



# Three new species of *Anacanthorus* Mizelle & Price, 1965 (Monogenea: Dactylogyridae) from *Markiana nigripinnis* Perugia (Actinopterygii: Characidae) in Pantanal wetlands, Brazil

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**Abstract** Three monogenean species, *Anacanthorus luquei* n. sp., *A. scholzi* n. sp. and *A. cohenae* n. sp. are described from the gills of the tetra fish *Markiana nigripinnis* (Perugia) (Characidae), collected in the Pantanal wetlands, State of Mato Grosso do Sul, Brazil. Among other differences, *Anacanthorus luquei* n. sp. differs from the most morphologically similar species, based on the structure of the accessory piece as follows: branches with smooth margins (*vs* with irregular margins in *A. cuticulovaginus*), without

pointed projections at distal end (*vs* with projections in *A. dipelecinus*) and with 2 branches (*vs* 3 in *A. quinquaramus*). *Anacanthorus scholzi* n. sp. is most morphologically similar to *A. luquei* n. sp., differing from it because one of the branches of the accessory piece is bifurcated at the distal portion. *Anacanthorus cohenae* n. sp. can be differentiated from the congeners based on the combination of the following features: MCO cylindrical and robust with sclerotised flanges on the extremities, accessory piece V-shaped, bearing two branches similar in length and with blunt distal ends, and hooks with a proximal bulb. This is the first parasitological study on *M. nigripinnis* and, currently, *Anacanthorus* allocates 88 species infesting characiform fishes in the Neotropical region, including the three new species described here.

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## Introduction

Pantanal is one of the richest biomes within the Neotropical region regarding animal species (Polaz et al., 2014). Its marked seasonal variations, characterised by distinct periods of flood and drainage, support a rich ichthyofauna (Corrêa et al., 2009; Britski et al., 2007). Regarding these fishes as hosts, it is possible to assume that they support communities of parasites also rich and abundant (Hechinger & Lafferty, 2005; Poulin, 2014). However, considering the high biodiversity potential of the Neotropics (and consequently of the Pantanal wetlands), our current knowledge of these organisms is incipient since less than 25% of the species of fish have been investigated for parasites (Luque & Poulin, 2007; Luque et al., 2017).

Among the helminth parasites of fishes, dactylogyrid monogeneans (Monogenea: Dactylogyridae) represent a large and speciose group (Boeger & Vianna, 2006; Cohen et al., 2013). Similarly, *Anacanthorus* Mizelle & Price, 1965 is a highly diverse genus of dactylogyrids, allocating a large number of host-specific species, which infect the gills of freshwater characiforms (Actinopterygii: Characiformes) in the Neotropical region (Cohen et al., 2013). Currently, *Anacanthorus* includes 85 species, of which 37 were originally described from fishes belonging to the Serrasalminidae, 21 to the Triportheidae, 15 to the Bryconidae, 11 to the Erythrinidae and one to the Characidae (Cohen et al., 2013; Leão et al., 2015; Monteiro et al., 2015; Moreira et al., 2019; Santos-Neto et al., 2019).

During the first parasitological study of *Markiana nigripinnis* (Perugia) (Characidae), collected in the Pantanal wetlands, State of Mato Grosso do Sul, Brazil, several helminths were recovered. Morphological observations of the monogeneans revealed that they belong to *Anacanthorus* and represent three new species described herein.

## Materials and methods

Fifty-seven specimens of *M. nigripinnis* were acquired from local fishermen from December 2013 to September 2014, at the municipality of Corumbá, Pantanal wetlands, State of Mato Grosso do Sul, Brazil. Fish were kept in water tanks with oxygen pumps prior to

necropsy. Host identification follows Britski et al. (2007); nomenclature and classification were updated according to the FishBase (Froese & Pauly, 2019). Monogeneans were removed from the gills of fresh killed hosts with aid of a dissecting microscope and fixed in hot 4% formalin. Some specimens were mounted unstained in Grey & Wess medium for observation of the sclerotised structures; others were stained with Gomori's trichrome and mounted in Canada balsam in order to study internal anatomy (Humason, 1979; Boeger & Viana, 2006). Light microscopic observations, drawings and measurements (obtained according to the procedures of Mizelle & Klucka, 1953) were made with the aid of a Leica DM 5500B equipped with Nomarski interference contrast, a camera Leica DFC495 and the software Leica LAS (version 4.0) with multifocus imaging capture system, as well as a Olympus BX51 with phase contrast and a drawing tube attached. Classification of hooks is according to Mizelle & Price (1963), the description of male copulatory organ (MCO) and morphological terminology follow Kritsky & Mizelle (1968), Mizelle et al. (1968) and Boeger & Vianna (2006). The filamentous hook is indicated as FH throughout the text. All measurements are in micrometres and are expressed as the range, followed by the mean and the number of measured structures (n) in parentheses.

The type-specimens were deposited in the “Coleção Zoológica da Universidade Federal de Mato Grosso do Sul” (acronym ZUFMS).

To comply with the regulations set out in Article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of all new taxa have been submitted to ZooBank. For each new taxon, the Life Science Identifier (LSID) is reported in the taxonomic summary.

### Family Dactylogyridae Bychowsky, 1933 Genus *Anacanthorus* Mizelle & Price, 1965

#### *Anacanthorus luquei* n. sp.

*Type-host*: *Markiana nigripinnis* (Perugia) (Characiformes: Characidae), tetra fish.

*Type-locality*: Marginal lake to the road MS184 (19°34.576'S, 57°00.823'W), Corumbá, State of Mato Grosso do Sul, Brazil.

**Type-material:** Holotype (ZUFMS-PLA00017) and 3 paratypes (ZUFMS-PLA00018–20).

**Site on host:** Gill filaments.

**Prevalence:** 12.3% (7 fish infected of 57 examined).

**ZooBank registration:** The Life Science Identifier (LSID) for *Anacanthorus luquei* n. sp. is urn:lsid:zoobank.org:act:38C6D732-DE8A-4C76-9C78-7BDD63A02402.

**Etymology:** The specific name honours Dr José Luis Luque for his contributions to the study of Neotropical parasites of fish.

## Description

[Based on 21 specimens: 15 mounted in Gray & Wess medium and 6 stained with Gomori's trichrome; Figs. 1–4.] Body fusiform, 315–1,340 (545; n = 21) long, with greatest width 87–155 (131; n = 21) at midlength (Fig. 1). Tegument smooth. Cephalic lobes 3, well defined, with Y-shaped cephalic organs: 1 medial bearing a pair of organs and 2 lateral bearing a single pair. Eyes 4, equidistant, with irregular shape, posterior pair larger than anterior; accessory granules absent or rarely present. Pharynx spherical to sub-spherical, 18–47 (34; n = 18) in diameter. Intestinal caeca 2, encircling internal organs, bifurcating slightly posterior to pharynx and joining slightly posterior to testis.

Haptor 38–85 (54; n = 19) long, 46–114 (80; n = 19) wide, bearing 7 pairs of hooks and 2 pairs (1 dorsal and 1 ventral) of 4A hooks with anacanthorine distribution; anchors and bars absent (Fig. 1). Hooks similar in shape and size, 23–26 (25; n = 17) long, with truncate broad thumb, slightly curved shaft and point, and thin, straight shank with expanded proximal bulbous portion (Fig. 4). Length of FH loop representing c.40% of shank length. Hooks 4A small, similar in shape and size, proximal end rounded, broader than distal thinner end, 8–12 (10; n = 8) long (Fig. 3).

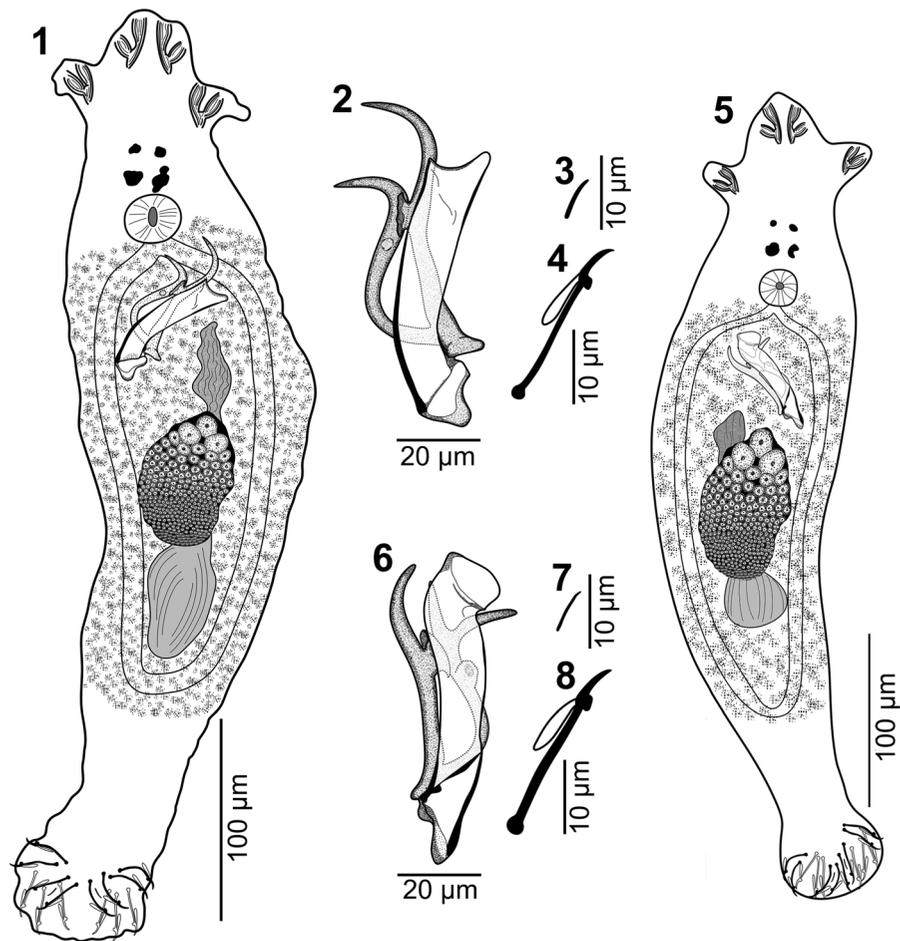
MCO forming conspicuous thick-walled and slightly L-shaped tube, 58–107 (74; n = 21) long, 12–20 (15; n = 21) wide; its proximal opening with flap-like projection (sclerotised flange). Accessory piece 47–107 (65; n = 21) long, slightly V-shaped, articulated with MCO base through proximal process; consisting of 2 unequal branches bifurcating near base, each branch with single tapering distally. Longer branch bearing 1 submedial reduced flat branch.

Shorter branch sigmoid, with blunt button-like projection on its distal third (Fig. 2). Gonads slightly overlapping, intercaecal; germarium sub-ovate with irregular edges, 60–142 (92; n = 7) long, 22–56 (37; n = 7) wide. Testis postgermarial, elongate-oval, 55–77 (67; n = 3) long, 32–45 (40; n = 3) wide (Fig. 1). Vitellarium composed of small follicles, evenly extending from pharyngeal level to slightly posterior to caeca loop (Fig. 1). Eggs, oviduct and oötype not observed.

## Remarks

The present specimens belong to *Anacanthorus* mainly by the absence of bars and anchors, as well as in the presence of 7 pairs of similar hooks and 2 pairs of distinct hooks denominated 4A (see Mizelle & Price, 1965; Kritsky et al., 1992).

The accessory piece of *A. luquei* n. sp. composed of two long branches in a somewhat V-shaped pattern, resembles the following congeners: *A. bicuspidatus* Cohen, Kohn & Boeger, 2012; *A. carinatus* Kritsky, Boeger & Van Every, 1992; *A. chaunophallus* Kritsky, Boeger & Van Every, 1992; *A. cuticulovaginus* Kritsky & Thatcher, 1974; *A. daulometrus* Cohen, Kohn & Boeger, 2012; *A. dipelecinus* Kritsky, Boeger & Van Every, 1992; *A. douradensis* Cohen, Kohn & Boeger, 2012; *A. furculus* Kritsky, Boeger & Van Every, 1992; *A. myleusi* Oliveira, Carneiro, Ruz & Luque, 2019; *A. neotropicalis* Mizelle & Price, 1965; *A. paradouradensis* Monteiro, Cohen & Brasil-Sato, 2015; *A. quinqueramus* Kritsky, Boeger & Van Every, 1992 and *A. tricornis* Kritsky, Boeger & Van Every, 1992. Besides several differences, e.g. in the morphology of MCO and hooks, none of these species have the longer branch of the accessory piece with an arising submedial, short and flat branch, referred to as “hatchet-like” by Kritsky et al. (1992), except for *A. cuticulovaginus*, *A. dipelecinus* and *A. quinqueramus*. However, the accessory piece of *A. cuticulovaginus* has one branch with irregular margins (Kritsky & Thatcher, 1974), that of *A. dipelecinus* has conspicuous pointed projections on the distal end of the branches, which are similar in size and each has a short branch, and that of *A. quinqueramus* has an additional small and median branch (Kritsky et al., 1992), features absent in the new species.



**Figs. 1–8** *Anacanthorus* spp. ex *Markiana nigripinnis*, from Pantanal wetlands, State of Mato Grosso do Sul, Brazil. 1–4, *Anacanthorus luquei* n. sp. 1, Whole mount, ventral view; 2, Copulatory complex, ventral view; 3, Hook 4A; 4, Hook. 5–8, *Anacanthorus scholzi* n. sp. 5, Whole mount, ventral view; 6, Copulatory complex, ventral view; 7, Hook 4A; 8, Hook

### *Anacanthorus scholzi* n. sp.

*Type-host*: *Markiana nigripinnis* (Perugia) (Characiformes: Characidae), tetra fish.

*Type-locality*: Marginal lake to the road MS184 (19°34.576'S, 57°00.823'W), Corumbá, State of Mato Grosso do Sul, Brazil.

*Type-material*: Holotype (ZUFMS-PLA00021) and 2 paratypes (ZUFMS-PLA00022, 23).

*Site on host*: Gill filaments.

*Prevalence*: 7% (4 fish infected of 57 examined).

*ZooBank registration*: The Life Science Identifier (LSID) for *Anacanthorus scholzi* n. sp. is urn:lsid:zooBank.org:act:D4A553F6-342D-48E0-BB47-037082FB5721.

*Etymology*: The specific name honours Dr Tomáš Scholz for his contributions to the taxonomy of helminth parasites of fish.

### Description

[Based on 4 specimens: 3 mounted in Gray & Wess medium and 1 stained with Gomori's trichrome; Figs. 5–8.] Body fusiform, 397–485 (471; n = 4) long, with greatest width 131–178 (163; n = 4) at midlength (Fig. 5). Tegument smooth. Cephalic lobes 3, well defined, with Y-shaped cephalic organs: 1 medial bearing a pair of organs and 2 lateral bearing a single pair. Eyes 4, equidistant, with irregular shape, posterior pair larger than anterior; accessory granules absent or rarely present. Pharynx spherical to

subspherical, 38–43 (41; n = 4) in diameter. Intestinal caeca 2, encircling internal organs, bifurcating slightly posterior to pharynx and joining slightly posterior to testis.

Haptor 43–100 (64; n = 4) long, 72–105 (89; n = 4) wide, bearing 7 pairs of hooks and 2 pairs (1 dorsal and 1 ventral) of 4A hooks, with anacanthorine distribution; anchors and bars absent (Fig. 5). Hooks similar in shape and size, 22–31 (28; n = 4) long, with truncate broad thumb, slightly curved shaft, straight point and straight shank with expanded proximal bulbous portion (Fig. 8). Length of FH loop representing c.70% of shank length. Hooks 4A small, similar in shape and size, thin and rod-shaped, 9–15 (11; n = 4) long (Fig. 7).

MCO forming sclerotised tube with slightly curved ends, 63–73 (70; n = 4) long, 10–20 (16; n = 4) wide (Fig. 6). Proximal opening of MCO bearing short flap-like projections (sclerotised flanges) (Fig. 6). Accessory piece 60–70 (65; n = 4) long, articulated with MCO base through proximal process; consisting of 2 main branches bifurcating near base: 1 evenly curved and 1 sigmoid (Fig. 6). Evenly curved branch bearing 1 submedial reduced flat branch, ending in single blunt tip; sigmoid branch bearing submedial blunt button-like projection and distal bifurcation with blunt ends (Fig. 6). Gonads slightly overlapping, intercaecal; germarium sub-ovate with irregular edges, 60–108 (85; n = 3) long, 36–55 (46; n = 3) wide. Testis postgermarial elongate-oval, 45–70 (53; n = 3) long, 18–40 (31; n = 3) wide. Vitellarium composed of small follicles, evenly extending from pharyngeal level to slightly posterior to caeca loop (Fig. 5). Eggs, oviduct and oötype not observed.

#### Remarks

The general morphology of *A. scholzi* n. sp. agrees with the generic diagnosis of *Anacanthorus* (see previous remarks). *Anacanthorus scholzi* n. sp. is distinct from *A. luquei* n. sp. in having one branch of the accessory piece bifurcated at the distal portion (vs absent in *A. luquei* n. sp.).

The following species are morphologically similar to *A. scholzi* n. sp. based on the shape of the accessory piece, i.e. with one of the two branches bifurcated at the distal end: *A. acuminatus* Kritsky, Boeger & Van Every, 1992; *A. bellus* Kritsky, Boeger & Van Every, 1992; *A. bicuspidatus*; *A. daulometrus*; *A. euryphallus*

Kritsky, Boeger & Van Every, 1992 and *A. tricornis*. However, none of these species possess a “hatchet-like” or a button-like projection on the accessory piece branches as in the new species (see Kritsky et al., 1992; Cohen et al., 2012). The copulatory complex of *A. quiqueramus* resembles that of *A. scholzi* n. sp. but differs in having an accessory piece with an additional small, median branch and the MCO with a pointed projection near the distal end (vs absence of additional branch and pointed projection in the accessory piece and MCO, respectively) (Kritsky et al., 1992).

#### *Anacanthorus cohena* n. sp.

*Type-host*: *Markiana nigripinnis* (Perugia) (Characiformes: Characidae), tetra fish.

*Type-locality*: Marginal lake to the road MS184 (19°34.576'S, 57°00.823'W), Corumbá, State of Mato Grosso do Sul, Brazil.

*Type-material*: Holotype (ZUFMS-PLA00024) and 2 paratypes (ZUFMS-PLA00025, 26).

*Site on host*: Gill filaments.

*Prevalence*: 3.5% (2 fish infected of 57 examined).

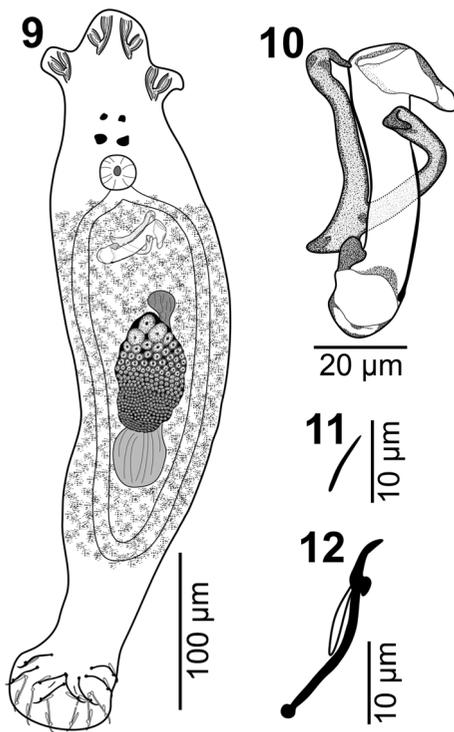
*ZooBank registration*: The Life Science Identifier (LSID) for *Anacanthorus cohena* n. sp. is urn:lsid:zoobank.org:act:0C843D36-8BE3-44D0-BD8E-5652534B064C.

*Etymology*: The specific name honours Dr Simone Chinicz Cohen for her contributions to the knowledge of the Neotropical fauna of monogeneans.

#### Description

[Based on 3 specimens: 2 mounted in Gray & Wess medium and 1 stained with Gomori's trichrome; Figs. 9–12.] Body fusiform, 206–611 (367; n = 3) long, with greatest width 69–104 (90; n = 3) at midlength (Fig. 9). Tegument smooth. Cephalic lobes 3, well defined, with Y-shaped cephalic organs: 1 medial bearing a pair of organs and 2 lateral bearing a single pair. Eyes 4, equidistant, with irregular shape, posterior pair larger than anterior; accessory granules absent. Pharynx spherical, 27–39 (34; n = 2) in diameter. Intestinal caeca 2, encircling internal organs, bifurcating slightly posterior to pharynx and joining slightly posterior to testis.

Haptor 38–72 (50; n = 3) long, 45–84 (68; n = 3) wide, bearing 7 pairs of hooks and 2 pairs (1 dorsal and 1 ventral) of 4A hooks, with anacanthorine



**Figs. 9–12** *Anacanthorus cohenae* n. sp. ex *Markiana nigripinnis*, from Pantanal wetlands, State of Mato Grosso do Sul, Brazil. 9, Whole mount, ventral view; 10, Copulatory complex, ventral view; 11, Hook 4A; 12, Hook

distribution; anchors and bars absent (Fig. 9). Hooks similar in shape and size, 26–27 (26;  $n = 3$ ) long, with truncate broad thumb, curved shaft and straight point, thin shank with expanded proximal bulbous portion (Fig. 12). Length of FH loop representing c.60% of shank length. Hooks 4A small, similar in shape and size, proximal end slightly rounded, broader than distal thinner end, 9–12 (11;  $n = 3$ ) long (Fig. 11).

MCO forming evenly curved sclerotised tube, thicker at proximal portion, 41–67 (55;  $n = 3$ ) long, 10–13 (12;  $n = 3$ ) wide (Fig. 10), with basal flap-like structures (sclerotised flanges); distal flange pronouncedly expanded (Fig. 10). Accessory piece 37–72 (50 long;  $n = 3$ ), slightly V-shaped, articulated with MCO base through proximal well-developed process; consisting of 2 long branches bifurcating near base, with blunt and folded distal ends (Fig. 8). Gonads slightly overlapping, intercaecal; germarium sub-ovate with irregular edges, 34–115 (61;  $n = 3$ ) long, 21–47 (34;  $n = 3$ ) wide. Testis postgermarial, elongate-oval, 43 ( $n = 1$ ) long, 20 ( $n = 1$ ) wide.

Vitellarium composed of small follicles, evenly extending from pharyngeal level to slightly posterior to caeca loop (Fig. 9). Eggs, oviduct and oötype not observed.

#### Remarks

As for *A. cohenae* n. sp., the following species have the accessory piece composed of two not bifurcated branches resembling a V-shaped pattern: *A. carinatus*; *A. cuticulovaginus*; *A. dipelecinus*; *A. douradensis*; *A. furculus*; *A. luquei* n. sp., *A. neotropicalis*; *A. paradouradensis* and *A. parakruidenieri* Cohen, Kohn & Boeger, 2012, but they differ from the new species as follows. *Anacanthorus douradensis*, *A. furculus* and *A. paradouradensis* have the MCO with other shapes than an evenly curved sclerotised tube as in *A. cohenae* n. sp. (see Kritsky et al., 1992; Cohen et al., 2012; Monteiro et al., 2015). *Anacanthorus carinatus* has a secondary accessory piece, which is absent in the new species (see Kritsky et al., 1992). *Anacanthorus cuticulovaginus* has one branch of the accessory piece with irregular edge, *A. luquei* n. sp. has a “hatchet-like” structure on one of the main branches (see previous remarks for details) and *A. dipelecinus* has “hatchet-like” structures on both branches, which end in pointed projections (*vs* smooth-edged branches, without “hatchet-like” structures and with blunt distal ends in *A. cohenae* n. sp.) (Kritsky & Thatcher, 1974; Kritsky et al., 1992). The accessory piece of *A. parakruidenieri* is thin, bifid near its mid-length and the MCO lacks terminal sclerotised flanges (*vs* robust accessory piece, bifid near its base and MCO with conspicuous terminal flanges in *A. cohenae* n. sp.) (Cohen et al., 2012). *Anacanthorus neotropicalis* has the accessory piece formed by two branches evidently different in length, with pointed ends and hooks with shank expanded from the mid-portion to proximal end (*vs* accessory piece with two branches of similar length and blunt ends, and hook shank with uniform width and a proximal bulb in the new species) (Mizelle & Price, 1965).

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures involving animals were according to the rules of the Sistema de Autorização e Informação em Biodiversidade (SISBIO 22119-2) and the Comissão de ética no uso de animais / Universidade Federal de Mato Grosso do Sul (CEUA/UFMS 572/2913).

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