and thick setulae, about 1.6 times shorter than seta (e); seta (e) bears thick setulae on lateral face; stiff seta armed laterally with tick spines, similar in length to posterior seta (d). Endite 1 with three posterior setae of similar length (g–i), which are bisegmented and densely setulated on the distal part, seta (j) plumose and approximately 2 times shorter than seta (i); ejector hooks of similar length among themselves and armed with spines; ventral face of the limb with 7–8 cluster of thick setulae. Gnathobase as a setulated setae.

Second limb (Fig. 4D). Exopodite armed with a seta about 1.8 times longer than the exopodite itself; inner limb portion armed with eight scrapers; scraper 1 about 0.9 of scraper 2 length; scraper 3 similar in length to scraper 2; scraper 4 about 0.8 of scraper 3 length; scraper 5 about 0.9 of scraper 4 length; scrapers 6–8 of similar length; scraper 7 armed with thick spines. Proximal portion of the gnathobase flattened, armed with 4–5 long setulae; distal portion armed with three elongated elements; filter comb with eight setulated setae.

Limb III (Fig. 4E–F). Epipodite not studied. Exopodite with four distal (1–4) and three lateral setae (5–7); seventh seta setulated, about 2.7 times longer than the sixth and fifth setae; fourth seta setulated, about 2.3 times longer than the third seta; third seta setulated and about 1.6 times shorter than the second seta; second seta setulated, longer than the first seta, about 0.68 of the length of the fourth seta. Distal endite with two setae (setae 1–2), slender and naked, seta 1 about 1.5 times longer than the seta 2; six setulated posterior setae similar in length (a–f). Basal endite with four setae (3–6) increasing in length towards the gnathobase. Gnathobase armed with a long and cylindrical sensillum (s) and two elements; filter comb with seven setulated setae.

Limb IV (Fig. 4G–H). Pre-epipodite oval and densely setulated. Epipodite with a short projection. Exopodite with seven marginal setae; setae 2–7 plumose; setae 7 and 6 similar in length; fifth seta about 1.2 times shorter than the setae 6 and 7; fourth seta about 1.8 times longer than the third seta; third seta about 1.4 times shorter than the second seta; first unfeathered seta about 0.6 of second seta length. Distal endite with four setae similar in length (1–4), seta 1 scraper-like armed with thin spinulae at its middle-length, setae 2–4 flaming-torch-like. Basal endite armed with four setae increasing in length towards the gnathobase. Gnathobase with one sensillum (s) and one element, armed with one curved setulated seta which is longer than the width of the endite itself; filter plate with five setae.

Limb V (Fig. 5A–B). Pre-epipodite densely setulated. Epipodite oval with a projection relatively long; exopodite wide, trilobed, armed with four plumose setae and two setulated hillocks implanted near the first seta; fourth seta about 2.7 times longer than first seta; seta 3 about 0.7 times shorter than seta 4; seta 2 about 0.7 times shorter than seta 3. Internal lobe elongated, with rounded apex armed with many setulae, two setae present; seta 1 densely setulated, about 1.2 times longer than seta 2; seta 1 with flaming-torch apex; filter comb with four setae.

Male and Ephippial female. Unknown.

Size. Length between 0.32–0.47 mm, height/length ratio 0.8

Differential diagnosis. *Chydorus tilhoi* has a unique set of morphological traits: a single, relatively large major head pore with a wide rim, elongated labral keel with a large spine and postabdomen with elongated postanal part, tapering distally, and with denticles near its anal margin organized in groups. It is distinguished from *C. dentifer* by presence of sharp denticles on the posteroventral margin on the carapace, one major head pore, and an elongated labral keel with a large spine. *Chydorus tilhoi* is differentiated from *C. nitidulus* by the presence of a single major head pore in adult females and an elongated labral keel with a large spine. *Chydorus tilhoi* can be distinguished from *C. breviceps* (Stingelin, 1905) because it has a large spine on the labral keel. Regarding limbs, the species are differentiated by the presence of seta (j) on the first limb of *C. tilhoi*, proportion of IDL setae 2–3, proportion of exopodite seta of the second limb and presence of an element in the corm of limb in *C. breviceps*, and the proportion of seta 1 in the exopodite of the fourth limb.

In contrast to *C. sphaericus*, *C. tilhoi* has groups of stiff setae on the corm of the first limb, while *C. sphaericus* has smooth setulae; *C. tilhoi* has a group of denticles on the IDL corm while *C. sphaericus* has setulae; the IDL 3 seta in *C. tilhoi* is bisegmented and relatively thin, while *C. sphaericus* has a distinctly IDL 3 seta clearly hook-shaped and thick. In *C. tilhoi*, the third limb exopodite is approximately twice as tall as its wide, while in *C. sphaericus* this

ratio is approximately 1.2; the setae on the basal endite are longer than the setae observed in *C. sphaericus,* similar in morphology to *Disparalona* species (Sousa *et al.* 2018; Neretina *et al*. 2018). The exopodite of the fifth limb of *C. tilhoi* is large and lobed, while in *C. sphaericus* the exopodite of the fifth limb is relatively small and rounded.

Distribution and biology. *Chydorus tilhoi* has exclusive geographic distribution in the Afrotropic zone. The presence of the species extends from the Chari River Basin to the Congo River Basin (Rey & Saint-Jean 1969; Korinek 1984). For a general limnological context for studied localities, see Borges *et al.* (2019).

Discussion

The original description of *Chydorus tilhoi* lacked many important morphological traits but is sufficient to indicate the validity of the proposed taxon (Rey & Saint-Jean 1969). At the same time, gaps in morphological knowledge prevent defining conclusions about phylogenetic affinities and differences from other taxa. The differentiation of *C. tilhoi* and *C. sphaericus* s.s. is based on several traits of habitus, postabdomen, and limbs. Most *Chydorus* species have a *sphaericus*-like sub-globular body, but *Chydorus tilhoi* shares a similar body shape to *C. dentifer* Daday, 1905, *Chydorus nitidulus* (Sars, 1901), *Chydorus breviceps* (Stingelin, 1905), and *Chydorus irinae* Smirnov & Sheveleva, 2010 (Paggi 1972; Smirnov 1996; Elmoor-Loureiro 1997; Smirnov & Sheveleva 2010; Sinev 2014).

The presence and peculiarities of denticles on the posteroventral margin of the carapace should be considered relevant, since species with a *sphaericus*-type subglobular body do not have them. In addition to *C. tilhioi*, denticles with a sharp apex are observed in *C. nitidulus* and *C. breviceps*, while *C. dentifer* has blunt denticles (Smirnov 1996; Elmoor-Loureiro 1997). It is important to note that *C. irinae* does not have denticles on the carapace (Smirnov & Sheveleva 2010; Kotov *et al*., 2012). Regarding the head, *Chydorus tilhoi* has a short rostrum similar to *C. nitidulus* and *C. breviceps*. Besides that, *Chydorus tilhoi* shares with *C. breviceps* the presence of a single major head pore (Sinev 2014). Both species have an elongated labral keel, but only *C. tilhoi* has a large denticle on it.

In a preliminary morphometric study on the postabdomens of *Chydorus* species from Brazil, Rosário *et al*. (2022) suggested the occurrence of two groups of *Chydorus* in the analyzed material: (group A) species with short postabdomen and (group B) species with elongated postabdomen. *Chydorus sphaericus* was part of group A, while *C. nitidulus* and *C. dentifer* were part of group B. *C. breviceps*, *Chydorus irinae*, and *C. tilhoi* could be considered as members of group B. The differences between species within group B are related to specificities in the shape and length of the preanal, anal, and postanal margins, as well as the number and organization of the marginal denticles.

The groups provided by the differences in the postabdominal morphometry of the animals seem to be consistent with the differences observed among the limbs of different taxa. Most species within the group A have the IDL armed with a seta 3 that is distinctly hook-shaped and broad, the exopodite of the third limb subquadrangular and broad, and the exopodite of the fifth limb relatively small and rounded, similarly to *C. sphaericus* (Frey 1980; Alonso 1996; Smirnov 1996; Sinev 2014). Species of the group B, including *C. tilhoi*, have IDL seta 3 bisegmented, relatively slender and thin (except in *C. irinae*), the exopodite of third limb rectangular, longer than wide, and a very large exopodite of fifth limb, which in *C. tilhoi* is lobed. The morphology of the third and fifth limbs in *C. dentifer*, *C. nitidulus* and *C. irinae* is unknown.

There are many morphological traits that support the translocation of *C. breviceps* and *C. tilhoi* to a separate genus (Sinev 2014). Our findings, including our previous morphometric study, contribute to support the hypothesis raised by Sinev (2014). The puzzle of the creation of a new genus and the changes in Chydorinae systematics are being assembled, but fundamental pieces related to the limbs of *C. nitidulus*, *C. dentifer* and *C. irinae* are still lacking. The search for lacking pieces is in progress!

Acknowledgments

The authors thank to Dr. Alexey A. Kotov and an anonymous reviewer for their valuable suggestions and criticism. The financial support for the execution of this study was granted by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 20/04047-5, 2022/16558-0). We are grateful to the Electron Microscopy Center (CME) of the Institute of Biosciences of Botucatu, UNESP, for their support with the equipment used in the SEM photographs. Sample collection in the Congo River was funded by the Fonds National de la Recherche Scientifique (FNRS) (T.0246.13). AVB is a Research Director at the FNRS.

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