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## Research article

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# Richness of *Dendrocephalus* (Branchiopoda, Anostraca) in Brazil with the description of two new species

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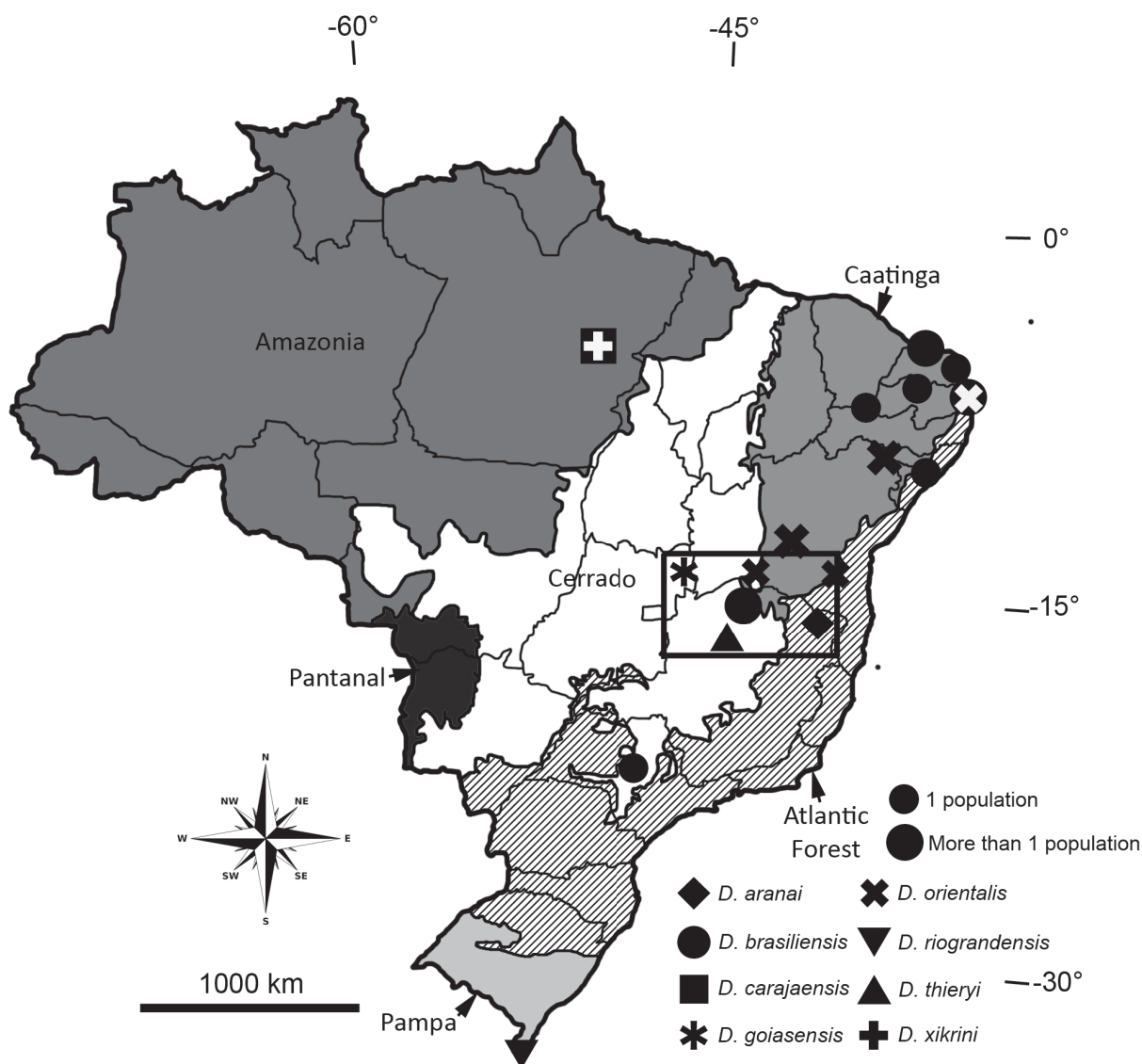
**Abstract.** We present an overview of the morphological diversity and geographical distribution of the anostracan genus *Dendrocephalus* Daday, 1908, and describe two new species: *D. aranai* sp. nov. from Jequitinhonha in the state of Minas Gerais and *D. xikrini* sp. nov. from the Carajás Mountains (Serra dos Carajás) in the state of Pará. These species have important similarities to *D. goiasensis* Rabet & Thiéry, 1996 and *D. thieryi* Rabet, 2006, respectively, but differ from them and each other through a combination of characters that are essentially unique to the endopods, and frontal appendage branch 2A and branch 2D. We also partly redescribe *D. carajaensis* Rogers, Gomes & Vieira, 2012, which shows a particular intra-populational variability in branch 2A and 2D III of the frontal appendage, a type of polymorphism that was also recently observed in *D. orientalis* Rabet & Thiéry, 1996 and which must now be taken into account in taxonomy. In terms of the distribution of species of *Dendrocephalus* in Brazil, we suggest that several other species are probably present in the Amazonian, Cerrado and Pantanal Biomes, which remain largely unexplored. A new taxonomic key for the identification of males of the Brazilian species is provided.

**Keywords.** Endemism, Minas Gerais, Pará, Serra dos Carajás, temporary ponds.

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## Introduction

Fairy shrimps (Anostraca) represent an important component of the diversity of temporary freshwater pools, yet only three genera have been recorded in Brazil: *Artemia* Leach, 1819, *Branchinecta* Verrill, 1869, and *Dendrocephalus* Daday, 1908 (Rabet & Thiéry 1998). Until now, the genus *Dendrocephalus* has included 18 species split into two subgenera (Rogers 2006): three in the subgenus *Dendrocephalinus* Rogers, 2006 distributed in the USA (Rogers 2006) and 15 species in the subgenus *Dendrocephalus* Daday, 1908 with a Neotropical distribution. Six species have been described in Brazil, but several authors have suggested that many other species might be present (Chaves *et al.* 2011; Rogers & Volcan 2016). Here, we synthesize the existing knowledge of the morphology and distribution of these different Brazilian species of *Dendrocephalus* and, thanks to new samples, we describe the seventh and eighth species from Brazil. We also review the intraspecific variability and discuss the taxonomic interest of some characters, as well as describing some details about the particular variability in *Dendrocephalus carajaensis* Rogers, Gomes & Vieira, 2012.



**Fig. 1.** Distribution of Brazilian *Dendrocephalus* Daday, 1908. The main biomes are indicated on the map, the empty black square delineates the hotspot of richness of *Dendrocephalus* in Brazil. The symbols are black except in the case of sympatry, where one of the species is in white.

## Material and methods

Specimens of *Dendrocephalus* were collected in two samples from different areas of Brazil. Their morphology revealed that they represent undescribed species: 15 specimens were collected by T. Chaves on 17 January 2009 in pool n° 4 in Jequitinhonha, Minas Gerais (16°25'10.47" S, 40°57'4.46" W) and 12 specimens were collected by R. Bozelli on 25 November 2013 in pool S11-BC in Serra dos Carajás, Pará (6°21'06.27" S, 50°23'43.66" W) (Figs 1, 7). These collections were stored in 70% ethanol and the specimens from Serra dos Carajás were placed in glycerine to facilitate handling. Morphological illustrations were prepared using a compound microscope with a camera lucida and eggs were prepared for scanning electron microscopy (JEOL JSM 6100) following Felgenhauer (1987), except that postfixation in osmium was not performed.

The holotypes, the allotypes and several paratypes were deposited at the Museu Nacional de Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); additional paratypes of *Dendrocephalus* are deposited at the Muséum national d'Histoire naturelle, Paris, France (MNHN), remaining material collected for this study is held in the personal collections of the first and third authors, N. Rabet (coll. NR) and R.L. Bozelli. The total length of the specimens was measured from the anterior margin of the head to the apices of the cercopods including the setae, and cercopod length was measured from the telson to the apex of the distal setae. Egg diameter was estimated as the average of three measurements made during SEM observations. Descriptive terminology of the frontal appendage follows Pereira (1983).

Other studied specimens were identified following adapted literature (Pereira 1983, 1984; Pereira & Belk 1987; Pereira & Ruiz 1995; Rabet & Thiéry 1996; Rabet 2006; Rogers *et al.* 2012; Hirose *et al.* 2015; Rogers & Volcan 2016; Barros-Alves *et al.* 2016).

## Results

All the known Brazilian populations of *Dendrocephalus* are geolocalized on the map of Brazil (Fig. 1).

### *New and updated species*

Class Branchiopoda Latreille, 1817  
Order Anostraca Sars, 1867  
Family Thamnocephalidae Packard, 1877  
Genus *Dendrocephalus* Daday, 1908  
Subgenus *Dendrocephalus* (*sensu stricto*) Daday, 1908

*Dendrocephalus aranai* Rabet & Lacau sp. nov.

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Figs 1–3, 7H

### Etymology

‘Aranã’ is the Portuguese (Brazil) name for an indigenous Brazilian tribe in the ‘Vale do Jequitinhonha’ region, which is near the type locality. Today, their population has been nearly extinguished, so the new species is named in honour of these people.

### Material examined

#### Holotype

BRAZIL: mature ♂, total length 24.1 mm, cercopods 4.2 mm, Minas Gerais, Jequitinhonha, 16°25'10.47" S, 40°57'4.46" W, temporary pool n° 4, 17 Jan. 2009, T. Chaves leg. (MNRJ 27970) (Fig. 7H).

### Allotype

BRAZIL: mature ♀, total length 21.7 mm, brood pouch 5.5 mm reaching the 5<sup>th</sup> abdominal segment, cercopods 4.1 mm, same collecting data as for holotype (MNRJ 27971).

### Paratypes

BRAZIL: 8 ♂♂, total length 14.5 to 25.1 mm, mean =  $19.55 \pm 2.95$  mm, cercopods 2.5 to 4.2 mm, mean =  $3.28 \pm 0.5$  mm, same collecting data as for holotype; 4 ♀♀, total length 17 to 20.6 mm, mean =  $18.33 \pm 1.58$  mm, brood pouch 4 to 4.7 mm, mean =  $4.43 \pm 0.31$  mm, cercopods 3.1 to 3.6 mm, mean =  $3.25 \pm 0.31$  mm, same collecting data as for holotype (2 ♂♂, 2 ♀♀ in MNHN (MNHN-IU-2016-3558); 2 ♂♂, 1 ♀ in MNRJ (MNRJ 27972)). Other specimens kept in NR's personal collection.

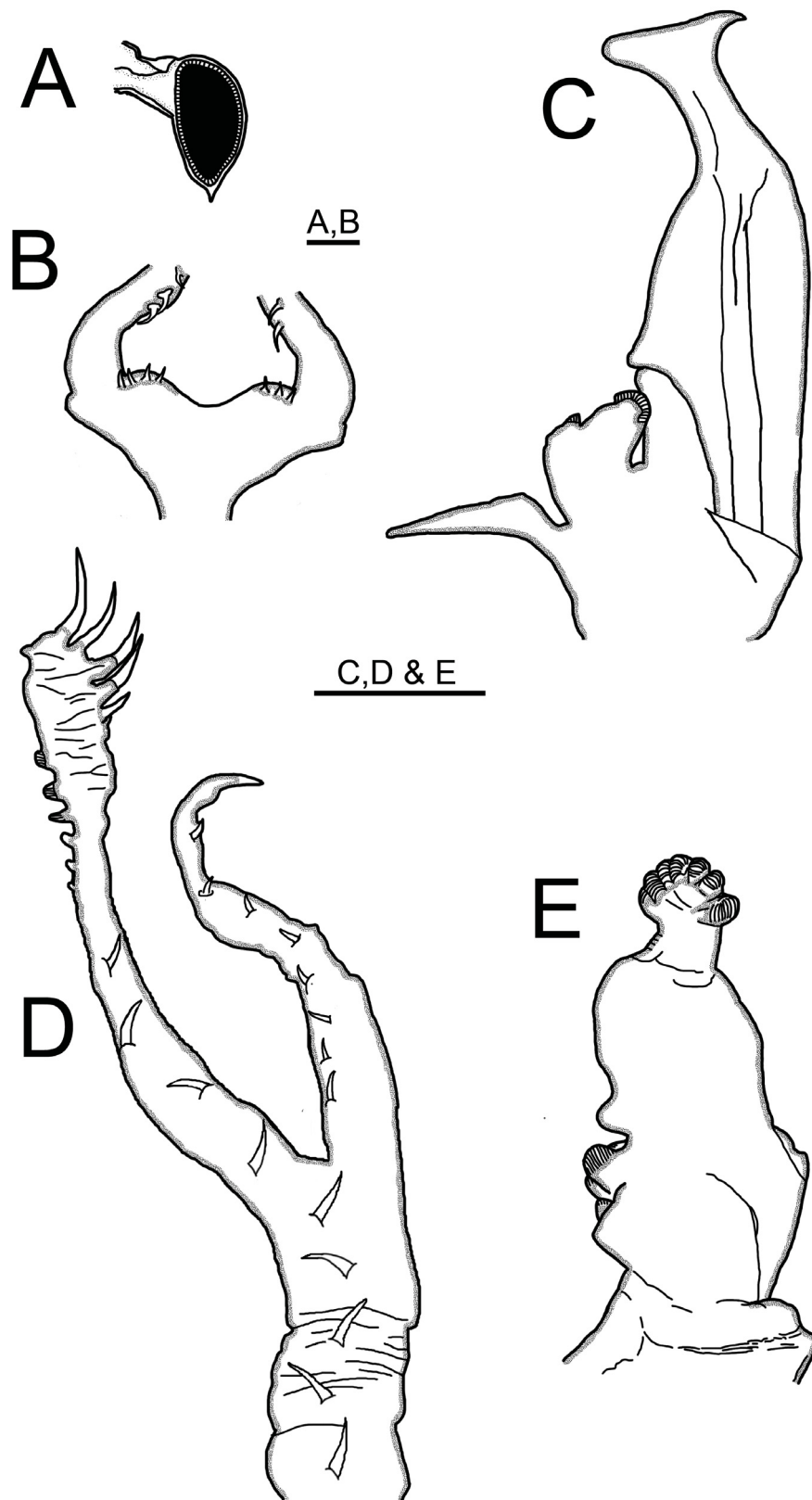
### Type locality

The pool (Fig.7H) is in a well-lit location at a climatic crossroads with influences from the Atlantic Forest, Cerrado and lesser Caatinga. The other large branchiopods sampled in the same ponds are *Eulimnadia colombiensis* Roessler, 1989.

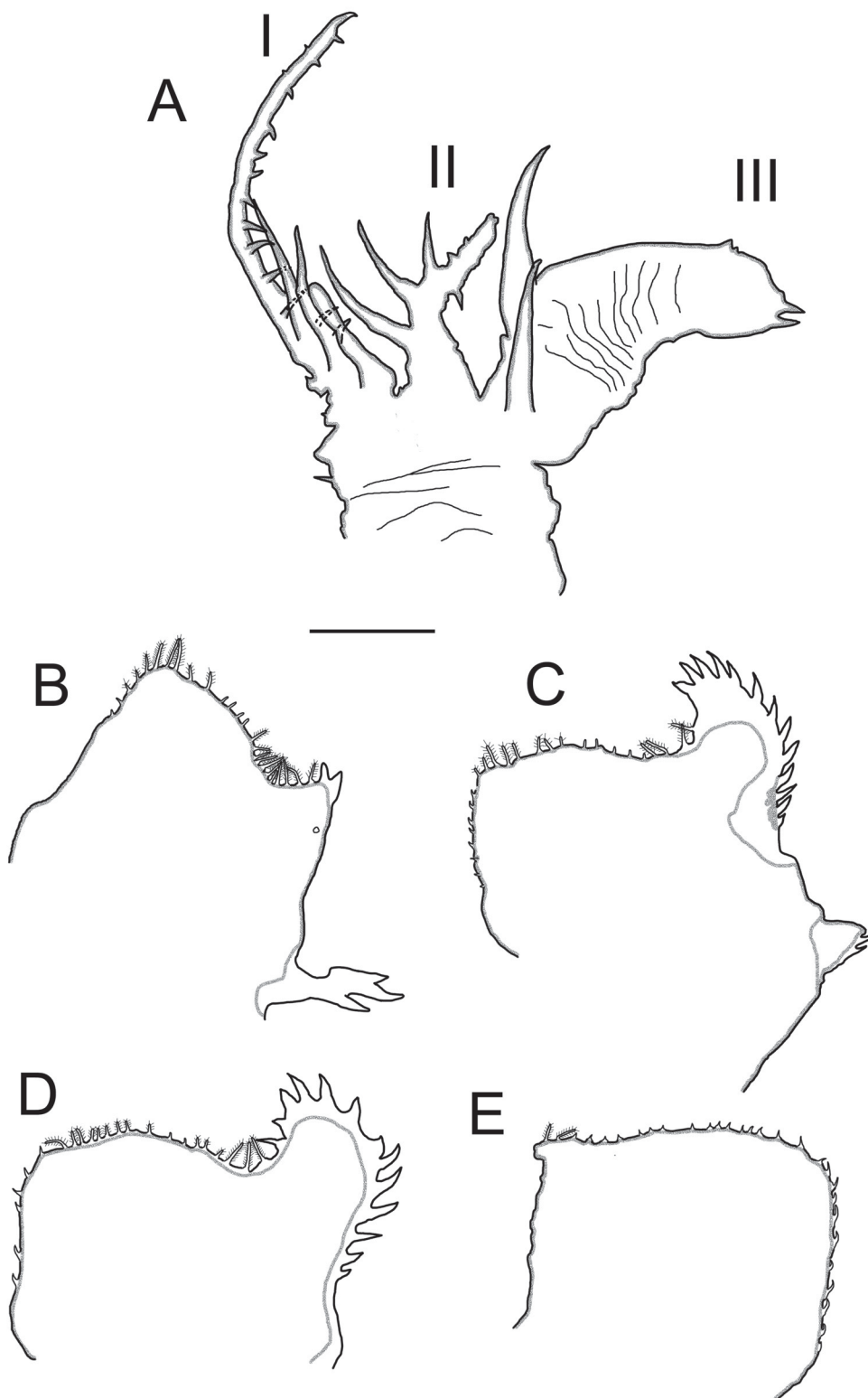
### Description

#### Male

Eye pedunculate, ovoid in lateral view with a prominent spine. Length of the spine relative to the eye between 10 and 20% (Fig. 2A). Antenna-like outgrowth slender, lying between first antennae and second antennae. Second antennae with proximal antennomeres fused basally on the anterior of the head. Proximal antennomere mediolaterally bearing a stout digitiform process ornamented with setae. Distal antennomere weakly sclerotized, evenly curved medially, and ornamented on the medial surface with scaliform transverse ridges; terminus acute. Frontal appendages with anterior margin of the base of the arms with three or four spines (Fig. 2B). Arms from the base to the terminal branches with spines (Fig. 2B). Frontal appendage complex with one ventral branch (1V) and three terminal branches (2V, 2D, 2A). The terminal appendage in the medial position, called branch 2A, with a podiform apex and one long cell pad present proximally, parallel to the main axis, and a more lateral structure resembling a 'cell pad' on a bulge. A long spiniform process is present basally (Fig. 2C). A ventral branch called branch 1V with two sub-branches. Sub-branch I (most proximal) with one row of spines on the medial side, a swollen extremity with five spines posteriorly oriented and three to five small cell pads anteriorly oriented. Branch 1V distally ramified in two sub-branches, both with a longitudinal row of medial spines (Fig. 2D). Sub-branch II  $\frac{3}{4}$  of the length of sub-branch I and with a row of short spines on the medial side with an acute extremity (Fig. 2D). Terminal and ventral branch called branch 2V cylindrical and ending in a cluster of five to seven cell-pads on a bulge, with two rows of cell pads and a long cell pad on the medial surface (Fig. 2E). Terminal branch in the dorsal position called branch 2D is subdivided into three sub-branches. Sub-branch I (most proximal) with a row of short spines on the medial side and with three long spines (length more than four times the diameter of the sub-branch) (Fig. 3A). Sub-branch II shorter than half of sub-branch I with three long spines (gradually decreasing in size towards the apical part from three times the length to twice the length of the diameter of the sub-branch) (Fig. 3A). Sub-branch III  $\frac{3}{4}$  shorter and five times wider than sub-branch I, bearing two large spiniform processes, and with a distally flattened portion with two spines on the posterolateral side (Fig. 3A). Endopodite of the first pair of thoracopods with a basolateral lobe ending in one to four smooth spines and a distolateral non-prominent border with two spines (Fig. 3B). Endopodites of limb 2 with a prominent distolateral border ornamented with 12–13 spines and a strong, small extension with two small spines (Fig. 3C). Endopodites of limb 3 with a prominent distolateral border ornamented with 12–13 spines (Fig. 3D). Endopodites of limb 4 with only eight small spines on the distolateral corner (Fig. 3E). No differentiation was observed in the fourth limbs (Fig. 3E). Abdominal segments smooth. Cercopods margined with plumose setae.



**Fig. 2.** *Dendrocephalus aranei* Rabet & Lacau sp. nov., ♂. **A.** Eye with a spine on the posterior edge. **B.** Basal part of the frontal appendage. **C.** Branch 2A of the frontal appendage. **D.** Branch 1V. **E.** Branch 2V. Scale bars: 1 mm.



**Fig. 3.** *Dendrocephalus aranai* Rabet & Lacau sp. nov., ♂. **A.** Branch 2D of the frontal appendage. **B.** Endopodite of the first limb. **C.** Endopodite of the second limb. **D.** Endopodite of the third limb. **E.** Endopodite of the fourth limb. Scale bar: 0.5 mm.

### Female

Typical of the genus.

### Resting egg

Subspherical with broad pentagonal or quadrangular facies. Diameter ranging from 222 to 247  $\mu\text{m}$ , mean =  $236.1 \pm 7.79$   $\mu\text{m}$  ( $n = 8$ , diameter based on three measurements for each egg). Depression number ranging from 7 to 8, mean =  $7.88 \pm 0.35$  ( $n = 8$ ).

### Distribution

This species is known from several pools in the area next to the *locus typicus*.

*Dendrocephalus carajaensis* Rogers, Gomes & Vieira, 2012  
Figs 4, 7B

### Material examined

BRAZIL: 12 animals, Pará, Serra dos Carajás, pool N1-A, 20 Nov. 2011, R. Bozelli leg. (Reinaldo Bozelli personal collection); 12 animals, Pará, Serra dos Carajás, pool N1-C, 22 Oct. 2013, R. Bozelli leg. (Reinaldo Bozelli personal collection); 12 animals, Pará, Serra dos Carajás, pool N7, 23 Oct. 2013, R. Bozelli leg. (Reinaldo Bozelli personal collection); 12 animals, Pará, Serra dos Carajás, pool S11-DC, 24 Nov. 2013, R. Bozelli leg. (Reinaldo Bozelli personal collection) (Fig. 7B).

### Description

Similar to the original description (see Rogers *et al.* 2012) including the following variations: branch 2V variable with one to three extremities, the tip of each with a cell pad. Branch 2A with a basal spine of varying shape from sinuate to straight (Fig. 4A–B). One or two basal cell pads produced. Anterior end sometimes arcuate or straight but always acute apically (Fig. 4A–B). Sub-branch III of branch 2D with varying shape from monoramal to biramal with various intermediate states. In some cases, three or five spiniform projections present in the posterior face of the sub-branch (Fig. 4C–D). In some individuals, two projections can fuse to form an elongate projection with two acute extremities (Fig. 4E–F) or form an elongate ramus sharing spiniform projections as in the original description (see Rogers *et al.* 2012).

### Habitat

The specimens studied in the present work were all collected in natural pools in an Amazonian savannah in the Serra dos Carajás (Canga deposits), see Fig. 7B.

*Dendrocephalus xikrini* Rabet & Bozelli sp. nov.  
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Figs 1, 5–6, 7I

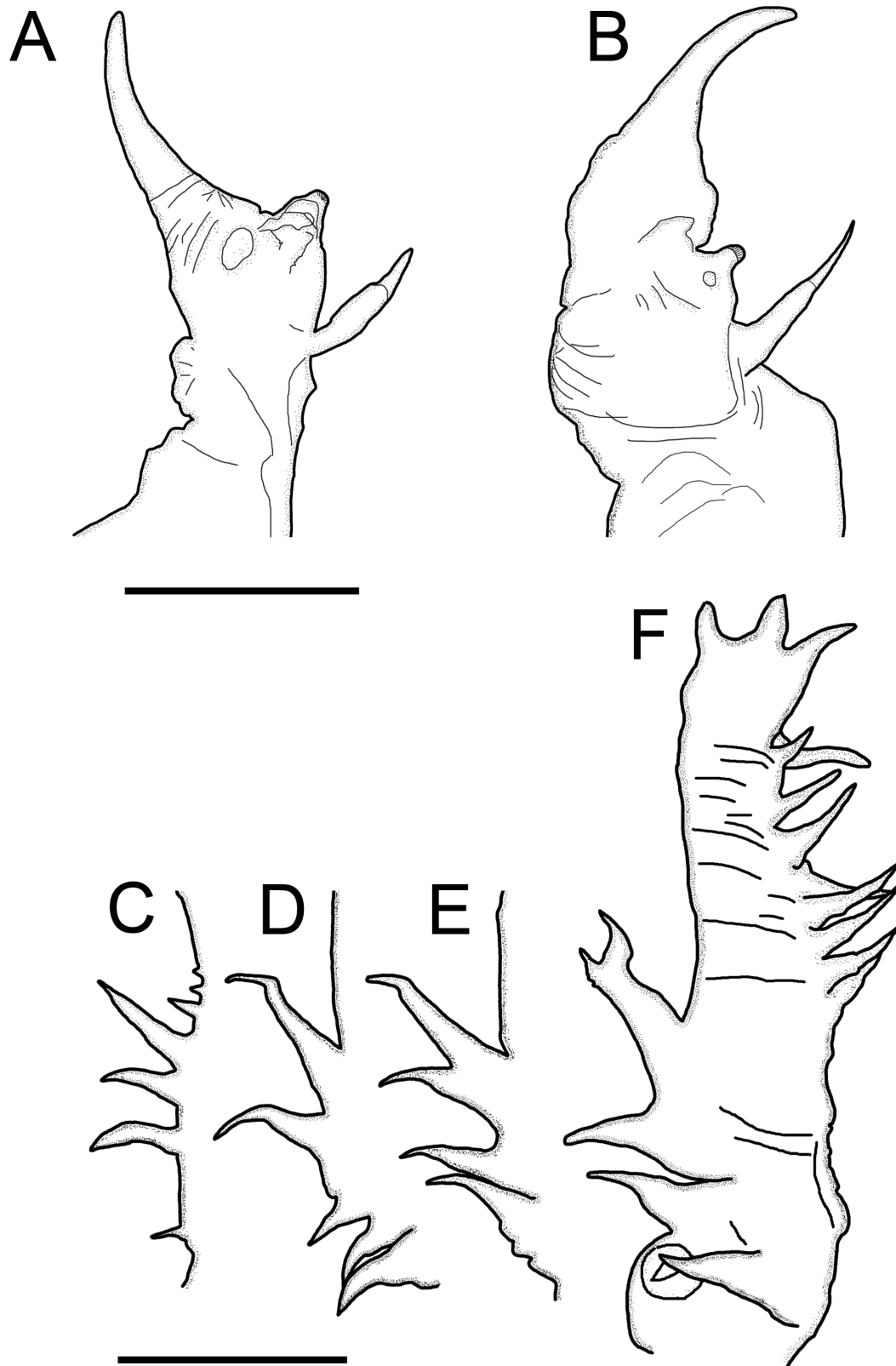
### Etymology

Named in honour of the Xikrin, a subgroup of indigenous people from the Kayapó tribes living in the area of the Serra dos Carajás.

### Material examined

#### Holotype

BRAZIL: mature ♂, total length 15.0 mm, cercopods 2.8 mm, Serra dos Carajás, Pará, 6°21'06.27" S, 50°23'43.66" W, pool S11-BC in an Amazonian savannah (Canga deposits), 25 Nov. 2013, R. Bozelli



**Fig. 4.** Morphological variation in the frontal appendage of *Dendrocephalus carajaensis* Rogers, Gomes & Vieira, 2012, ♂. **A–B.** Branch 2A from two specimens. **C–F.** Sub-branch III of branch 2D from different specimens. Scale bars: 0.5 mm.



leg. (MNRJ 2973). In the same study period, approximately 30 phytoplankton species, 30 zooplankton species and 15 aquatic macrophyte species were recorded in the pool, but no fish were observed.

### Allotype

BRAZIL: mature ♀, total length 12.8 mm, brood pouch 2.9 mm reaching the extremity of the 6<sup>th</sup> abdominal segment (including genital segments), cercopods 2.7 mm, same collecting data as for holotype (MNRJ 2974).

### Paratypes

BRAZIL: 8 ♂♂, same collecting data as for holotype, total length 15.1 to 16.5 mm, mean =  $15.75 \pm 0.58$  mm, cercopods 2.3 to 2.7 mm, mean =  $2.56 \pm 0.15$  mm; 1 ♀, same collecting data as for holotype, length 13.5 mm, brood pouch 3 mm, cercopods 3 mm (2 ♂♂, 1 ♀ MNHN (MNHN-IU-2016-3559); 2 ♂♂ in MNRJ (MNRJ 2975)). Other specimens are kept in personal collections of NR and RLB.

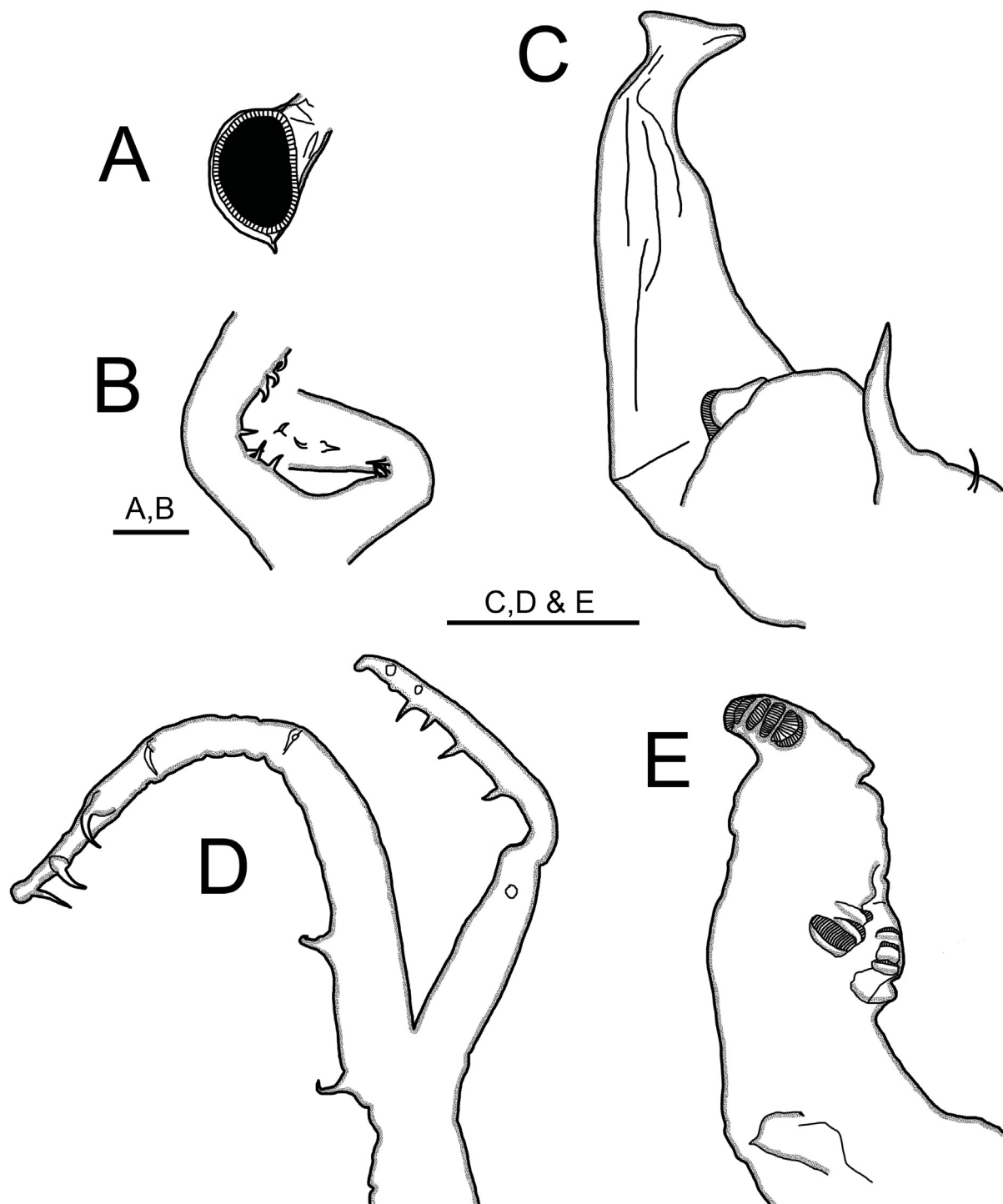
### Type locality (Figs 1, 7I)

Pool S11-BC is the smallest (average area of 0.13 ha) among the previously studied lentic aquatic environments in the Serra dos Carajás inside the Carajás National Forest. Although very shallow (0.10 to 0.25 m) during our sporadic explorations from 2005–2013, the pool was never found completely dry, and during this period, its volume ranged from 56 to 140 m<sup>3</sup>. Nevertheless, it is a temporary pool because it was possible to verify that the pool dried up in Google Earth images from August 2006. The electrical conductivity of the water varies between 4 and 64  $\mu\text{S}/\text{cm}$  and pH varied between 4.89 and 5.63. The recorded turbidity values were between 1 and 64 NTU, but the water was always completely transparent.

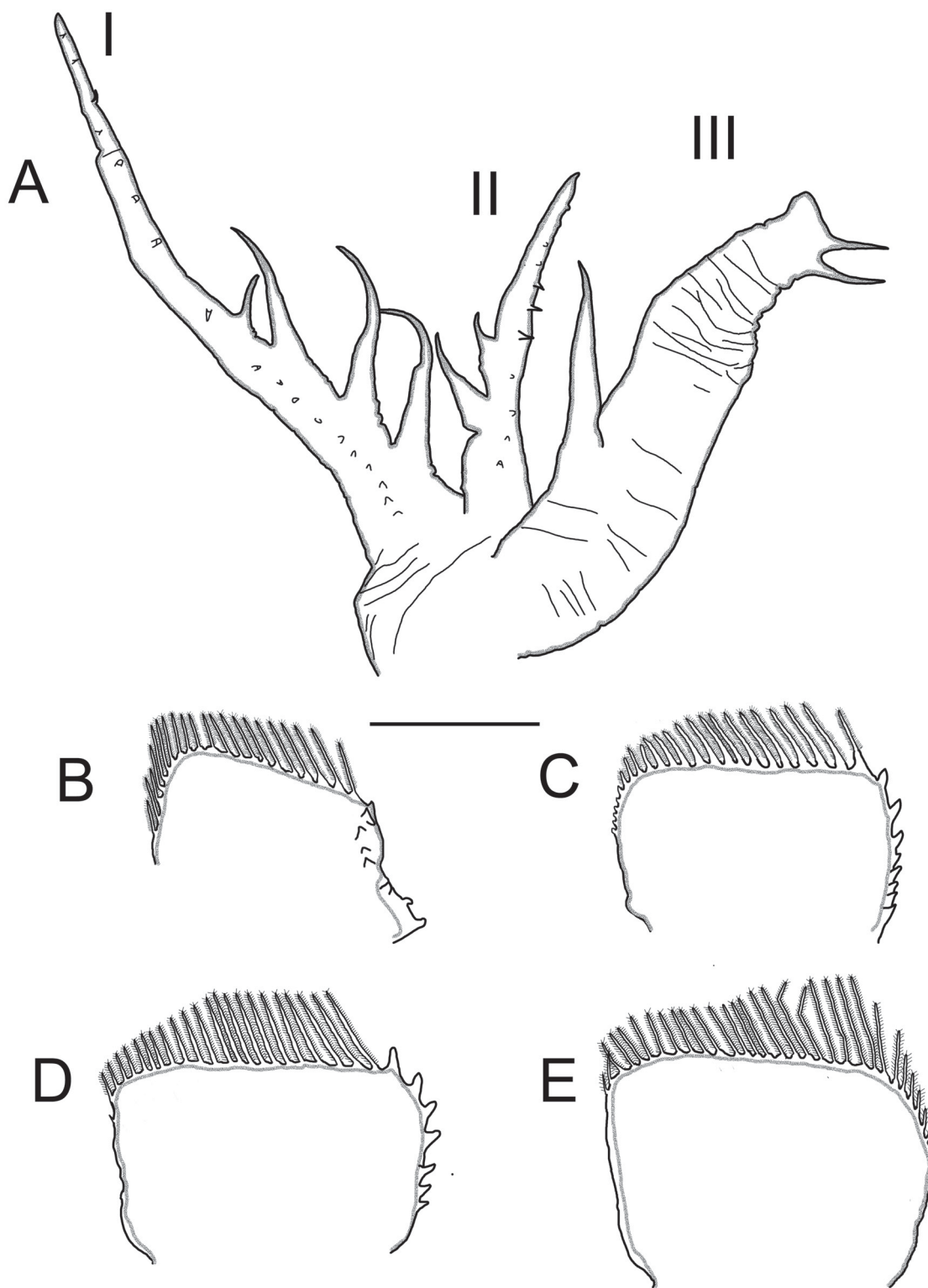
## Description

### Male

Eye pedunculate, ovoid in lateral view with a prominent spine. Length of the spine relative to that of the eye between 10 and 20% (Fig. 5A). Antenna-like outgrowth slender, lying between the first antennae and second antennae. Second antennae with proximal antennomeres fused basally on the anterior of the head. Proximal antennomere mediolaterally bearing a stout digitiform process ornamented with setae. Distal antennomere weakly sclerotized, evenly curved medially, and ornamented on the medial surface with scaliform transverse ridges; terminus acute. Frontal appendages with anterior margin of the base of the arms with three or four spines (Fig. 5B). Arms from the base to the terminal branches with spines (Fig. 5B). Frontal appendage complex with one ventral branch (1V) and three terminal branches (2V, 2D, and 2A). The terminal appendage in the medial position called branch 2A with a podiform apex and one well-developed cell-pad on the first third of the branch. A long spiniform process is present basally (Fig. 5C). Ventral branch called branch 1V with two sub-branches. Sub-branch I (most proximal) with one row of spines on the medial side with an acute extremity (Fig. 5D). Sub-branch II  $\frac{3}{4}$  of the length of sub-branch I and with a row of short spines on the medial side with an acute extremity (Fig. 5D). Terminal ventral branch called branch 2V cylindrical and ending in a cluster of four to five cell-pads on a bulge and four rows of cell pads on the medial surface (Fig. 5E). Terminal branch in the dorsal position called branch 2D subdivided into three sub-branches. Sub-branch I (most proximal) with a row of short spines on the medial side and with three or four long spines (length of which is more than twice the diameter of the sub-branch) in the first half of the sub-branch (Fig. 6A). Sub-branch II longer than half of sub-branch I, with a row of small spines on the medial side and with two long spines (the length of which is greater than or equal to the diameter of the sub-branch) in the first half and half the length of the sub-branch (Fig. 6A). Sub-branch III slightly shorter than sub-branch I (80%) with twice the average diameter, proximally cylindrical and distally flattened and bearing a large spiniform process; distal flattened portion with two or three long spines (equalling the width of the sub-branch) on the posterolateral side (Fig. 6A). Endopodite of the first pair of limbs with a reduced basolateral lobe sharing



**Fig. 5.** *Dendrocephalus xikrini* Rabet & Bozelli sp. nov., ♂. **A.** Eye with a spine on the posterior edge. **B.** Basal part of the frontal appendage. **C.** Branch 2A of the frontal appendage. **D.** Branch 1V. **E.** Branch 2V. Scale bars: 1 mm.



**Fig. 6.** *Dendrocephalus xikrini* Rabet & Bozelli sp. nov., ♂. **A.** Branch 2D of the frontal appendage. **B.** Endopodite of the first limb. **C.** Endopodite of the second limb. **D.** Endopodite of the third limb. **E.** Endopodite of the fourth limb. Scale bar: 0.5 mm.

small spines that form a row extending to the distal corner (Fig. 6B). Endopodite of the second pair of limbs with a border sharing large spines (Fig. 6C). Endopodite of the third pair of limbs similar to the second with slightly larger spines (Fig. 6D). No differentiation was observed in the fourth pair of limbs (Fig. 6E). Abdominal segments smooth. Cercopods margined with plumose setae.

#### **Female**

Typical of the genus.

#### **Resting egg**

Similar to those of *D. aranai* sp. nov., subspherical with broad pentagonal or quadrangular facies.

#### **Distribution**

Known only from the *locus typicus*. Other pools prospected in the Carajás Mountains are inhabited by *D. carajaensis* only.

#### ***Other Brazilian species examined***

From the studied samples and the literature (Pesta 1921; Lutz 1929; Lemos de Castro & Lima 1986; Rabet & Thiéry 1996; Rabet 2006; Chaves *et al.* 2011; Rogers *et al.* 2012; Hirose *et al.* 2015; Rogers & Volcan 2016; Barros-Alves *et al.* 2016), we analysed the morphological characters shared by the frontal appendage and the limbs of all Brazilian species of *Dendrocephalus* and summarized these observations in Tables 1–3. All the known locations of *Dendrocephalus* are indicated on the map of Brazil (Fig. 1).

#### ***Dendrocephalus brasiliensis* Pesta, 1921**

##### **Material examined**

BRAZIL: 10 animals, Rio Grande do Norte, Caicó, fish tank, 6 Aug. 1993, N. Rabet leg.; 2 animals, Rio Grande do Norte, Mossoró, ESAM, 27 Feb. 1980, Lemos de Castro and P. Coelho leg.; 10 animals, Paraíba, João Pessoa, temporary pools, 18 Jul. 1993, N. Rabet leg.; 2 animals, Minas Gerais, Januária, 15°05'03.3" S, 44°05'21.4" W, temporary pool, 7 Feb. 1994, W. Costa, G. Brasil and C. Campinha leg.; 2 animals, Minas Gerais, Januária, temporary pool, 6 Feb. 2003, J. Goma Pinto and D. Pillet leg.; 4 animals, Ceará, Brejo Santo, Açude Atalhos, 6 Dec. 1998, U. Caramaschi, R.N. Feio and H. De Nieweyer leg. (MNRJ 15893) (Fig. 7A). All specimens kept in NR's personal collection.

#### ***Dendrocephalus goiasensis* Rabet & Thiéry, 1996**

##### **Material examined**

BRAZIL: 4 paratypes, Goiás, Iaciara, temporary pool, 16 Jan. 1989, W. Costa and J. C. Oliveira leg.; 2 animals, Goiás, Iaciara, temporary pool, 2 Feb. 2003, J. Goma Pinto and D. Pillet leg. (Fig. 7C). All specimens kept in NR's personal collection.

#### ***Dendrocephalus orientalis* Rabet & Thiéry, 1996**

##### **Material examined**

BRAZIL: 5 paratypes, Paraíba, João Pessoa, Cabo Branco, temporary pond, 21 Jul. 1993, N. Rabet leg.; 4 animals, Bahia, near Oliveira dos Brejinhos, temporary pond along the Caturama-Macaúbas and Boquira-Beira Rio roads, 25–26 Jan. 1994, P.S. Young and M.C. Britto-Pereira leg.; 4 animals, Bahia, Jequié, 23 Jan. 2002, S. Lacau leg. (Fig. 7D); 4 animals, Bahia, Palmas de Monte Alto, rock pool, 23 Dec. 2014, M.L. Oliveira, N.S. Silva, H. Gonçalves and J.G. Neto leg. (Fig. 7E). All specimens kept in NR's personal collection.



**Fig. 7.** Photographs of different temporary pools inhabited by *Dendrocephalus* Daday, 1908 in Brazil. **A.** *Dendrocephalus brasiliensis* Pesta, 1921 from Ceará. **B.** *D. carajensis* Rogers, Gomes & Vieira, 2012 from Serra dos Carajás, Pará. **C.** *D. goiásensis* Rabet & Thiéry, 1996 from Iaciara, Goiás. **D.** *D. orientalis* Rabet & Thiéry, 1996 from Jequié, Bahia. **E.** *D. orientalis* from Palmas de Monte Alto, Bahia. **F–G.** *D. thieryi* Rabet, 2006 from Buritizeiro, Minas Gerais. **H.** *Locus typicus* of *D. aramai* Rabet & Lacau sp. nov. from Jequitinhonha, Minas Gerais. **I.** *Locus typicus* of *D. xikrini* Rabet & Bozelli sp. nov. from Serra dos Carajás, Pará.

**Table 1.** Cephalic richness in Brazilian *Dendrocephalus* Daday, 1908. The bold characters correspond to characters allowing a direct determination of species in Brazil. Sub-branch II is indicated as short when it was less than half of sub-branch I.

Brazilian species	Eye spines	Spines on Primary branch	Primary branch from base to terminal branches with spines	Branch IV		Shape of the extremity of branch 2A
				Extremity of Sub-branch I	Sub-branch II	
<i>D. aranaei</i>	1	3–4	Yes	Swollen	Long	podiform
<i>D. brasiliensis</i>	0	0	Yes	Swollen	Short	podiform
<i>D. carajaensis</i>	0	0	<b>No</b>	Swollen	Short	<b>acute</b>
<i>D. goiasensis</i>	1	3–4	Yes	Acute	Long	podiform
<i>D. orientalis</i>	<b>2 (0–2)</b>	<b>1 (0–3)</b>	Yes	Swollen	Long	<b>unguiform</b>
<i>D. riograndensis</i>	1	3	Yes	Acute	Short	podiform
<i>D. thieryi</i>	1	3–4	Yes	Swollen	Long	podiform
<i>D. xikrini</i>	1	3–4	Yes	Acute	Long	podiform

*Dendrocephalus thieryi* Rabet, 2006**Material examined**

BRAZIL: 4 paratypes, Minas Gerais, Buritizeiro, temporary pond, 6 Feb. 1994, W. Costa, G. Campinha and G. Campelo leg.; 4 animals, Buritizeiro, pool 10, temporary pond, 5 Dec. 2008, L. Godinho leg. (Fig. 7F); 4 animals, Minas Gerais, Buritizeiro; pool 20, artificial temporary pond, 6 Dec. 2008, L. Godinho leg. (Fig. 7G). All specimens kept in NR's personal collection.

**Discussion****Habitat and distribution**

Species of *Dendrocephalus* inhabit temporary aquatic habitats in different climatic zones (see Figs 1, 7), particularly pools with variable types of sediment including granitic rock (Fig. 7E). Therefore, there are at least eight species of *Dendrocephalus* that live in tropical or subtropical areas in Brazil (this study), while *Branchinecta*, the sole other freshwater genus of Anostraca living in the country, is rather restricted to the subtropical zones of Southern Brazil (Lilljeborg 1889; Rabet & Thiéry 1998) or at lower latitudes in the mountains of Minas Gerais (Rogers & Ferreira, 2007).

Sympatry of species of *Dendrocephalus* does not appear to be widespread in Brazil and to date has been observed around João Pessoa, where *D. orientalis* and *D. brasiliensis* have even been found in the same pools (Rabet & Thiéry 1996), as well as in the Serra dos Carajás, where *D. carajaensis* sp. nov. and *D. xikrini* sp. nov. live in the same area (this paper). However, in the latter case, the species have not yet been found in the same pool.

Distribution of *Dendrocephalus* reflects that Brazil has not yet been completely surveyed. Indeed, surveys for anostracans have still to be conducted in the Pantanal Biome or in a large part of the Cerrado and Amazonian savannah, which remain unexplored. It also appears that dense forest environments do not favour the fairy shrimps, which would explain their rarity in the east which has traditionally been more intensively studied. Fairy shrimps seem to have only been collected in forest margins or highland areas of savannah, such as in the Serra dos Carajás, or in environments influenced more by seasonal dry climates, such as in the Northern Atlantic Forest (João Pessoa) or the interior of Minas Gerais (Jequitinhonha).

**Table 2.** Richness in Brazilian *Dendrocephalus* Daday, 1908. The bold characters correspond to characters allowing a direct determination of species in Brazil.

Brazilian species	Extremity 2V	Branch 2D				
	Number of cell pads	Long spines at base of sub-branch I	Relative length of sub-branch II vs sub-branch I	Long spines at base of sub-branch II	Shape of the extremity of sub-branch III	Long spines at base of sub-branch III
<i>D. aranai</i>	> 4	2	More than ½	3	Thick and flat with 2 terminal small spines	2
<i>D. brasiliensis</i>	> 4	3	<b>Less than ½</b>	0	Thick and flat, small spines in internal and external part	1
<i>D. carajaensis</i>	1–3	0	<b>Very reduced</b>	0	<b>Thick and flat with many small spines in external part</b>	<b>0</b>
<i>D. goiasensis</i>	> 4	3	More than ½	4	Thick and flat with 3 external spines	2
<i>D. orientalis</i>	> 4	0	More than ½	0	<b>Cylindrical</b>	2
<i>D. riograndensis</i>	2	4	More than ½	2	<b>Apex asymmetrical, sub-truncate</b>	2
<i>D. thieryi</i>	> 4	0	More or less ½	0	Thick and flat with external and some internal small spines	1
<i>D. xikrini</i>	> 4	3	More than ½	2	Thick and flat with 2 external long spines	1

### Hotspot richness

The distribution of *Dendrocephalus* also indicates that there is a hotspot of Anostraca richness in the climate interface between the Cerrado, Caatinga and the Atlantic Forest. Indeed, we have listed five species in an area of approx. 400 x 800 km that includes Iaciara, Jequié, Palmas de Monte Alto and Buritizeiro (approximately 400 km by 800 km), which means that more than 60% of the known species for Brazil can be found in less than 4% of the surface area of the country (Fig. 1).

Another interesting point is the importance of rugged terrain culminating in isolated ferruginous rock plateaux, called Canga, in the diversification of fairy shrimps. Indeed, the discovery of *D. xikrini* sp. nov. brings the total number of fairy shrimps present on this type of substrate in Brazil to three; *Branchinecta*

**Table 3.** Endopodite richness in Brazilian *Dendrocephalus* Daday, 1908. The bold characters correspond to characters allowing a direct determination of species in Brazil.

Brazilian species	1 <sup>st</sup> endopodite		2 <sup>nd</sup> endopodite		3 <sup>rd</sup> endopodite	
	Exterior proximal corner	Exterior distal corner	Exterior proximal corner	Exterior distal corner	Exterior proximal corner	Exterior distal corner
<i>D. aranai</i>	Expansion longer than wide, ending with a few spines	Small spiny lobe	<b>Expansion shorter than wide, ending with a few short spines</b>	Pronounced spiny lobe	No expansion	Pronounced spiny lobe
<i>D. brasiliensis</i>	No expansion	Spiny border	No expansion	Spiny border	No expansion	Spiny border
<i>D. carajaensis</i>	No expansion	Small spiny lobe	No expansion	Pronounced spiny lobe	No expansion	Small spiny lobe
<i>D. goiasensis</i>	Expansion longer than wide, ending with a few spines	Small spiny lobe	No expansion	Small spiny lobe	No expansion	Pronounced spiny lobe
<i>D. orientalis</i>	Expansion longer than wide, ending with a few spines	Spiny border	No expansion	Spiny border	No expansion	Spiny border
<i>D. riograndensis</i>	<b>Spiniform expansion</b>	Pronounced spiny lobe	No expansion	No differentiation	No expansion	No differentiation
<i>D. thieri</i>	Expansion longer than wide, ending with a few spines	Small spiny lobe	<b>Spiniform expansion</b>	Pronounced spiny lobe	No expansion	Pronounced spiny lobe
<i>D. xikrini</i>	Expansion shorter than wide	Spiny border	No expansion	Spiny border	No expansion	Spiny border

*ferrolimneta* Rogers & Ferreira, 2007 was discovered in Minas Gerais (Rogers & Ferreira 2007) and *D. carajaensis* was also collected in Serra dos Carajás (Rogers *et al.* 2012). This type of temporary pool is particularly interesting, especially because mining activities in these areas threaten these highly vulnerable species with habitat destruction (Rogers & Ferreira 2007; Rogers *et al.* 2012).

### Differential diagnosis

The two new species described here bear an eye spine, three or four spines at the base of the frontal appendage primary branches, a podiform extremity on branch 2A and a relatively massive sub-branch III of branch 2D, which is similar to *D. cornosuris* Pereira & Ruiz, 1995, *D. cornutus* Pereira & Belk, 1987, *D. spartanovae* Margalef, 1961 and the Brazilian species *D. goiasensis* Rabet & Thiéry, 1996, *D. thieri* Rabet, 2006 and *D. riograndensis* Rogers & Volcan, 2016. Essentially, the differences between these species are therefore in the shapes of branch 1V, branch 2D and the limbs; *D. cornosuris*, *D. cornutus*, *D. spartanovae* and *D. riograndensis* have a smooth and acute spiniform expansion at the base of the endopodite of limb I whereas *D. aranai* sp. nov. and *D. xikrini* sp. nov. have a basal expansion with small spines. The two new species are relatively similar, but they can be easily separated by the shape of the extremity of branch 1V, which is acute in *D. xikrini* sp. nov. and swollen in *D. aranai* sp. nov., as well as by the shape of the limbs. The endopodites of limb I in *D. xikrini* sp. nov. have a short basal expansion,



whereas those in *D. aranai* sp. nov. have a long basal expansion. Additionally, the endopodites of limb II and III in *D. aranai* sp. nov. have a spinous lobe that is absent in *D. xikrini* sp. nov. *D. aranai* sp. nov. and *D. goiasensis* have similar limbs and 2D branches, but differ in the end of branch 1V which is acute in *D. goiasensis* but swollen in *D. aranai* sp. nov. *D. aranai* sp. nov. is also very similar to *D. thieryi*, with which it was compared in the key by *Chaves et al.* (2011), notably at the level of branch 1V. However, *D. thieryi* is very different due to the absence of long spines at the base of the endopodites of sub-branches I and II of branch 2D, which also distinguishes it from *D. xikrini* sp. nov. Finally, *D. xikrini* sp. nov. is also different from *D. goiasensis* in the shape of its limbs. Indeed, the expansion of the limb I endopodite is short in *D. xikrini* sp. nov., but long in *D. goiasensis*. The spinous lobe on the endopodites of limb II and III of *D. goiasensis* are also absent in *D. xikrini* sp. nov.

### Variability and difficulty of identification

Recently, *Hirose et al.* (2015) reported variable characters of the eye and the base of the branches in *D. orientalis*. In *D. carajaensis*, we found variations in branch 2A, branch 2V and sub-branch III of branch 2D within a population across several populations (Fig. 4). However, we have not found such variations in other species, so we believe that this species may have a greater morphological variability than others. This demonstrates that a population approach is needed for the delineation of species of *Dendrocephalus*.

The morphological richness within *Dendrocephalus* requires a combination of diagnostic characters rather than a single discriminating trait. Many of these characters are present in several taxa, but only a particular combination is species specific. Nevertheless, some of the salient characters given in Tables 1–3 could be sufficient to directly identify Brazilian species, but in most cases they are present in other species outside of Brazil. For example, in Brazil only the frontal appendage of *D. carajaensis* possesses primary branches without spines from the base to the terminal branches, but this character is present in other species, particularly in *D. cervicornis* (Weltner, 1890) (Weltner 1890; Daday 1910; Pereira 1983). The unguiform extremity of branch 2A is only present in *D. orientalis* in Brazil, but it is also found in *D. venezolanus* Pereira, 1984 and *D. argentinus* Pereira & Belk, 1987. The spiniform expansion in the endopodite I described in *D. riograndensis* in Brazil is also found in *D. cornutus*, *D. cornosuris*, *D. spartanovae* and *D. venezolanus* (Margalef 1961; Pereira 1984; Pereira & Belk 1987; Pereira & Ruiz 1995), while the spiniform expansion in the endopodite II described in *D. thieryi* is also present in *D. venezolanus* or *D. cornutus* (Pereira 1984; Pereira & Belk 1987). Similarly, the acute shape of the extremity of branch 2A in *D. carajaensis* is unique in Brazil but found elsewhere, such as in *D. cervicornis* or *D. sarmentosus* (Pereira 1983; Pereira & Belk 1987).

However, some of these characters are quite significant and are not present in other species, such as the presence of a single spine at the base of the frontal appendage primary branches or the presence of two eye spines that are only reported in the Brazilian species *D. orientalis*. Unfortunately, the use of this trait is problematic, as it has been shown to be particularly variable (*Hirose et al.* 2015).

The branch 2D seems to be taxonomically interesting because it has a large number of points of variation that can be used as diagnostic in combination, but a precise, comparative study of multiple populations is necessary. Thus far, only *D. carajaensis* has such a reduced sub-branch II.

In the future, we believe that genetic studies will be very useful in better delineating species of *Dendrocephalus*, especially widely distributed species such as *D. brasiliensis* (see *Barros-Alves et al.* 2016), to detect possible cryptic species as well as to better understand the diversification of species with restricted distributions, such as *D. aranai* sp. nov. or *D. xikrini* sp. nov. We also believe that collection efforts should be intensified in the Pantanal, in the Amazonian savannah and in the Cerrado, where it is likely that new species will be discovered in the future.

## Key for all known species of Brazilian *Dendrocephalus*

A key based on male characters is presented for all known species of the Brazilian *Dendrocephalus*, and we recommend verifying the coherence of the determination with Tables 1–3.

1. Proximal anterior surface of frontal appendage primary branches without spines ..... 2
  - Proximal anterior surface of frontal appendage primary branches with at least one spine ..... 3
2. Primary branches of frontal appendage with spines on medial surface, thoracopod I with endopodite without expansion ..... *D. brasiliensis* Pesta, 1921
  - Primary branches of frontal appendage without spines, thoracopod I with endopodite with spiny expansion ..... *D. carajaensis* Rogers, Gomes & Vieira, 2012
3. Proximal anterior surface of frontal appendage primary branches generally with one spine and branch 2A extremity with a strongly sclerotized hook ..... *D. orientalis* Rabet & Thiéry, 1996
  - Proximal anterior surface of frontal appendage primary branches with three to four spines and branch 2A extremity podiform ..... 4
4. Branch 1V anterior sub-branch with swollen distal portion ..... 5
  - Branch 1V anterior sub-branch with acute distal portion ..... 6
5. Branch 2D, sub-branch I and II with several long spines ..... *D. aranai* Rabet & Lacau sp. nov.
  - Branch 2D, sub-branch I and II without long spines ..... *D. thieri* Rabet, 2006
6. Thoracopod III with endopodite producing distolateral corner .....
  - ..... *D. goiasensis* Rabet & Thiéry, 1996
  - Thoracopod III with endopodite not producing distolateral corner ..... 7
7. Thoracopod I with endopodite with basolateral spiniform projection; thoracopods II and III with endopod without large spines ..... *D. riograndensis* Rogers & Volcan, 2016
  - Thoracopod I with endopodite with small basolateral extension with spines; thoracopods II and III with endopod with large marginal spines ..... *D. xikrini* Rabet & Bozelli sp. nov.

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