

# A new species of *Cacatuocotyle* (Monogenea, Dactylogyridae) parasitizing *Astyanax* spp. (Characiformes, Characidae) from Brazil, including molecular data and a key to species identification

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## Abstract

The present study describes *Cacatuocotyle papilionis* n. sp. (Monogenea, Dactylogyridae) from the skin of the characid fishes *Astyanax lacustris* (Lütken, 1875) (= *Astyanax altiparanae* Garutti & Britski, 2000) and *Astyanax fasciatus* (Cuvier, 1819) (Characiformes, Characidae) from the Southeast of Brazil, supported by morphological and molecular data. The new species differs from all congeners, mainly due to the morphology of the ventral bar (resembling a butterfly), accessory piece, and the number of rings of the male copulatory organ (MCO), comprising a coiled tube with 4.5–5.5 counterclockwise rings. The first molecular data for this monogenean genus is provided in this study, using the partial sequences of the ribosomal gene (28S), as well as providing an identification key to the species.

## Keywords

Characid fishes, monogenean, ectoparasite, 28S rDNA fragments, Neotropical region

## Introduction

Characiformes (*sensu* Fink and Fink, 1996) represents one of the largest groups of teleost fishes, including 23 families and around 2,247 species (Eschmeyer *et al.* 2016). They inhabit freshwater environments, and many species are popular aquarium fishes or an important source of human food (Nelson 2006). Characidae Buckup, 1998 is one of the largest families included in this order, with 1,126 described species (Eschmeyer *et al.* 2016).

Several species of characid fishes are known in Brazil, with those belonging to the genus *Astyanax* Baird and Girard, 1854 the richest in species (Nelson 2006). *Astyanax lacustris* (Lütken, 1875) (= *Astyanax altiparanae* Garutti and Britski, 2000) popularly known as “*lambari-do-rabo-amarelo*”, is native to Eastern Brazil; while *Astyanax fasciatus* (Cuvier, 1819) known as “*lambari-do-rabo-vermelho*”, is distributed in freshwater basins in North, Central and South America (Argentina, Belize, Brazil, Colombia, Costa Rica, Mexico, Panama, Uruguay, and Venezuela) (Reis *et al.* 2003).

Monogeneans are common fish parasites, and a significant number of new species has been described in the recent years,

mainly in the Neotropics (Mendoza-Palmero *et al.* 2012; Monteiro and Brasil-Sato 2014; Rossin and Timi 2014; Moreira *et al.* 2016; Acosta *et al.* 2017; Franceschini *et al.* 2017; Zago *et al.* 2017). Dactylogyridae is the most abundant taxon in South America (Cohen *et al.* 2013), which accommodates the genus *Cacatuocotyle*, parasites of Characidae fishes in the Neotropical region.

To date, four species have been placed in this genus, two of which were described in Brazil: *Cacatuocotyle paranaensis* Boeger, Domingues and Kritsky, 1997 (type-species), which was found parasitizing *Characidium lanei* Travassos, 1967 and *Characidium pterostictum* Gomes, 1947 in the Cacatu and 2 de Fevereiro Rivers, in the state of Paraná (Boeger *et al.* 1997); and *Cacatuocotyle guaibensis* Gallas, Callegaro-Marques and Amato, 2014, originally described parasitizing specimens of *Astyanax* aff. *fasciatus* and *Astyanax jacuhiensis* (Cope, 1894) from Lake Guaíba, in the state of Rio Grande do Sul (Gallas *et al.* 2014). After that, two species have been described in Mexico: *Cacatuocotyle chajuli* Mendoza-Franco, Caspeta-Mandujano and Salgado-Maldonado, 2013, and *Cacatuocotyle exiguum* Mendoza-Franco, Caspeta-Mandujano and Salgado-Maldonado, 2013, parasitizing *Astyanax aeneus* (Günther, 1860) from the La-

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cantún River Basin in the Montes Azules Biosphere Reserve in the state of Chiapas (Mendoza-Franco *et al.* 2013).

The use of molecular techniques has become increasingly common in descriptions of new species or redescriptions, as it is complementary to and supports morphological analysis (Cunningham *et al.* 2001; Huyse *et al.* 2004; Řehulková *et al.* 2013; Franceschini *et al.* 2017). Thus, the aim of the present study was therefore to describe a new species of *Cacatuocotyle* from the Sapucaí-Mirim River, in the southeast of Brazil, supported by morphological and molecular characterization. An identification key to the genus *Cacatuocotyle* is also provided.

## Materials and Methods

### Collection of host and parasite specimens

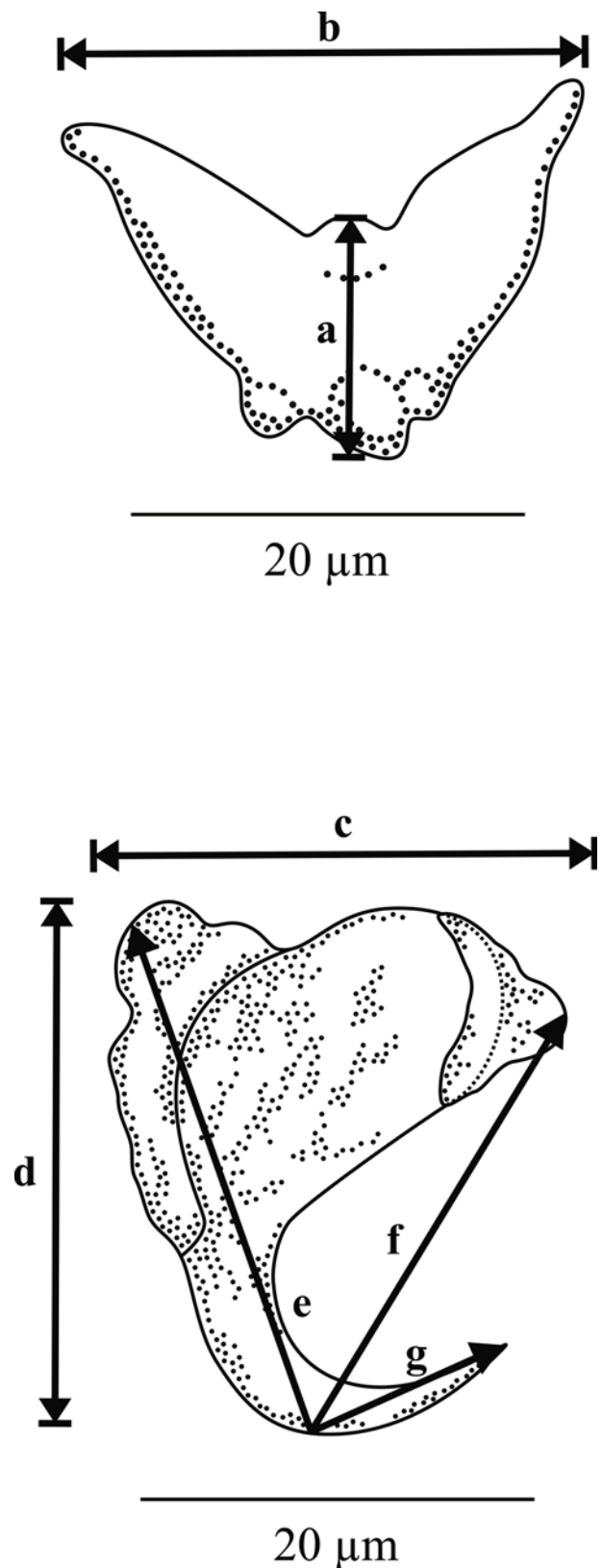
Forty specimens of *A. lacustris* and 40 of *A. fasciatus* were collected from the Sapucaí-Mirim River, in the state of São Paulo, Brazil (20°26'12.5"S, 47°53'18.59"W) between March 2012 and July 2013 using gillnets. The capture of fish specimens was authorized under a Permanent License for the Collection of Zoological Material (SISBio 13794-1). The fish specimens collected were stored individually in plastic bags in a Styrofoam box with ice, for immediate transportation to the laboratory.

The monogeneans were collected and stored in 70% ethanol solution. Samples of these parasites were stained with Gömöri's trichrome and mounted in Canada balsam for analysis of the internal organs. Some specimens were mounted in Hoyer's or in Gray and Wess medium for the study of sclerotized structures (Kritsky *et al.* 1986).

The morphological and morphometric analysis of the parasites was carried out using a computerized system for image analysis with differential interference contrast (DIC) – LAS V3 (Leica Application Suite V3; Leica Microsystems, Wetzlar, Germany). The illustrations of the sclerotized structures were performed with the aid of a camera lucida mounted on a Leica DMLS microscope with phase contrast optics.

All the measurements were presented in micrometers ( $\mu\text{m}$ ) and expressed as means, followed by the range and number of specimens measured (n) in parentheses. Measurements of the (a) bar length, (b) bar width, (c) anchor width, (d) anchor length, (e) extern length, (f) intern length and (g) tip length were performed according to the scheme shown in Figure 1. The prevalence and mean intensity of infestation were calculated in accordance with Bush *et al.* (1997).

Voucher host fish specimens were deposited in the fish collection of the Laboratório de Biologia e Genética de Peixes (the Fish Biology and Genetics Laboratory) (LBP), Universidade Estadual Paulista (São Paulo State University - UNESP), in the municipality of Botucatu, in the state of São Paulo, Brazil (*A. lacustris* - LBP 18794 and *A. fasciatus* - LBP 18795). Holotype and the paratypes of the new species proposed were deposited in the Coleção Helmintológica do Instituto Oswaldo



**Fig. 1.** Scheme of measurements for the bar and anchor of *Cacatuocotyle papilionis* n. sp. **a.** Bar length. **b.** Bar width. **c.** Anchor width. **d.** Anchor length. **e.** Extern length. **f.** Intern length. **g.** Tip length

Cruz (the Helminthological Collection of the Oswaldo Cruz Institute) (CHIOC), in the state of Rio de Janeiro, Brazil. Voucher specimens were deposited in the zoological collection of the Instituto Nacional de Pesquisas da Amazônia (Amazonas National Research Institute) (INPA), in the state of Amazonas, Brazil, and in the Coleção Helminológica do Instituto de Biociências (the Helminthological Collection of the Institute of Biosciences of Botucatu) (CHIBB) of the Universidade Estadual Paulista (São Paulo State University - UNESP), in the municipality of Botucatu, in the state of São Paulo, Brazil.

#### DNA extraction, amplification and sequencing

For separation to be performed correctly and identification confirmed, each parasite specimen subjected to molecular analysis was mounted on a slide with glycerin and photographed. The same specimens were then used for molecular characterization. The total DNA genomic was extracted using the Qiagen Dneasy® Blood and Tissue Kit, according to the manufacturer's protocol, with a final volume of 30 µl. Conventional PCR amplifications were performed in 25 µl reactions containing 5 µl of DNA extract. A quantity of 0.5 µl of each PCR primer was added using Ready-to-Go PCR beads (Pure Taq™ Ready-to-Go™ beads, GE Healthcare, Chicago, USA), with the solution consisting of stabilizers, BSA, dATP, dCTP, dGTP, dTTP, ~2.5 units of puReTaq DNA polymerase and reaction buffer. The beads were reconstituted to a final volume of 25 µl, and the concentration of each dNTP was 200 µM in 10 mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl and 1.5 mM MgCl<sub>2</sub>. The thermocycling profile employed was: initial denaturation of DNA at 94°C for 3 min, followed by 34 amplification cycles at 94°C for 30 s, 56°C for 30 s and 72°C for 1:30 min, and a final extension at 72°C for 7 min (Mendoza-Palmero *et al.* 2015). The primers used for amplification and for sequencing were the partial 28S rDNA (LSU) fragments U178 (5' - GCA CCC GCT GAA YTT AAG - 3') and L1642 (5' - CCA GCG CCA TCC ATT TTC A - 3') (Lockyer *et al.* 2003), and L1200R (5' - GCA TAG TTC ACC ATC TTT CGG - 3') for sequencing (Littlewood *et al.* 2000).

The PCR products were run on agarose gel using GelRed and loading buffer and purified using the QIAquick PCR Purification Kit (Qiagen®, CA, USA). Automated sequencing was performed directly on the purified PCR products from specimens using BigDye v.3.1 Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) for cycle sequencing. Sequences were run on an Applied Biosystems ABI 3500 DNA genetic analyzer.

Contiguous sequences were assembled and edited in Sequencher v. 5.2.4 (Gene Codes, Ann Arbor, MI) and subjected to BLAST analysis (<http://blast.ncbi.nlm.nih.gov>) to confirm their identity. Unambiguous alignment was carried out using the MUSCLE software implemented in Geneious version 7.1.3 (Kearse *et al.* 2012) with dactylogyrids available in GenBank (Table I). The outgroup chosen was *Ancyrocephalus percae* (KF499080).

The genetic divergence between the sequences was calculated within the aligned portion using the Kimura-2-parameter distance model (Kimura 1980) in the MEGA6 program (Tamura *et al.* 2013). The NJ analyzes were performed using the Kimura-2-parameter model and 2000 bootstrap replicates.

## Results

### Morphological description

Dactylogyridae Bychowsky, 1933

*Cacatuocotyle* Boeger, Domingues and Kritsky, 1997

#### *Cacatuocotyle papilionis* (Fig. 2)

**Diagnosis:** Based on 14 specimens. Body 728 (383–1,100; n = 11) long; greatest width 178 (113–286; n = 12) near mid-length; tegument smooth. Cephalic lobe well defined and cephalic glands not observed. Two pair of eyes, with the anterior smaller than the posterior pair; one or both members of each pair sometimes absent; numerous accessory granules in cephalic and anterior trunk region. Pharynx spherical, 38 (28–53; n = 12) in diameter; esophagus long: 78 (50–149; n = 8) length, 16 (9–23; n = 8) width; caeca confluent and not sinuous. Haptor 142 (115–203; n = 14) wide, 77 (55–109; n = 14) long, with thickened muscular on anterior margins; one pair of anchors, (d) 38 (34–42; n = 13) long, (c) 28 (23–34; n = 13) wide, (f) 35.5 (30.1–40.1; n = 14) intern length, (e) 34.6 (31.1–37.8; n = 14) extern length, (g) 13.8 (10.8–16.1; n = 14) tip length, robust, with broad superficial root, short deep root, regularly curved shaft and point; presence of protuberances on both superficial and deep root. Bar (a) 10 (8–12; n = 14) long, (b) 24 (21–25; n = 14) wide, with variable opening of its ends and a shape resembling a butterfly; presence of medial enlargement and tapered ends, with one protuberance in the anterior midportion margin; irregular margins and some indents in the posterior midportion, with the presence of several protuberances. Hooks similar, with uniform shank and protruding thumb; hook 17 (14–19; n = 13) long; FH loop nearly one half of shank length. Testis elongated, 34 (21–73; n = 9) long, 11 (8–16; n = 9) wide; ovary elongated, 79 (60–120; n = 10) long, with greatest width 22 (14–32; n = 10) at anterior end. Male copulatory organ (MCO), a coiled tube of 4.5–5.5 counterclockwise rings with a spherical base surrounded by 2 *tandem* circular flanges; articulation process of the accessory piece present; ring diameter 18 (14–20; n = 14). Accessory piece 21 (17–24; n = 13) long, with greatest width 11 (8–14; n = 13), with distal portion tweezer-shaped. Vaginal aperture sinistral; vagina comprising an elongated, sclerotized and delicate tube. Seminal receptacle observed. Vas deferens looping left intestinal caecum anterior to the ovary. Seminal vesicle pyriform; small prostatic reservoir posterior to the base of the MCO. Oviduct, ootype and uterus not observed. Vitelline follicles coextensive with intestinal caecum and absent near esophagus, pharynx and reproductive organs. An egg without

**Table I.** Host, locality, and GenBank accession numbers of Dactylogyridae parasites used in the present study

Parasite	Host	Locality	GenBank*	Reference
<i>Ameloblastella chavarriai</i>	<i>Rhamdia quelen</i>	Catemaco Lake, Mexico	KP056251	Mendoza-Palmero <i>et al.</i> (2015)
<i>Ancyrocephalus percae</i>	<i>Perca fluviatilis</i>	Germany	KF499080	Behrmann-Godel <i>et al.</i> (2014)
<i>Aphanoblastella</i> sp. 3	<i>Goeldiella eques</i>	Nanay River, Peru	KP056238	Mendoza-Palmero <i>et al.</i> (2015)
<b><i>Cacatuocotyle papilionis</i> n. sp.</b>	<b><i>Astyanax lacustris</i> and <i>A. fasciatus</i></b>	<b>Sapucaí-Mirim River, Brazil</b>	<b>MG832889</b>	<b>Present study</b>
<i>Cosmetocleithrum bulbocirrus</i>	<i>Pterodoras granulosus</i>	Aguapeí River, Brazil	MG001326	Acosta <i>et al.</i> (2018)
Dactylogyridae sp.9	<i>Platynemachthys notatus</i>	Santa Clara, Peru	KP056224	Mendoza-Palmero <i>et al.</i> (2015)
<i>Demidospermus</i> sp.11	<i>Brachyplatystoma vaillantii</i>	Nanay River, Peru	KP056235	Mendoza-Palmero <i>et al.</i> (2015)
<i>Demidospermus anus</i>	<i>Loricariichthys platymetopon</i>	Rio Paraná, Brazil	KY766957	Franceschini <i>et al.</i> (2017)
<i>Demidospermus rhinelepisi</i>	<i>Rhinelepis aspera</i>	Aguapeí River, Brazil	MG001324	Acosta <i>et al.</i> (2018)
<i>Demidospermus spirophallus</i>	<i>Loricaria prolixa</i>	Brazil	KY766954	Franceschini <i>et al.</i> (2017)
<i>Demidospermus prolixus</i>	<i>Loricaria prolixa</i>	Brazil	KY766955	Franceschini <i>et al.</i> (2017)
<i>Heteropriapulus simplex</i>	<i>Pterygoplichthys ambrosettii</i>	Aguapeí River, Brazil	MF116372	Acosta <i>et al.</i> (2017)
<i>Heteropriapulus heterotylus</i>	<i>Pterygoplichthys ambrosettii</i>	Aguapeí River, Brazil	MF116370	Acosta <i>et al.</i> (2017)
<i>Paracosmetocleithrum trachydorasi</i>	<i>Trachydoras paraguayensis</i>	Aguapeí River, Brazil	MG001323	Acosta <i>et al.</i> (2018)
<i>Parasciadicleithrum octofasciatum</i>	<i>Rocio octofasciata</i>	Mexico	KY305885	Mendoza-Palmero <i>et al.</i> (2017)
<i>Sciadicleithrum meeki</i>	<i>Rocio octofasciata</i>	Mexico	KY305889	Mendoza-Palmero <i>et al.</i> (2017)
<i>Sciadicleithrum mexicanum</i>	<i>Rocio octofasciata</i>	Mexico	KY305887	Mendoza-Palmero <i>et al.</i> (2017)
<i>Thaparocleidus</i> sp.	<i>Pangasius</i> sp.	Aquarium from Czech Republic, origin Asia	KP056238	Mendoza-Palmero <i>et al.</i> (2017)
<i>Unibarra paranoplatensis</i>	<i>Aguarunichthys torosus</i>	Santa Clara, Peru	KP056219	Mendoza-Palmero <i>et al.</i> (2015)
<i>Unilatus unilatus</i>	<i>Pterygoplichthys ambrosettii</i>	Aguapeí River, Brazil	MF102106	Acosta <i>et al.</i> (2017)
<i>Vancleaveus januacaensis</i>	<i>Pterodoras granulosus</i>	Itaya River, Peru	KP056247	Mendoza-Palmero <i>et al.</i> (2015)

\*GenBank Accession number.

polar filament was observed in one specimen, which was 39 long and 30 wide.

#### Taxonomic summary

**Type host:** *Astyanax lacustris* (Lütken, 1875) (= *Astyanax altiparanae* Garutti and Britski, 2000) (Characiformes: Characidae).

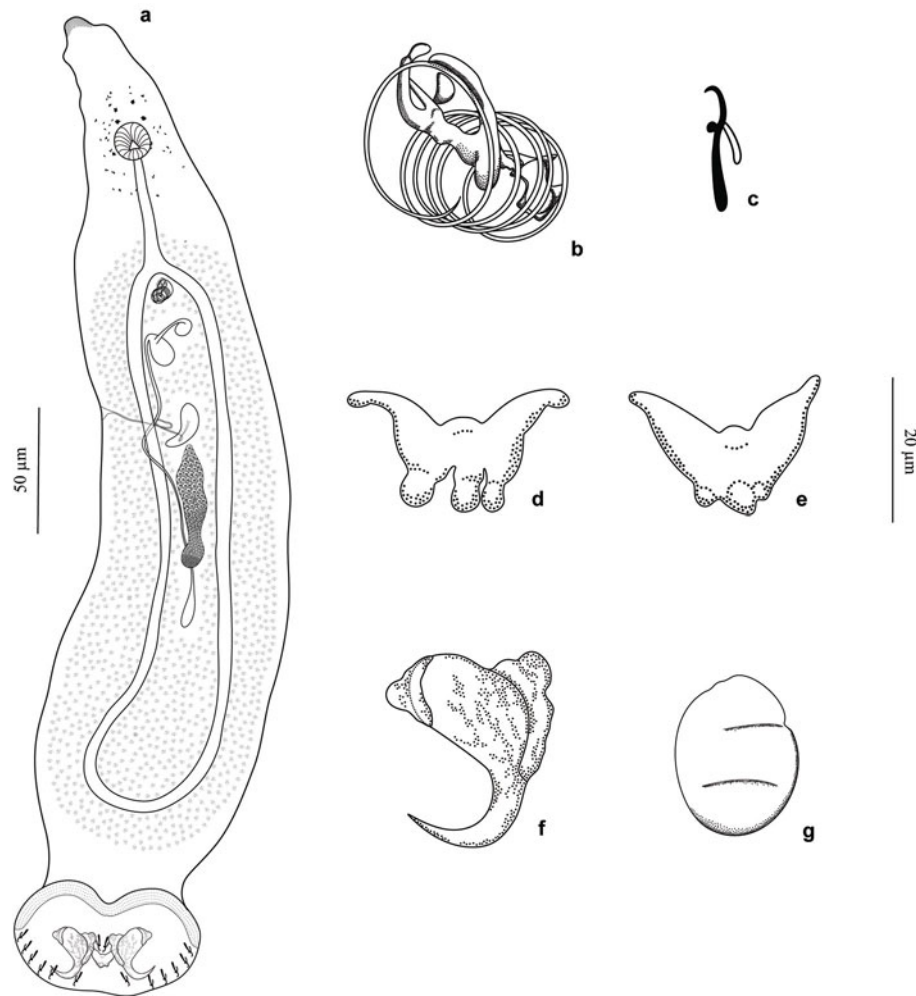
**Other hosts:** *Astyanax fasciatus* (Cuvier, 1819) (Characiformes: Characidae).

**Site of infestation:** Skin.

**Type locality:** Sapucaí-Mirim River, Grande River Basin, state of São Paulo, Brazil (20°29'38.38"S, 47°51'33.11"W).

**Prevalence and mean intensity of infestation:** *A. lacustris* - 9 fish from 40 examined (22.5%), and 1.8 ± 0.3 (1-4), respectively; and *A. fasciatus* - 2 fish from 40 examined (5%), and 1, respectively.

**Specimens deposited:** Holotype CHIOC (39028) and Paratypes CHIOC (39029, 39030); Vouchers INPA (758, 759) and CHIBB (345L).



**Fig. 2.** *Cacatuocotyle papilionis* n. sp. from *Astyanax lacustris* (= *Astyanax altiparanae*). **a.** Whole mount (composite, dorsal view). **b.** Copulatory complex (ventral view). **c.** Hook. **d, e.** Variations in the bar shape. **f.** Anchor. **g.** Egg

**Specimens examined:** Paratypes (CHIOC 37965b and 37965c) of *Cacatuocotyle guaibensis* Gallas, Callegaro-Marques and Amato, 2014 deposited by Gallas *et al.* (2014).

**Etymology:** the specific name is derived from the Latin (*papilio* = butterfly) and refers to the ventral bar that resembles a butterfly.

#### Remarks

Based on the presence of a convex haptor with thickened muscular anterior margins, one anchor-bar complex (ventral), seven pairs of ventral hooks (one pair associated with the anchor shafts; one central pair anterior to the bar; five submarginal bilateral pairs) and a sinistral vaginal aperture (Boeger *et al.* 1997), the new species proposed in this study is considered a member of *Cacatuocotyle*.

*Cacatuocotyle papilionis* n. sp. can be distinguished from most of its congeners mainly by the morphology of the ventral bar (resembling a butterfly) and accessory piece, and the number of rings of the male copulatory organ. The new species re-

sembles *C. paranaensis* due to the shape of the ventral anchor (robust and with the presence of protuberances on both the superficial and deep root) and the eyes (one or both members of each pair is sometimes absent); but can be differentiated by the shape of the accessory piece (distal portion tweezer-shaped in *C. papilionis* n. sp.), the number of rings of the MCO (3.5 in *C. paranaensis* and 4.5–5.5 in *C. papilionis* n. sp.), body length (400–555 in *C. paranaensis* and 383–1,100 in *C. papilionis* n. sp.), bar (U-shaped in *C. paranaensis*, and with the presence of one protuberance in the anterior midportion margin, irregular margins and some indents in the posterior midportion with the presence of several protuberances, and the shape resembling a butterfly in *C. papilionis* n. sp.) and the intestinal caecum (sinuous in *C. paranaensis* and not sinuous in *C. papilionis* n. sp.).

*Cacatuocotyle papilionis* n. sp. is similar to *C. guaibensis* in relation to body length (580–1,010 for *C. guaibensis* and 383–1,100 for *C. papilionis* n. sp.), but differs in relation to haptor width (72.5–112.5 in *C. guaibensis* and 115–203 in *C. papilionis* n. sp.), protuberances on both superficial and deep

**Table II.** Genetic divergence (Kimura-2-parameter, expressed in %) estimated for the partial 28S rDNA within species of Dactylogyridae retrieved from GenBank and *Cacatuocotyle papilionis* n. sp. compared in this study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
<b>1. <i>Ancyrocephalus percae</i></b>		38	33	32	29	20	25	24	27	28	27	36	36	35	35	37	36	33	32	29	32
<b>2. <i>Parasciadicleithrum octofasciatum</i></b>	38		33	26	40	38	36	36	37	37	38	38	38	38	36	37	40	36	35	34	38
<b>3. <i>Sciadicleithrum mexicanum</i></b>	33	26		03	36	34	35	35	34	37	36	36	34	32	34	36	40	32	32	29	35
<b>4. <i>Sciadicleithrum meeki</i></b>	32	26	03		36	35	36	36	34	36	37	36	32	32	35	37	40	31	32	29	36
<b>5. <i>Cacatuocotyle papilionis</i> n. sp.</b>	<b>29</b>	<b>40</b>	<b>36</b>	<b>36</b>		<b>26</b>	<b>27</b>	<b>27</b>	<b>30</b>	<b>34</b>	<b>32</b>	<b>38</b>	<b>41</b>	<b>36</b>	<b>37</b>	<b>36</b>	<b>38</b>	<b>36</b>	<b>36</b>	<b>34</b>	<b>35</b>
<b>6. <i>Unilatus unilatus</i></b>	20	38	34	35	26		14	14	28	30	28	36	38	36	34	37	36	34	33	30	32
<b>7. <i>Heteropriapulus simplex</i></b>	25	36	35	36	27	14		01	30	30	27	34	38	37	35	39	35	31	32	29	31
<b>8. <i>Heteropriapulus heterotylus</i></b>	24	36	35	36	27	14	01		32	30	28	35	38	37	35	39	35	31	33	30	31
<b>9. <i>Unibarra paranoplatensis</i></b>	27	37	34	34	30	28	30	32		25	24	37	41	35	33	41	38	33	34	33	33
<b>10. <i>Vancleaveus janaucaensis</i></b>	28	37	37	36	34	30	30	30	25		23	38	37	34	34	44	38	35	34	31	34
<b>11. <i>Ameloblastella chavarriai</i></b>	27	38	36	37	32	28	27	28	24	23		37	36	37	34	37	38	35	35	35	35
<b>12. <i>Thaparocleidus</i> sp.</b>	36	38	36	36	38	36	34	35	37	38	37		38	34	34	31	32	29	31	28	28
<b>13. <i>Aphanoblastella</i> sp.</b>	36	38	34	32	41	38	38	38	41	37	36	38		36	37	37	39	33	32	30	32
<b>14. <i>Demidospermus</i> sp.</b>	35	38	32	32	36	36	37	37	35	34	37	34	36		16	31	31	27	28	27	27
<b>15. Dactylogyridae sp. 9</b>	35	36	34	35	37	34	35	35	33	34	34	34	37	16		30	29	26	27	24	27
<b>16. <i>Paracosmetocleithrum trachydorasi</i></b>	37	37	36	37	36	37	39	39	41	44	37	31	37	31	30		32	29	28	27	28
<b>17. <i>Cosmetocleithrum bulbocirrus</i></b>	36	40	40	40	38	36	35	35	38	38	38	32	39	31	29	32		27	26	24	25
<b>18. <i>Demidospermus spirophallus</i></b>	33	36	32	31	36	34	31	31	33	35	35	29	33	27	26	29	27		12	12	14
<b>19. <i>Demidospermus anus</i></b>	32	35	32	32	36	33	32	33	34	34	35	31	32	28	27	28	26	12		08	10
<b>20. <i>Demidospermus prolixus</i></b>	29	34	29	29	34	30	29	30	33	31	35	28	30	27	24	27	24	12	08		09
<b>21. <i>Demidospermus rhinelepisi</i></b>	32	38	35	36	35	32	31	31	33	34	35	28	32	27	27	28	25	14	10	09	

root in the ventral anchor (absent in *C. guaibensis* and present in *C. papilionis* n. sp.), shaft of the ventral anchor (more robust and short in *C. papilionis* n. sp. than in *C. guaibensis*), prominent tips in the inner and outer roots of the ventral anchor (present in *C. guaibensis* and absent in *C. papilionis* n. sp.), bar (U-shaped with regular anterior margin and small irregularities in the posterior margin at midportion in *C. guaibensis*, and with the presence of one protuberance in the anterior midportion margin, irregular margins and some indents in the posterior midportion with the presence of several protuberances, and the shape resembling a butterfly in *C. papilionis* n. sp.), hooks (smaller in *C. papilionis* n. sp.: 14–19 µm long than in *C. guaibensis*: 17.5–25 µm long), the shape of the accessory piece (distal portion tweezer-shaped in *C. papilionis* n. sp.), and the eyes (one or both members of each pair is sometimes absent only in *C. papilionis* n. sp.).

The new species differs from *C. chajuli* and *C. exiguum* through its body length (270–418 for *C. chajuli*, 270–275 for *C. exiguum*, and 383–1,100 for *C. papilionis* n. sp.), haptor width (54–95 in *C. chajuli*, 60 in *C. exiguum*, and 115–203 in *C. papilionis* n. sp.), bar (V-shaped in *C. chajuli*, U-shaped in *C. exiguum*, and with the presence of one protuberance in the anterior midportion margin, irregular margins and some indents in the posterior midportion with the presence of several protuberances, and the shape resembling a butterfly in *C. papilionis* n. sp.), and the shape of the accessory piece (distal portion tweezer-shaped in *C. papilionis* n. sp.)

### Key to species of *Cacatuocotyle*

1. Vagina sclerotized ..... 2
- 1'. Vagina slightly visible and apparently unsclerotized ..... *Cacatuocotyle exiguum*
2. Bar U-shaped ..... 3
- 2'. Bar not U-shaped ..... 4
3. Anchor with protuberances on deep root and proximal margin of the superficial root; MCO with up to 3.5 counterclockwise rings ..... *Cacatuocotyle paranaensis*
- 3'. Anchor without protuberances on deep root and proximal margin of the superficial root; MCO with 4-5 counterclockwise rings ..... *Cacatuocotyle guaibensis*
4. Bar V-shaped ..... *Cacatuocotyle chajuli*
- 4'. Bar resembling a butterfly, with irregular margins and some indents in the posterior midportion, with the presence of several protuberances; MCO with 4.5–5.5 counterclockwise rings ..... *Cacatuocotyle papilionis* n. sp.

### Molecular characterization

The 28S ribosomal gene sequence of *C. papilionis* n. sp. was 1,490bp and was aligned with 20 dactylogyrids retrieved from the GenBank database. The alignment was 883bp long and the data obtained, based on the K2P distance matrix (Table II), identified a lowest nucleotide interspecific distance of 26% with *Unilatus unilatus* (Mizelle and Kritsky,

1967) and a greatest interspecific distance of 41% with *Aphanoblastella* sp.3.

## Discussion

*Astyanax* spp. exhibit a great diversity of parasite fauna, and have been found parasitized by species of Nematoda, Monogenea, Digenea, Trematoda, Acanthocephala, Myxozoa, Branchiura, Isopoda, and Copepoda (Eiras *et al.* 2010). According to Mendoza-Franco *et al.* (2013), some reports indicate that species of *Astyanax* are considered the most diverse suite of monogeneans species in the Neotropics, because of the large number of this parasite group that they can harbor. Until now 13 genera of monogeneans have been reported in *Astyanax* spp., including: *Amphithecium*, *Anacanthocotyle*, *Cacatuocotyle*, *Characithecium*, *Cyclopectanum*, *Diaphorocleidus*, *Gyrodactylus*, *Jainus*, *Notozothecium*, *Palombitrema*, *Pseudorhabdosynochus*, *Trinibaculum*, and *Urocleidoides* (Kritsky and Fritts 1970; Kritsky and Leiby 1972; Boeger *et al.* 1997; Thatcher 2006; Mendoza-Franco *et al.* 2009; Cohen *et al.* 2013; Narciso *et al.* 2014).

Only two fish genera of the family Characidae have been found parasitized by *Cacatuocotyle* to date: *Astyanax* and *Characidium*, demonstrating high specificity for the host or closely related species. According to Buchmann and Lindenstrøm (2002), the selection of a certain host species by an ectoparasitic monogenean must be governed mainly by factors related to the host surface, and it has been suggested that the chemical stimuli emitted from the host attract parasites and even initiate certain behavioral and physiological changes in the parasite. Furthermore, anatomical structures of certain host surfaces are likely to exhibit greater compatibility with some parasite attachment mechanisms (Buchmann and Lindenstrøm 2002).

Although only five species of the genus *Cacatuocotyle* have been described to date (including the new species described in the present study), they exhibit broad, preferably micro-habitats, as they can be found in the gills (*C. paranaensis* and *C. exiguum*), skin (*C. guaibensis* and *C. papilionis* n. sp.) or external surface of the anal aperture (*C. chajuli*). According to Euzet and Combes (1998), it is estimated that more than 95% of monogeneans parasite the gills or skin of fish, and these ectoparasitic modes of life are generally supposed to be "ancestral" traits. In the course of evolution, some species or groups have changed their habitat type, and these changes by monogeneans in fish could may represent a tendency to abandon the ectoparasitic mode of life for a meso- or endoparasitic form, in order to avoid competition, or to have access to better resources, or even reduce the pressures exerted by predators of ectoparasites (Euzet and Combes 1998).

The occurrence of *C. papilionis* n. sp. in *A. lacustris* and *A. fasciatus* expands the list of hosts of *Cacatuocotyle*, as well as presenting a new register of occurrence in the Sapucaí-Mirim River in the southeast of Brazil. In addition, we pres-

ent for the first time molecular data of a species of the genus *Cacatuocotyle*, which will contribute to studies on the phylogenetics and diversity of Dactylogyridae in the Neotropical region. As there are few molecular studies regarding monogeneans in Brazil (Fehlauer-Ale *et al.* 2011; Gasques *et al.* 2016; Müller *et al.* 2016; Acosta *et al.* 2017; Franceschini *et al.* 2017) it is hoped that this work will encourage others to sequence more data on *Cacatuocotyle* species and study host associations and interrelationships among monogeneans from the Neotropical region.

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## References

- Acosta A.A., Franceschini L., Zago A.C., Scholz T., Silva R.J. 2017. Six new species of *Heteropriapulius* (Monogenea: Dactylogyridae) from South American fishes with an amended diagnosis to the genus. *Zootaxa*, 4290, 459–482. DOI: 10.11646/zootaxa.4290.3.3
- Acosta A.A., Scholz T., Blasco-Costa I., Alves P.V., Silva R.J. 2018. A new genus and two new species of dactylogyrids monogeneans from gills of Neotropical catfishes (Siluriformes: Doradidae and Loricariidae). *Parasitology International*, 67, 4–12. DOI: 10.1016/j.parint.2017.09.012
- Behrmann-Godel J., Roch, S., Brinker, A. 2014. Gill worm *Ancyrocephalus percae* (Ergens, 1966) outbreak negatively impacts the Eurasian perch *Perca fluviatilis* L. stock of Lake Constance, Germany. *Journal of Fish Diseases*, 37, 925–930, DOI: doi:10.1111/jfd.12178
- Boeger W.A., Domingues M.V., Kritsky D.C. 1997. Neotropical Monogenoidea. 32. *Cacatuocotyle paranaensis* n. g., n. sp. (Dactylogyridae, Ancyrocephalinae) from *Characidium* spp. (Teleostei, Characidae) from the State of Paraná, Brazil. *Systematic Parasitology*, 36, 75–78. DOI: 10.1023/A:1005796027406
- Buchmann K., Lindenstrøm T. 2002. Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology*, 32, 309–319. DOI: 10.1016/S0020-7519(01)00332-0
- Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology*, 83, 575–583
- Cohen S.C., Justo M.C., Kohn A. 2013. South American Monogenoidea parasites of fishes, amphibians and reptiles. *Oficina de livros*, Rio de Janeiro, pp. 663
- Cunningham C.O., Mo T.A., Collins C.M., Buchmann K., Thiery R., Blanc G., Lutraite A. 2001. Redescription of *Gyrodactylus teuchis* Lutraite, Blanc, Thiery, Daniel & Vigneulle, 1999 (Monogenea: Gyrodactylidae); a species identified by ribosomal RNA sequence. *Systematic Parasitology*, 48, 141–150. DOI: 10.1023/A:1006407428666
- Eiras J.C., Takemoto R.M., Pavanelli G.C. 2010. Diversidade dos parasitas de peixes de água doce do Brasil. *Clichetec*, Maringá, pp. 333
- Eschmeyer W.N., Fricke R., van der Laan R. (Eds) 2016. Catalog of fishes: genera, species, references. Available at: <<http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>> Accessed in: June, 2017
- Euzet L., Combes C. 1998. The selection of habitats among the monogenea. *International Journal for Parasitology*, 28, 1645–1652. DOI: 10.1016/S0020-7519(98)00062-9
- Fehlauer-Ale K.H., Littlewood D.T.J. 2011. Molecular phylogeny of *Potamotrygonotyle* (Monogenea, Monocotylidae) challenges the validity of some of its species. *Zoologica Scripta*, 40, 638–658
- Franceschini L., Zago A.C., Müller M.I., Francisco C.J., Takemoto R.M., Silva, R.J. 2017. Morphology and molecular characterization of *Demidospermus spirophallus* n. sp., *D. prolixus* n. sp. (Monogenea: Dactylogyridae) and a redescription of *D. anus* in siluriform catfish from Brazil. *Journal of Helminthology*. DOI: 10.1017/S0022149X17000256
- Gallas M., Calegari-Marques C., Amato S.B. 2014. A new species of *Cacatuocotyle* (Monogenea, Dactylogyridae) parasitizing two species of *Astyanax* (Ostariophysi, Characidae) in southern Brazil. *Acta Parasitologica*, 59, 638–642. DOI: 10.2478/s11686-014-0283-6
- Gasques L.S., Graca R.J., Prioli S.M.A.P., Takemoto R.M., Prioli, A.J. 2016. Molecular characterization of *Urocleidoides cuiabai* and *U. malabaricus* (Monogenea: Dactylogyridae) from the trahira fish *Hoplias aff. malabaricus* in the Parana River, Brazil. *Journal of Helminthology*, 90, 693–697. DOI:10.1017/S0022149X15000966
- Huysse T., Malmberg G., Volckaert F.A.M. 2004. Four new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea, Gyrodactylidae) on gobiid fishes: combined DNA and morphological analyses. *Systematic Parasitology*, 59, 103–120. DOI: 10.1023/B:SYPA.0000044427.81580.3
- Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., *et al.* 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649. DOI: 10.1093/bioinformatics/bts199
- Kritsky D.C., Fritts T.H. 1970. Monogenetic trematodes from Costa Rica with the proposal of *Anacanthocotyle* gen. n. (Gyrodactylidae: Isoancistrinae). *Proceedings of the Helminthological Society of Washington*, 37, 63–68
- Kritsky D.C., Leiby, P.D. 1972. Dactylogyridae (Monogenea) from the Freshwater Fish, *Astyanax fasciatus* (Cuvier), in Costa Rica, with descriptions of *Jainus hexops* sp. n., *Urocleidoides costaricensis*, and *U. heteroancistrum* combs, n. *The Helminthological Society of Washington*, 39, 227–230
- Kritsky D.C., Thatcher V.E., Boeger W.A. 1986. Neotropical Monogenea. 8. Revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proceedings of the Helminthological Society of Washington*, 53, 1–37
- Littlewood D.T.J., Curini-Galletti M., Herniou E.A. 2000. The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution*, 16, 449–466. DOI: 10.1006/mpev.2000.0802
- Lockyer A.E., Olson P.D., Littlewood D.T.J. 2003. Utility of complete large and small subunit rRNA genes in resolving the



- phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society*, 78, 155–171. DOI: 10.1046/j.1095-8312.2003.00141.x
- Mendoza-Franco E., Reina R.G., Torchin M.E. 2009. Dactylogyrids (Monogonoidea) parasitizing the gills of *Astyanax* spp. (Characidae) from Panama and southeast Mexico, a new species of *Diaphorocleidus* and a proposal for *Characithecium* n. gen. *Journal of Parasitology*, 95, 46–55. DOI: 10.1645/GE-1592.1
- Mendoza-Franco E., Caspeta-Mandujano J.M., Salgado-Maldonado G. 2013. New species of *Cacatuocotyle* (Monogonoidea, Dactylogyridae) parasitizing the anus and the gill lamellae of *Astyanax aeneus* (Pisces, Ostariophysi: Characidae) from the Rio Lacantún basin in the Biosphere Reserve of Montes Azules, Chiapas, Mexico. *Parasitology Research*, 12, 199–205. DOI: 10.1007/s00436-012-3126-0
- Mendoza-Palmero C.A., Scholz T., Mendoza-Franco E.F., Kuchta R. 2012. New species and geographical records of dactylogyrids (Monogonoidea) of catfish (Siluriformes) from the Peruvian Amazonia. *Journal of Parasitology*, 98, 484–497. DOI: 10.1645/GE-2941.1
- Mendoza-Palmero C.A., Blasco-Costa I., Scholz T. 2015. Molecular phylogeny of Neotropical monogeneans (Platyhelminthes: Monogonoidea) from catfishes (Siluriformes). *Parasite & Vectors*, 8, 164. DOI: 10.1186/s13071-015-0767-8
- Mendoza-Palmero C.A., Blasco-Costa I., Henández-Mena, D., Pérez-Ponce de León, G. 2017. *Parasciadiclithrum octofasciatum* n. gen., n. sp. (Monogonoidea: Dactylogyridae), parasite of *Rocio octofasciata* (Regan) (Cichlidae: Perciformes) from Mexico characterised by morphological and molecular evidence. *Parasitology International*, 66, 152–162. DOI: 10.1016/j.parint.2017.01.006
- Monteiro C.M., Brasil-Sato M.C. 2014. A new species of *Anacanthoroides* and redescription of *Apedunculata discoidea* (Monogonoidea) parasitizing *Prochilodus argenteus* (Actinopterygii) from the São Francisco River, Brazil. *Zootaxa*, 3784, 259–266. DOI: 10.11646/zootaxa.3784.3.5
- Moreira J., Scholz T., Luque J. L. 2016. A new species of *Diaphorocleidus* (Monogonoidea: Ancyrocephalinae) from the gills of *Argonectes robertsi* (Characiformes) and new records of dactylogyrids parasitic on fishes from the Xingu River, Amazon Basin, Brazil. *Zoologia*, 33, 1–6. DOI: 10.1590/S1984-4689zool-20160022
- Müller M.I., Ceccarelli P.S., Ueta M.T. 2016. Supplementary studies on *Anacanthorus penilabiatius* and *Mymarothecium viatorum* (Monogonoidea: Dactylogyridae) from *Piaractus mesopotamicus* (Characiformes: Serrasalminidae) in Brazil. *Acta Parasitologica*, 61, 508–515. DOI: 10.1515/ap-2016-0067
- Narciso R.B., Brandão H., Yamada F.H., Benine, R.C., Silva R.J. 2014. A new species of *Trinibaculum* (Monogonoidea: Dactylogyridae) parasite of the gills of *Astyanax fasciatus* (Cuvier, 1819) (Characiformes: Characidae) in a neotropical River, São Paulo State, Brazil. *Neotropical Helminthology*, 8, 85–95
- Nelson J.S. 2006. Fishes of the world. 4th edn. John Wiley & Sons, United States of America, pp. 601
- Řehulková E., Mendlová M., Šimková A. 2013. Two new species of *Cichlidogyrus* (Monogonoidea: Dactylogyridae) parasitizing the gills of African cichlid fishes (Perciformes) from Senegal: morphometric and molecular characterization. *Parasitology Research*, 112, 1399–1410. DOI: 10.1007/s00436-013-3291-9
- Reis R.E., Kullander S.O., Ferraris Jr. C.J. 2003. Check List of the Freshwater Fishes of South and Central America. EDIPU-CRS, Porto Alegre, pp. 742
- Rossin M.A., Timi J. T. 2014. *Characithecium* (Monogonoidea: Dactylogyridae) parasitic on the Neotropical fish *Oligosarcus jenynsii* (Teleostei: Characidae) from the Pampasic region, Argentina, with the emendation of the genus. *Zootaxa*, 3893, 382–396. DOI: 10.11646/zootaxa.3893.3.4
- Tamura K., Stecher G., Peterson D., Filipowski A., Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 12, 2725–2729. DOI: 10.1093/molbev/mst197
- Thatcher V.E. 2006. Amazon fish parasites. 2nd Edn. Pensoft, Bulgaria, pp. 508

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