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Research Article

Description of *Tresuncinidactylus wilmienae* gen. et sp. n. (Monogenea: Gyrodactylidae), from the gills of the bulldog, *Marcusenius macrolepidotus* (Peters) from Lake Kariba, Zimbabwe

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Abstract: The African continent has a rich diversity of fish and amphibians in its inland water systems that serve as hosts for monogeneans of seven genera of the Gyrodactylidae van Beneden et Hesse, 1832. In August 2011, eight gyrodactylid parasites were collected from the gills of two specimens of bulldog, *Marcusenius macrolepidotus* (Peters), from Lake Kariba, Zimbabwe. Morphometric evaluation and sequencing of 18S rDNA confirmed that the specimens represented a species of a new viviparous genus, *Tresuncinidactylus wilmienae* gen. et sp. n. The attachment apparatus consists of a single pair of large slender hamuli with prominently flattened roots that are connected by a simple, narrow dorsal bar. The ventral bar is small and possesses a thin lingulate membrane but no evident anterolateral processes. There are 16 marginal hooks of one morphological type, but of three different sizes, with large falculate sickles that are proportionaly equal in length to the length of their handles. The two largest pairs of marginal hooks are positioned closest to the opisthaptoral peduncle, the neighbouring two pairs of medium-sized marginal hook sickles are situated along the lateral margins of the opisthaptor. Four pairs of smallest marginal hooks are positioned along the posterior margin of the opisthaptor. The male copulatory organ consists of a muscular pouch armed with approximately 30 gracile spines. Phylogenetic analyses of partial sequences of the 18S rDNA using Maximum Likelihood and Bayesian Inference placed the new genus within the lineage of solely African genera and suggests *Afrogyrodactylus* Paperna, 1968, *Citharodactylus* Přikrylová, Shinn et Paladini, 2017 and *Mormyrogyrodactylus* Luus-Powell, Mashego et Khalil, 2003 as genera most closely related to the new genus.

Key words: 18S rDNA, phylogeny, Mormyridae, Africa

The family Gyrodactylidae van Beneden et Hesse, 1832 currently contains 24 valid genera of viviparous monogeneans (Boeger et al. 2020). Until recently, both viviparous and oviparous genera were grouped into one family. However, Harris (1983) who had originally proposed a separate family for oviparous genera, the Oogyrodactylidae Harris, 1983, was recently reinstated by Boeger et al. (2020) to include all South American oviparous genera. Species of more than 90% of the genera (i.e., n = 22) in the Gyrodactylidae primarily parasitise fish hosts with only two exceptions, i.e., species of *Gyrdicotylus* Vercammen-Grandjean, 1960 and *Insancistrum* de Beauchamp, 1912, which infect frogs and cephalopods, respectively (de Beauchamp 1912, Vercammen-Grandjean 1960). However, some North American species of the genus *Gyrodactylus* von Nord-

mann, 1832, have been recorded from amphibian hosts, notably *G. aurorae* Mizelle, Kritsky et McDougal, 1969, *G. catesbeianae* Wootton, Ryan, Demaree et Critchfield, 1993, and *G. jennyae* Paetow, Cone, Huyse, McLaughlin et Marcogliese, 2009 (see Paetow et al. 2009).

Seven gyrodactylid genera are currently known from Africa of which six are geographically limited to this continent (Přikrylová et al. 2017a, Řehulková et al. 2018). These genera are *Afrogyrodactylus* Paperna, 1968, *Citharodactylus* Přikrylová, Shinn et Paladini, 2017, *Diplogyrodactylus* Přikrylová, Matějusová, Musilová, Gelnar et Harris, 2009, *Gyrdicotylus*, *Macrogyrodactylus* Malmberg, 1957, and *Mormyrogyrodactylus* Luus-Powell, Mashego et Khalil, 2003. Four of these African genera remain monotypic (Vercammen-Grandjean 1960, Luus-Powell et al.

2003, Přikrylová et al. 2009, 2017a), but the true species diversity of this family and its genera remains hidden. This concealed diversity is evidenced by the description of two new species of *Afrogyrodactylus* 45 years after the original description and representing the only other species within the genus (Přikrylová and Luus-Powell 2014).

In the late 1990s, the need for methodologies permitting the confident discrimination of *Gyrodactylus salaris* Malmberg, 1957 from its comparatively benign congeners led to the development of several molecular markers with utility for discrimination of species within the genus (Cunningham et al. 1995a, b). The internal transcribed spacers (ITS), 5.8S and 18S rDNA regions have become most used in studies on taxonomy, phylogeny and co-phylogeny (Huyse et al. 2002, Gilmore et al. 2012, Přikrylová et al. 2013, García-Vásquez et al. 2018, 2019, Boeger et al. 2020). The study of Cunningham et al. (1995a) was the first that used gyrodactylid 18S rDNA sequences to compare their relationship to other monogenean genera. The number of 18S rDNA sequences of gyrodactylid species increased (Matějusová et al. 2003, Přikrylová et al. 2013). Most notably with relevance to the current study focused on African representatives was that of Přikrylová et al. (2013) and Zahradníčková et al. (2016). While ITS and 5.8S rDNA sequences have limited utility for investigating phylogeny within the Gyrodactylidae at the intergeneric level (Vanhove et al. 2011, Přikrylová et al. 2013), they are suitable for inferring relationships within the genus *Gyrodactylus* (see Cable et al. 1999, Matějusová et al. 2001, Gilmore et al. 2012). At present, 18S rDNA sequences are available for 14 of the 23 genera within the Gyrodactylidae, including five African genera (Kritsky et al. 2013, Přikrylová et al. 2013).

Eschmeyer's catalog of fishes (Fricke et al. 2021); lists 35,797 species worldwide; the Mormyridae Bonaparte are listed as containing 229 species divided into 22 genera. The mormyrids, or African electric elephantfish, are distributed across tropical Africa and the Nile catchment basin only. Parasites have been reported for only 39 mormyrid species of 13 genera (Carvalho Schaeffner 2018). Most of the available information regarding the parasite fauna of mormyrids is linked to species belonging to the genera *Marcusenius* Gill and *Mormyrops* Müller, most notably *Marcusenius macrolepidotus* (Peters) with 16 parasite species documented with representatives of the Protozoa, Monogenea, Crustacea and Trematoda (Carvalho Schaeffner 2018). To date, three monogenean genera, *Archidiplectanum* Mizelle et Kritsky, 1969, *Bouixella* Euzet et Dossou, 1976, and *Mormyrogyrodactylus* have been found on mormyrid hosts (Řehulková et al. 2018), but only *Mormyrogyrodactylus gemini* Luus-Powell, Mashego et Khalil, 2003 has been found on *M. macrolepidotus*. Here we describe a new species from the gills of the bulldog, *M. macrolepidotus*, for which another new genus is proposed.

MATERIAL AND METHODS

Two specimens of bulldog, *Marcusenius macrolepidotus* (TL = 25–28 cm), were collected using gill nets in a side channel of Lake Kariba, Zimbabwe (-16.5270, 28.847344) in August 2011. The fish were caught with the help of the staff from the University of Zimbabwe Lake Kariba Research Station and transported to the station's laboratory where the fish were kept in aerated lake water. Fish were euthanised by severing the spinal cord following euthanasia through the administration of an overdose of anesthetic MS222 (tricaine methane sulphonate) (Neiffer and Stamper 2009) as per protocols for the Ethic Handling for Ectothermic Vertebrates (SANS 10386, 2008).

Monogeneans attached to the gills were removed using mounted needles and either prepared as whole mounts in ammonium picrate glycerine (APG; Malmberg 1970) or their haptors were excised, fixed with APG and mounted on a slide for subsequent morphological analysis while their corresponding anterior portions were stored, individually, in molecular grade ethanol (Sigma-Aldrich, St. Louis, USA). In November 2014, additional sampling of *M. macrolepidotus* (TL = 18–19.6 cm, n = 8) was undertaken at Nwanedi-Luphephe Dam, Limpopo Province, South Africa (-22.661386, 30.375597) to collect *Mormyrogyrodactylus gemini* for molecular identification.

Morphological analysis of the collected parasites was performed in the Laboratory of Parasitology, Department of Botany and Zoology, Masaryk University, Brno, Czech Republic, and in the Department of Biodiversity, University of Limpopo, South Africa. Morphology of the hard haptoral elements was examined using a phase-contrast microscope (Olympus BX51, Olympus, Tokyo, Japan) equipped with camera and Image Analysis Software, Stream Motion Software (Olympus).

The hard parts of the haptor were drawn with the aid of a drawing attachment. Measurements of the hamuli and associated bars were taken as suggested by Přikrylová et al. (2008); body size parameters were taken based on those presented by Christison et al. (2005), and marginal hook measurements followed Přikrylová et al. (2009; Figure 1D). All measurements are presented in micrometres, unless otherwise stated, and are presented as the range with the mean with in parentheses.

Prior to depositing the specimens in museum collections, the specimens in APG were transferred into Canada balsam following the procedure proposed by Ergens (1969). To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature, details of the new species have been submitted to ZooBank (International Commission on Zoological Nomenclature, 2012). A dichotomous key to African genera of the Gyrodactylidae was produced using software package DELTA (Description Language for Taxonomy) (Coleman et al. 2010).

For the parallel molecular analysis, ethanol was evaporated in a vacuum centrifuge, after which DNA was extracted from the anterior portion of each specimen using the Qiagen Blood and Tissue Isolation kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. DNA was eluted in 50 µl. The ITS region of the rDNA was amplified with the primers ITS-1F (5′-GTTTCCGTAGGT-GAACCT-3′; Rokicka et al. 2007) and ITS-2R (5′-TCCTCCGCT-TAGTGATA-3′; Matějusová et al. 2001). For amplification of the 18S rDNA region the primers SSU F (5′-GATCCTTCTGCA GGT-TCACCTAC-3′) and SSU R (5′-AAGCTGGTTGATCCTGCCAGT-3′) (Cunningham et al. 1995a) in combination with internal primers for SSU, 18S F-INT (5′-CTATTGGAGGGCAAGTCTGG-3′) and 18S R-INT (5′-CCGAACATCTAAGGCATCA-3′) (Přikrylová et al. 2013) were used.

Table 1. List of species included in the phylogenetic analyses (newly generated sequences in bold).

Species	Accession number	Host (Family)	Reference
Afrogyrodactylus girgifae Přikrylová et Luus-Powell, 2014	HF548672	*Brycinus nurse* (Rüppell) (Alestidae)	Přikrylová et al. 2013
Aglaiogyrodactylus forficulatus Kritsky, Vianna et Boeger, 2007	KX981461	*Kronichthys lacerta* (Nichols) (Loricariidae)	Patella et al. 2017
Aglaiogyrodactylus forficuloides Kritsky, Vianna et Boeger, 2007	KX981462	*Schizolecis gunther* (Miranda Ribeiro) (Loricaridae)	Patella et al. 2017
Citharodactylus gagei Přikrylová, Shinn et Paladini, 2017	HF548670	*Citharinus citharus* (Geoffroy Saint-Hilaire) (Citharinidae)	Přikrylová et al. 2013
Diplogyrodactylus martini Přikrylová, Matějuová, Musilová, Gelnar et Harris, 2009	HE858426	*Polypterus senegalus* Cuvier (Polypteridae)	Přikrylová et al. 2013
Fundulotrema prolongis (Hargis, 1955)	HF548673	*Fundulus heteroclitus* (Linnaeus) (Fundulidae)	Přikrylová et al. 2013
Gyrodactyloides bychowskii Albova, 1948	AJ566379	*Salmo salar* Linnaeus (Salmonidae)	Matějusová et al. 2003
Gyrodactyloides sp.	HF548677	*Mallotus villosus* (Müller) (Osmeridae)	Přikrylová et al. 2013
Gyrodactylus alekosi Přikrylová, Blažek et Vanhove, 2012	FR850683	*Clarias gariepinus* Burchell (Clariidae)	Přikrylová et al. 2013
Gyrodactylus carassii Malmberg, 1957	AJ566377	*Carassius carassius* (Linnaeus) (Cyprnidae)	Matějusová et al. 2003
Gyrodactylus corydori Bueno-Silve et Boeger, 2009	JX840356	*Corydoras ehrhardti* Steindachner (Callichthyidae)	Kritsky et al. 2013
Gyrodactylus ergensi Přikrylová, Matějuová, Musilová et Gelnar, 2009	HF548668	*Sarotherodon galilaeus* (Linnaeus) (Cichlidae)	Přikrylová et al. 2013
Gyrodactylus gobiensis Gloser, 1974	AJ566375	*Gobio gobio* (Linneaus) (Cyprinidae)	Matějusová et al. 2003
Gyrodactylus malalai Přikrylová, Blažek et Gelnar, 2012	FR695485	*Oreochromis niloticus* (Linneaus) (Cichlidae)	Přikrylová et al. 2013
Gyrodactylus rysavyi Ergens, 1973	FR850680	*Clarias anguillaris* (Linneaus) (Clariidae)	Přikrylová et al. 2013
Gyrodactylus salaris Malmberg, 1957	Z26942	*Salmo salar* Linnaeus (Salmonidae)	Cunningham et al. 1995
Gyrodactylus sedelnikowi Gvosdev, 1950	AJ566378	*Barbatula barbatula* (Linneaus) (Nemacheilidae)	Matějusová et al. 2003
Gyrodactylus superbus (Szidat, 1973)	JX840357	*Corydoras ehrhardti* Steindachner (Callichthyidae)	Kritsky et al. 2013
Ieredactylus rivuli Schelkle, Paladini, Shinn, King, Johnson, Oosterhout, Mohammed et Cable, 2011	JX840358	*Rivulus hartii* Poey (Rivulidae)	Kritsky et al. 2013
Laminiscus gussevi (Bychovsky et Polyansky, 1953)	HF548679	*Mallotus villosus* (Müller) (Osmeridae)	Přikrylová et al. 2013
Macrogyrodactylus congolensis (Prodhoe, 1957)	HF548680	*Clarias gariepinus* Burchell (Clariidae)	Přikrylová et al. 2013
Macrogyrodactylus polypteri Malmberg, 1957	AJ567671	*Polypterus senegalus* Cuvier (Polypteridae)	Matějusová et al. 2003
Mormyrodactylus geminii* Luus-Powell, Mashego et Khalil, 2003	MZ474666	*Marcusenius macrolepidotus* (Peters) (Mormyridae)	present study
Onychogyrodactylus hydaticus Kritsky, Vianna et Boeger, 2007	KX981457	*Ancistrus multispinis* (Regan) (Loricariidae)	Patella et al. 2017
Oogyrodactylus sp.	MT521666	*Farlowella* sp. (Loricariidae)	Boeger et al. 2020
Phanerothecium spinulatum Kritsky, Viann et Boeger, 2007	JX840360	*Hypostomus* sp. (Loricariidae)	Kritsky et al. 2013
Phanerothecium sp.	KX981456	*Rineloricaria lima* (Kner) (Loricariidae)	Patella et al. 2017
Scleroductus sp.	JX840361	*Rhamdia* sp. (Heptapteridae)	Kritsky et al. 2013
Swingleus ancistrus Billeter, Klink et Maugel, 2000	HF548675	*Fundulus heteroclitus* (Linnaeus) (Fundulidae)	Přikrylová et al. 2013
***Tresunincidactylus wilminae* gen. et sp. n.**	MZ474665	*Marcusenius macrolepidotus* (Peters) (Mormyridae)	present study

Each amplification reaction contained 1 µl of template DNA, 2 µl 1× PCR buffer, 1.25 mM MgCl₂, 100 µM dNTPs, 0.1 mg/ml µl bovine serum albumin, 0.5 µM of each primer (Generi Biotech, Hradec Králové, Czech Republic) and 1.5 U of Taq polymerase in a total volume of 20 µl and was run in Mastercycler eP gradient thermocycler (Eppendorf, Hamburg, Germany). Five microliters of PCR product were visualised on a Gold View (Ecoli, Bratislava, Slovakia) stained agarose gel (1%) and the remaining 15 µl was purified using the High Pure PCR Product Purification Kit (Roche, Basel Switzerland). Sequencing, using identical primers as in initial amplification, was carried out with the Big Dye Chemistry Cycle Sequencing Kit v.3.1 and an ABI 3130 Genetic Analyser automated sequencer (Applied Biosystems, Foster City, USA).

Published sequences for genera belonging to the Gyrodactylidae and Oogyrodactylidae were included in the 18S rDNA alignment, together with several representatives of *Gyrodactylus* (Table 1). Sequences were then aligned using MEGA X (Kumar et al. 2018) under ClustelW algorithm distance measures, followed by trimming using trimAl v.1.2 (Capella-Gutierrez et al. 2009). The optimal model of molecular evolution was selected based on the Bayesian information criterion (BIC; Schwarz 1978) and the corrected Akaike information criterion (AICc; Hurvich and Tsai 1989) implemented in Model Test v.0.1.1 (Posada 2008).

Based on the corrected AIC, the best transversional model (TVM) (Posada 2003) + Γ + I model was selected. To allow subsequent implementation in the phylogenetic software, the model with the second best corrected Akaike score was chosen, namely the generalised time-reversible model (GTR) (Tavaré 1986, Rodriguez et al. 1990) + Γ + I model, with a gamma-shape parameter of 0.43 and a proportion of invariable sites of 0.64.

Reconstruction of a model-averaged phylogeny showed that tree topology did not display phylogenetic uncertainty because of model selection. Hence, model choice should not influence the results. Bayesian inference (BI) was performed using the selected models with MrBayes v.3 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Posterior probabilities were calculated over 2×10^6 generations, sampling the Markov chain every 100 generations. One-fourth of the samples was discarded as "burn-in". In PhyML v.3.0 (Guindon and Gascuel 2003), a maximum likelihood (ML) search was performed under the optimised model. Nodal support was assessed through 1,000 bootstrap samples using the nearest-neighbour interchange branch-swapping algorithm. Conversion of alignment files was carried out using ALTER v.1.2 (Glez-Pena et al. 2010) and a tree was drawn in FigTree v.1.3 (http://tree.bio.ed.ac.uk/software/figtree).

RESULTS

Two specimens of the bulldog were collected from a side channel of Lake Kariba (Sanyati basin near Kariba town, Zimbabwe) and studied for the presence of parasites. Both fish specimens were infected with gill monogeneans (n = 8).

The presence of a developing embryo within the uterus of a few specimens indicated that the specimens were viviparous and that they belong to the Gyrodactylidae, but the overall arrangement of the opisthaptoral hard parts, as well as the unique morphology of the male copulatory organ (MCO), indicated that the discovery represented a new genus. Morphological and molecular descriptions of the parasite are provided below.
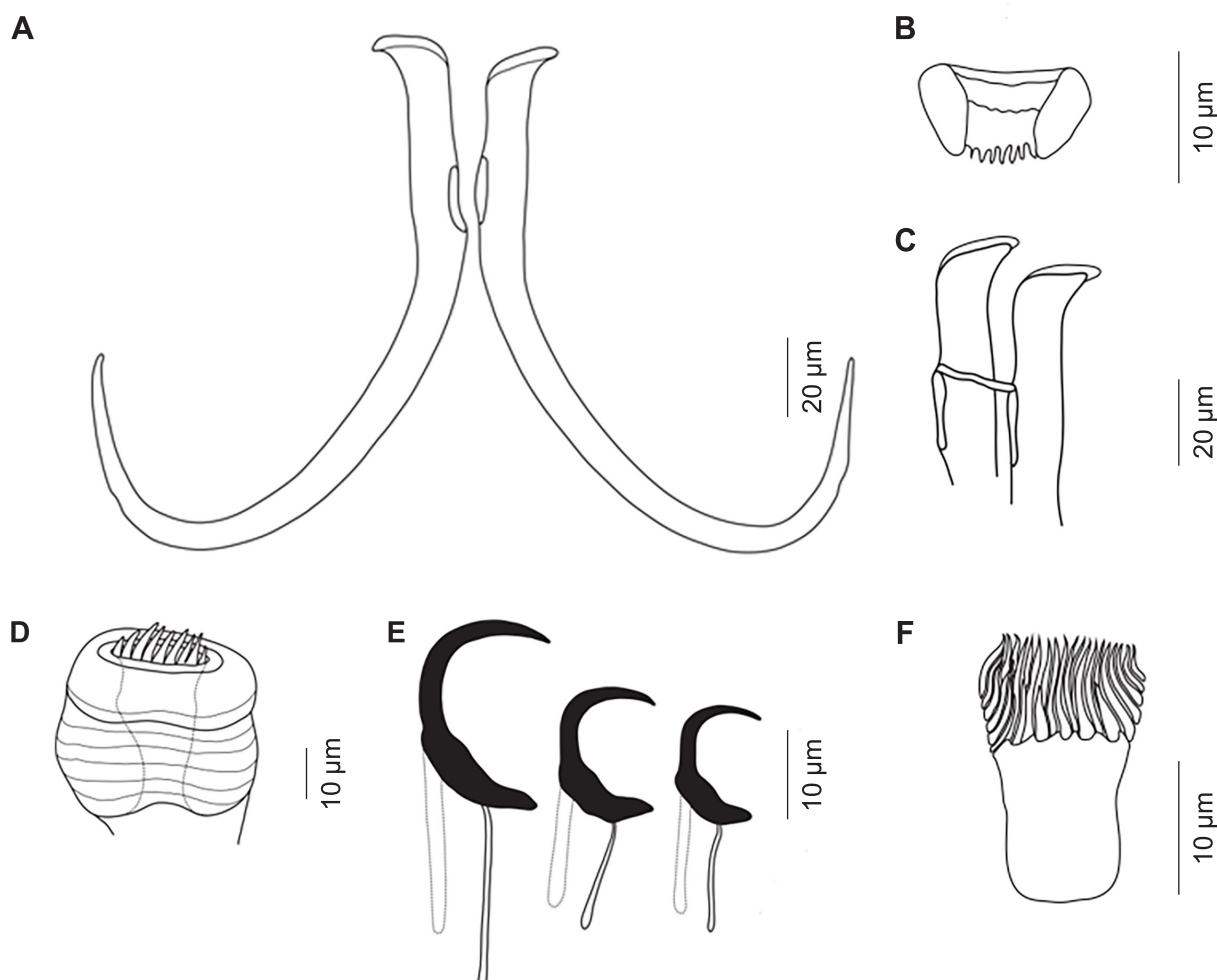
Fig. 1. Line drawings of *Tresuncinidactylus wilmienae* gen. et sp. n. from *Marcusenius macrolepidotus* (Peters). **A** – hamuli; **B** – ventral bar; **C** – details of hamuli roots and dorsal bar; **D** – pharynx; **E** – marginal hooks; **F** – male copulatory organ.

Subclass Polyonchoinea Bychowsky, 1937

Order Gyrodactylidea Bychowsky, 1937

Gyrodactylidae van Beneden et Hesse, 1832

***Tresuncinidactylus* gen. n.**

ZooBank number for species:
urn:lsid:zoobank.org:act:E36CE744-A54F-4531-8BA5-ECAFD4A4DFE3

Type and only species: *Tresuncinidactylus wilmienae* sp. n.

Diagnosis (based on 8 specimens). Body fusiform, comprising body proper (cephalic regions, trunk and peduncle) and opisthaptor. Body wall thin and smooth. Cephalic region bilobed, each lobe bears a spike sensillum. Cephalic gland present. Eye spots absent. Mouth situated ventrally. Pharynx spherical, comprised of two bulbs, anterior bulb presents finger-like pharyngeal processes. Oesophagus branching into two simple, blind-ended intestinal crura that extend beyond uterus. Viviparous, only one embryo *in utero* observed. Male copulatory organ consists of muscular pouch, positioned ventrally, close to bifurcation of intestinal crura, armed with approximately 30 gracile spines.

Opisthaptor delineated from body, bearing a single pair of large, slender hamuli with constriction on outer edge between shaft and point regions on each hamulus. Hamuli root prominently flattened; ventral terminus of hamulus cap tapered. Large muscle masses and tendons associated with hamulus root caps evident. Small and simple ventral bar, without anterolateral processes; thin lingulate membrane present. Thin, simple dorsal bar. Sixteen marginal hooks with large falculate sickles of one morphological type, but of three different sizes, length of each sickle being approximately equal in length to that of their handle. Two largest pairs of marginal hooks positioned closest to opisthaptoral peduncle, neighbouring two pairs of medium-sized marginal hook sickles situated along lateral margins of opisthaptor, and remaining four pairs and smallest marginal hooks positioned along posterior margin of opisthaptor.

E t y m o l o g y : Generic name refers to the presence of marginal hooks of three differing sizes.

***Tresuncinidactylus wilmienae* gen. et sp. n**   Figs. 1, 2

ZooBank number for species:
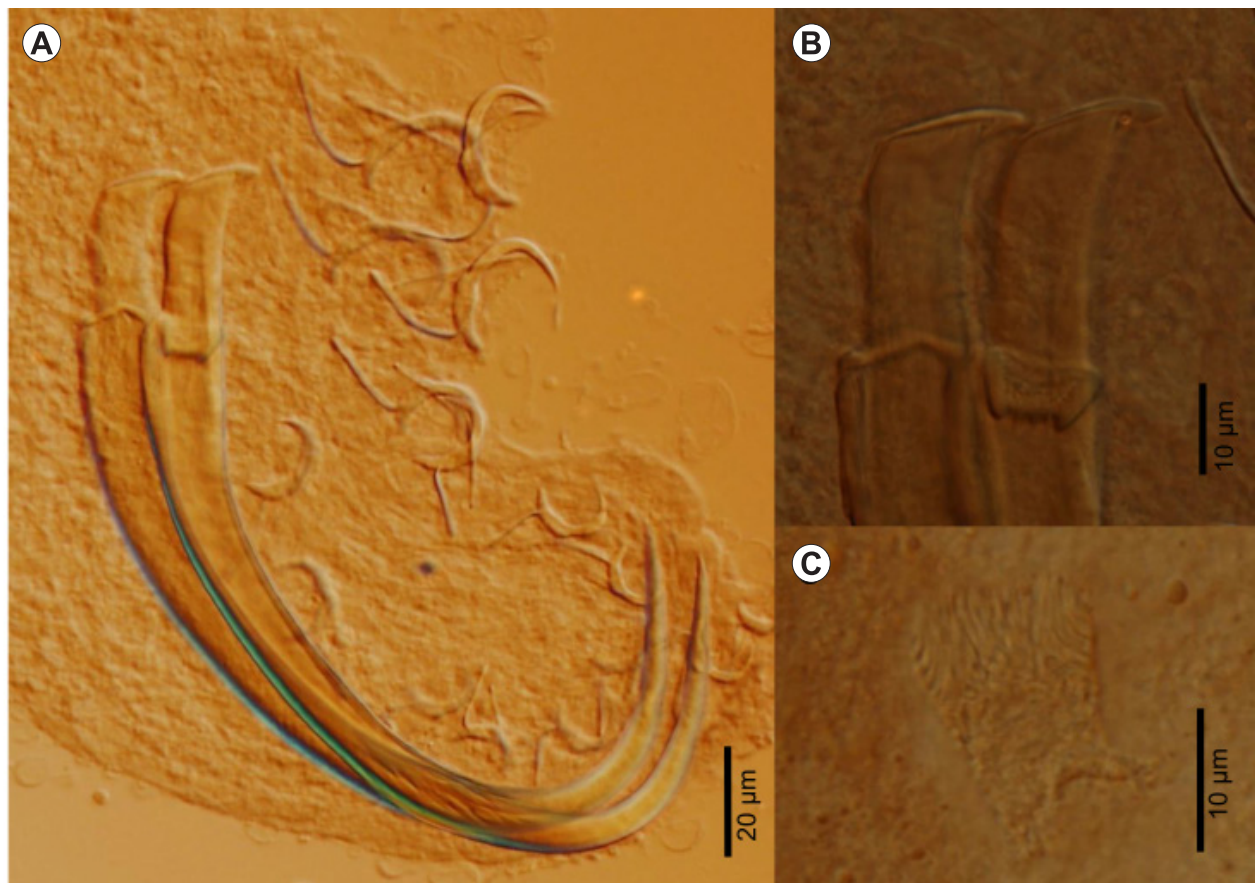urn:lsid:zoobank.org:act:E36CE744-A54F-4531-8BA5-ECAFD4A4DFE3

Fig. 2. Light microscope photographs of *Tresuncinidactylus wilmienae* gen. et sp. n from *Marcusenius macrolepidotus* (Peters). **A** – opisthaptor; **B** – hamuli root, ventral and dorsal bars details; **C** – male copulatory organ.

Description. Coverslip-flattened specimens 550–755 (606, n = 6) long, 103–127 (114, n = 6) wide at level of the uterus. Pharynx muscular bulb 35–48 (43, n = 7) long and 38–45(41) wide; anterior bulb of pharynx with eight, short pharyngeal processes. Excretory bladders present. MCO observed in three specimens; MCO muscular pouch, 19–24 (21, n = 3) long, 13–14 (13, n = 3) wide, armed with 30 gracile spines. Hamuli total length 140–154 (146, n = 8), point length 45–53 (50, n = 8), shaft length 125–134 (129, n = 8), root length 28–32 (30, n = 8). Dorsal bar 16–19 (18, n = 6) long and 2–3 (2, n = 6) width. Ventral bar 13–16 (14, n = 7) wide, 7–10 (8, n = 7) long. Marginal hooks (large anterior positioned pairs): handle length 19–23 (22, n = 21); sickle length 20–23 (21 n = 22); ratio handle: sickle 1.0–1.1 (1.0); sickle loop length 25–28 (26, n = 22); sickle proximal width 12–16 (14, n = 22); sickle point length 10–12 (11, n = 22). Marginal hooks (median lateral positioned pairs): handle length 13–17 (15, n = 16); sickle length 14–16 (15, n = 18); ratio handle: sickle 1.0–1.1 (1.0); sickle loop length 18–21 (19, n = 17); sickle proximal width 9–12 (10, n = 17); sickle point length 7–9 (8, n = 16). Marginal hooks (small posterior positioned pairs): handle length 11–13 (12, n = 17); sickle length 11–13 (12, n = 16); ratio handle: sickle 1–1.1 (1.0); sickle loop length 15–17 (16, n = 17); sickle proximal width 8–12 (9, n = 17); sickle point length 6–7 (7, n = 17).

T y p e   h o s t : *Marcusenius macrolepidotus* (Peters) (Osteoglossiformes: Mormyridae).

T y p e   l o c a l i t y : Lake Kariba, Zimbabwe (-16.5270, 28.847344)

T y p e   m a t e r i a l : Holotype, one paratype and one hologenophore deposited in the Helminthological Collection of the Institute of Parasitology, the Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (M-758). Another two specimens, one paratype and one hologenophore, in the parasitological collection in the National Museum, Bloemfontein, South Africa (NMB P 798 and 799), and two additional paratype specimens deposited in the parasite collection of the Royal Museum for Central Africa, Tervuren, Belgium (RMCA VERMES 43423 and 43424).

S i t e   o n   t h e   h o s t : Gill filaments.

M o l e c u l a r   s e q u e n c e   d a t a : Three specimens were successfully sequenced and the longest sequence for each region was deposited in the nucleotide database GenBank under accession numbers MZ479697 and MZ474665 for ITS and 18S rDNA, respectively. The 18S rDNA sequence was 1,864 bp long and ITS rDNA 669 bp; the latter consists of partial ITS1-291 bp, 5.8S-157 bp, and partial ITS2-221bp.

E t y m o l o g y : The species is named in honour of Wilmien Luus-Powell from the University of Limpopo, a close friend and colleague of the principal author, for her endless support and enormous contribution to research on the Monogenea in African freshwater fishes.
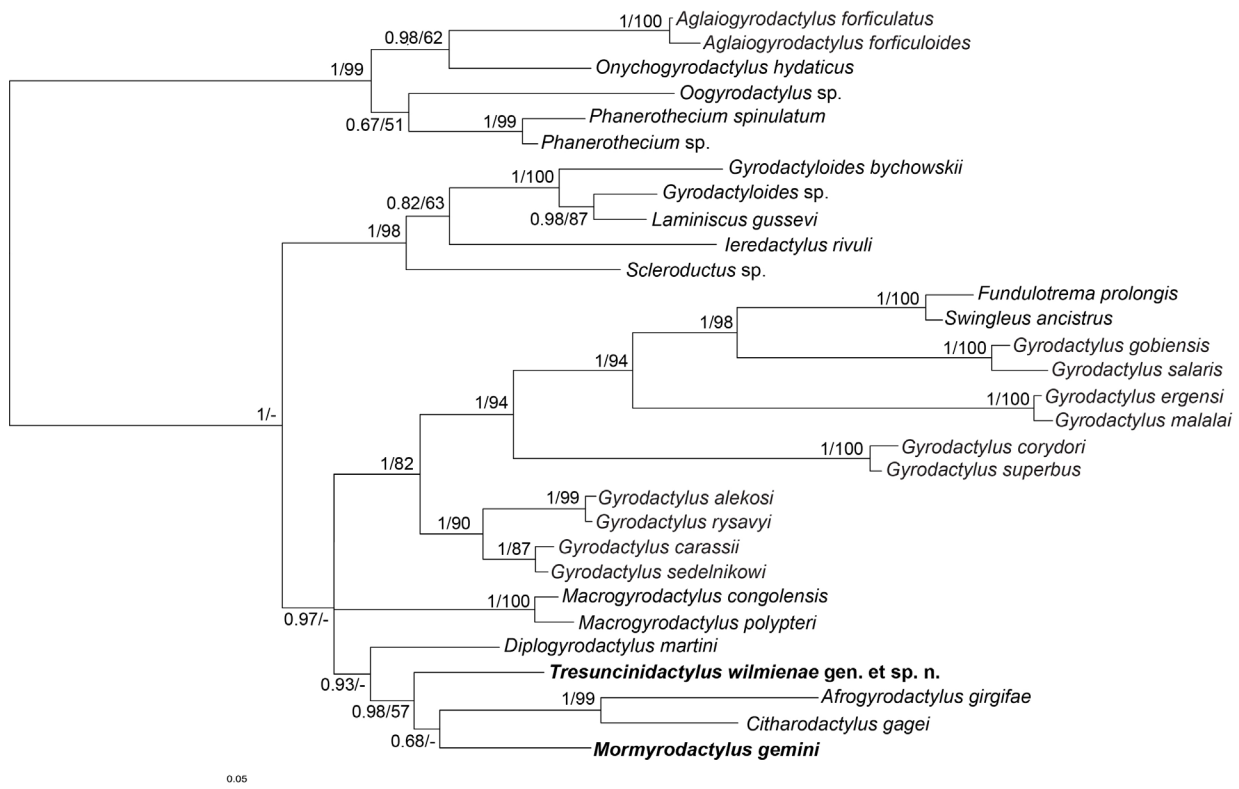
Fig. 3. Phylogenetic tree inferred from Bayesian inference (BI) based on 18S rDNA sequences alignment of 1,434 bp. Nodal support values are given as BI/ML (maximum likelihood). Support values lower than 50 (ML) are not presented. Newly generated sequences are highlighted in bold.

Phylogenetic relationships

Oviparous genera of the Oogyrodactylidae grouped in a separate and well-defined cluster with a very high support (Fig. 3). The observed distances between the new genus and the representatives of this cluster were 14.78–16.99% (Table 2). From all representatives of the Gyrodactylidae considered in this study, most closely related to any of oogyrodactylid genera were species of *Diplogyrodactylus*, *Mormyrogyrodactylus* and *Phanerothecium* sp. with 14.27%, and the most distant were *Gyrodactylus malalai* Přikrylová, Blažek et Gelnar, 2012 and *Oogyrodactylus* sp. (19.82%).

Within the Gyrodactylidae, *Gyrodactyloides* Bychowsky, 1947, *Ieredactylus* Schelkle, Paladini, Shinn, King, Johnson, Oosterhout, Mohammed et Cable, 2011, *Laminiscus* (Bychovsky et Polyansky, 1953) and *Scleroductus* Jara et Cone, 1989 form a very well supported, separate lineage with distances to the new genus of 9.12–10.71%. Between the genera of this cluster, the observed distances were 2.79–9.04%, with 2.79% being the lowest observed distance between species of different viviparous genera, i.e., *Laminiscus gussevi* (Bychowsky et Polyansky, 1953) and *Gyrodactyloides* sp. Species of *Gyrodactylus* formed a separate lineage that also includes *Fundulotrema prolongis* (Hargis, 1955) and *Swingleus ancistrus* Billeter, Klink et Maugel, 2000. The relationship of *T. wilmienae* gen. et sp. n. to the representatives of the genus *Gyrodactylus* varied from 6.4% to 13.87%, with *Gyrodactylus sedelnikowi* Gvosdev, 1950 being the closely related species.

Tresuncinidactylus gen. n. clustered as a part of the lineages including solely African genera, i.e., *Afrogyrodactylus*, *Citharodactylus*, *Diplogyrodactylus* and *Mormyrogyrodactylus*, in the range of distances 6.19–8.94%. The new genus appeared as a sister taxon to *Afrogyrodactylus* + *Citharodactylus* and *Mormyrogyrodactylus* with *Diplogyrodactylus* being the sister of these four taxa. The position of *Macrogyrodactylus* remains unresolved but is closely related to the new genus together with the other African genera. The overall lowest distance among the species of the Gyrodactylidae was 0,35% between *Gyrodactylus alekosi* Přikrylová, Blažek et Vanhove 2012 and *Gyrodactylus rysavyi* Ergens, 1973, and the most distant of 17.01% between *Gyrodactylus salaris* and *Afrogyrodactylus girgifae* Přikrylová et Luus-Powell, 2014.

Key to genera of the Gyrodactylidae from African freshwater fish

1 (2) Opisthaptor with 16 marginal hooks of one type 3
2 (1) Opisthaptor with 16 marginal hooks of differing morphologies; five pairs of marginal hooks with large falculate sickles and three pairs of smaller hooks with well articulated sickles …..............................***Diplogyrodactylus***
3 (4) Marginal hooks of one morphological type, but of differing sizes, length of each sickle being approximately equal in length to that of their handle ....
.... ***Tresunicidactylus* gen. n.**
4 (5) Marginal hook of one type and of equal size and equally distributed around the periphery of the opisthaptor.......... 6

Table 2. Uncorrected pairwise genetic distances between taxa included in the phylogenetic analysis (partial sequences of 18S rDNA)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1 *Tresuncinidactylus wilmienae* gen. et sp. n.																													
2 *Afrogyrodactylus girgifae*	8.94																												
3 *Aglaiogyrodactylus forficulatus*	16.23	16.62																											
4 *Aglaiogyrodactylus forficuloides*	16.99	17.29	0.78																										
5 *Citharodactylus gagei*	8.48	7.23	15.68	16.57																									
6 *Diplogyrodactylus martini*	6.19	10.99	16.97	17.77	8.98																								
7 *Fundulotrema prolongis*	11.35	14.39	17.47	18.48	13.05	11.44																							
8 *Gyrodactyloides bychowskii*	10.71	12.57	16.54	17.34	11.35	10.39	14.9																						
9 *Gyrodactyloides sp.*	9.62	12.44	17.71	18.42	10.92	10.01	13.1	5.43																					
10 *Gyrodactylus alekosi*	7.92	11.14	15.55	16.33	9.59	6.81	10.49	10.74	9.89																				
11 *Gyrodactylus carassii*	6.82	10.88	16.15	16.93	9.5	7.21	10.37	11.28	10.52	3.84																			
12 *Gyrodactylus corydori*	12.14	13.59	17.56	18.58	13.35	12.18	12.62	14.39	13.97	10.51	10.58																		
13 *Gyrodactylus ergensi*	12.25	14.48	18.8	19.49	13.81	12.81	11.75	14.49	14.37	11.29	11.83	13.49																	
14 *Gyrodactylus gobiensis*	13.04	15.71	16.66	17.53	14.1	13.23	8.65	15.35	14.69	10.97	10.9	12.76	12.06																
15 *Gyrodactylus malalai*	12.34	14.56	19.01	19.71	13.99	12.89	11.94	14.67	14.28	11.29	11.82	13.38	0.57	12.34															
16 *Gyrodactylus rysavyi*	8.01	11.35	15.99	16.78	9.69	6.9	10.78	11.03	10.17	0.35	3.83	10.7	11.5	11.17	11.48														
17 *Gyrodactylus salaris*	13.87	17.01	17.99	18.88	14.76	14.45	9.58	16.39	15.62	11.96	11.89	13.67	12.58	1.74	12.75	12.17													
18 *Gyrodactylus sedelnikowi*	6.4	11.17	16.69	17.49	9.68	6.63	10.27	11	10.79	3.99	0.71	10.51	11.63	11.08	11.62	3.83	12.08												
19 *Gyrodactylus superbus*	11.85	13.58	17.52	18.54	13.62	11.8	12.41	13.8	13.66	10.06	10.48	0.92	13.28	12.55	13.18	10.24	13.45	10.32											
20 *Ieredactylus rivuli*	10.76	11.05	16.55	17.22	10.98	10.48	12.47	8.75	8.19	8.97	10.05	13.83	14.04	14.39	14.12	9.25	15.36	10.32	13.56										
21 *Laminiscus gussevii*	9.4	12.19	18.06	18.88	10.6	9.51	13.62	5.52	2.76	9.61	10.13	14.12	14.1	14.55	14.28	9.88	15.31	10.41	13.63	8.25									
22 *Macrogyrodactylus congolensis*	8.11	10.63	15.71	16.69	8.67	7.02	11.64	10.56	9.54	7.47	7.12	11.53	12.46	12.43	12.64	7.22	13.22	6.54	11.33	10.08	9.24								
23 *Macrogyrodactylus polypteri*	8.15	10.49	15.79	16.67	9.22	7.69	11.76	10.95	10.03	7.66	6.97	11.47	12.15	12.78	12.23	7.48	13.49	6.47	11.27	10.51	9.55	1.43							
24 *Mormyrogyrodactylus gemini*	6.47	9.57	14.52	15.35	8.36	7.03	12.09	10.91	9.39	8.8	8.71	11.72	12.98	12.63	12.96	8.81	13.74	8.54	11.52	10.33	10.05	7.88	8.41						
25 *Onychogyrodactylus hydaticus*	15.59	17.06	7.67	8.28	16.48	16.39	17.45	17.4	16.7	16.03	16.36	18.23	19.07	17.25	19.08	16.28	18.71	16.81	18.2	16.08	16.53	15.9	16.17	14.58					
26 *Oogyrodactylus sp.*	15.89	17.57	11.05	11.71	17.31	16.17	17.32	17.91	18.34	15.64	15.71	18.82	19.86	16.96	19.89	15.77	18.17	16.05	18.89	16.51	17.53	15.99	16.16	15.7	8.73				
27 *Phanerothecium spinulatum*	15.81	17.76	9.55	10.47	16.43	15.69	16.82	18.91	18.73	15.95	15.5	18.72	18.62	16.06	18.74	15.96	17.24	15.91	18.39	17.08	18.09	16.05	16.44	15.38	8.37	9.04			
28 *Phanerothecium sp.*	14.78	16.58	8.71	9.6	15.09	14.27	15.96	17.57	17.2	14.71	14.48	17.81	17.51	15.14	17.52	14.63	16.09	14.88	17.48	15.94	16.59	14.62	14.99	14.27	7.48	8.13	1.87		
29 *Scleroductus sp.*	9.12	11.28	14.93	15.81	9.78	9.78	12.43	8.75	8.36	10.1	9.66	13.36	14.43	12.53	14.53	10.3	13.57	10.04	13.43	9.04	7.91	8.58	8.96	9.93	15.53	16.27	16.13	14.5	
30 *Swingleus ancistrus*	11.17	14.19	16.76	17.76	12.56	11.16	1.51	13.72	12.62	10.4	10.09	12.25	11.17	8.5	11.45	10.69	9.33	10	12.04	12.4	13.14	11.09	11.2	11.9	17.17	16.92	16.76	15.8	11.69

5 (4) Distribution of the marginal hooks unequal; 14 marginal hooks located along the posterior margin of the opisthaptor, two marginal hooks located on anterolateral lobes; ventral bar with two pairs of rods .... ***Macrogyrodactylus***

6 (7) Opisthaptor with additional suction discs; ventral bar complex consists of an inverted U-shaped piece with two semi-attached bars .... ***Mormyrogyrodactylus***

7 (8) Hamuli with only one (inner) developed root 9

8 (7) Hamuli with two developed roots; outer root conspicious .... ***Afrogyrodactylus***

9 (10) Bulbous MCO; MCO equipped with one apical spine and one to several rows of small spines; one pair of hamuli connected by a simple dorsal bar; ventral bar with or without anterolateral processes, ventral bar membrane present .... ***Gyrodactylus***

10 (9) MCO muscular; MCO consists of central curved cone, muscular pouch armed with numerous small spines; one pair of hamuli with a notable constriction at the junction between the shaft and point regions on each hamulus; simple dorsal and ventral bars; parasite on Citharinidae .. ***Citharodactylus***

DISCUSSION

Tresuncinidactylus wilmienae gen. et sp. n. is a gyrodactylid parasite with unique morphological features which is supported by molecular data and its comparative phylogenetic position within the family. Together with the new taxon, the African continent hosts species of seven viviparous gyrodactylid genera thus far (Řehulková et al. 2018). Taking into consideration the diversity of fish in African freshwater systems and the limited number of hosts and countries where fish parasites have been reported to date, there is undoubtedly a high probability of finding many more new species and genera. The South American fauna is comparable in this, with six genera belonging to the Gyrodactylidae and eight belonging to the Oogyrodactylidae (see Boeger et al. 2020) having been described from the diverse fish fauna.

Typically, only a single genus of the Gyrodactylidae is found on a genus of host. *Tresuncinidactylus* gen. n., however, is the second genus of the Gyrodactylidae, together with *Mormyrogyrodactylus,* to be identified from its host *M. macrolepidotus*. In addition to the latter species, 10 species of *Bouixella* and a single species of *Archidiplectanum* represent all the monogeneans known from mormyrid hosts (Řehulková et al. 2018). In Africa, the highest number of monogenean genera are recorded on the Cichlidae (i.e., five dactylogyrid genera and a single genus of gyrodactylid) and on the Clariidae (i.e., three genera of dactylogyrids and two gyrodactylid genera) (Řehulková et al. 2018).

It is highly probable that our current understanding of the monogenean fauna on these host families of fish results from sampling effort focused on these important food fish easy to capture, and from their utility as models for speciation (Kornfield and Smith 2000, Kocher 2004, Barson and Avenant-Oldewage 2006, Barson et al. 2008, Rahmouni et al. 2017, Jorissen et al. 2018, Geraerts et al. 2020).

Five of the seven known African genera of the Gyrodactylidae remain monotypic, but it is likely that this will change with future studies of new hosts and watercourses. This is exemplified by the genus *Afrogyrodactylus* which had remained monotypic for almost half a century until two studies discovered three new species and additionally referred to another potential new species (Přikrylová and Luus-Powell 2014, Přikrylová et al. 2017b).

Morphologically, species of six out of the seven African genera of gyrodactylids typically possess marginal hooks of a single type, with the notable exception being that of *Diplogyrodactylus*, species of which possess marginal hooks of two very marked and differing morphologies. Within the genus *Macrogyrodactylus*, *M. simentiensis* Přikrylová et Gelnar, 2008 also possesses marginal hook sickles with two different morphologies (Přikrylová and Gelnar 2008). The opisthaptoral armature of *Macrogyrodactylus* and that of *Mormyrogyrodactylus* with their additional accessory structures, however, permit their ready discrimination from the other gyrodactylid species discussed here.

The marginal hooks of *T. wilmienae* gen. et sp. n. are quite different from those of the other genera, not only because of the different size of hooks in the typical opisthaptoral complement but also because of their markedly differing morphology. Gyrodactylid species possessing marginal hooks of three differing sizes have, however, been recorded before, e.g., *Gyrodactylus milleri* Harris et Cable, 2000 (see Rubio-Godoy et al. 2010). *Tresuncinidactylus wilmienae* gen. et sp. n. has marginal hooks with large falculate sickles of three different sizes making them quite unique within the family. While the opisthaptoral armature of species belonging to *Afrogyrodactylus* and *Citharodactylus* is quite similar to that of species belonging to *Gyrodactylus*, all three genera, differ in the morphology and armature of their MCOs. In addition, species belonging to *Afrogyrodactylus* have two pronounced roots (i.e., an inner and outer root) of species of their hamuli facilitating their ready discrimination from all other gyrodactylid genera found in Africa.

The large hamuli of *T. wilmienae* gen. et sp. n. (mean length 145 μm), over double the typical size of those found in species of *Gyrodactylus* (i.e., < 70 μm), and the differing morphology of the marginal hook sickles to those of *Gyrodactylus*, provide the first indication that the process of attachment differs from that known in species of *Gyrodactylus* parasitising the skin of their hosts, with their marginal hooks serving as the principal means of attachment (Shinn et al. 2003).

The large falculate sickles of *T. wilmienae* gen. et sp. n. may function in a manner like that observed in *Diplogyrodactylus* (see Přikrylová at al. 2009), where the sickle proper of two neighbouring marginal hooks operate to clamp gills filaments between them. The direct observation of live specimens *in situ* on the gill prior to their detachment suggested that the hamuli operate in a similar clamping-like fashion. This might explain the presence of the prominently flattened hamuli root caps, which permits the attachment of muscles and tendons to the hamuli and facilitates their operation.

Tresuncinidactylus wilmienae gen. et sp. n. clustered in the phylogenetic tree with African lineages comprised of
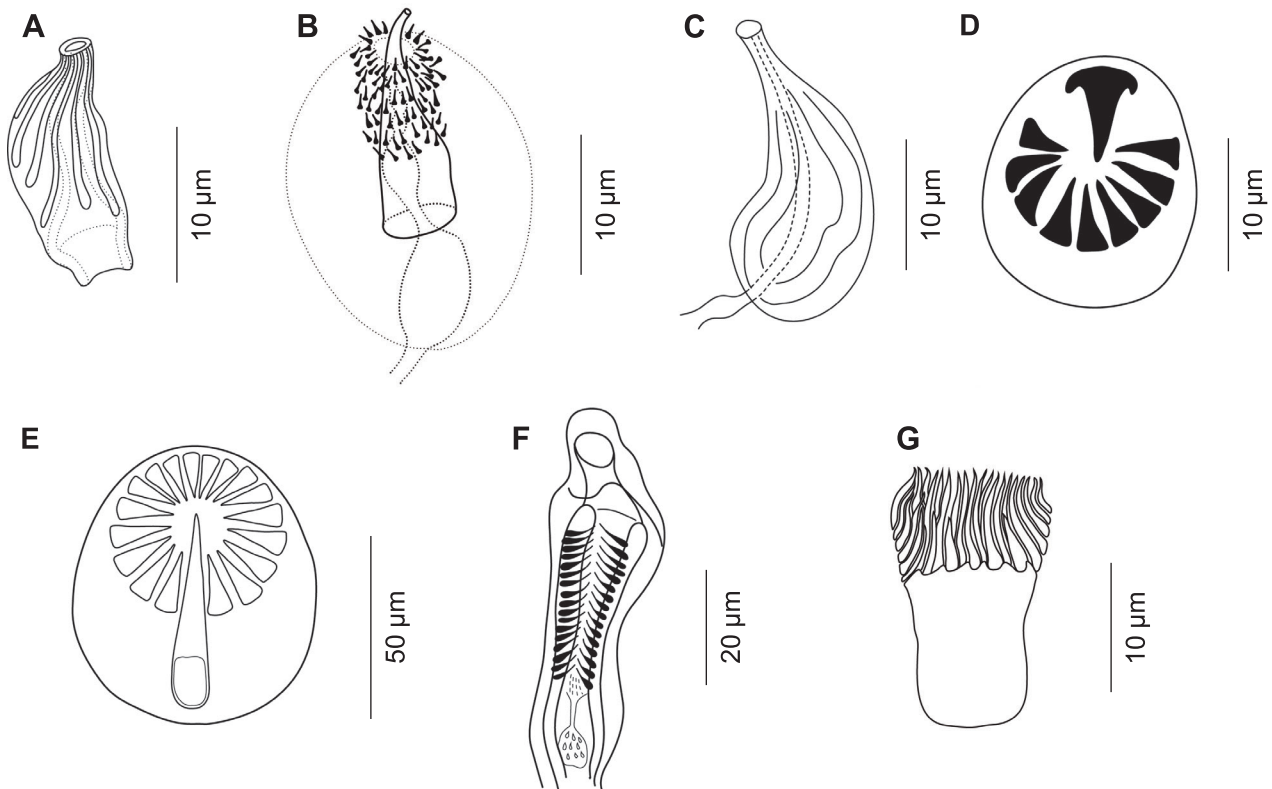
Fig 4. Line drawings of male copulatory organs of genara of Gyrodactylidae parasitizing African freshwater fishes. **A** – *Afrogyrodactylus* Paperna, 1968 (modified from Přikrylová and Luus-Powell 2014); **B** – *Citharodactylus* Přikrylová, Shinn et Paladini, 2017 (original drawing from Přikrylová et al. 2017a); **C** – *Diplogyrodactylus* Přikrylová, Matějusová, Musilová, Gelnar et Harris, 2009 (modified from Přikrylová et al. 2009); **D** – *Gyrodactylus* von Nordmann, 1832 (modified from Přikrylová et al. 2012); **E** – *Macrogyrodactylus* Malmberg, 1957 (modified from Řehulková et al. 2018); **F** – *Mormyrogyrodactylus* Luus-Powell, Mashego et Khalil, 2003 (modified from Vianna et al. 2007); **G** – *Tresuncinidactylus* gen. n. (present study).

five genera. The position of another African representative, *Macrogyrodactylus*, for which genetic data are available, however, remains unresolved. In the study of Přikrylová et al. (2013), *Macrogyrodactylus* spp. formed a separate lineage with high support. The present study including sequences for another two African genera contributed to the formation of a well-defined group of African representatives. The sister taxon to *Macrogyrodactylus*, however, remains to be discovered. While the MCO of species of *Macrogyrodactylus* is similar in its morphology to that of species of *Gyrodactylus*, the remaining five African gyrodactylid genera (see Fig. 4), which form this cluster (see Fig. 3), have non-typical *Gyrodactylus*-like MCOs (i.e., bulbous structure with a few, small spines accompanied with one an apical spine). The MCOs of species *Afrogyrodactylus* and *Diplogyrodactylus* are unarmed. The MCOs of species belonging to the genus *Citharodactylus* are bulbous and possesse a central, sclerotised, curved cone tapering to a hollow tip which protrudes through an opening onto the tegument. The opening is surrounded by approximately 40 small, upwardly facing, splinter-like spines (Přikrylová et al. 2017a). The MCOs of species of *Mormyrogyrodactylus* and *Tresuncinidactylus* gen. n. are muscular armed pouches, with that of the former being notably elongated and additionally bearing a long central spine. From a tree built using sequences of the mitochondrial

region presented by Boeger et al. (2020), it appears that *Paragyrodactylus* Gvozdev et Matrechov, 1953 might be a sister taxon to *Macrogyrodactylus*. Species of both genera possess similar MCOs and accessory pieces within their opisthaptoral attachment apparatus.

The current study indicates that the closest relatives to *Tresuncinidactylus* gen. n. appear to be the genera *Afrogyrodactylus*, *Citharodactylus* and *Mormyrogyrodactylus*. *Diplogyrodactylus* emerged as a sister taxon to this cluster, but showed the lowest uncorrected genetic distance of 6.19% towards the new genus from all genera of this African lineage. *Diplogyrodactylus* is the only genus within the family that shares a number of similar features, i.e., marginal hooks with large falculate sickles, the length of which is approximately equal in length to that of their handles.

Mormyrogyrodactylus, the other genus of the Gyrodactylidae parasitising *M. macrolepidotus,* is also very closely related (6.47%). Although species of both genera infect a common host, there are distinct differences between them. Species of *Mormyrogyrodactylus* occur on the body surface and fins, whereas *T. wilmienae* gen. et sp. n. is described from the gills. The site of infection of each parasite is mirrored in the arrangement of its opisthaptoral armature. The haptoral armature of species of *Mormyrogyrodactylus* is supplemented with four pairs of accessory bars and three supporting rods which facilitates the haptor's disk-like

suctorial attachment to its host, providing intimate contact with its host over the entire ventral surface of the opisthaptor (Luus-Powell et al. 2003, Vianna et al. 2007). In contrast, *T. wilmienae* gen. et sp. n. uses its complement of marginal hooks and hamuli to either clamp to or to pierce the gill epithelium to ensure its attachment to its host.

Unfortunately, only a partial sequence of the 18S rDNA region of *Paragyrodactylus variegatus* You, King, Ye et Cone, 2014, consisting of 448 bp, is available (You et al. 2014), and this has not been included in the present study. For the same reason, the short 451 bp sequence for *Gyrocerviceanseris passamaquoddyensis* Cone, Abbott, Gilmore et Burt, 2010 was also omitted (Gilmore et al. 2012) as it cannot be compared with the 1,500 bp long sequences used for the species considered here. It is evident, however, that obtaining new "long" sequences of the 18S rDNA region for species of the gydactylid genera which are currently unavailable can bring new insights into the phylogeny and relationships within the family. The study of Přikrylová et al. (2013) included representatives of nine genera and Kritsky et al. (2013) added an additional two genera belonging to the Gyrodactylidae together with a few genera belonging to the Oogyrodactylidae. The recent resurrection of the family Oogyrodactylidae by Boeger et al. (2020) contributed to defining the lineages of four genera, i.e., the *Gyrodactyloides*, *Laminiscus*, *Ieredactylus* and *Scleroductus*. The current study helps define an African lineage. To date, sequencing effort means that sequences are now available for species of 16 of the 24 genera within the Gyrodactylidae.

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