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Morphological and molecular characterisation of a new species of *Gyrodactylus* von Nordmann, 1832 (Monogenoidea: Gyrodactylidae) of cichlid fishes (Perciformes) from Mexico

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ABSTRACT

Gyrodactylus mojarrae n. sp. is described from the gills of the Neotropical cichlids *Thorichthys maculipinnis, Rocio octofasciata, Vieja zonata* and *V. fenestrata* from several localities across southern Mexico. The new species is erected on the basis of the morphology of the haptoral elements (anchors, bars and hooks), and its phylogenetic position within the Gyrodactylidae is evaluated based on the analysis of partial sequences of the ITS1, 5.8 rRNA gene and ITS2. *Gyrodactylus mojarrae* n. sp. differs from other congeneric species by having hooks with a straight shaft and recurved point, pointed toe and convex heel, presence of reduced superficial anchor roots, by the number and disposition of spinelets of the male copulatory organ, and the absence of shield and lateral processes of the superficial bar. Molecular circumscription of isolates of *G. mojarrae* n. sp. from different host and hydrological basins showed that these isolates are conspecific and represent a distinct lineage from other congeners, including newly sequenced isolates of *Gyodactylus* sp. A and *Gyrodactylus* sp. B from *Astyanax mexicanus* (Characidae) and *Gobiomorus dormitor* (Eleotridae), respectively. Genetic affinities of *Gyodactylus* sp. A and B indicate that they might represent undescribed species infecting freshwater fish from the Americas.

1. Introduction

Gyrodactylus von Nordmann, 1832 (Gyrodactylidae) represents one of the most challenging monogenoidean groups for taxonomists due to not only the huge species diversity but also, to the conservative morphology of particular structures such as hooks, anchors and bars that have been traditionally used to distinguish among species [1]. However, the use of molecular data in gyrodactylid systematics has shown to be very useful for species identification and delimitation, particularly in those species that seem to represent species complexes [2–5]. Since the first two species of gyrodactylid were described as parasites of native fish in Mexico, i.e., *Gyrodactylus mexicanus* Mendoza-Palmero, Sereno-Uribe and Salgado-Mandonado, 2009 and *G. lamothei* Mendoza-Palmero, Sereno-Uribe and Salgado-Mandonado, 2009 [6], a remarkable advance in the knowledge on this parasitic group infecting freshwater fishes in Mexico has been achieved. Of the 23 species of *Gyrodactylus* recorded in native freshwater fish across Mexico, 17 were described in the last decade [7–9]). These species infect 18 native fish species of the families Catostomidae, Characidae, Goodeidae, Ictaluridae, Profundulidae and Poecillidae across Mexico [5,6,8–14]. Additionally, exotic species of gyrodactylids, i.e., *G. yacatli* García-Vásquez, Hansen, Christison, Bron and Shinn, 2011, *G. cichlidarum* Paperna, 1968, *G. salmonis* (Yin and Sproston, 1948) and *G. sprostonae* Ling, 1962 have also been reported from introduced African cichlids (*Oreochromis* spp.), salmonids (*Oncorhynchus mikiss*) and Asian cyprinids (*Cyprinus carpio*) in Mexico [7,15,16]. So far, < 9% (45 species) of approximately 500 native fish species inhabiting continental waters of Mexico [17] have been reported as hosts of gyrodactylids. Thus, the richness of this parasitic group in Mexican freshwater fish is likely underestimated and could increase with further study effort in the near future [5,6,8–10,13,14].

Cichlid fish (Cichlidae: Perciformes) represent one of the most diverse freshwater fish groups in Mexico with approximately 54 species, of which 25 are known to host five dactylogyrid species within two genera: *Parasciadicleithrum octofasciatum* Mendoza-Palmero, Blasco-Costa, Hernández-Mena and Pérez-Ponce de León, 2017, *Sciadicleithrum*

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bravohollisae Kritsky, Vidal-Martínez and Rodríguez-Canul, 1994, *S. meekii* Mendoza-Franco, Scholz and Vidal-Martínez, 1997, *S. mexicanum* Kritsky, Vidal-Martínez and Rodríguez-Canul, 1994 and *S. splendidae* Kritsky, Vidal-Martínez and Rodríguez-Canul, 1994 [18–20]. Additionally, three gyrodactylid species recorded as *Gyrodactylus* sp. 1, *Gyrodactylus* sp. 2 and *Gyrodactylus* sp. 3 have been reported parasitizing cichlids from the Yucatan Peninsula in south eastern Mexico. However, these species were never formally described due to the low number of sampled specimens [21].

In the present survey of parasites of freshwater fish in Mexico, a new species of *Gyrodactylus* was found on the gills of three species of cichlids *Thorichthys maculipinnis* (Steindachner), *Rocio octofasciata* (Regan) and *Vieja zonata* (Meek) in several localities of southern Mexico. The new species is herein described on the basis of the morphology (external and internal anatomy, haptoral elements and male copulatory organ) and its comparison to other *Gyrodactylus* spp., and molecular data of the Internal Transcribed Spacers of the ribosomal gene (ITS1 and ITS2 regions and 5.8 rRNA gene). The new species is genetically different from isolates of *Gyrodactylus* sp. A and *Gyrodactylus* sp. B newly sequenced from freshwater fish from the Americas, and their genetic affinities to congeneric species of *Gyrodactylus* are explored.

2. Material and methods

2.1. Specimen collection and morphological analyses

Fish were captured by electrofishing and sine nets in freshwater environments such as creeks, rivers and lakes located in Veracruz, Oaxaca and Chiapas states, southern Mexico in 2010 and 2015 (see Table 1). Additional samples from non-cichlid fish were obtained from *Astyanax mexicanus* (De Filippi) (Characidae) (Ojo de Agua San Juan, Durango, Mexico, 23°57'06"N; 104°16'09"W) and from *Gobiomorus dormitor* Lacépède (Eleotridae) (Palo de Aquita, Nicaragua, 11°07'12"N; 84°36'05"W) (see Table 1). Fish were transported to the laboratory in plastic containers filled up with water with aeration and kept alive until their parasitological examination. Hosts were euthanized via spinal cord severance and immediately placed in Petri dishes with tap water. Fins and skin were examined for ectoparasites using a stereomicroscope (Leica Zoom 2000, Germany). Then after, gills were removed from the carcasses and placed individually in Petri dishes for examination. Gyrodactylids found were fixed in GAP (a mixture of glycerine and ammonium picrate) [22] to visualize their haptoral hard structures (anchors, bars and hooks) and male copulatory organ (MCO). After their morphological evaluation, these specimens were remounted in Canada balsam as permanent slides following Ergens' procedure [23]. Some individuals were fixed in hot water (~80 °C) and stored in vials with 96% ethanol for morphological study of their internal and external anatomy and for molecular characterisation [20,24].

To study the external morphology and internal organs, selected specimens were stained with Gomori's trichrome and mounted in Canada balsam. Drawings were made with the aid of an optical microscope (Olympus BX51, Japan) equipped with a drawing tube. Measurements were obtained with an ocular micrometre. Metrical data are in micrometres and presented as the mean followed by the range and number of specimens measured (n) in parentheses. Measurements are provided only for specimens collected from *T. maculipinnis* because only few specimens were found on *R. octofasciata* and on *V. zonata*, respectively. Morphological terminology used herein for gyrodactylids followed the proposal of Boeger and Popazoglo [25]. Measurements of haptoral elements followed that of Kritsky et al. [26], except those of the hooks which followed Christison et al. [27].

Type and voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico, in the helminthological collection of the Institute of Parasitology, České Budějovice, Czech Republic (IPCAS) and in the Natural History Museum of Geneva, Switzerland (MHNG) as indicated in the species description. For comparative purposes, the following museum specimens were studied: *Gyrodactylus anisopharhynx* Popazoglo and Boeger, 2000 (IPCAS M-358, two paratypes), *G. bueni* Bueno-Silva and Boeger, 2014 (IPCAS M-546, three paratypes), *G. corydori* Bueno-Silva and Boeger, 2009 (IPCAS M-471, two paratypes), *G. geophagensis* Boeger and Popazoglo, 1995 (only microphotographs were studied of this species); holotype (CHOIC 33144a), two paratypes ([CHOIC 33144b-c]), *G. major* Bueno-Silva and Boeger, 2014 (IPCAS M-545, two paratypes), *G. scleromystaci* Bueno-Silva and Boeger, 2014 (IPCAS M-547, two paratypes), *Gyrodactylus* sp. 3 (CNHE 10027, one voucher) and *Gyrodactylus* sp. (CNHE 6172, one voucher). Scientific host names followed those of FishBase [28].

2.2. DNA extraction, amplification and sequencing

Molecular characterisation of gyrodactylids obtained from *T. maculipinnis*, *V. zonata* and *R. octofasciata*, and those from *A. mexicanus* and *G. dormitor* followed the procedure described by Mendoza-Palmero et al. [20,24]. Individual specimens were digested in a solution containing 10 mM Tris-HCl (pH 7.6), 200 mM NaCl, 0.5 M EDTA (pH 8.0), 10% Sarkosyl, 0.1 mg/ml proteinase K and H_2O (100 µl per sample) during 3 h at 56 °C. Then, the proteinase was denaturized at 95 °C for 15 min. Genomic DNA was extracted using DNAzol™ (Molecular Research Centre, Cincinnati, Ohio) according to manufacturer instructions. Polymerase chain reaction (PCR) amplifications were performed in 25 µl reactions containing 2.5 µl of extraction supernatant (~10–20 ng of template DNA), and 0.4 µM of each PCR primer. A partial fragment of the Internal Transcribed Spacers (ITS1 and ITS2) and the 5.8S rDNA regions were amplified using the primers ITS1-fm (5′-TAGAGGAAGTACAAGTCG-3′) and ITS2-rm (5′-GCTYGAATCGAGGTCAGGAC-3′) [14]. The following thermocycling profile was utilized: denaturation of DNA (95 °C for 4 min); 34 cycles of amplification (94 °C for 30s, 54 °C for 45 s and 72 °C for 1 min); and a final extension hold for 7 min at 72 °C [14]. PCR products were purified prior to sequencing using exonuclease I and shrimp alkaline phosphatase enzymes [29]. Amplicons were cycle-sequenced from both strands with PCR primers [14], using an ABI BigDye™ Terminator v3.1 Ready Sequencing Kit, alcohol-precipitated and run on an ABI 3730xl Genetic Analyser (Applied Biosystems). Contiguous sequences were assembled and edited using Geneious® (Biomatters Ltd. v. 8.1) and submitted to GenBank under ID codes: MK573785-MK573789.

Table 1
Gyrodactylid parasites and fish hosts examined in this study.

Parasite	Host species	Site on host	Locality	Examined/Infected
Gyrodactylus mojarrae n. sp.	*Thorichthys maculipinnis*	Gills	Tlacotalpan, Veracruz, Mexico	2/2
	Rocio octofasciata	Gills	Ejido Reforma Agraria, Chiapas, Mexico	3/3
	Vieja zonata	Gills	Santa María Guienagati, Oaxaca, Mexico	4/3
	V. fenestrata	Gills	Río Máquinas, Veracruz, Mexico	2/2
			Lago de Catemaco, Veracruz, Mexico	5/4
Gyrodactylus sp. A	*Astyanax mexicanus*	Fins	Ojo de Agua San Juan, Durango, Mexico	14/3
Gyrodactylus sp. B	*Gobiomorus dormitor*	Fins	Palo de Aquita, Nicaragua	1/1

Table 2
Species of *Gyrodactylus* and related genera included in the phylogenetic analysis.

Species	Host	Host family	Distribution	GenBank ID	References
G. actzu	*Poecilia mexicana*	Poeciliidae	Veracruz, Mexico	KM514476	[10]
G. alekosi	*Clarias gariepinus*	Clariidae	Mozambique, Africa	FR850682	[44]
G. anguillae	*Anguilla anguilla*	Anguillidae	Valencia, Spain	AB063294	[45]
G. apazapanensis	*Poecilia mexicana*	Poeciliidae	Veracruz, Mexico	KM514468	[10]
G. arcuatus	*Gasterosteus aculeatus*	Gasterosteidae	Baltic Sea, Finland	AF328865	[46]
G. branchicus	*Gasterosteus aculeatus*	Gasterosteidae	Baltic Sea, Finland	AY061977	[47]
G. bueni	*Scleromystax macropterus*	Callichthyidae	Paraná, Brazil	KF767475	[48]
G. bullatarudis	*Poecilia reticulata*	Poeciliidae	Lopinot River, Trinidad	AY692024	[49]
G. carassii	*Carassius carassius*	Cyprinidae	Baltic Sea basin, Finland	AY278033	[50]
G. carolinae	*Characidium pterostictum*	Crenuchidae	Paraná, Brazil	KF673399	[40]
G. chileani	*Helcogrammoides chilensis*	Tripterygiidae	Valparaiso, Chile	JQ045347	[51]
G. cichlidarum	*Oreochromis niloticus*	Cichlidae	Scotland, UK	DQ124228	[52]
G. corydori	*Corydoras paleatus*	Callichthyidae	Paraná, Brazil	HQ636621	[48]
G. ergensi	*Sarotherodon galilaeus*	Cichlidae	Senegal	FN394985	[53]
G. eyipayipi	*Syngnathus acus*	Syngnathidae	Cape Town, South Africa	FJ040183	[54]
G. granoei	*Cobitis granoei*	Cobitidae	Central China	HM185817	[55]
G. hildae	*Oreochromis niloticus*	Cichlidae	Ethiopia	FJ231869	[16]
G. iunuri	*Goodea atripinnis*	Goodeidae	Jalisco, Mexico	KR815853	[9]
G. jarocho	*Xiphophorus hellerii*	Poeciliidae	Veracruz, Mexico	KJ621984	[13]
*G. jennyae**	*Rana catesbiana*	Ranidae	Missouri, USA	EU678357	[56]
G. katamba	*Goodea atripinnis*	Goodeidae	Querétaro Mexico	KR815854	[8]
G. lhkahuili	*Poecilia mexicana*	Poeciliidae	Veracruz, Mexico	KM514478	[10]
G. lamothei	*Girardinichthys multiradiatus*	Goodeidae	Estado de México, Mexico	KX555666	[14]
G. longipes	*Sparus aurata*	Sparidae	Latina Province, Italy	GQ150536	[57]
G. major	*Scleromystax macropterus*	Callichthyidae	Paraná, Brazil	KF767478	[48]
G. malalai	*Oreochromis niloticus*	Cichlidae	Turkana Lake, Kenya	FR695484	[58]
G. microdactylus	*Poecilia mexicana*	Poecillidae	Veracruz, Mexico	KM514474	[10]
G. micropsi	*Pomatoschistus microps*	Gobiidae	North Sea, Belgium	AF328868	[46]
***Gyrodactylus mojarrae* n. sp.**	***Thorichthys maculipinnis***	**Cichlidae**	**Veracruz, Mexico**	**MK573785**	**Present study**
	Vieja zonata	**Cichlidae**	**Oaxaca, Mexico**	**MK573786**	**Present study**
	Rocio octofasciata	**Cichlidae**	**Chiapas, Mexico**	**MK573787**	**Present study**
G. montealbani	*Profundulus oaxacae*	Profundulidae	Oaxaca, Mexico	MG883696	[9]
G. nigritae	*Synodontis nigrita*	Mochokidae	Niokolo Koba River, Senegal	FR850686	[58]
G. ostendicus	*Pomatoschistus microps*	Gobidae	Ostend, Belgium	AY338439	[59]
G. pakan	*Astyanax aeneus*	Characidae	Veracruz, Mexico	KR733205	[5]
G. pictae	*Micropoecilia picta*	Poeciliidae	Lower Marianne River, Trinidad	AY692023	[49]
G. poeciliae	*Poecilia caucana*	Poeciliidae	La Concepción, Venezuela	AJ001844	[60]
G. pseudobullatarudis	*Poecilia mexicana*	Poeciliidae	Veracruz, Mexico	KM514441	[10]
G. pungitii	*Pungitius pungitius*	Gasterosteidae	White Sea, Finland	AF484543	[61]
G. rarus	*Pungitius pungitius*	Gasterosteidae	Baltic Sea, Finland	AY061976	[47]
G. rivularae	*Abbottina rivularis*	Cyprinidae	Shaanxi Province, China	HM185818	[55]
G. rugiensis	*Pomatoschistus microps*	Gobiidae	North Sea, Belgium	AF328870	[61]
G. rysavyi	*Clarias anguillaris*	Clariidae	Niokolo Koba National Park, Senegal	FR850679	[58]
G. salaris	*Salmo salar*	Salmonidae	United Kingdom	Z72477	[62]
G. scleromystaci	*Scleromystax barbatus*	Callichthyidae	Paraná, Brazil	KF767472	[48]
G. sedelnikovi	*Barbatula barbatula*	Nemacheilidae	Vlára River, Czech Republic	AJ407935	[63]
G. sprostonae	*Carassius gibelio*	Cyprinidae	River Martwa Wisla, Poland	AY278044	[50]
G. sturmbaueri	*Simochromis diagramma*	Cichlidae	Lake Tanganyika, Zambia	HQ214479	[64]
G. synodonti	*Synodontis nigrita*	Mochokidae	Niokolo Koba National Park, Senegal	FR850684	[44]
G. takoke	*Heterandria bimaculata*	Poeciliidae	Veracruz, Mexico	KM514460	[10]
G. teken	*Astyanax aeneus*	Characidae	Veracruz, Mexico	KR733210	[5]
G. tepari	*Goodea atripinnis*	Goodeidae	Guanajuato, Mexico	KR815856	[9]
G. thysi	*Simochromis diagramma*	Cichlidae	Lake Tanganyika, Zambia	HQ214481	[64]
G. tomahuac	*Goodea atripinnis*	Goodeidae	Querérato, Mexico	KJ621983	[14]
G. turnbulli	*Poecilia reticulata*	Poeciliidae	Poland	AJ001846	[65]
G. ulinganisus	*Oreochromis mossambicus*	Cichlidae	Stellenbosch, South Africa	FJ231870	[16]
G. unami	*Poeciliopsis gracilis*	Poeciliidae	Veracruz, Mexico	KM514473	[10]
G. xalapensis	*Poeciliopsis gracilis*	Poeciliidae	Veracruz, Mexico	KJ621985	[13]
G. xtachuna	*Poeciliopsis gracilis*	Poeciliidae	Veracruz, Mexico	KM514443	[10]
G. zapoteco	*Profundulus oaxacae*	Profundulidae	Oaxaca, Mexico	MG883704	[9]
G. zimbae	*Simochromis diagramma*	Cichlidae	Lake Tanganyika, Zambia	HQ214482	[64]
***Gyrodactylus* sp. A**	***Astyanax mexicanus***	**Characidae**	**Durango, Mexico**	**MK573788**	**Present study**
***Gyrodactylus* sp. B**	***Gobiomorus dormitor***	**Eleotridae**	**Palo de Aquita, Nicaragua**	**MK573789**	**Present study**
Gyrodactylus sp.	*Scleromystax barbatus*	Callichthyidae	Paraná, Brazil	KF767481	[48]
Gyrodactylus sp. 1	*Clarias gariepinus*	Clariidae	Nile, Sudan	FR850688	[44]
Gyrodactylus sp. 2	*Hemichromis bimaculatus*	Cichlidae	Nile, Sudan	HF548666	[66]
Gyrodactylus sp. 3	*Citharinus citharus*	Citharinidae	Kenya	HF548669	[66]
Other genera					
Acanthoplacatus sp.	*Siganus* sp.	Siganidae	Australia	AF465784	[67]
Afrogyrodactylus girgifae	*Brycinus nurse*	Alestidae	Nile, Sudan	HF548671	[66]
Diplogyrodactylus martini	*Polypterus senegalus*	Polypteridae	Niokolo Koba National Park, Senegal	AM943008	[68]
Fundulotrema foxi	*Fundulus heteroclitus*	Fundulidae	Nova Scotia, Canada	GQ918278	[69]
F. prolongis	*Fundulus heteroclitus*	Fundulidae	Nova Scotia, Canada	GQ918279	[69]
*Gyrdicotylus gallieni**	*Xenopus laevis*	Pipidae	KwaZulu-Natal, South Africa	AJ001843	[65]

(*continued on next page*)

Table 2 (*continued*)

Species	Host	Host family	Distribution	GenBank ID	References
Gyrodactyloides bychowskii	*Salmo salar*	Salmonidae	Scotland, UK	AJ249348	[70]
Gyrodactyloides sp.	*Mallotus villosus*	Osmeridae	Iceland	HF548676	[66]
Ieredactylus rivuli	*Rivulus hartii*	Rivulidae	Trinidad and Tobago	HQ738514	[71]
Laminiscus gussevi	*Mallotus villosus*	Osmeridae	Iceland	HF548678	[66]
Macrogyrodactylus clarii/ heterobranchii (A)[a]	*Heterobranchus bidorsalis*	Clariidae	Africa	HF548683	[66]
M. congolensis	*Clarias gariepinus*	Clariidae	Lake Turkana, Kenya	GU252716	[72]
M. polypteri	*Polypterus senegalus*	Polypteridae	Africa	AJ567672	[73]
M. simentiensis	*Polypterus senegalus*	Polypteridae	Nile, Sudan	HF548681	[66]
Swingleus ancistrus	*Fundulus heteroclitus*	Fundulidae	Maryland, USA	HF548674	[66]

Species newly sequenced in this study are in bold.
* Asterisk indicated species parasites of amphibians.
[a] This species was retrieved from GenBank as *Macrogyrodactylus* sp.

2.3. Alignment and phylogenetic analyses

Nineteen newly generated partial sequences for the ITS1, 5.8 rRNA gene and ITS2 of specimens of *Gyrodactylus* infecting cichlids (15 sequences), one characid (3) and one eleotrid (1) were aligned together with 77 published sequences retrieved from GenBank (see Table 2). Sequences were aligned using default parameters of MAFFT implemented in Guidance [30]. The extremes of the alignment were trimmed to match the shortest sequences and large gaps and ambiguously aligned positions were excluded. A total of 516 bp were retained in the alignment, which mostly corresponded to the 5.8S rDNA and ITS2 regions. Phylogenetic analyses were run under Maximum Likelihood (ML) and Bayesian Inference (BI) criteria, employing the model of nucleotide evolution GTR + Γ in both cases (estimated using jModelTest 2.1.1 [31,32]). ML analyses were conducted using the program RAxML v. 7.3 [33,34]. All model parameters and bootstrap support values (1000 repetitions) were estimated using RAxML. BI trees were constructed using MrBayes v. 3.2 [35], running two independent MCMC runs of four chains for 10 million generations and sampling tree topologies every 1000 generations. Burn-in periods were set to the first 2500 generations. A consensus topology and nodal support estimated as posterior probability (PP) values [36] were calculated from the remaining trees. All MrBayes and RAxML analyses were performed on the computational resource CIPRES [37]. Genetic divergences were calculated for the alignment as uncorrected p-distances using MEGA v.6 [38].

3. Results

3.1. Nomenclatural acts

This work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. 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:act:7111FF8A-88E1-4C35-9937-F97D41B27799.

3.2. A new species (Monogenoidea: Gyrodactylidae)

3.2.1. Morphological description

Gyrodactylus mojarrae n. sp. (Fig. 1A–G)

Morphological description based on 10 specimens fixed in GAP and 21 specimens stained with Gomori's trichrome, all collected from *Thorichthys maculipinnis*. Body elongate, 293 (255–337; n = 21) long, 66 (51–85; n = 18) wide. Cephalic lobes well developed, each with spike sensilla, two to three head organs, cephalic glands mostly posterolateral to pharynx. Distal pharyngeal bulb muscular 21 (19–23; n = 12) wide; no digitiform projections of distal pharyngeal bulb; proximal pharyngeal bulb glandular 25 (23–26; n = 17) wide. Intestinal caeca not confluent posterior to uterus. Male copulatory organ (MCO) 10 (9–11; n = 12) wide, armed with a central spine and two rows of spinelets; external row with four spinelets, two large basal and two small lateral, each with wide and truncate base; internal row with three small spinelets. Seminal vesicle double, dorsal to the MCO. Prostatic reservoir large, located posterior to MCO. Testis ovate 19 (15–25: n = 12) long, 19 (15–23; n = 11) wide, posterior to germarium. Germarium ovate, 17 (12–22; n = 19) long, 18 (13–22; n = 16) wide. Uterus with up to 2 generations of embryos. Large syncytial glandular masses located lateral and immediately posterior to the gonads, partially invading the peduncle. Anchor 44 (40–47; n = 10) total length, point 21 (20–22; n = 10), shaft 36 (35–39; n = 10), base 19 (18–20; n = 10); deep root poorly developed, knob-like; superficial root short, truncate forming nearly a 45° angle; shaft slightly recurved and straight point. Deep bar 12 (11–14; n = 8) length, rod-shaped with no posterior projections. Superficial bar 17 (15–19; n = 7) length, 9 (5–12; n = 7) wide, rectangular and finely striated in its medial portion without anterolateral projections; no shield observed in any specimen studied (either fixed in GAP or stained). Hooks with straight shaft, point moderately recurved not exceeding toe; heel convex; pointed toe; knob conspicuous; FH loop about half of shank length. Total length 24 (23–24; n = 10); shank length 16 (15–17; n = 10); hooklet length 8 (8–9; n = 10); hooklet distal width 5 (4–6; n = 10); hooklet proximal width 6 (5–6; n = 10).

3.2.2. Taxonomic summary

Synonyms: *Gyrodactylus* sp. (CNHE 6172) on the gills of *Vieja fenestrata* (Günther) from Río Máquinas, Los Tuxtlas, Veracruz, Mexico. *Gyrodactylus* sp. 3 (CNHE 10027) on the gills of *Rocio octofasciata* from Chiapas, Mexico reported by Mendoza-Palmero et al. [20] and newly sequenced in this study.

Type host: *Thorichthys maculipinnis* (Cichlidae: Perciformes).

Other hosts: *Rocio octofasciata*, *V. zonata* and *V. fenestrata*.

Site of infection: gills.

Type locality: Tlacotalpan, Papaloapan River basin, Veracruz, Mexico (18°41'04"N; 95°38'48"W).

Other localities and records: Santa María Guienagati, Oaxaca (16°44'29"N; 95°21'31"W) (*V. zonata*); unnamed creek at Ejido Reforma Agraria, Municipio Marqués de Comillas, a tributary of the Lacantún River basin, Chiapas (16°13'11"N; 90°50'34"W) (*R. octofasciata*); Río Máquinas at Los Tuxtlas (18°37'21"N; 95°05'29"W) (*V. fenestrata*), and Lago de Catemaco (18°25'02"N; 95°06'47"W) (*V. fenestrata*), both latter localities in Veracruz, Mexico.

Type specimens: Holotype (CNHE 10811), eight paratypes (CNHE 10812), four paratypes (IPCAS M-679), two paratypes (MNHG – PLAT –

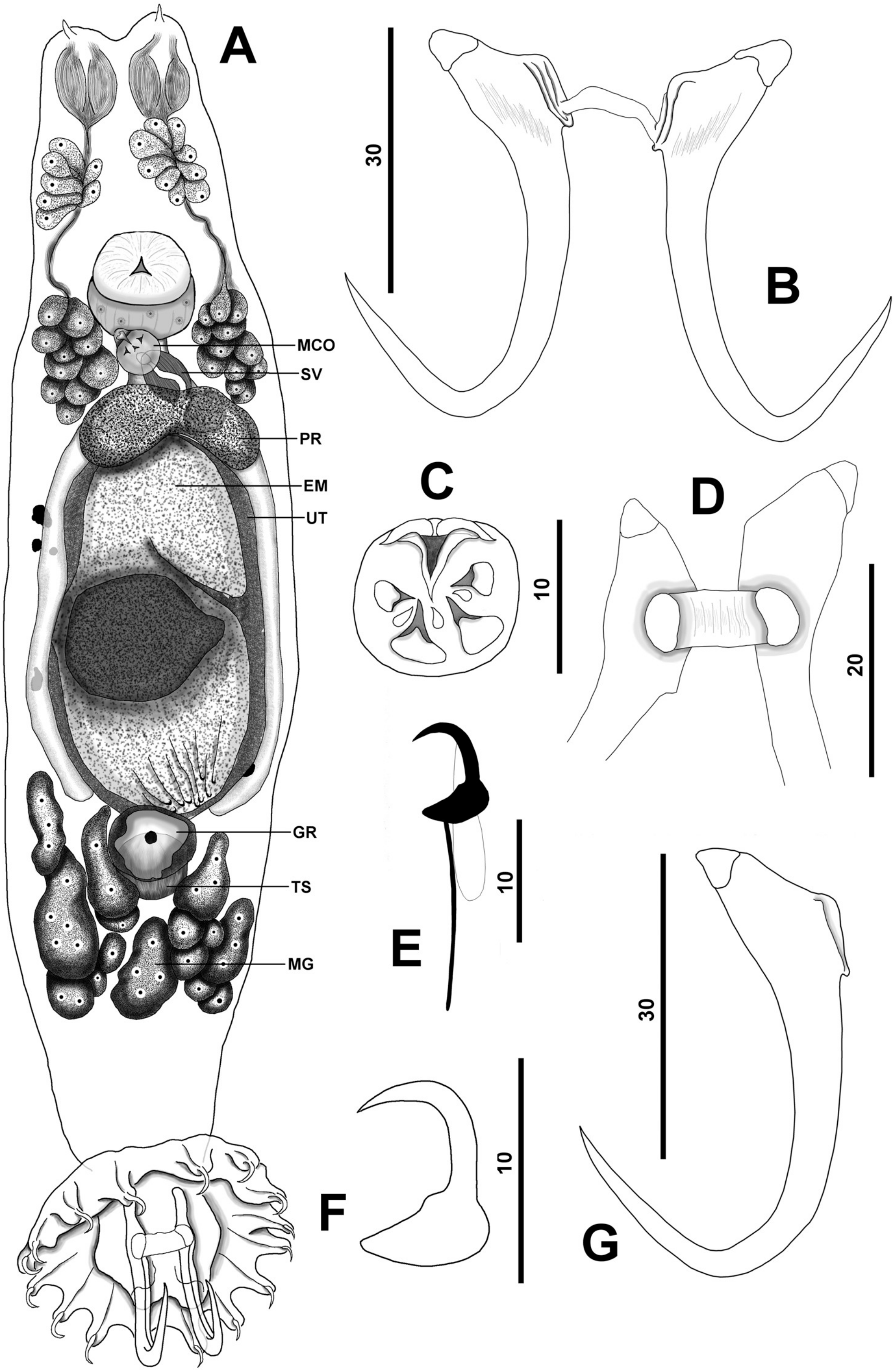

Fig. 1. Holotype of *Gyrodactylus mojarrae* n. sp. from the gills of *Thorichthys maculipinnis*. A – Whole specimen (composite, ventral view); B – Anchors and deep bar (superficial bar omitted to show the morphology of the deep bar); C – Male copulatory organ; D – Superficial bar (deep bar omitted); E – Hook; F – Hooklet; G – Anchor. Abbreviations: MCO – Male copulatory organ; SV – Seminal vesicle; PR – Prostatic reservoir; EM – Embryos in uterus; UT – Uterus; GR – Germarium; TS – Testis; MG – Mass glands. Scale bars: A, 100 μm; B, G, 30 μm; C, E and F, 10 μm; D, 20 μm.

97906), 15 vouchers (CNHE 6172, 10,813) and four vouchers (IPCAS M-679). Thirteen hologenophores at CNHE 10814.

Etymology: The specific epithet refers to the common name "*mojarra*" of the hosts that are used to designate several species of cichlids in Mexico.

3.2.3. Remarks

To date, the only gyrodactylid described from native cichlid fishes in the Americas is *Gyrodactylus geophagensis* Boeger and Popazoglo, 1995, a parasite of *Geophagus brasiliensis* (Quoy and Gaimard) from Brazil [25,39]. *Gyrodactylus mojarrae* n. sp. exhibits similar hook morphology to that of *G. geophagensis*. In both species, hooks have a straight shaft and recurved point, not exceeding toe, convex heel, conspicuous knob, pointed toe and slender shank. Nevertheless, in *G. mojarrae* n. sp. the shaft is comparatively more robust than that in *G. geophagensis*, the point in the new species reaches the toe, whereas in *G. geophagensis* the point is shorter and comprises only half of hooklet width.

The new species possesses shorter anchors (total anchor length,

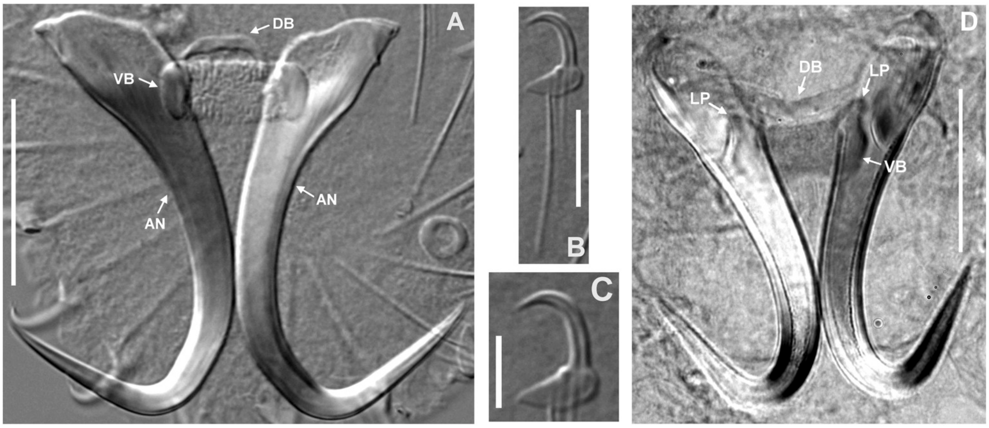

Fig. 2. Microphotograph of the haptoral structures of *Gyrodactylus mojarrae* n. sp. from the gills of *Thorichthys maculipinnis* (A–C), and *Gyrodactylus geophagensis* Boeger and Popazoglo, 1995 (Paratype CHOIC 33144a) from *Geophagus brasiliensis* (Cichlidae) from Brazil (D), taken using differential interphase contrast. Abbreviations: DB: Dorsal bar; VB: Ventral bar; AN: Anchors; LP: Lateral process. Scale bars: A, D, 20 μm; B, 10 μm; C 5 μm.

range of 40–47 μm) compared to those of *G. geophagensis* (55–58 μm). In *G. mojarrae* n. sp., the superficial anchor roots are extremely reduced forming an angle of about 45 ° (see Fig. 1B, G), whereas *G. geophagensis* possesses short and robust superficial roots terminally rounded (Fig. 2D). The superficial bar of *G. mojarrae* n. sp. lacks antero-lateral projections and shield (Fig. 1D), whereas that of *G. geophagensis* has short antero-lateral projections and conspicuous U-shaped shield ([25] and Fig. 2D).

Other differential characteristics between *G. mojarrae* n. sp. and *G. geophagensis* are the number and distribution of spinelets of MCO. In the new species, there are two rows of spinelets, an external row with four spinelets and an internal row with three spinelets (Fig. 1C); whereas in *G. geophagensis*, the MCO possesses a single row with eight spinelets [25]. Additionally, the site of infection, the host species, and geographical distribution of the two species are different. *Gyrodactylus mojarrae* n. sp. was found on the gills of its hosts, conversely to *G. geophagensis* that has been reported from the body surface. The new species was found on *T. maculipinnis, R. octofasciata, V. fenestrata* and *V. zonata*, whereas the host of *G. geophagensis* is *Geo. brasiliensis*. Furthermore, the new species is distributed in southern Mexico (Veracruz, Oaxaca and Chiapas states), whereas *G. geophagensis* was found in Rio da Guarda, state of Rio de Janeiro, Brazil [25].

To date, *G. mojarrae* n. sp. and *Gyrodactlyus inesperatus* Boeger, Ferreira, Vianna and Patella, 2014, a parasite of *Characidium* sp. (Characiformes: Crenuchidae) from Brazil, are the only members of *Gyrodactylus* reported in the Neotropical region bearing no shield of the superficial bar [40]. Both species, *G. mojarrae* n. sp. and *G. inesperatus*, can be separated from each other with respect to the distinct morphology of the hooks; in *G. inesperatus* the shaft is robust, point is perpendicular to the shaft and exceeds toe, toe is blunt and slightly erected, and the heel is subrectangular [37], whereas in *G. mojarrae* n. sp. the shaft is slender, point is recurved and does not exceed toe, toe is pointed and tilted, and the heel is convex. The ventral bar in *G. mojarrae* n. sp. does not possess lateral process, whereas in *G. inesperatus* lateral process are present on the ventral bar.

3.3. Molecular circumscription of *G. mojarrae* n. sp.

Newly obtained partial sequences of the ITS region varied in length between 1008 and 1119 bp. Three haplotypes were retrieved from the 15 sequenced isolates of *G. mojarrae* n. sp. Each haplotype was associated to a host and locality combination (haplotype 1, 7 isolates in *T. maculipinnis* from Veracruz; haplotype 2, 6 isolates in *V. zonata* from Oaxaca; and haplotype 3, 2 isolates in *R. octofasciata* from Chiapas). Divergence ranged 0.4/1.9% between haplotypes, the highest divergence was found between haplotype 2 and 3. For the Neotropical species, *Gyrodactylus pakan* Razo-Mendivil, García-Vásquez and Rubio-Godoy, 2016 and *Gyrodactylus carolinae* Boeger, Ferreira, Vianna and Patella, 2004 low values of intraspecific genetic variation for the ITS region have also been reported (0/0.82% and 0.33/0.99%, respectively) [5]. *Gyrodactylus mojarrae* n. sp. appeared genetically distant to *Gyrodactylus* spp. of Mexican goodeid (36.9/39.7% divergence), poeciliid (37.2/40.3%), profundulid (36.2/37.6%) and characid fish (38.3/38.6%).

Results of the phylogenetic analyses of the ITS data set (Fig. 3) showed that *G. mojarrae* n. sp. represents a distinct lineage from its congeners. However, support for most nodes in the trees was generally low, likely due to high divergence among sequences and the relatively short region analysed for the number of taxa considered (80 taxa and 516 bp for the ITS). The position of the new species in the phylogeny of the Gyrodactylidae was therefore unresolved.

3.4. *Gyrodactylus* spp. from non-cichlid fish hosts

ITS sequences of the three newly sequenced isolates from the Mexican tetra *A. mexicanus* from northern Mexico, namely *Gyrodactylus* sp. A (Figs. 3 and 4A–F) were 909 bp long and identical. Genetic sequences of *Gyrodactylus* sp. A diverged 3.5/3.6% from those of *Gyrodactylus teken* Razo-Mendivil, García-Vásquez and Rubio-Godoy, 2016, and *Gyrodactylus pakan* Razo-Mendivil, García-Vásquez and Rubio-Godoy, 2016, parasites of *A. aeneus* (Günther) from southern Mexico [5]. The position of *Gyrodactylus* sp. A in the ITS tree suggests that it could represent a distinct species yet undescribed. Additionally, one isolate referred in this study as *Gyrodactylus* sp. B (Figs. 3 and 5A–D) from *G. dormitor* (Eleotridae) from Central America also seems to represent an undescribed species based on its low affinity to other sequenced species in the ITS tree (Fig. 3). Both, *Gyrodactylus* sp. A and B appeared genetically different from *G. mojarrae* n. sp.

The limited number of specimens found of each morphotype, *Gyrodactylus* sp. A and *Gyrodactylus* sp. B (3 and 1, respectively), precluded their description as new taxa infecting Neotropical perciform (Perciformes) freshwater fish. However, we provided microphotographs of the hard structures of each isolate for the purpose of vouchering our sequences and to show their morphology (voucher specimens deposited at IPCAS; *Gyrodactylus* sp. A M-692, *Gyrodactylus* sp. B M-693). Sampling more specimens is required to properly characterise and describe these two putative new species.

4. Discussion

Gyrodactylus mojarrae n. sp. is proposed based on the morphological features of the haptoral elements and MCO, and supported by genetic data for the ITS region, which allowed to distinguish it from other sequenced *Gyrodactylus* spp. infecting perciforms and other fish orders. One of the most distinctive morphological characteristics of *G. mojarrae* n. sp. is the lack of shield of the superficial bar. The absence of this

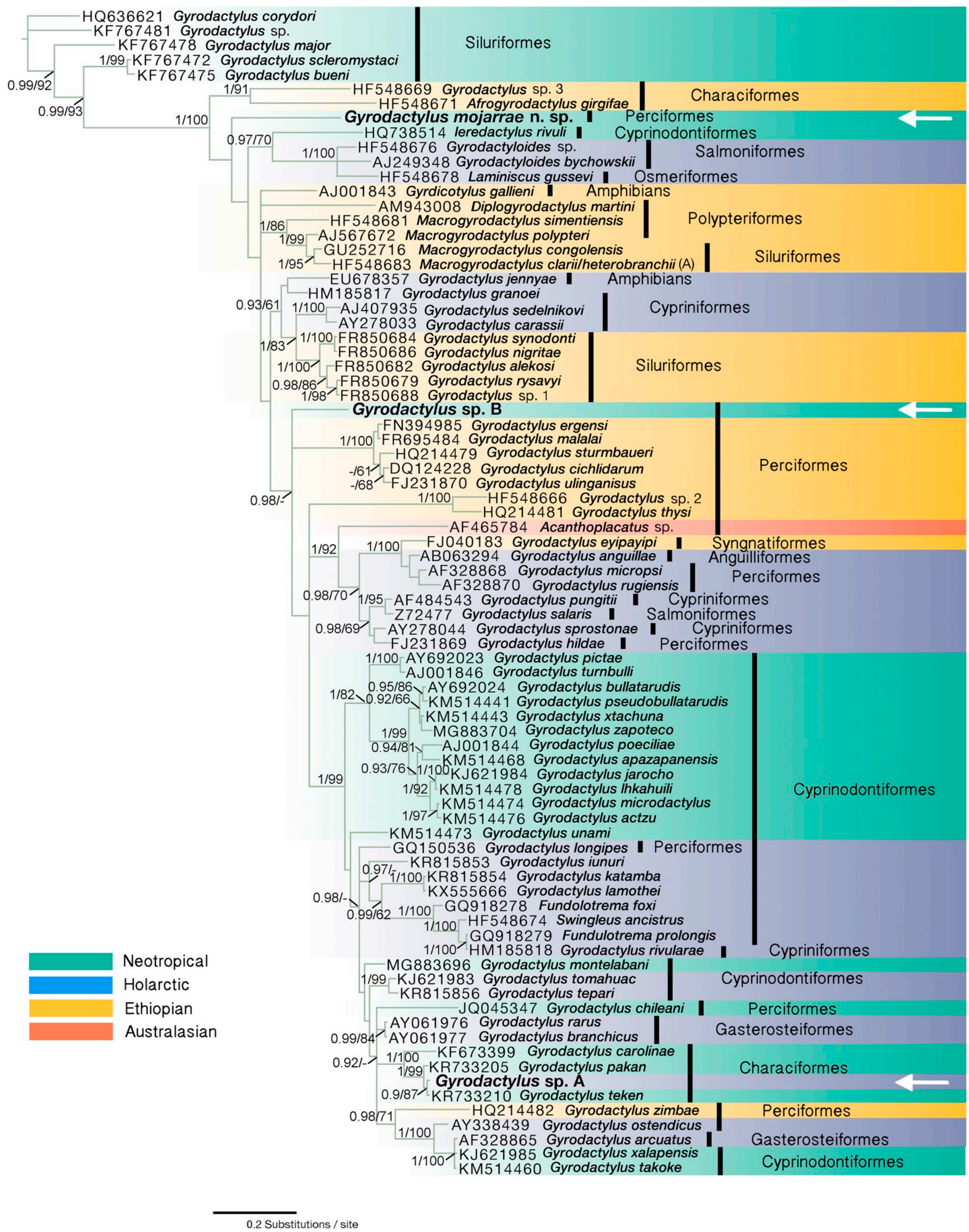

Fig. 3. Molecular phylogeny of the Gyrodactylidae estimated by Bayesian Inference using partial sequences of the Internal Transcribed Spacers (ITS1 and ITS2) and 5.8S rDNA (516 bp) showing the affinities of *Gyrodactylus mojarrae* n. sp. parasite of Neotropical cichlids, and two undescribed species, *Gyrodactylus* sp. A and *Gyrodactylus* sp. B from characid and eleotrid fish, respectively, newly sequenced for this study. New isolates sequenced herein are in bold and highlighted by arrows. Species of *Gyrodactylus* parasites of the Siluriformes were used as outgroups. GenBank sequence ID follows species name. Posterior probabilities and Maximum Likelihood support values are given above the branches (posterior probabilities < 0.90 and bootstrap values < 60 not reported).

structure has been previously documented in *G. inesperatus*, as well as in oviparous gyrodactylids from South America [41,42]. Shields may become transparent and inconspicuous in specimens fixed in GAP (personal observations). Therefore, we confirmed the lack of shield in specimens of *G. mojarrae* n. sp. stained with Gomori's trichrome. Boeger et al. [40] mentioned that the lack of shield could be interpreted as retention of a primitive character state (in oviparous gyrodactylids) or as secondary loss.

Some species of gyrodactylids described from freshwater fish across the Neotropical biogeographic region infecting siluriforms, characiforms, perciforms and cyprinodontiforms, possess well-developed superficial roots, conspicuous shields (except for *G. inesperatus*, see above) and lateral processes in their superficial bars [5,8–10,13,14,39,43]. In this regard, *G. mojarrae* n. sp. can be readily differentiated from those species because its anchor roots are highly reduced, and the superficial bar does not possess lateral projections or shield.

A detail observation of the illustrations and morphometric data of an unidentified species reported as *Gyrodactylus* sp. 3 from the gills of *Thorichthys aureus* (Günther) (syn. *Cichlasoma aureum*) from the cenote (sinkhole) Noc-choncunchey, Yucatan, Mexico [21] (and not referred as being deposited in a parasitological collection), suggests that these

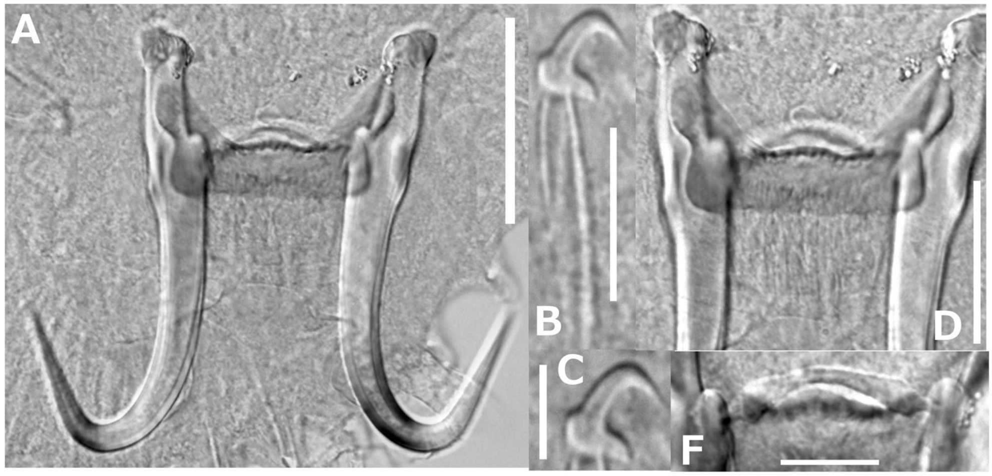

Fig. 4. Haptoral elements of *Gyrodactylus* sp. A of *Astyanax mexicanus* from northern Mexico. A – Anchors; B – Hook; C – Hooklet; D – Superficial bar; F – Deep bar. Scale bar: A, 30 μm; B,F, 10 μm; C, 5 μm; D, 20 μm.

Fig. 5. Haptoral elements of *Gyrodactylus* sp. B of *Gobiomorus dormitor* from Central America. A – Anchors; B – Superficial bar (deep bar not shown); C – Hook; D – Hooklet. Scale bar: A, 30 μm; B, C, 20 μm; D, 10 μm.

specimens likely belong to *G. mojarrae* n. sp. However, additional specimens of this locality and host are needed to confirm their identity, both morphologically and molecularly.

In this study, genetic sequences were obtained for the fist time for gyrodactylids infecting Neotropical cichlids and used to complement the characterisation of *G. mojarrae* n. sp. The results showed that isolates from different hosts and geographical regions were genetically closely related and distinct from congeneric species. However, the relationships of the new species to other members of the Gyrodactylidae (especially those infecting African cichlids) could not be established due to the low support value for most nodes in the phylogenetic reconstruction. Considering previous records, it is possible that the sister taxon of the newly described species would be *G. geophagensis* from the South American cichlid *Geo. brasiliensis*, although this needs to be further corroborated when sequence data of that species is generated.

Isolates of *Gyrodactylus* sp. B from the eleotrid *G. dormitor* were included in the phylogenetic analyses to rule out the possibility of being conspecific with *G. mojarrae* n. sp., due to the fact that eleotrids and cichlids can be found in similar environments (brackish and freshwater), their geographic distribution overlaps along the Atlantic coast (southern Mexico and Central America), and both groups belong to the same fish order. However, our results showed that they represent distinct lineages although their phylogenetic relationships with other gyrodactylids remain unresolved.

The presence of *G. mojarrae* n. sp. in different species of cichlids allocated to different genera from independent hydrological systems across southern Mexico, i.e., *T. maculippinis* and *V. fenestrata* from the Papaloapan River basin, *V. zonata* from the Coatzacoalcos River basin, and *R. octofasciata* from the Usumacinta River basin, may suggest a complex history of colonisation of host species by the new species. Additional parasitological surveys focused on cichlids that have been identified to harbour *Gyrodactylus* spp. in other regions of Mexico are required to properly describe the diversity of gyrodactylid parasites in this highly diversified freshwater fish group, and to elucidate evolutionary patterns of these parasites in the Neotropics regarding those of their hosts.

5. Conclusion

Gyrodactylus mojarrae n. sp. is described based on the morphological characteristics of haptoral elements and the MCO. The new species can be distinguished from other congeners parasitizing Neotropical freshwater fish particularly by morphological features such as anchors with short superficial roots, shape of hooks, and the absence of shield and lateral processes in the ventral bar. Genetic divergence of isolates of *G. mojarrae* n. sp. from different hosts and hydrological basins showed that these isolates are genetically closely related and distinct from other congeneric species, however phylogenetic relationships of *G. mojarrae* n. sp. within the Gyrodactylidae remain unresolved. Two putative species from characids and eleotrids remain undescribed.

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