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ORIGINAL ARTICLE

Populations of Flavalona setigera (Brehm, 1931) in Brazil belong to a new species: *Flavalona asymmetrica* (Cladocera: Chydoridae: Aloninae)

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Abstract

Flavalona asymmetrica sp. nov. belongs to the *costata*-clade. This species has two main head pores, a feature shared only with *Flavalona setigera* (Brehm, 1931). Morphological differences between them are related to main and lateral head pores, armature of scrapers on the second limb, and proportion of setae on the third and fourth limbs, number of denticles on the postabdomen. Males of *F. asymmetrica* sp. nov. has genital process about ten times shorter than length of postabdominal claw, which is an exclusive morphological trait. *Flavalona asymmetrica* sp. nov. is a Neotropical species, so far, it occurs only in Brazil and can be found together with *Flavalona iheringula* (Sinev & Kotov, 2004) and *Flavalona margipluma* (Sousa, Santos, Güntzel, Diniz-Filho, de Melo-Júnior & Elmoor-Loureiro, 2015).

Key words

Amphi-Pacific distribution, *Biapertura*, main head pores, postabdomen.

Introduction

The genus *Flavalona* Sinev & Dumont, 2016 was created to allocate species of the *costata*-group from the Hexalona-branch (Sinev and Dumont, 2016). Until now, the genus has been composed of 11 valid species which share, as a main morphological trait, lateral head pores with deep sacs underneath them (Sinev, 1999a; 2001; 2008; 2009; Kotov and Sinev, 2004; Van Damme *et al.*, 2011a; Van Damme and Eggermont, 2011; Sousa *et al.*, 2015a; Sinev and Dumont, 2016). In the Neotropics, there are four species: *Flavalona hudeci* (Sinev, 1999), *Flavalona iheringula* (Kotov & Sinev, 2004), *Flavalona*

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margipluma (Sousa, Santos, Güntzel, Diniz-Filho, de Melo-Júnior & Elmoor-Loureiro, 2015), and *Flavalona setigera* (Brehm, 1931). The last three species co-occur in Brazilian water bodies (Sousa and Elmoor-Loureiro, 2012; 2013; Sousa *et al.*, 2013; 2014).

Flavalona setigera was reported for the first time in the Neotropics by Santos-Wisniewski *et al.* (2001) from samples collected in São Paulo state, Brazil (Santos-Wisniewski *et al.*, 2002; Rocha *et al.*, 2011). Later, it was reported from other localities in southeastern and central Brazil (Sousa and Elmoor-Loureiro, 2012; 2013; Sousa *et al.*, 2013; 2014). However, the validity of Neotropical populations of *F. setigera* is questionable (Sinev and Dumont, 2016) because it is an Australasian species and this fact does not agree with the concept of non-cosmopolitanism in cladocerans (Frey, 1982). Thus, the aim of this study is to evaluate putative *F. setigera* specimens from Brazil and to describe a new species of *Flavalona*.

Material and Methods

Morphological analyses

The animals selected for this study were taken to a binocular stereomicroscope, where they were put in glycerin slides and studied under a phase contrast Olympus BX41 microscope. Several individuals were dissected for the observation of the appendages. The organization of morphological structures followed suggestions from 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 Cladocera (Kotov *et al.*, 2010). All drawings were made in a *camera lucida*.

Abbreviations in the text, figures and table

as - accessory seta;
CBS - copulatory brush seta;
en - endite; ep - epipodite;
ex - exopodite;
fc - filter comb;
gfp - gnathobasic filter plate;
gn - gnathobase;
IP - interpore distance (distance between anterior and posterior major head pores);
IDL - inner distal lobe;
il - inner lobe; ms - male seta;
ODL - outer distal lobe;
PA - postabdomen;
pep - pre-epipodite;
PP - postpore distance (distance between the posterior major head pore and the posterior border of the head shield);
P1 - first limb;
P2 - second limb;
P3 - third limb;
P4 - fourth limb;
P6 - sixth limb;
s - sensillum;
EL - Personal collection of Lourdes M. A. Elmoor-Loureiro;
FDRS - Personal collection of Francisco Diogo Rocha Sousa;
MZUSP - Museu de Zoologia da Universidade de São Paulo, Brazil.

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 *Flavalona* Sinev & Dumont, 2016

***Flavalona asymmetrica* sp. nov.**
(Figs. 1–4)

Alona setigera—Santos-Wisniewski *et al.*, 2001: p. 702, fig. 1; Santos-Wisniewski *et al.*, 2002: p. 604; Rocha *et al.*, 2011: p. 289; Sousa and Elmoor-Loureiro, 2012: p. 356; Sousa and Elmoor-Loureiro, 2013: p. 7; Sousa *et al.*, 2013: p. 226; Sousa *et al.*, 2014: p. 147 [not *Flavalona setigera* (Brehm, 1931)].

Type locality. Inhacica River, Parque Nacional das Sempre Vivas, Diamantina, Minas Gerais, Brazil (17°50'11"S 43°45'58"W).

Material examined. Holotype: Undissected, adult parthenogenetic female in a tube with 92%

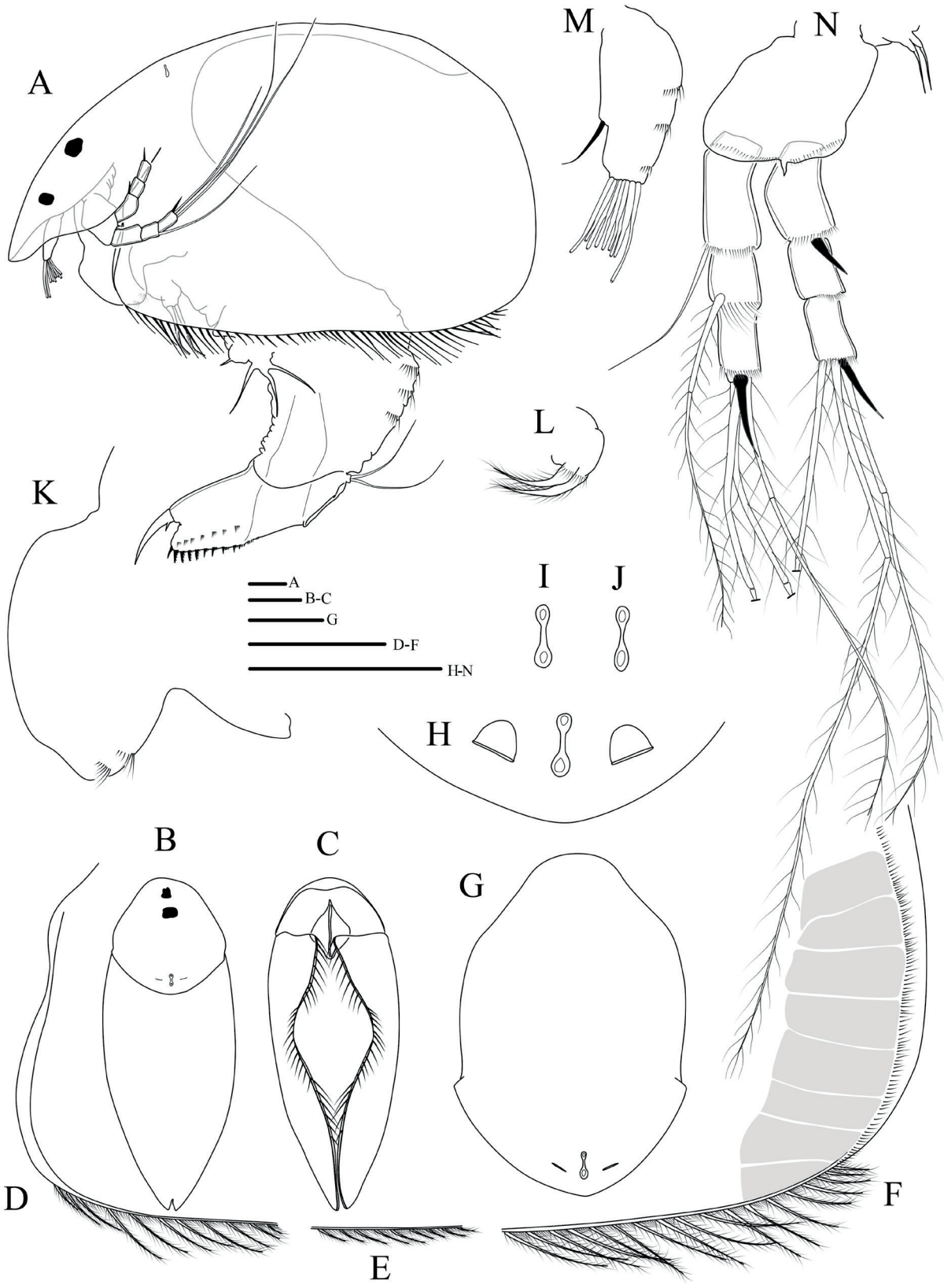

Figure 1. *Flavalona asymmetrica* sp. nov. from Sempre Vivas National Park, Minas Gerais, Brazil, parthenogenetic female. A, Habitus of parthenogenetic female; B, Dorsal view of carapace; C, Ventral view of carapace; D, Anterior portion of ventral margin of carapace; D, Median portion of ventral margin of carapace; E, Median portion of carapace; F, Posterior portion and posteroventral corner of carapace; G, Head shield; H–I, Head pores of adult; J, Main head pore of juvenile; K, Labrum; L, Maxilla; M, Antennules; N, Antenna. Scale bars = 50 µm.

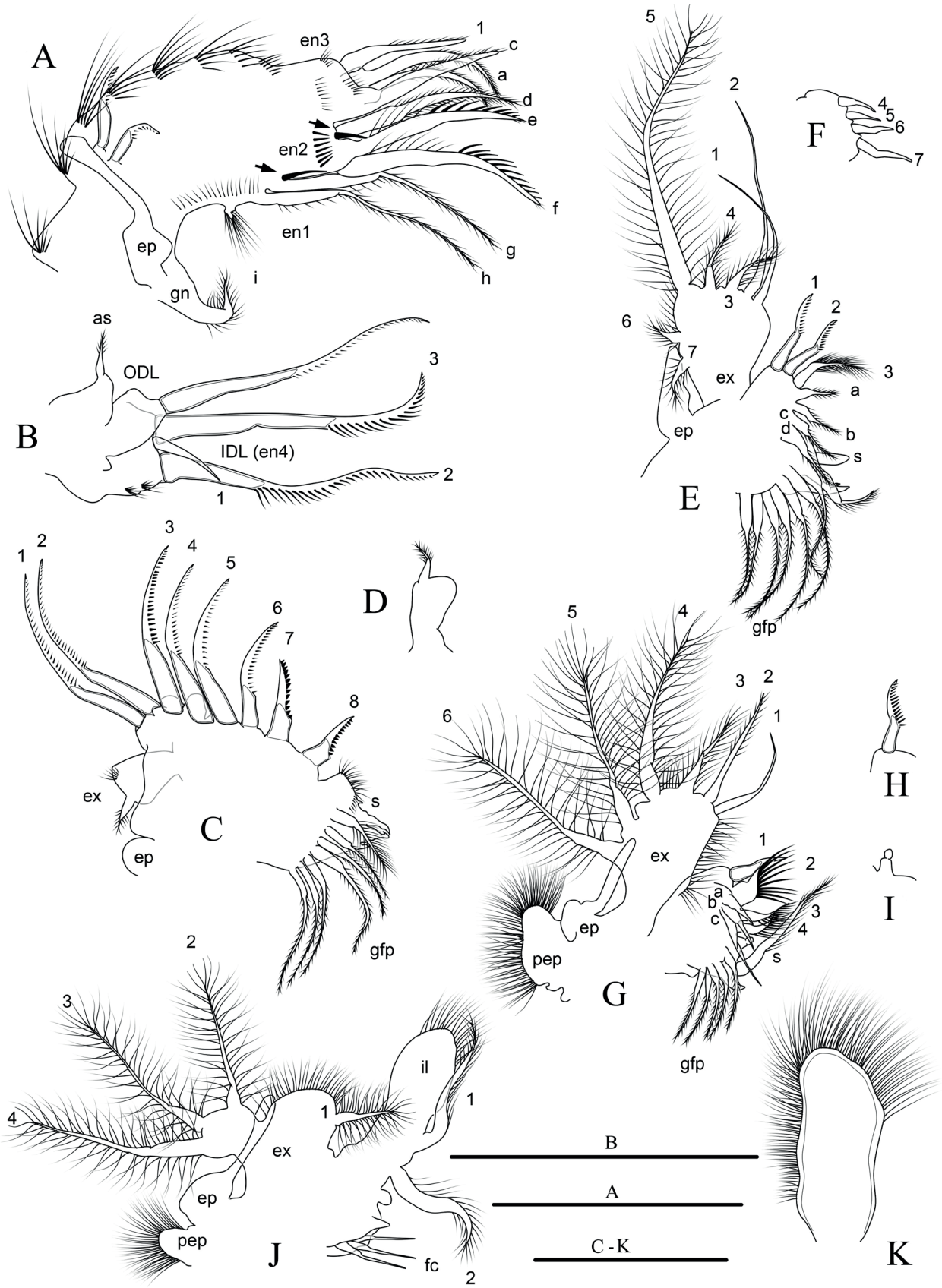

Figure 2. *Flavalona asymmetrica* sp. nov. from Sempre Vivas National Park, Minas Gerais, Brazil, parthenogenetic female. A, First limb, arrows indicate stiff setae; B, *idem* - ODL and IDL; C, Second limb; D, *idem* - exopodite; E, Third limb; F, *idem* - setae of basal endite; G, Fourth limb; H, *idem* - first seta of distal endite; I, *idem* - sensillum; J, Fifth limb; K, Sixth limb. Scale bar = 50 μm.

ethanol deposited at the Museu de Zoologia da Universidade de São Paulo, Brazil under access number MZUSP 35347. The label of holotype is: *Flavalona asymmetrica*, 1 parthenogenetic ♀ from Inhacica River, Parque Nacional das Sempre Vivas, Brazil. Holotype. *Paratypes*. 6 parthenogenetic females, Inhacica River, Parque Nacional das Sempre Vivas, Diamantina, Minas Gerais, Brazil (17º50'11"S 43º45'58"W), Francisco Diogo Rocha Sousa coll., 14/IX/2010 (FDRS0508); 2 parthenogenetic females, Jequitaí River, Parque Nacional das Sempre Vivas, Diamantina, Minas Gerais, Brazil (17°54'8.64"S 43°45'40.28"W), Adriana Marinho Fernandes coll., 03/V/2010 (EL0186); 3 parthenogenetic females, Jequitaí River, Parque Nacional das Sempre Vivas, Diamantina, Minas Gerais, Brazil (17°54'8.64"S 43°45'40.28"W), Adriana Marinho Fernandes coll., 03/V/2010 (FDRS0507); 4 parthenogenetic females, Preto River, Parque Nacional das Sempre Vivas, Diamantina, Minas Gerais, Brazil (17°55'54.01"S 43°48'50.33"W), Adriana Marinho Fernandes coll., 02/V/2010 (EL01850); 2 parthenogenetic females, Exército Pond, Parque Nacional de Brasília, Distrito Federal, Brazil (15°44'44.30"S 47°58'49.10"W), Francisco Diogo Rocha Sousa coll., 1/IV/2008 (EL01724); 2 parthenogenetic females, Exército Pond, Parque Nacional de Brasília, Distrito Federal, Brazil (15°44'44.30"S 47°58'49.10"W), Francisco Diogo Rocha Sousa coll., 16/VIII/2009 (FDRS0471). 1 parthenogenetic female, Peito de Moça wetland, Parque Nacional de Brasília, Distrito Federal, Brazil (15°45'5.8"S 48°01'33.2"W), Francisco Diogo Rocha Sousa coll., 17/VIII/2009 (FDRS473); 7 parthenogenetic females, Peito de Moça wetland, Parque Nacional de Brasília, Distrito Federal, Brazil (15°45'5.8"S 48°01'33.2"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 15/IV/2009 (EL01727); 1 parthenogenetic female. Henrique Pond, Parque Nacional de Brasília, Distrito Federal, Brazil (15°41'18"S 47°56'26.10"W), Francisco Diogo Rocha Sousa coll., ?/VIII/2009 (FDRS0474); 1 parthenogenetic female, Murunduns Wetlands, Parque Nacional de Brasília, Distrito Federal, Brazil (15°46'42"S 47°58'34"W), material collected by Grupo de Estudos de Ecossistemas Aquáticos, 17/VII/2009 (EL01684); 4 parthenogenetic females, Cedro Pond, Park Way, Distrito Federal, Brazil (15°53'50.2"S 47°56'37.7"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 21/III/2008 (EL01637); 2 parthenogenetic females, Rio dos Couros, Alto Paraíso, Goiás, Brazil (14°09'36.5"S 47°35'37.6"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 10/VII/2004 (EL02439); 10 parthenogenetic females, pond, Alto Paraíso, Parque Nacional da Chapada dos Veadeiros, Goiás, Brazil (14°6'5.84"S 47°42'4.89"W), Luciana Mendonça de Galvão, Bárbara M. Fonseca, and Ricardo L. Pinto colls., 17/VI/2012 (EL02241); 7 parthenogenetic females and 1 male, pond, Alto Paraíso, Parque Nacional da Chapada dos Veadeiros, Goiás, Brazil (14°6'8.57"S 47°42'7.35"W), Luciana Mendonça de Galvão, Bárbara M. Fonseca and Ricardo L. Pinto colls., 17/VI/2012 (EL02265); 3 parthenogenetic females, pond, Alto Paraíso, Parque Nacional da Chapada dos Veadeiros, Goiás, Brazil (14°6'8.37"S 47°42'12.32"W), Francisco Diogo Rocha Sousa coll., 12/III/2013 (EL02541); 21 parthenogenetic females and 4 males, pond, Alto Paraíso, Parque Nacional da Chapada dos Veadeiros, Goiás, Brazil (14°6'8.37"S 47°42'12.32"W), Luciana Mendonça de Galvão, Bárbara M. Fonseca and Ricardo L. Pinto colls., 17/VI/2012 (EL02269); 2 parthenogenetic females, Estiva Stream, Alto Paraíso, Parque Nacional da Chapada dos Veadeiros, Goiás, Brazil (14°06'40.3"S 47°44'02.2"W), Francisco Diogo Rocha Sousa coll., 13/III/2013 (EL02376); 3 parthenogenetic females, Pico Itapeva Dam, Campos do Jordão, São Paulo, Brazil (22°46'25.2"S 45°33'24.1"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 15/XI/2002 (EL0754); 1 parthenogenetic females, Pico Itapeva Dam, Campos do Jordão, São Paulo, Brazil (22°46'25.2"S 45°33'24.1"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 15/XI/2002 (EL01361); 1 parthenogenetic females, Pico Itapeva Dam, Campos do Jordão, São Paulo, Brazil (22°46'25.2"S 45°33'24.1"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 15/XI/2002 (EL01362); 1 parthenogenetic females, Pico Itapeva Dam, Campos do Jordão, São Paulo, Brazil (22°46'25.2"S 45°33'24.1"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 15/XI/2002 (EL01363); 1 parthenogenetic females, Pico Itapeva Dam, Campos do Jordão, São Paulo, Brazil (22°46'25.2"S 45°33'24.1"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 15/XI/2002 (EL01364); 1 parthenogenetic females, Pico Itapeva Dam, Campos do Jordão, São Paulo, Brazil (22°46'25.2"S 45°33'24.1"W),

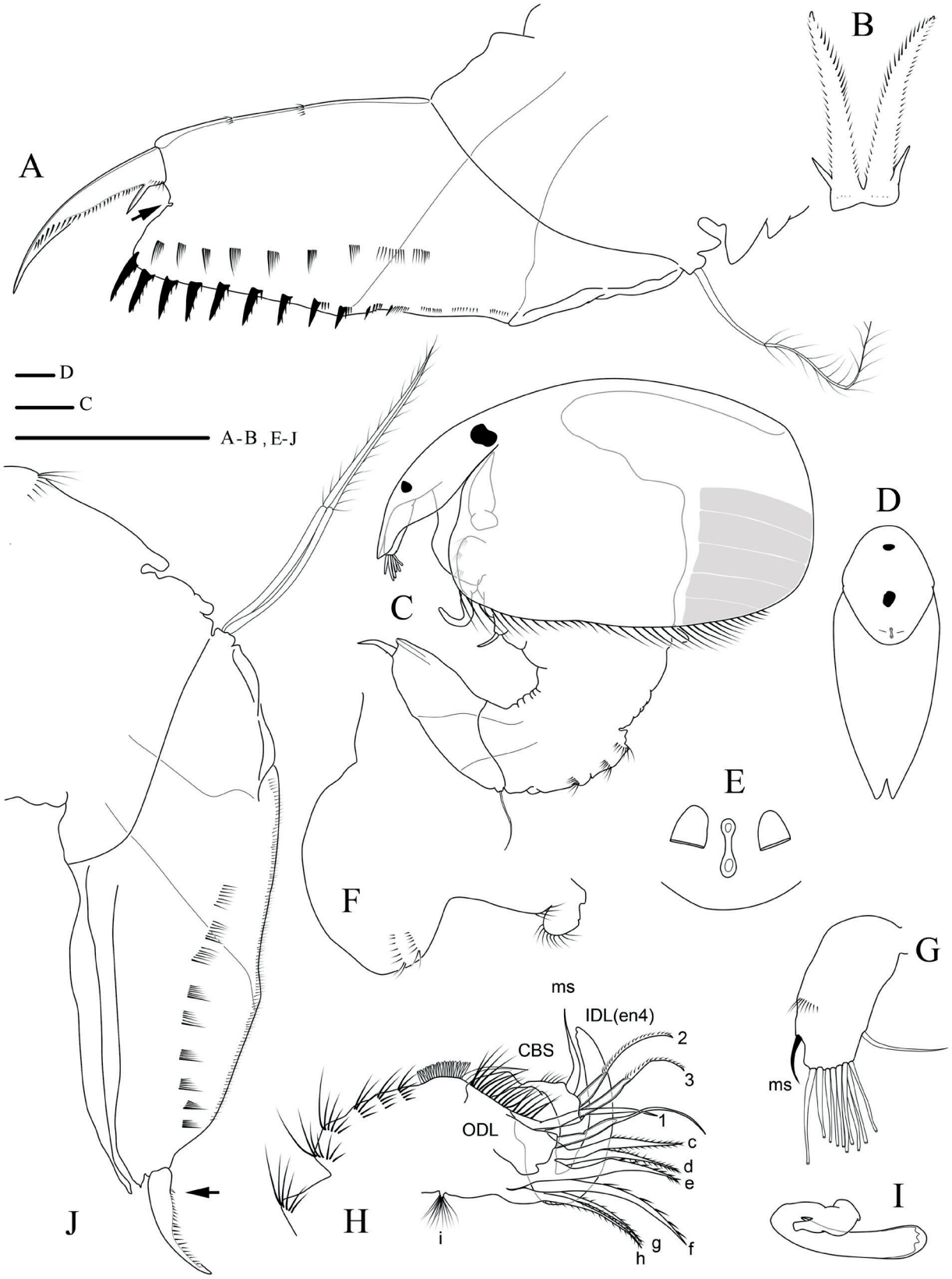

Figure 3. *Flavalona asymmetrica* sp. nov. from Sempre Vivas National Park, Minas Gerais, Brazil, parthenogenetic female A–B. A, Postabdomen; B, Postabdominal claw, dorsal view; Male from Chapada dos Veadeiros National Park C–J. C, Habitus; D, Dorsal view of carapace; E, Head pores; F, Labrum; G, Antennule; H, First limb; I, Ventral view of copulatory hook; J, Postabdomen, arrows indicate the basal spine. Scale bar = 50 μm.

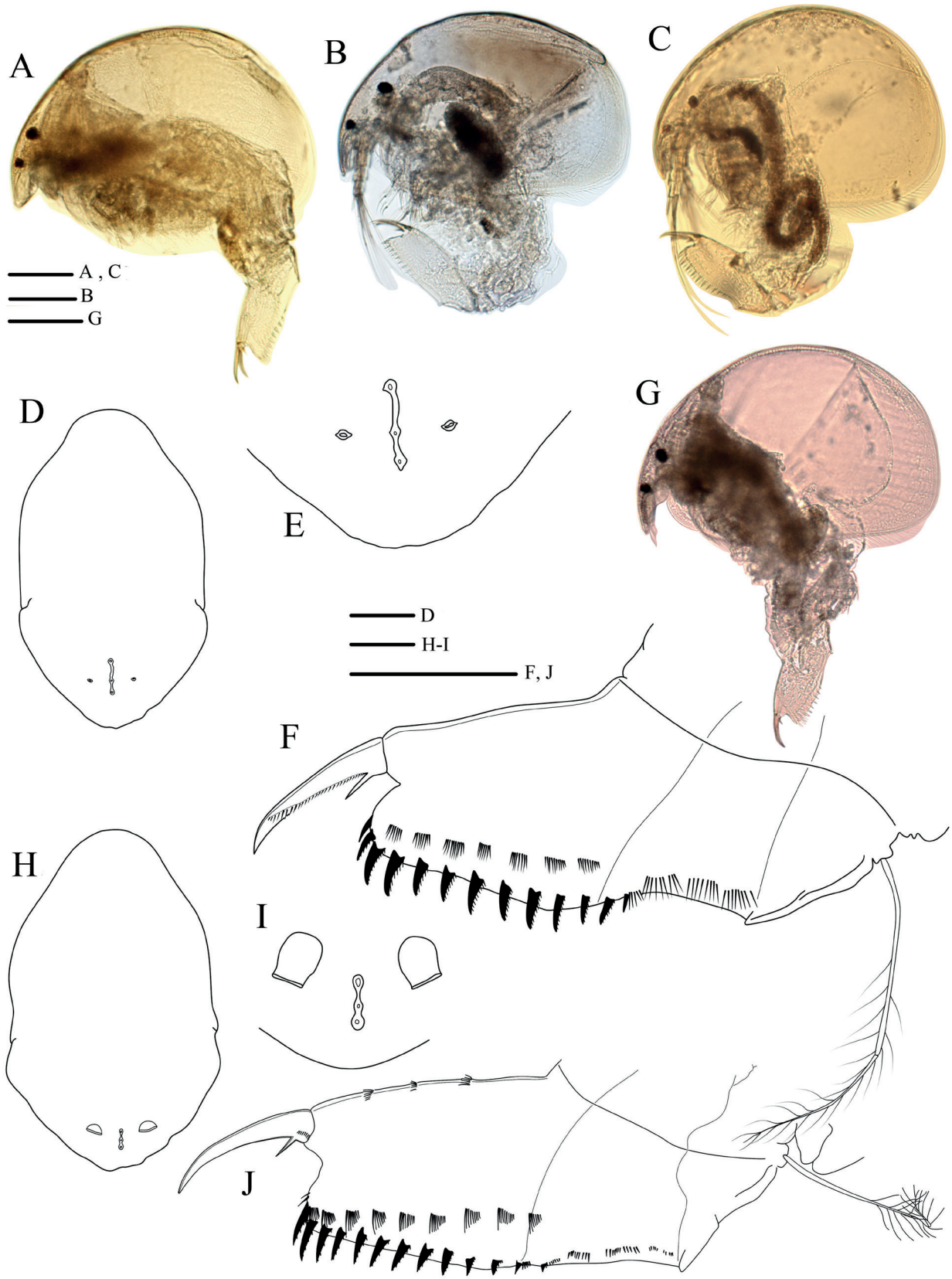

Figure 4. Species of *Flavalona* co-occurring in water bodies from Brazil. A, *Flavalona asymmetrica* sp. nov., parthenogenetic female; B, *idem* - ephippial female; C, *Flavalona iheringula* (Sinev & Kotov, 2004); D, *idem* - head shield; E, *idem* - head pores; F, *idem* - postabdomen; G, *Flavalona margipluma* (Sousa, Santos, Guntzel, Diniz-Filho, de Melo-Júnior & Elmoor-Loureiro, 2015); H, *idem* - head shield; I, *idem* - head pores; J, *idem* - postabdomen. Scale bars to figures A–C, G = 100 μm; figures D, E–F, H–J = 50 μm.

Lourdes Maria Abdu Elmoor-Loureiro coll., 15/XI/2002 (EL01365); 1 parthenogenetic females, Pico Itapeva Dam, Campos do Jordão, São Paulo, Brazil (22°46'25.2"S 45°33'24.1"W), Lourdes Maria Abdu Elmoor-Loureiro coll., 15/XI/2002 (EL01366).

Etymology. The epithet comes from joining the Greek prefix "*a*" (absence) and Latin word "*symmetria*" (symmetry). The name makes reference to the rim around the posterior pore, which is broader than the connection around the anterior one.

Differential diagnosis. Flavalona asymmetrica sp. nov. has two main head pores, differently from *Flavalona costata* (Sars, 1862), *Flavalona cheni* (Sinev, 1999), *Flavalona natalensis* (Sinev, 2008), *F. margipluma*, *Flavalona weltneri* (Kilhack, 1905), *Flavalona rustica* (Scott, 1895), *Flavalona bicolor* (Frey, 1965), *F. hudeci*, *F. iheringula* and *Flavalona sphagnophila* (Van Damme & Eggermont, 2011). Regarding the females, *F. asymmetrica* sp. nov. is differentiated from *F. setigera* because it has the rim around the posterior pore broader than anterior one, PP about 0.7 IP, deep and elliptic sacs about 0.6 of length of the lateral pores; long seta 1 of IDL on the first limb; second limb with scrapers 7–8 armed with denticles; third limb with first exopodite seta about 0.7 of length of the second seta; fourth limb with exopodite setae 4–5 of similar lengths; epipodite of the fifth limb with long projection; sixth limb about 2.4 times longer than wide with setulae on the margins; postabdomen armed with up to nine marginal denticles. Regarding the males, *F. asymmetrica* sp. nov. is differentiated from *F. setigera* because it has seta of the copulatory brush markedly shorter than setae 2–3 of IDL, postabdomen armed with marginal setulae on the distal and anal margins not separated in groups, basal spine present and genital process about ten times shorter than length of postabdominal claw.

Description of parthenogenetic female. General Habitus (Fig. 1A–C). Body more elongate than spherical, length 0.37–0.45 mm, maximum height at middle of body, height/length ratio about 0.6; dorsal margin arched, without dorsal keel or lateral projection; in dorsal and ventral view body not laterally compressed.

Carapace (Fig. 1A–F). With slight longitudinal lines; ventral margin almost straight; each valve with 49–52 plumose setae and relatively long setulae between them; setae arranged in three groups, anterior and posterior groups longer than median group; internal spinulae of the posterior part not arranged in groups, proximal spinulae exceeding the line of valves.

Cephalic structures (Fig. 1G–N). Ocellus shorter than eye; rostrum short and not sharp in lateral view. *Head shield* (Fig. 1G–J). About 1.6 times longer than wide, posterior part (posterior to mandibular articulations) about 0.52 of length of the anterior part, posterior margin strongly arched; rostrum short with margin rounded; two connected main head pores, rim around the posterior pore about 1.3 times broader than the anterior one; PP about 0.7 IP; transverse lateral head pores about 0.7–0.9 IP and located at the level of posterior main head pore or between posterior and anterior main head pores, deep and elliptic sacs underneath about 0.6 of length of the lateral pores. *Labrum* (Fig. 1K). Of moderate size; keel without a notch, anterior margin convex, posterior margin with two clusters of setulae. *Maxilla* (Fig. 1L). Well developed, with two long setulated setae. *Antennule* – A1 (Fig. 1M). Antennular body length about 2.2 times the width, does not exceeding tip of rostrum, with three rows of setulae; antennular sensory seta slender, about three times shorter than antennular body, inserted at the second third of the antennular body; nine aesthetascs projecting beyond the tip of rostrum, two aesthetascs about 1.4 times longer than the others. *Antenna* – A2 (Fig. 1N). Coxal setae of similar length; basal segment thick with a short spine; first exopodite segment slightly longer than first segment of endopodite; second exopodite segment armed with long setulae on the tip and a seta about 0.45 of the length of longest apical seta; first endopodite segment armed with a spine slightly shorter than second segment; apical spines longer than length of apical segments. Antennal formula (exo/endo): spines 001/101, setae 113/003.

Thoracic limbs (Fig. 2A–K)**.** Six pairs of thoracic limbs. *First limb* (Figs. 2A, B). Epipodite with long projection; ODL bears a thin seta armed with fine

setulae, similar in length to IDL setae; IDL (en 4) with three setae, first seta about 3.5 times shorter than setae 2–3, setae 2–3 bisegmented and armed with hard setulae; endite 3 with four setae, seta 1 markedly shorter than posterior setae (a–c), setae (a–b) of similar length and slightly longer than seta (c); endite 2 with a row of long spinulae, three posterior setae present (d–f), setae (d–e) of similar length and about 0.8 of the length of seta (f), setae (e–f) bearing spinulae on the lateral face, anterior stiff seta short, about 0.16 of setae (d–e); endite 1 with three posterior setae (g–i), two being bisegmented and densely setulated from to distal part (g–h), a short flat plumose seta (i), anterior stiff seta about 0.24 of setae (d–e); ejector hooks of different length and armed with short spines; ventral face of the limb with seven groups of setulae organized in clusters, decreasing in length towards the distal portion; gnathobase elongated, apex sharp and setulated. *Second limb* (Fig. 2C, D). Exopodite elongated with a row of short setulae, seta on the exopodite about 0.57 of length of exopodite itself; inner limb portion armed with eight scrapers; scrapers 1–2 of similar length and armed with fine and short spinulae, scrapers 3–8 decreasing in length towards to gnathobase; scraper 3 armed with spines; scraper 7 armed with strong denticles, about 1.4 times shorter than scraper 6; scraper 8 armed with strong denticles, about 1.8 shorter than scraper 6; proximal portion of gnathobase wide and short, armed with short setulae; distal portion armed with four elements, first as a short sensillum, second and third elements with blunt distal portion, fourth element short and obtuse; filter comb with seven setae, first one short and densely setulated, others long and slightly setulated. *Third limb* (Fig. 2E, F). Epipodite elongated, without projection; exopodite relatively large, subquadrangular, with five distal and two lateral setae; sixth and seventh setae setulated, short and of similar length; fifth seta about 1.8 times longer than first seta and 1.4 times longer than second seta; fourth seta slightly longer than third seta; First seta about 0.7 of length of the second, both naked; distal endite with three setae (1–3), seta (1–2) scraper-like, seta (3) curved and armed with many setulae bilaterally implanted; four long and plumose posterior setae increasing in length toward to posterior part of the endite (a–d); basal endite with four soft anterior setae slightly increasing in length towards the gnathobase (4–7); gnathobase armed with three elements, the first being a cylindrical sensillum, the second a geniculated and relatively short seta, third and fourth elements naked and with acute tip; filter comb with seven setae. *Fourth limb* (Fig. 2G–I). Pre-epipodite rectangular, densely setulated; epipodite oval with a long projection; exopodite subquadrangular with six marginal setae; setae 3–6 plumose; sixth seta about two times longer than the third and slightly longer than fourth and fifth setae; third seta about 0.53 of length of the fourth and fifth setae and about 0.7 of length of the second seta; first seta markedly shorter than second seta; distal endite with four setae (1–4), one scraper-like (1), three flaming-torch-like (3–4); first flaming-torch ticker than the others and armed with long setulae; gnathobase armed with a bottle-shaped sensillum and a setulated seta shorter than width of endite; filter comb with five setae. *Fifth limb* (Fig. 2J). Pre-epipodite short and rounded, densely setulated; epipodite oval with long projection; exopodite clearly divided in two lobes; four plumose setae; third seta about 0.9 of length of the fourth seta; second seta about 0.75 of length of the fourth seta; first seta about 0.46 of length of the second seta; internal lobe elongated, relatively oval and armed with many setulae, two setulated setae of different length on inner face of the lobe, seta 1 about 0.9 of length of the seta 2; gnathobase armed with two elements; filter comb with three setae about two times shorter than seta 1 of internal lobe. *Sixth limb* (Fig. 2K). An elongated lobe, about 2.4 times longer than wide; apical and lateral margins armed with long setulae.

Abdominal and postabdominal structures. *Abdomen* (Figs. 1A, 3J). Shorter than thorax, armed with three rows of abdominal setae. *Postabdomen* (Fig. 3A). About 3 times as long as wide, ventral margin almost straight with two rows of short spinulae; preanal margin slightly longer than anal margin; anal margin armed with 4–5 groups spines; postanal margin about 1.3 times longer than preanal and anal margin, straight or slightly concave, distal angle slightly projected, a short incision is present near to base of postabdominal claw; eight-nine merged marginal denticles with width at base/height ratio about 0.3; eight-ten lateral fascicles armed with thin spinulae which do not exceed the margin of postabdomen; postabdominal seta about 2.2

times shorter than postabdomen length. *Postabdominal claw* (Fig. 3A, B). About 0.3 of the length of the postabdomen and longer than anal margin, base armed with short spinulae; inner pecten with distalmost spinulae longer than proximalmost ones; outer pecten with spinulae of similar length. *Basal spine* (Fig. 3A, B). Naked, not inserted on the base of postabdominal claw, about 0.1 of length of the postabdominal claw, similar to width of postabdominal claw at its base.

Description of ephippial female (Fig. 4B). Length 0.37–0.46 mm, maximum height at middle of body, height/length ratio about 0.6; dorsal margin arched, without dorsal keel, not laterally compressed; carapace on the region of ephippium covered by longitudinal lines, yellow-brown.

Description of male. **General Habitus and Carapace** (Fig. 3C, D). Lower than female, length up 0.35 mm, about 1.7 times longer than height; maximum height near to middle of body; without dorsal keel; not laterally compressed; carapace with slight longitudinal lines; ventral margin almost straight; each valve with 40–44 plumose setae and relatively long, setulae between of them; setae arranged in three groups, anterior and posterior groups longer than median group.

Cephalic structures (Fig. 3E–G). Ocellus shorter than eye; rostrum short and not sharp in lateral view. *Head shield* (Fig. 3E). Two connected main head pores, rim around the posterior pore about 1.3 times broader than the anterior one; PP about 0.5 IP; transverse lateral head pores about 0.62 IP and located at the level between posterior and anterior main head pores, deep and elliptic sacs underneath of similar length to the lateral pores. *Labrum* (Fig. 3F). Shorter than in female; keel more rounded than in female, without a notch, anterior margin convex, posterior margin with two clusters of setulae. *Antennule* – A1 (Fig. 3G). Antennular body length about two times width, does not exceeding tip of rostrum; antennular sensory seta slender, about two times shorter than antennular body, inserted at the last third of the antennular body; twelve aesthetascs projecting beyond the tip of rostrum; male seta about 3 times shorter than antennular body.

Thoracic structures (Fig. 3H, I). First limb with copulatory hook U-shaped; arms of different lengths, not projected one over the other, scales present on the tip; seta of the copulatory brush about 0.8 of the length of the male seta on the IDL (en 4); seta of the ODL about 1.3 times longer than IDL setae (2–3); IDL with two setae (2–3) of similar length and armed with short hard setulae; male seta about 0.7 of length of the IDL setae; five clusters of long setulae inserted on the body of the limb.

Abdominal and Postabdnominal structures (Figs. 3C, 3J). *Abdomen* (Fig. 3C). Shorter than thorax, armed with three rows of abdominal setae. *Postabdomen* (Fig. 3J). About 2.9 times as long as wide, narrowing distally; anal margin about 1.3 times longer than postanal; anal and postanal margin armed with short setulae not arranged in groups; nine lateral fascicles armed with thin spinulae; genital process about ten times shorter than length of postabdominal claw; postabdominal seta about 1.4 times shorter than postabdomen length. *Postabdominal claw* (Fig. 3J). Smaller and more robust than in the female, about 0.2 of the length of the postabdomen, slightly shorter than preanal margin; inner pecten with distalmost spinulae longer than proximalmost ones; outer pecten with spinulae of similar length. *Basal spines* (Fig. 3J). Very short and thin.

Remarks. The juveniles of *F. asymmetrica* sp. nov. presented variability in the morphology of main head pores (Fig. 1J). *Flavalona asymmetrica* sp. nov. can be found associated with macrophytes in different types of water bodies, such as dams, shallow lakes, ponds and lotic systems. *Flavalona asymmetrica* sp. nov. co-occurs with *F. iheringula* (Fig. 4C–F) and *F. margipluma* (Fig. 4G–J) (Santos-Wisniewski *et al.*, 2002; Sousa and Elmoor-Loureiro, 2012; 2013; Sousa *et al.*, 2013).

Distribution. So far, *Flavalona asymmetrica* sp. nov. was observed from Southeast to Central Brazil (Distrito Federal, Goiás, Minas Gerais and São Paulo). A wider distribution in Neotropics is expected. To distribution on the hydrographic regions, see Fig. 5.

Discussion

When revising the *costata*-group of Hexalonabranch, Sinev and Dumont (2016) separated *Flavalona* into two great clades which are differentiated by the

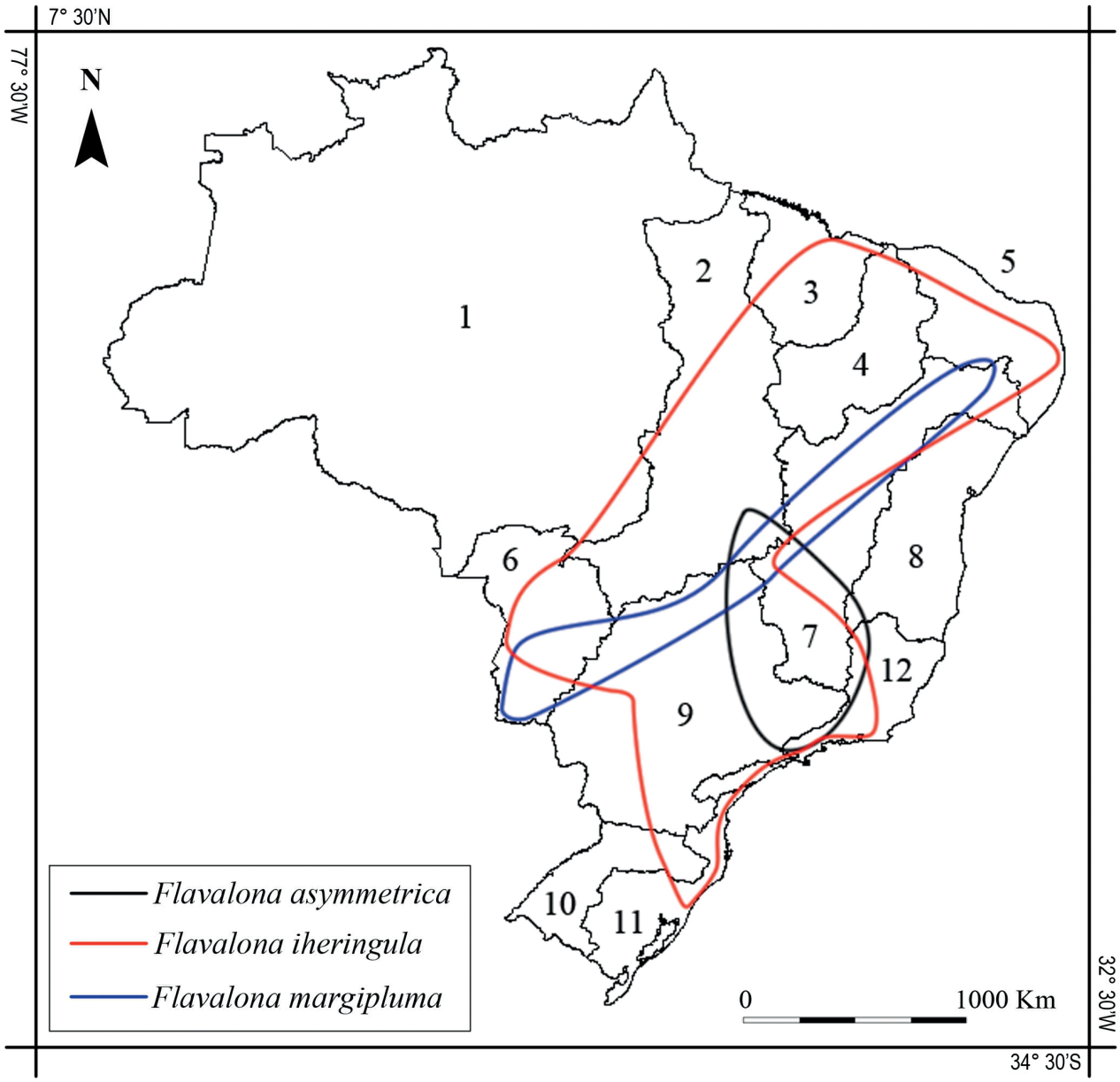

Figure 5. Approximated geographical range of *Flavalona* species in Brazil. The map presents the delimitation of Hydrographic Regions according to Resolution 32 from Conselho Nacional de Recursos Hídricos. 1 - Amazônica; 2 - Tocantins/Araguaia; 3 - Atlântico Nordeste Ocidental; - 4 Parnaíba; 5 - Nordeste Oriental; 6 - Paraguai; 7 - São Francisco; 8 - Atlântico Leste; 9 - Paraná; 10 - Uruguai; 11 - Atlântico Sul; 12 - Atlântico Sudeste.

morphology of lateral head pores and postabdomen of both females and males. *Flavalona asymmetrica* sp. nov. shares many important morphological traits with the *costata*-clade: (i) valves with pronounced linear sculpture, (ii) very elongated lateral head pores with length over 0.5 IP, (iii) distalmost setulae of each fascicle of the postabdomen thicker than others, (iv) genital process of the postabdomen male is short. The *costata*-clade is also composed of *F. costata*, *F. cheni*, *F. natalensis*, *F. margipluma*, and *F. weltneri* which are easily differentiated from *F. asymmetrica* sp. nov. in the morphology of main head pores. *Flavalona asymmetrica* sp. nov. has two main head pores while the aforementioned species have three (see Sinev and Dumont, 2016).

Within the *costata*-clade, the presence of two main head pores is only shared by *F. asymmetrica* sp. nov. and *F. setigera* (Sinev, 1999a; Sinev and Dumont, 2016). The presence of two main head pores was already used to justify the inclusion of *F. setigera* in the *Biapertura*

Smirnov, 1971 genus by Smirnov and Tims (1983). Currently, it is a consensus that *Biapertura* correspond to an artificial assemblage with many different lineages (see Frey, 1987; Sinev, 1999a; Van Damme *et al.*, 2011b; Sinev and Dumont, 2016; Sousa *et al.*, 2015b; Sousa *et al.*, 2016a). *Flavalona asymmetrica* sp. nov. and *F. setigera* are distributed along trans-Pacific areas, with *F. setigera* occurring in Australia, Indonesia, Tasmania, New Zealand, and some Pacific oceanic islands (Smirnov and Tims, 1983; Sinev, 1999a; Van Damme and Sinev, 2013; Sinev and Dumont, 2016). *Flavalona asymmetrica* sp. nov. is endemic from the Neotropics. Trans-Pacific distribution is not common in Cladocera, however, a similar pattern known as Amphi-Pacific disjunction was mentioned as a strong hypothesis of divergence between species of *Leydigiopsis* Sars, 1901 (Van Damme and Sinev, 2013), *brandorffi*-clade of *Anthalona* Van Damme, Sinev & Dumont, 2011 (Tiang-nga *et al.*, 2016), and *Magnospina* Sousa, Elmoor-Loureiro & Santos, 2016 (Sousa *et al.*, 2016b; Van Damme and Sinev, 2013).

The antiquity of Cladocera taxa of different ranks (Frey, 1987; Kotov and Taylor, 2011; Van Damme and Kotov, 2016) suggest that important morphological differences are expected between groups with divergence related to large-scale biogeographical pattern, for instance, Neotropical *Magnospina dentifera* (Sars, 1901) *versus* South-East Asian *Magnospina siamensis* (Sinev & Sanoamuang, 2007) (Sinev *et al.*, 2004; Sinev and Sanoamuang, 2007; Sousa *et al.*, 2016b). In the case of *F. asymmetrica* sp. nov. and *F. setigera*, morphological differences are observed in many structures and might be in part explained by biogeographical divergence. The rim of posterior main head pore about 1.3 times broader than anterior, PP about 0.7 IP, deep and elliptic sacs about 0.6 of length of the lateral pores (Fig. 1G, H). In *F. setigera* the main head pores has similar morphology, PP about 1.1–1.3 IP, semicircular sacs with length similar to lateral pores (Sinev and Dumont, 2016). The most important differences in the limbs of *F. setigera* and *F. asymmetrica* sp. nov. are related to length of seta 1 of IDL on the first limb, armature of scraper 3, 7–8 on the second limb, and ratio between first and second setae of third limb exopodite (Tab. 1). The main difference observed in the postabdomen of *F. asymmetrica* sp. nov. is the presence of eight to nine marginal denticles, while *F. setigera* has 10–14 marginal denticles (Sinev, 1999a; Sinev and Dumont, 2016).

Some studies have confirmed the potential of male morphology to distinguish species, and it is considered very important to the systematics of Chydoridae (Sinev, 1999b; 2013; 2015; Sinev and Sanoamuang, 2011; Sinev and Shiel, 2012; Kotov and Fuentes-Reines, 2015; Sousa *et al.* 2016b; 2016c). Males of *F. asymmetrica* sp. nov. have important differences from *F. setigera* males, for example: (i) they differ in length (0.37–0.46 in *F. asymmetrica* sp. nov. and 0.55–0.58 *in F. setigera*); (ii) setae 2–3 of IDL in *F. asymmetrica* sp. nov. are about 1.5 times shorter than females while in *F. setigera* this proportion is about 2; (iii) in *F. asymmetrica* sp. nov. the seta of the copulatory brush is markedly shorter than setae 2–3 of IDL, while in *F. setigera* this seta has similar length to IDL setae (Sinev, 1999a; Sinev and

Table 1. Main morphological differences between *Flavalona setigera* (Brehm, 1931) and *Flavalona asymmetrica* sp. nov.

	Flavalona setigera	*Flavalona asymmetrica* sp. nov.
Size (mm)	0.39–0.49	0.37–0.45
Rim around posterior main head pore	similar to anterior	1.3 times broader than anterior
Main head pores - PP	1.1–1.3 IP	about 0.7 IP
Lateral head pores - Depth of pockets/ length lateral pores	0.9–1	0.6
Lateral head pores – shape of pockets	semicircular	elliptic
P1 - IDL setae 1/2 ratio	0.25	0.3
P2 - denticles on scrapers 7-8	absent	present
P3 - exopodite setae 1/2 ratio	about 0.9	about 0.7
P4 - exopodite setae 4 and 5	different length	similar in length
P6 - height/width	2	2.4
PA - number of marginal denticles	10–14	8–9
Males - PA, marginal setulae	arranged in groups	not arranged in groups
Males - PA, basal spine	absent	present
Males - PA, genital process	about three times shorter than postabdominal claw	about ten times shorter than postabdominal claw

Dumont, 2016). The postabdomen of *F. asymmetrica* sp. nov. differs from *F. setigera* because it has setulae on the distal and anal margins not separated in groups and genital process about ten times shorter than length of postabdominal claw (Fig. 3J).

Even though a wide distribution in the Neotropics is expected, until now, *F. asymmetrica* sp. nov. has only been observed in Brazil (Fig. 5). This species can be found together with *F. margipluma* and *F. iheringula* (Santos-Wisniewski *et al.,* 2002; Sousa and Elmoor-Loureiro, 2012; 2013; Sousa *et al.,* 2013; 2014). *Flavalona margipluma* is part of the *costata*-clade but is easily differentiated from *F. asymmetrica* sp. nov. because it has three main head pores and postabdomen with 11–13 marginal denticles (Fig. 4G, J). *Flavalona iheringula* belongs to another clade, named as *rustica*-clade by Sinev and Dumont (2016). Differently from *F. asymmetrica* sp. nov., individuals of *F. iheringula* sometimes retain valves from the previous molts, presents three main head pores, lateral head pores relatively short with shallow circular sacs underneath them, postabdomen with distal angle rounded and markedly prominent (Fig. 4C–F).

In conclusion, populations from the Neotropics previously identified as *F. setigera* belong to a new species described here. The important morphological differences and speciation process involving *F. asymmetrica* sp. nov. and *F. setigera* can be associated with the Amphi-Pacific disjunction. Currently, this small group from the *costata*-clade has a trans-Pacific geographical distribution pattern. *Flavalona asymmetrica* sp. nov. can be found together with *F. iheringula* and *F. margipluma*, however, it is easily differentiated by external morphological traits. The effort to increase knowledge about cladoceran taxonomy in the Neotropics, especially in South America, is in progress, and our results represent just a small part of the necessary advance when taking account of the wide geographical extension of subcontinent.

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