

Research Paper

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# Neotropical sisterhood: new species of *Gyrodactylus* von Nordmann, 1832 (Platyhelminthes: Monogenea) infecting *Rhamdia guatemalensis* and *Rhamdia laticauda* (Siluriformes: Heptapteridae) in Mexico

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## Abstract

We describe two new species of monogenean parasites of the genus *Gyrodactylus* von Nordmann, 1832 infecting Neotropical catfishes (Siluriformes) in southern Mexico: *Gyrodactylus chulini* n. sp. from ‘chulín’, *Rhamdia laticauda* collected in Oaxaca; and *Gyrodactylus juili* n. sp. from ‘juil’, *Rhamdia guatemalensis* from Veracruz. Morphologically, both new taxa are similar to *Gyrodactylus* spp. infecting catfishes (Siluriformes) in South America. Sequences of the internal transcribed spacers (ITS1-5.8S–ITS2 rDNA), the D2+D3 domains of the large ribosomal subunit (28S rDNA) and the cytochrome oxidase II (COII) gene were obtained from multiple parasite specimens and analysed using Bayesian inference. Phylogenetic hypotheses using ITS rDNA and COII genes, recovered two new *Gyrodactylus* species from *Rhamdia* spp.: *G. chulini* n. sp.; and *Gyrodactylus juili* n. sp., which are sister species to *Gyrodactylus liliana*, a parasite of *Rhamdia quelen* in Brazil, and show strong affinity to other gyrodactylids infecting Neotropical catfishes. This suggests that these new taxa, the first gyrodactylids described from *Rhamdia* spp. in Mexico, co-migrated to Tropical Middle America with their Neotropical catfish hosts, after the emergence of the Isthmus of Panama.

## Introduction

Neotropical freshwater catfishes from the genus *Rhamdia* are found throughout the Americas, from Mexico to Argentina. *Rhamdia* is taxonomically complex and no consensus exists on the total number of species that the genus contains, with hypotheses spanning a huge range, from 11 to 16 species (Silfvergrip, 1996; Perdices *et al.*, 2002; Hernández *et al.*, 2015; Arroyave & De La Cruz-Fernández, 2021). There is agreement, however, that the genus originated in South America and expanded northwards by colonizing Central America after the rise of the Isthmus of Panama – and stopped at the Isthmus of Tehuantepec in Mexico, the northernmost distribution limit for several Neotropical organisms. This region between Tehuantepec and Panama corresponds to Tropical Middle America *sensu* Choudhury *et al.* (2017), which is inhabited by two species of *Rhamdia* supported by morphometric and phylogenetic analyses, the pale catfish or ‘juil’, *Rhamdia guatemalensis*; and the filespine ‘chulín’, *Rhamdia laticauda* (Perdices *et al.*, 2002; Arroyave & De La Cruz-Fernández, 2021).

The distinction of these two species is supported by the studies by Perdices *et al.* (2002), in which the mtDNA analyses place the trans-Andean ‘*Rhamdia quelen*’ of Central America in a different clade from the typical cis-Andean *R. quelen* of South America (Hernández *et al.*, 2015). *Rhamdia quelen* found in South America is known to be a complex of cryptic species, containing at least seven allopatric molecular operational taxonomic units associated to different hydrological basins (Ribolli *et al.*, 2021), which could become separate species if shown to be independent evolutionary lineages.

Although clearly understudied in the Neotropics (Mendoza-Palmero *et al.*, 2015; García-Prieto *et al.*, 2022), some monogenean parasites from the speciose Gyrodactylidae have been described infecting catfishes (Siluriformes). Members of *Scleroductus* (Jara & Cone, 1989) were identified from this group of fish: *Scleroductus yuncensis* Jara & Cone, 1989 from *Pimelodella yuncensis*, Steindachner in Peru (Jara & Cone, 1989); *Scleroductus lyr-ocleithrum* Kritsky, Boeger, Mendoza-Franco & Vianna, 2013, from *R. guatemalensis* in Mexico; *Scleroductus angularis* Kritsky, Boeger, Mendoza-Franco & Vianna, 2013 from barred surobim *Pseudoplatystoma fasciatum* in Brazil; and undescribed *Scleroductus* sp. from *R.*

*quelen* and other wild heptaterid and pimelodid catfishes in Brazil (Kritsky et al., 2013). A few species of *Gyrodactylus* have also been described from siluriformes in Brazil: *Gyrodactylus any-sopharynx* Popazoglo & Boeger, 2000, *Gyrodactylus samirae* Popazoglo & Boeger, 2000 and *Gyrodactylus superbis* (Szidat, 1973) Popazoglo & Boeger, 2000, all three from armored catfishes *Corydoras paleatus* and *Corydoras ehrhardti* (Popazoglo & Boeger, 2000); *Gyrodactylus bueni* Bueno-Silva & Boeger, 2014, *Gyrodactylus major* Bueno-Silva & Boeger, 2014 and *Gyrodactylus scleromistaci* Bueno-Silva & Boeger, 2014, all from sailfin corydoras, *Scleromystax macropterus* and banded corydoras, *Scleromystax barbatus* (Bueno-Silva & Boeger, 2014); and *Gyrodactylus lilianae* Razzolini, Murari, Baldisserotto & Boeger, 2019 from *R. quelen* (Razzolini et al., 2019). Finally, *Gyrodactylus* sp. have been recorded from both *R. guatemalensis* and *R. laticauda* in Mexico (Salgado-Maldonado et al., 2005, 2011; Mendoza-Garfias et al., 2017).

In the present study, two new species of *Gyrodactylus* from *R. guatemalensis* and *R. laticauda* collected in Mexico are described using morphological and molecular analyses.

## Materials and methods

### Sample collection and preparation

Surveys were conducted between 2013 and 2018 to collect two species of heptapterids in southern Mexico: pale catfish or 'juil' *Rhamdia guatemalensis* (Günther, 1864); and 'filespine chulin' *R. laticauda* (Kner, 1858). Fish were collected by electro-fishing from Río La Antigua, Apazapan, Veracruz, and Río Chiquito, Cuicatlán, Oaxaca, respectively. Fish were anaesthetized with an overdose of 2-phenoxyethanol (Sigma-Aldrich, St. Louis, Missouri) prior to their preservation in 95% ethanol. Monogenean parasites were removed using surgical needles and processed individually. Two lots of worms were processed following different techniques to visualize their morphology: (a) Grey & Wess was used to examine internal structures such as the male copulatory organ (MCO) and the pharynx – for this, whole worms were cleared and mounted in a drop of Grey & Wess solution, coverslipped and incubated overnight at 60°C (Vidal-Martínez et al., 2001); and (b) proteinase-K based digestion was employed to better visualize haptor hook morphology – for this, haptors were excised using a scalpel, and a partial proteolytic digestion was completed to remove tissue enclosing the haptor armature (Rubio-Godoy et al., 2012). Digestion was arrested by the addition of a 50:50 formaldehyde-glycerine solution, and specimens were then coverslipped and sealed with nail varnish. For further molecular analyses, the bodies whose haptors had been excised were preserved in 95% ethanol and stored individually at –20°C in 1.5 ml Eppendorf tubes.

### Morphological analysis

To study the digested haptor hard parts, these were placed on a Leica DM750 compound microscope (magnification of 10 × 100 for the hamuli with oil immersion 100 objective lens for the marginal hooks, in phase contrast). Photographs were taken using imaging analysis software LAS V4.12 V4 in a Leica ICC50 HD camera, attached to a Leica DM 750 compound microscope (magnification of 10 × 100 with oil immersion lens). Using the ImageJ 1.46r software, attachment hook measurements were taken from images. A total of 29 point-to-point measurements detailed by

**Table 1.** Morphological measurements of the new *Gyrodactylus* species collected from Cuicatlán, Oaxaca and Apazapan, Veracruz, Mexico infecting 'chulin', and *Rhamdia laticauda* 'juil' *Rhamdia guatemalensis*, respectively.

Measurement	<i>Gyrodactylus chulini</i> n. sp., n = 20	<i>Gyrodactylus juili</i> n. sp., n = 17
HTL	53.9 ± 3.84 (51.2–66.8)	50.5 ± 3.33 (43.6–54.8)
HA	15.9 ± 1.53 (13.7–18.7)	17.0 ± 1.50 (13.9–19.9)
HPSW	7.2 ± 0.68 (6.2–8.3)	6.5 ± 0.43 (5.8–7.4)
HPL	28.6 ± 2.45 (25.0–36.2)	24.95 ± 1.65 (21.2–27.1)
HDSW	3.7 ± 0.48 (3.1–5.0)	3.0 ± 0.25 (2.6–3.4)
HSL	32.8 ± 2.39 (30.7–40.4)	30.8 ± 2.29 (27.3–34.8)
HICL	1.0 ± 0.24 (0.6–1.5)	0.9 ± 0.40 (0.4–1.7)
HAA°	29.7 ± 2.78 (24.7–33.4)	34.8 ± 3.90 (30.1–45.3)
HPCA°	2.6 ± 1.08 (0.2–4.5)	3.1 ± 3.12 (1.3–5.7)
IHAA°	34.0 ± 2.85 (28.9–37.8)	30.6 ± 3.36 (34.1–45.2)
HRL	20.2 ± 2.04 (17.5–26.8)	19.4 ± 1.18 (17.0–21.5)
VBL	20.1 ± 1.27 (18.3–23.5)	19.6 ± 1.54 (17.1–22.4)
VBW	19.8 ± 1.63 (16.9–24.0)	17.5 ± 1.48 (14.6–19.5)
VBPML	2.8 ± 0.47 (2.2–3.5)	2.5 ± 0.59 (1.0–3.3)
VBML	6.8 ± 0.58 (5.7–7.9)	5.4 ± 0.77 (4.0–6.9)
VBPL	1.8 ± 0.30 (1.4–2.5)	1.3 ± 0.40 (0.6–2.2)
VBMemL	11.1 ± 1.20 (9.4–13.6)	11.7 ± 1.46 (9.5–15.5)
DBL	2.1 ± 0.36 (1.4–2.7)	1.7 ± 0.27 (1.1–2.2)
DBW	15.4 ± 2.83 (10.9–21.9)	15.5 ± 2.81 (10.6–21.8)
DBAPTL	7.5 ± 0.49 (6.4–8.5)	6.2 ± 0.39 (5.6–6.7)
MHTL	20.2 ± 1.96 (17.4–25.4)	17.5 ± 1.57 (14.4–19.2)
MHSL	13.8 ± 1.66 (10.7–17.8)	12.1 ± 1.18 (9.4–13.4)
MHSiL	6.5 ± 0.41 (6.0–7.7)	5.6 ± 0.41 (4.9–6.2)
MHSiPW	3.9 ± 0.28 (3.4–4.6)	3.3 ± 0.31 (2.6–3.8)
MHToEL	2.4 ± 0.21 (2.2–2.7)	2.0 ± 0.33 (1.0–2.5)
MHSiDW	3.1 ± 0.45 (2.6–4.1)	3.0 ± 0.42 (2.0–3.8)
MHA	6.5 ± 0.45 (5.2–7.5)	5.5 ± 0.53 (4.0–6.3)
MHI/AH	0.3 ± 0.06 (0.2–0.4)	0.4 ± 0.09 (0.2–0.6)
MHFL	11.5 ± 1.09 (10.4–14.5)	10.3 ± 0.61 (9.3–11.3)

HTL, hamulus total length; HA, hamulus aperture; HPSW, hamulus point shaft width; HPL, hamulus point length; HDSW, hamulus distal shaft width; HSL, hamulus shaft length; HICL, hamulus inner curve length; HAA°, hamulus aperture angle; HPCA°, hamulus point curve angle; IHAA°, inner hamulus aperture angle; HRL, hamulus root length; VBL, ventral bar length; VBW, ventral bar width; VBPML, ventral bar process to mid length; VBML, ventral bar median length; VBPL, ventral bar process length; VBMemL, ventral bar membrane length; DBL, dorsal bar length; DBW, dorsal bar width; DBAPTL, dorsal bar attachment point length; MHTL, marginal hook total length; MHSL, marginal hook shaft length; MHSiL, marginal hook sickle length; MHSiPW, marginal hook sickle point width; MHToEL, marginal hook toe length; MHSiDW, marginal hook sickle distal width; MHA, marginal hook aperture; MHAA, marginal hook aperture angle; MHI/AH, marginal hook instep/arch height; and MHFL, marginal hook filament loop.

García-Vásquez et al. (2015) were obtained from each specimen (see table 1). All measurements are given in micrometres, showing average ± standard deviation, minima and maxima in parentheses. Micrographs of the following *Gyrodactylus* specimens (*pers. comm.* Walter A. Boeger 2022) were used to compare the marginal hook morphology of previously described species with

those of the current study: *G. anisopharynx*; *G. bueni*; *G. corydori*; *G. liliana*; *G. major*; *G. samirae*; *G. scleromystaci*; and *G. superbus*.

### Molecular analysis

#### DNA extraction, amplification and sequencing

Total genomic DNA was isolated from eight gyrodactylids fixed in 96% ethanol. Genomic DNA of each individual was extracted (following two protocols) using the DNeasy® Blood and Tissue Kit (QIAGEN, Valencia, California) according to the manufacturer's protocol (for specimens collected from Veracruz) and DNAzol Molecular Research Center, INC. (for specimens collected from Oaxaca). Individual worms were digested overnight at 56°C in a solution containing 10 µl of 100 mM Tris-hydrochloride (pH = 7.6), 10 µl of 200 mM sodium chloride, 20 µl of 0.5 M ethylenediaminetetraacetic acid (pH = 8.0), 10 µl of 10% Sarkosyl, 1.4 µl of 20 mg/µl proteinase K and 48.6 µl of sterile distilled water. Following digestion, DNA was isolated from the supernatant using DNAzol reagent (Molecular Research Center, Cincinnati, Ohio, USA) according to the manufacturer's instructions. Three fragments of DNA were amplified: the mitochondrial cytochrome oxidase subunit II gene (COII) using the primer COII F1 (5'-TACATAYCGCCCGTCAATYT-3') and COII R1 (5'-TCARTAYCACTGDCGCCYA-3') (Xavier *et al.*, 2015); the ribosomal region spanning the 3' end of the 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, and the 5' end of the 28S rRNA gene (ITS rDNA) using the primers BD1 (5'-GTCGTAACAAGGTTTCCGTA-3') and BD2 (5'-ATCTAGACCGGACTAGGCTGTG-3') (Bowles & McManus, 1993; Bowles *et al.*, 1995); and the D1 + D2 region of the 28S rDNA using the primers C1 (5'-ACCCGCTGAATTTAAGCAT-3') and D2 (5'-TGGTCCGTGTTTCAAGAC-3') (Wu *et al.*, 2005; Mandeng *et al.*, 2015). Polymerase chain reaction (PCR) amplifications were carried out in 25 µl reactions, containing 1 µl of each primer (10 µM), 2.5 µl of 10× buffer (Promega, Madison, WI, USA), 1.5 µl of MgCl<sub>2</sub> (25 mM), 0.5 µl of dNTP mixture (10 mM), 0.125 µl of Platinum Taq DNA polymerase (5 U/µl) (Invitrogen Corporation, São Paulo, Brazil) and 2 µl of diluted template DNA. The following amplification profiles for COII/ITS rDNA/28S rDNA were used, respectively: denaturation at 95/94/94°C for 1.5/1/2 min, 40/35/40 cycles of 95/94/93°C for 0.5/1/0.5 min; annealing at 50/50/56°C for 1.5/1/0.5 min; extension at 72°C for 1.5/1/1 min; and a final extension of 72/75/72°C for 7/10/10 min. Unincorporated nucleotides and primers of each PCR amplicon were removed using ExoSap-IT (USB Corporation, Ohio). PCR amplicons for COII, ITS rDNA and 28S rDNA were cycle sequenced from both strands using the PCR primers. Sequencing reactions were performed in a final volume of 10 µl, using 1 µl of sequencing buffer 2.5×, 0.8 µl of the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California), 2.5 µl of purified amplicons, 1 µl of primer (3 pmol) used in the amplification, and 4.7 µl of sterile distilled water. Sequencing products were purified by filtration with Sephadex™ G50 (Sigma-Aldrich, St. Louis, MO) and analysed on an ABI PRISM 310 genetic analyser (Applied Biosystems). Contigs were assembled and base-calling differences resolved using Geneious 8.1.8 (<https://www.geneious.com>). Sequences were deposited in the GenBank data set.

#### Alignment and phylogenetic analyses

Sequences of COII, ITS rDNA obtained in this study were aligned with sequences of other *Gyrodactylus* spp. available in the GenBank database (see table 2). Very few 28S rDNA sequences

are available for *Gyrodactylus*, so no phylogenetic analyses are presented for the sequences generated in this study. All the sequences generated for ITS rDNA and COII were run in the Basic Local Alignment Search Tool, and species with the highest percentage of identity were included in the alignment, for example, *G. liliana* with the maximum score. Data of the two genes were aligned separately using the software ClustalW (Thompson *et al.*, 1994) with default parameters implemented in MEGA version 7.0 (Kumar *et al.*, 2016). COII sequences were adjusted manually and checked for correct amino acid translation. Nucleotide substitution models were selected for each molecular marker using jModeltest 2.1.10 (Darriba *et al.*, 2012) and applying Akaike's information criterion for each dataset (COII = GTR + I + G; ITS rDNA = TIM2 + I + G). Individual data sets were analysed using Bayesian inference (BI). Trees under BI analyses were inferred with MrBayes v3.2 (Ronquist *et al.*, 2012), where Metropolis-coupled Markov chain Monte Carlo (MC3) simulations were run for 10 million generations and sampled every 1000 generations, and the first 2500 samples were discarded as burn-in (25%). To search for the convergence of different parameters, to estimate the approximate number of generations at which log likelihood values stabilized, to recognize the effective sample size (EES > 200) for each parameter, and to estimate the magnitude of model parameters in individual and combined runs, the outputs were examined with Tracer v1.4 (Rambaut & Drummond, 2007). The initial 25% of MC3 was verified to include all the generations before stationarity was achieved. We obtained posterior probabilities of clades from the 50% majority rule consensus of sampled trees after we excluded the initial 25% as burn-in for MrBayes. The genetic divergence among species of *Gyrodactylus* was estimated using uncorrected 'p' distances with MEGA 7.0 (Kumar *et al.*, 2016).

### Results

Two new species of *Gyrodactylus* were identified from two States in Mexico: *Gyrodactylus chulini* n. sp. was found infecting *R. laticauda* in Río Chiquito, Cuicatlán, Oaxaca; and *Gyrodactylus juili* n. sp. was found on *R. guatemalensis* in Río La Antigua, Apazapan, Veracruz. These are the first two species of *Gyrodactylus* described from *Rhamdia* spp. in Mexico. Morphological descriptions of each new species are presented in alphabetical order and their measurements are shown in table 1 (average ± standard deviation, and minima and maxima in parentheses).

**Nomenclatural acts:** This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the International Code of Zoological Nomenclature. The ZooBank Life Science Identifiers (LSIDs) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix 'http://zoobank.org/'. The LSID for this publication is: urn:lsid:zoobank.org:pub:86AA1B2C-B9A3-4DD7-8B2C-E6CD4DC0B570.

#### *Gyrodactylus chulini* n. sp. (figs 1a and 2, table 1)

LSID urn:lsid:zoobank.org:act:0EEEDD35-2EB5-4C20-A2BF-3FD7DE5ADE82

*Type host:* *Rhamdia laticauda* (Kner, 1858)

*Site of infection:* Fins.

*Type locality:* Río Chiquito, Cuicatlán, Oaxaca, Mexico (17° 48' 41.7" N, 96° 57' 38.7" W)

*Type material:* Holotype (accession nos. CNHE11703), two paratypes (accession nos. CNHE11704, CNHE11705) and three vouchers (accession nos. CNHE11706, CNHE11707, CNHE11708)

**Table 2.** Newly generated sequences and sequences available on GenBank for *Gyrodactylus* spp.

Species	Host	Country: Locality	GenBank			Reference
			Internal transcribed spacer	Cytochrome oxidase II	28S	
<i>Gyrodactylus juili</i> n. sp.	<i>Rhamdia guatemalensis</i>	Mexico: Río La Antigua, Apazapan, Veracruz	OP023865-OP023867	OP009448-OP009449	OP023897-OP023898	Present study
<i>Gyrodactylus chulini</i> n. sp.	<i>Rhamdia laticauda</i>	Mexico: Río Chiquito, Cuicatlán, Oaxaca	OP023860-OP023864	OP009445-OP009447	OP023895-OP023896	Present study
<i>Gyrodactylus anisopharynx</i>	<i>Characidium lanei</i>	Brazil: Rio Marumbi, Morretes, Parana	MH035872	–	–	Razzolini <i>et al.</i> (2019)
	<i>Corydoras paleatus</i>	Brazil: Rio Iguacu, Agua Azul, Lapa, Parana	–	MH043345-MH043347	–	Razzolini <i>et al.</i> (2019)
<i>Gyrodactylus corydori</i>	<i>Corydoras ehrhardti</i>	Brazil:	HQ636619, HQ636620	GU131197, GU131198	–	Bueno-Silva <i>et al.</i> (2011)
<i>Gyrodactylus superbus</i>	<i>Corydoras</i> sp.	Brazil: Barragem do Chasqueiro, Pelotes, Rio Grande do Sul	MH035869	–	–	Razzolini <i>et al.</i> (2019)
	<i>C. ehrhardti</i>	Brazil:	–	GU131202, GU131203, GU131205	–	Unpublished
<i>Gyrodactylus liliana</i>	<i>Rhamdia quelen</i>	Brazil: Barragem do Chasqueiro, Pelotes, Rio Grande do Sul	MH035870, MH035871	MH043348, MH043349	–	Razzolini <i>et al.</i> (2019)
<i>Gyrodactylus major</i>	<i>Scleromystax macropterus</i>	Brazil:	KF767478-KF767480	GU131209, GU131210, KF751720, MK395591	–	Bueno-Silva & Boeger (2014)
<i>Gyrodactylus scleromystaci</i>	<i>Scleromystax barbatus</i>	Brazil:	KF767472, KF767474	GU131216, GU131217, GU131219, GU131220, KF751709, MK395588, MT521674	–	Bueno-Silva & Boeger (2014)
<i>Gyrodactylus bueni</i>	<i>S. macropterus</i>	Brazil:	KF767475-KF767477	GU131214, GU131215, KF751721	–	Bueno-Silva & Boeger (2014)
	<i>S. macropterus</i>	Brazil:	–	MK395607	–	Boeger <i>et al.</i> (2020)
<i>Gyrodactylus samirae</i>	<i>C. ehrhardti</i>	Brazil:	–	GU131200	–	Unpublished
	<i>C. ehrhardti</i>	Brazil:	–	KF751718	–	Bueno-Silva & Boeger (2014)
<i>Gyrodactylus bullatarudis</i>	–	Trinidad and Tobago: Lopinot (Arouca) River	AY692024	–	–	Cable <i>et al.</i> (2005)

(Continued)

Table 2. (Continued.)

Species	Host	Country: Locality	GenBank			Reference
			Internal transcribed spacer	Cytochrome oxidase II	28S	
	<i>Poecilia reticulata</i>	Trinidad and Tobago:	–	KP168350	–	Xavier <i>et al.</i> (2015)
<i>Gyrodactylus turnbulli</i>	<i>P. reticulata</i>	Poland: Gdansk aquarium	EF445942	–	–	Lumme & Ziętara (2018)
	<i>P. reticulata</i>	Trinidad and Tobago:	–	KP168404	–	Xavier <i>et al.</i> (2015)
<i>Gyrodactylus anaspidus</i>	–	Brazil: Rio do Nunes, bacia Cacatu, Antonina, PR	–	MT521677	–	Boeger <i>et al.</i> (2021)
<i>Gyrodactylus carolinae</i>	<i>Characidium lanei</i>	Brazil: Rio Marumbi, Morretes, Parana	–	MH043352	–	Razzolini <i>et al.</i> (2019)
	<i>C. lanei</i>	Brazil: Rio Marumbi, Morretes, Parana	KF673402	–	–	Boeger <i>et al.</i> (2014)
<i>Gyrodactylus</i> sp.	<i>Aspidoras eurycephalus</i>	Brazil: Ribeirao Manduca, Porto Nacional, TO	–	MT521673	–	Boeger <i>et al.</i> (2021)

deposited in the Colección Nacional de Helminthos (CNHE), Mexico City.

**DNA reference sequences:** Sequences obtained from five individuals are deposited in GenBank (ITS accession nos. OP023860–OP023864; COII accession nos. OP009445–47; and 28S accession nos. OP023895, OP023896).

**Etymology:** This species is named after its type host, *R. laticauda*, commonly known in Central America as ‘chulín’.

**Description** (average (range), in micrometers): Morphological description based on 18 specimens, ten whole worms cleared in Grey & Wess solution, and eight haptors proteolytically digested. Body 411 (352–463) long; 108 (89–133) wide at uterus level. Pharynx ( $n = 8$ ) 28 (22–33) long  $\times$  29 (24–32) wide (fig. 1a). Haptor 68 (63–85) length  $\times$  67 (48–82) width. Hamuli 53 (51–66) long, 3 (3–5) wide; almost the same thickness throughout the hook, except at the dorsal bar attachment point, which becomes wider; shaft 32 (30–40) long; slim point 28 (25–36) long, comprising more than half of the shaft length, slightly curved outward at the point proper; proximal shaft width 7 (6–8) wide; slightly curved shaft; hamulus aperture distance 15 (13–18) long; hamulus aperture angle 29° (24°–33°) long; hamulus root 20.2 (17–26) long (fig. 2b, i). Ventral bar 19 (16–24) wide with pointed edges, 20 (18–23) long; rounded, short ventral bar processes 2 (1–3) long; trapezoidal ventral bar median portion 6 (6–8) long, short triangular ventral bar membrane 11 (9–13) long, acute edge slightly pointed (fig. 2b, k). Dorsal bar straight 15 (10–21) wide, slightly elongated in the middle 2 (1–2) long,

oval attachment points 7 (6–8) long (fig. 2c, j). Marginal hook 20 (17–25) long; shaft 14 (10–17) long (fig. 2d, l). Marginal hook sickle 6 (6–7) long, tilted forward, when reaching the point is barely curved (fig. 2e, f, m). Marginal hook distal width 3 (2–4), long and slim. Blunt toe 2 (2–3) long, sickle base wavy. Heel pointed. Marginal hook shaft entirely curved and angled towards the toe, short point ending well forward toe level. Short and curved bridge. Marginal hook aperture 6 (5–7) long. Marginal hook instep height almost straight 0.3 (0.2–0.4) deep (fig. 2d–f). Filament loop 11 (10–14) long, almost the same length of shaft. MCO visible and completely developed in three specimens. Globular 17 (15–18) long  $\times$  14 (12–15) wide, consisting in one large principal hook 6 (5–7) long, seven spines distributed next to each other forming a circle, points facing to the principal hook point, as follows (clockwise direction): (a) slender and long spine 1 (0.7–2) long, which is closer to the principal hook point, with a narrow base; (b) big spine 4 (3–4) long, with a wide base and long slim point; (c) big spine 3.5 (3.8–4.6) long, with a wider base than the second spine, point being the thickest of all spines; (d) resembling the second spine, but with shorter point and slightly wider base, 4 (3–5) long; (e) wider base and short point, being the biggest of all spines 4 (4–5) long; (f) almost identical to the second spine, but thicker point 4 (2–4) long; and (g) small and narrow base with a long point 2 (1–3) long (fig. 2g, h).

**Remarks:**

The marginal hook morphology of *G. chulini* n. sp. roughly resembles those of *G. juili* n. sp. (see below), *G. lilianae* and *G. superbus* (fig. 3). However, these species can be discriminated.



**Fig. 1.** Light micrographs of *Gyrodactylus* species described from *Rhamdia guatemalensis* and *Rhamdia laticauda* in Mexico: (a) *Gyrodactylus chulini* n. sp. at a glance; (b) *Gyrodactylus juili* n. sp. at a glance. Scale bars: 100  $\mu$ m.

Although similar, in *G. juili* n. sp. the sickle base has a more compact, pointed toe; and the sickle shaft is more upright than in *G. chulini* n. sp. Both *G. lilianae* and *G. superbus* are generally similar to *G. chulini* n. sp., but the sickle base is slightly stouter and rounded at the toe, in the new species; and the sickle shaft allows to differentiate the species, as this structure is clearly more robust and tilted forward in *G. lilianae* (fig. 3r), and at a more upright angle in *G. superbus* (fig. 3z).

***Gyrodactylus juili* n. sp.** (figs 1b and 4, table 1)

LSID urn:lsid:zoobank.org:act:8764053B-4862-45A7-B9D6-03A75183C9D1

Type host: *Rhamdia guatemalensis* (Günther, 1864).

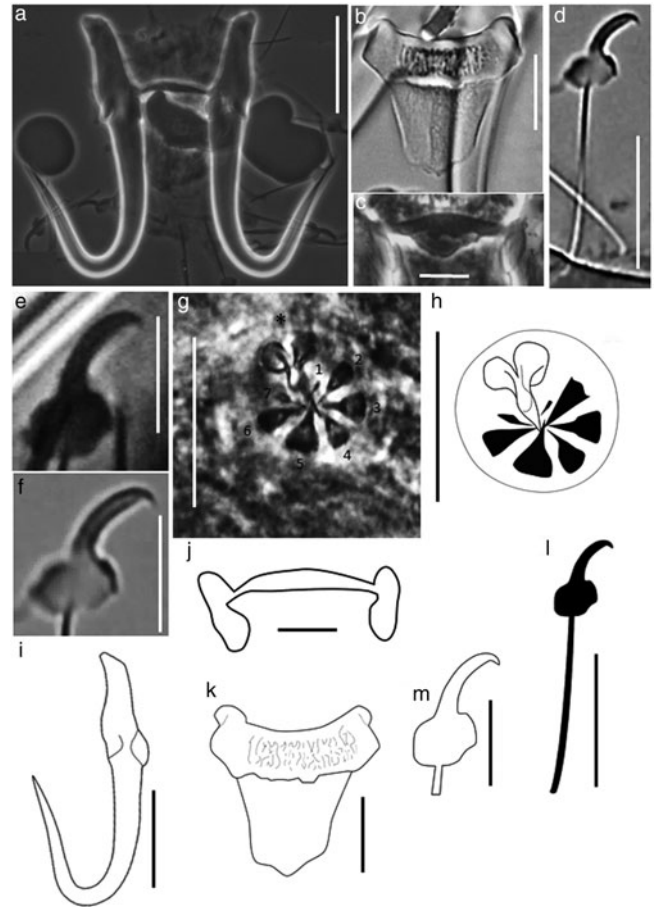
Site of infection: Fins.

Type locality: Río La Antigua, Apazapan, Veracruz, Mexico (19° 19'31.49" N, 96°43'31.57" W)

Type material: Holotype (accession no. CNHE10254), three paratypes (accession nos. CNHE10255, CNHE11701, CCNHE11702) and two vouchers (accession nos. CNHE10256, CNHE11700) deposited in the Colección Nacional de Helminthos (CNHE), Mexico City.

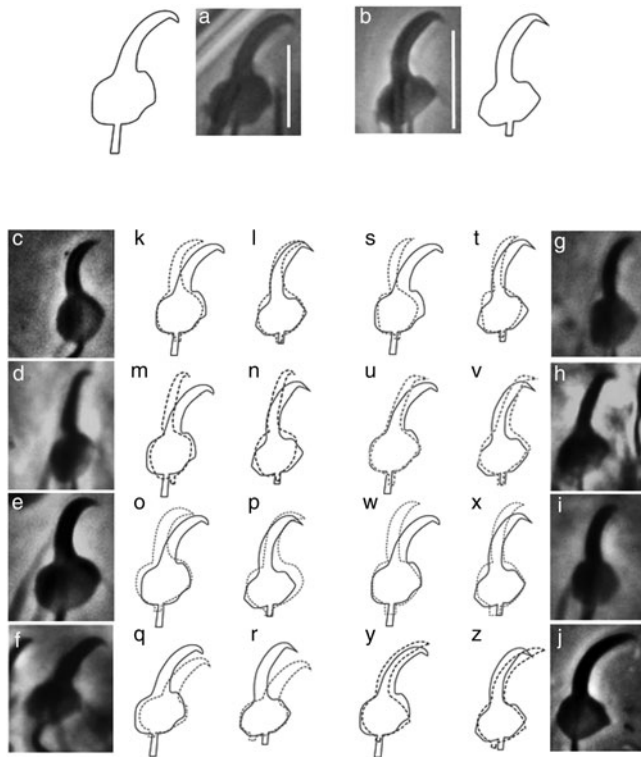
DNA reference sequences: Sequences obtained from three individuals are deposited in GenBank (accession nos. OP023865-OP023867, for ITS; COII accession nos. OP009448, OP009449; and 28S accession nos. OP023897, OP023898).

Etymology: This species is named after its type host, *Rhamdia guatemalensis*, commonly known in Mexico as 'juil'. The word probably comes from the Aztec 'xohuilin', meaning 'spotted, trout-like fish which lays many eggs'.



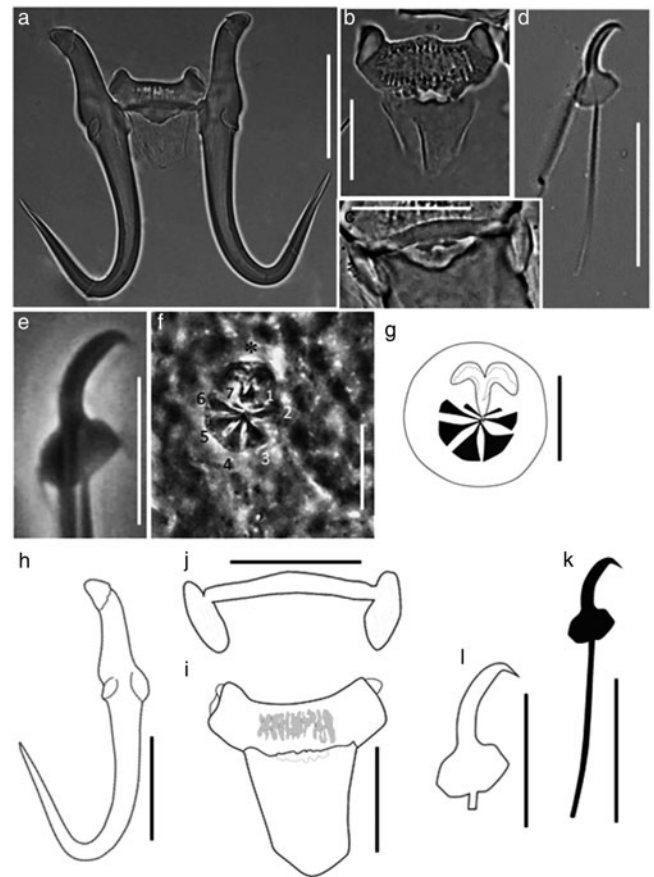
**Fig. 2.** Light micrographs and drawings of *Gyrodactylus chulini* n. sp. described from 'chulin', *Rhamdia laticauda* from Río Chiquito, Oaxaca, Mexico: (a) hamuli complex; (b, k) ventral bar; (c, j) dorsal bar; (d, l) marginal hook at a glance; (e, f, m) marginal hook sickle; (g, h) micrographs and drawing of male copulatory organ showing the principal hook (asterisk) and the spines (1–7 in clockwise direction); (i) hamulus. Scales bars: (a) 20  $\mu$ m; (b, d, g–i, k, l) 10  $\mu$ m; (c, e, f, j, m) 5  $\mu$ m.

**Description** (average (range), in micrometers): Morphological description based on 17 specimens, 11 whole worms cleared in Grey & Wess solution, and six haptors proteolytically digested. Body ( $n = 6$ ) Body 454 (271–554) long; 106 (73–157) wide at uterus level (fig. 1b). Pharynx ( $n = 11$ ) 26 (20–36) long  $\times$  27 (20–36) width (fig. 1b). Haptor ( $n = 6$ ) 66 (61–75) length  $\times$  57 (37–72) width. Hamuli 50 (44–55) long, 3 (3–4) wide, being thicker in the ventral bar attachment point; shaft 31 (27–35) long; slim point 25 (21–27) long, comprising almost the same shaft length; proximal shaft width 6 (6–7) wide; slightly curved shaft; hamulus aperture distance 17 (14–20) long; hamulus aperture angle 35° (30°–45°) long and wide; hamulus root 19 (17–21) long, ends pointed and facing to hamulus point (fig. 4a). Ventral bar 17 (15–19) wide, 19 (17–22) long; triangular ventral bar processes 2 (1–3) long, facing upwards to the hamuli roots; ventral bar median portion 5 (4–7) long, slightly curved on the edges, triangle-shaped middle notch, ventral bar membrane 12 (9–15) long, V-shaped with a stretch mark in the middle (fig. 4b). Dorsal bar 15 (11–24) wide, 2 (1–2) long and straight, oval attachment points 6 (6–7) long, (fig. 4c). Marginal hook 17 (14–19) long; shaft 12 (9–13) long, barely curved at the end (fig. 4d). Marginal hook sickle 6 (5–6) long, upright shaft bending distally ending in short point facing sickle base, just after the toe level.



**Fig. 3.** Micrographs and drawings of the marginal hooks of the new *Gyrodactylus* species collected in Mexico from *Rhamdia* spp., compared with those of gyrodactylids described from Silurid hosts in Brazil (micrographs kindly provided by Dr Walter A. Boeger, Universidade Federal do Paraná): (a) marginal hook sickle of *Gyrodactylus chulini* n. sp. from *Rhamdia laticauda*; (b) marginal hook sickle of *Gyrodactylus juili* n. sp. from *Rhamdia guatemalensis*; (c) marginal hook sickle of *Gyrodactylus anisopharynx* from *Corydoras paleatus*; (d) marginal hook sickle of *Gyrodactylus bueni* from *Scleromystax macropterus*; (e) marginal hook sickle of *Gyrodactylus corydori* from *Corydoras ehrhardti*; (f) marginal hook sickle of *Gyrodactylus lilianae* from *Rhamdia quelen*; (g) marginal hook sickle of *Gyrodactylus major* from *S. macropterus*; (h) marginal hook sickle of *Gyrodactylus samirae* from *C. ehrhardti*; (i) marginal hook sickle of *Gyrodactylus scleromystaci* from *Scleromystax barbatus*; (j) marginal hook sickle of *Gyrodactylus superbus* from *C. ehrhardti*; (k) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. anisopharynx* (dotted line); (l) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. anisopharynx* (dotted line); (m) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. bueni* (dotted line); (n) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. bueni* (dotted line); (o) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. corydori* (dotted line); (p) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. corydori* (dotted line); (q) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. lilianae* (dotted line); (r) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. lilianae* (dotted line); (s) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. major* (dotted line); (t) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. major* (dotted line); (u) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. samirae* (dotted line); (v) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. samirae* (dotted line); (w) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. scleromystaci* (dotted line); (x) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. scleromystaci* (dotted line); (y) overlay of the marginal hook sickle of *G. chulini* n. sp. (solid line) with that of *G. superbus* (dotted line); and (z) overlay of the marginal hook sickle of *G. juili* n. sp. (solid line) with that of *G. superbus* (dotted line). Scale bars: 5  $\mu$ m.

Marginal hook distal width 3.3 (2.6–3.8) long and slim. Toe 2 (1–2) long. Marginal hook aperture 5 (4–6) long. Heel ovate. Marginal hook bridge short and pointed. Marginal hook instep height barely noticeable 0.4 (0.2–0.6). Filament loop 10 (9–11) long, almost same length of the shaft (fig. 4d, e). MCO ( $n = 3$ )



**Fig. 4.** Micrographs of *Gyrodactylus juili* n. sp. described from 'juil', *Rhamdia guatemalensis* from Río La Antigua, Veracruz, Mexico: (a) hamuli complex; (b, i) ventral bar; (c, j) dorsal bar; (d, k) marginal hook at a glance; (e, l) marginal hook sickle; (f, g) male copulatory organ showing the principal hook (asterisk) and the spines (1–7 in clockwise order); and (h) hamulus. Scale bars: (a, h) 20  $\mu$ m; (b–d, f, g, i–k) 10  $\mu$ m; (e, l) 5  $\mu$ m;

globular to ovoid 12 (10–14) long  $\times$  12 (10–13) wide. Consisting of one large principal hook 5 (5–6) long (fig. 2f, g), two tiny and thin spines, five robust triangular spines almost same length, all distributed around the principal hook (clockwise direction): two tiny spines (1: 1 and 7: 1), located to each side of the base point of the principal hook (only visible on one specimen) (fig. 3g); two slim spines 2: 4 (3–4) long, 6: 3 (3–4) long, positioned to each side of the principal hook, points facing the centre of MCO; three thick base spines: 3: 5 (4–5) long; 4: 4 (4–5); and 5: 4.3 (4.2–4.4) long, gradually narrowing into an acute point when reaching the centre (fig. 4f, g).

#### Remarks:

In *G. juili* n. sp., the marginal hook morphology closely resembles that of gyrodactylids infecting catfishes (Callichthyidae) in southern Brazil: *G. anisopharynx*; *G. bueni* and *G. corydori* (fig. 3). Nonetheless, these species can be discriminated. Compared to *G. anisopharynx*, *G. juili* n. sp. has a more angular marginal hook base, and the sickle shaft although upright, begins lower and is comparatively tilted forward (fig. 3k). The marginal hook base of *G. juili* n. sp. is clearly more angular than in *G. bueni*, and the sickle shafts markedly differ in width and their distal end (fig. 3m). The marginal hook of *G. corydori* is the most similar, but this structure is overall more rounded and robust in this species, enabling discrimination from *G. juili* n. sp. (fig. 3o).

### Molecular characterization and genetic divergence

The ITS rDNA sequences were similarly long in the two new species: *G. juili* n. sp. (1341 bp) and *G. chulini* n. sp. (1391 base pairs (bp)) showed an ITS1 of 536 and 588 bp, respectively, whereas ITS2 was 649 and 646 bp long, respectively. The length of the 5.8S rDNA gene in *G. juili* n. sp. was of 156 bp while in *G. chulini* n. sp. it was of 157 bp. With respect to COII, the partial length of the sequences was of 262 bp. The partial length of the 28S rDNA was of 752 bp. The genetic divergence estimated with the three molecular markers for *G. chulini* n. sp. and *G. juili* n. sp. ranged from 14.59 to 14.58% for ITS rDNA, from 10.92 to 11.55% for COII, and from 3 to 3.5% for 28S rDNA; and between *G. liliana*e and the two new species, ITS rDNA and COII divergence ranged from 23.28 to 30.65% and from 17.20 to 19.78%, respectively. Intraspecific genetic divergence in *G. juili* n. sp. and *G. chulini* n. sp. was of 1.8% and 0% for ITS rDNA, 3.3% and 0% for COII, respectively, and null for 28S rDNA (table 3).

### Phylogenetic analyses

We obtained sequence data from three loci (ITS rDNA, COII and 28S rDNA) for *G. juili* n. sp. ( $n = 3$ ) from Veracruz and *G. chulini* n. sp. ( $n = 5$ ) from Oaxaca, Mexico. Phylogenetic analyses of the sequences of the ITS rDNA and COII gene fragments support the erection of two new gyrodactylid species, each forming independent clades with high posterior probability support (see fig. 5). *Gyrodactylus chulini* n. sp. and *G. juili* n. sp. were found to be reciprocally monophyletic in all analyses, and to be related as sister species; both with strong nodal support ( $\geq 0.96$ ). These two gyrodactylids are recovered as sister species to *G. liliana*e (fig. 5).

### Discussion

The Amazon basin is one of the most diverse biomes for fish and parasite species in the world (Luque & Poulin, 2007; Nelson et al., 2016), and a place where evolutionary novelties/oddities occurred in the Monogenea. For instance, continental waters in Brazil are the only place where oviparous gyrodactylids have been recorded, with 23 species described from armoured (Loricariidae) and long-whiskered (Pimelodidae) catfishes; and it has recently been suggested that Oogryodactylidae diversified following the Gondwana breakup and the separation of Africa and South America (Boeger et al., 2020). Also, the speciose family Dactylogyridae, which primarily includes gill-infecting species and has undergone a marked evolutionary radiation in Africa (e.g. Tanganyika) (Vanhove et al., 2015), occasionally evolved in the Neotropics to an endoparasitic habit more commonly

**Table 3.** Pairwise differences ( $p$ -distances) of internal transcribed spacers (ITS1–5.8S–ITS2) rDNA (above the diagonal) and cytochrome oxidase II (COII) mtDNA (below the diagonal) among new species of *Gyrodactylus*.

	1	2	3
1. <i>Gyrodactylus juili</i> n. sp.	3.30/0–1.81	14.59–14.84	23.28–29.10
2. <i>Gyrodactylus chulini</i> n. sp.	10.92–11.55	0/0	25.00–30.65
3. <i>Gyrodactylus liliana</i> e	17.20–19.78	18.04	0/3.49

Genetic divergence values are expressed as a percentage. Values in the diagonal (grey fill) represent the intraspecific genetic divergence (COII/ITS).

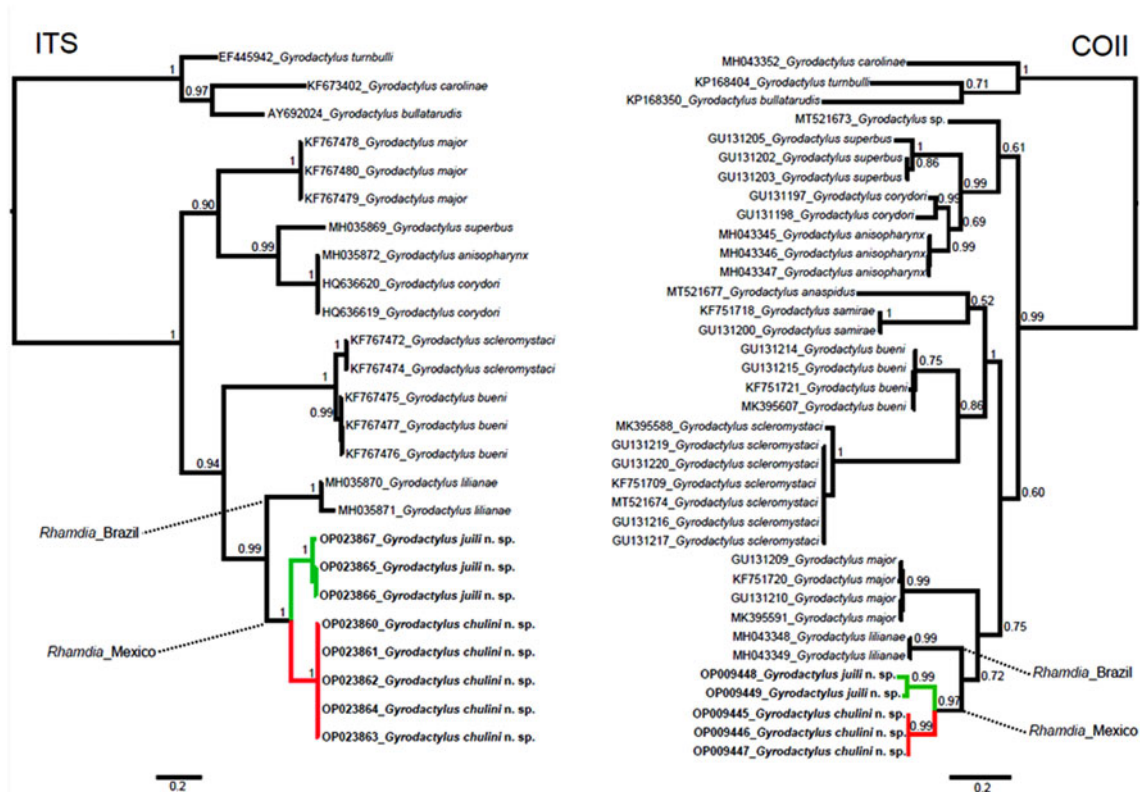
associated to Polystomatids, as a handful of monogeneans inhabit the urinary bladder and ureters of their fish hosts – examples include: *Kristkyia moravec*i Kohn, 1990, infecting *R. quelen* in Brazil (Kohn, 1990); *Kristkyia annakohnae* Boeger, Tanaka & Pavanelli, 2001 recorded from piranhas *Serrasalmus marginatus* and *Serrasalmus spilopleura* in Brazil (Boeger et al., 2001); *Kristkyia boegeri* Takemoto, Lizama & Pavanelli, 2002 from streaked prochilod, *Prochilodus lineatus* in Brazil (Takemoto et al., 2002); *Acolpenteron australe* Viozzi & Brugni, 2003 from Creole perch, *Percichthys trucha* in Patagonia, Argentina (Viozzi & Brugni, 2003); or *Philureter trigoniopsis* Viozzi & Gutiérrez, 2001 infecting common galaxias, *Galaxias maculatus*, also in Patagonia, Argentina (Viozzi & Gutiérrez, 2001) – although this is not unique to the Neotropics, as a few species with this habit are known from Cameroon, Russia and the United States. Considering these examples, it is generally interesting to further characterize the species-rich and relatively understudied Monogenea infecting Neotropical fishes. Furthermore, scrutinizing parasites of fishes in the Americas also sheds light on the validity of the ‘Great American Biotic Interchange’ as a bidirectional exchange of Neotropical and Nearctic faunas, a concept that has recently been challenged regarding freshwater fishes and their associated parasites, as many more fish host–parasite associations seem to have travelled northward from the Neotropics than in the other direction, southwards from the Nearctic (Choudhury et al., 2017).

The two new species of *Gyrodactylus* we describe in this work contribute to these two aspects: we characterize the parasites of a representative fish family originating and widely distributed in the Neotropics; and support the notion that the parasites were co-distributed with their fish hosts as they migrated through Central America following the emergence of the Isthmus of Panama.

Both *G. chulini* n. sp. and *G. juili* n. sp. are morphologically similar to gyrodactylids that infect siluriform catfishes in South America, when contrasting the taxonomically-informative marginal hooks of the new parasite species found on *Rhamdia* spp. in Mexico: *G. chulini* n. sp. resembles *G. liliana*e and *G. superbus*; while *G. juili* n. sp. is akin to *G. anisopharynx*, *G. bueni* and *G. corydori*. Similarly, the molecular analysis of ITS rDNA and COII genes shows that the *Gyrodactylus* species collected in the present study from *Rhamdia* in Mexico are a sister group to *G. liliana*e from South America. A phylogenetic hypothesis based on both ITS rDNA and COII sequences suggests that *G. chulini* n. sp. and *G. juili* n. sp. are sister species and unequivocally recognized as two new species; and the ITS rDNA and COII divergence among the two new taxa is consistent with species-level divergence for other species pairs within *Gyrodactylus* (Razzolini et al., 2019). We also show that all the gyrodactylids infecting Heptapteridae are related to other *Gyrodactylus* spp. found on Siluriformes from South America (fig. 5). This suggests that the two new species of *Gyrodactylus* infecting *Rhamdia* in Mexico have South American origins; and that, as suggested by Choudhury et al. (2017), they seem to have co-migrated through Central America with their catfish hosts.

Northward co-migration of Neotropical parasites with *Rhamdia* hosts could have also occurred with other monogeneans, as members of dactylogyridean genera such as *Amelloblastella* Kritsky, Mendoza-Franco & Scholz, 2000, *Aphanoblastella* Kritsky, Mendoza-Franco & Scholz, 2000, *Pavanelliella* Kritsky & Boeger, 1988, and the gyrodactylid *Scleroductus* Java & Cone, 1989, have been recorded infecting these catfishes throughout southern





**Fig. 5.** Phylogenetic hypothesis inferred from internal transcribed spacer 1, 5.8S rRNA, internal transcribed spacer 2 and cytochrome oxidase II for two new species of *Gyrodactylus* infecting native fishes from Oaxaca and Veracruz, Mexico. Numbers near internal nodes show the posterior probability clade frequencies. Scale bars indicate the number of substitutions per site. For new species described in the present study, the clades are dashed in green (*Gyrodactylus julli* n. sp.), and red (*Gyrodactylus chulini* n. sp.).

Mexico, Central and South America, and Trinidad in the Caribbean (Kohn *et al.*, 2006; Salgado-Maldonado, 2006, 2008; Mendoza-Garfias *et al.*, 2017). To corroborate this hypothesis, extensive surveys are needed to close the parasitological knowledge gap still existing in Central America (e.g. Santacruz *et al.*, 2022); this would also contribute to address the overall knowledge shortfalls identified for biodiversity data (Hortal *et al.*, 2015).

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**Conflicts of interest.** None.

**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of wild animals.

**Author contributions.** MRG conceived and designed the study; AGV, CDPP, IGV, MCR and MRG carried out the field and laboratory work; AGV took the microphotographs and did the drawings; AGV, CDPP, IG, and MRG analysed the data; AGV, CDPP and MRG wrote the manuscript. All authors checked and agreed with the final manuscript.

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