

Research Article

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Morphological description and molecular characterisation of *Dactylogyrus matlopong* sp. n. (Monogenea: Dactylogyridae) from the South African endemic *Labeobarbus aeneus* (Cyprinidae: Torinae)

Aline A. Acosta¹, Marliese Truter^{1,2}, Wynand Malherbe¹, Nico J. Smit¹

¹ Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa;

² DSI/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African Institute for Aquatic Biodiversity, Makhanda, South Africa;

Abstract: *Dactylogyrus* Diesing, 1850 is the most species-rich genus in Platyhelminthes, with over 900 documented species, that are mostly strictly specific to freshwater cyprinoids. The morphological *Dactylogyrus* groups afrobarbae-type, carpathicus-type, pseudoanchoratus-type, and varicorhini-type are known to occur in Africa. This study describes a new species of *Dactylogyrus* of the varicorhini-type from the gills of the endemic smallmouth yellowfish *Labeobarbus aeneus* (Burchell) from the Vaal River, Free State Province, South Africa. *Dactylogyrus matlopong* sp. n. is unique among its varicorhini-type congeners mainly by the accessory piece of the male copulatory complex that presents a hook-shaped subunit with defined round base not reaching the male copulatory organ, combined with the presence of a conspicuous medial projection on the anterior margin of the ventral bar. Sequences of the partial 28S and 18S rRNA genes, together with entire ITS1 region, were generated for the first time for a species of *Dactylogyrus* from South Africa. Concatenated phylogenetic analyses of selected *Dactylogyrus* spp. showed that these parasites group according to their morphological types.

Key-words: yellowfish, new species, taxonomy, ribosomal genes, ITS1 region

Dactylogyrus Diesing, 1850 (Monogenea: Dactylogyridae) is currently the most species-rich genus in Platyhelminthes, with over 900 species documented (Gibson et al. 1996). Its species are morphologically characterised by possessing a single pair of anchors pointed to the dorsal side of the haptor, one dorsal bar in some groups, or ventral and dorsal bars in others (Pugachev et al. 2009). Species of *Dactylogyrus* are mostly strictly specific to freshwater cyprinoids, reflecting the ecology and natural distribution of their hosts, being recorded in Asia, Africa, Europe and North America (Šimková et al. 2004, Benovics et al. 2018). Strict specificity to cyprinoid hosts have been confirmed, with most species of *Dactylogyrus* being specific to a single host species, although the degree of host specificity may vary for other species, with one species parasitising two or more related hosts (Šimková et al. 2006).

The current diversity of *Dactylogyrus* spp. is estimated to be even higher, considering that many endemic cyprinoid hosts have not had their ecto-parasite fauna surveyed yet (Řehulková et al. 2021). Most *Dactylogyrus* spp. can be allocated into morphological groups that are defined according to the shape of their sclerotised structures (an-

chors, marginal hooks, bars, male copulatory complex and vagina) (Pugachev et al. 2009).

The morphological *Dactylogyrus* groups afrobarbae-type, carpathicus-type, pseudoanchoratus-type, and varicorhini-type are the ones known to occur in Africa (Paperna 1973, 1979). It was postulated by Paperna (1979) that the varicorhini-type species occurring in Africa show affinities with large-scaled cyprinoids such as the yellowfishes of the genus *Labeobarbus* Rüppell, while the afrobarbae-type species show affinities with small host species such as the barbs of the genus *Enteromius* Cope.

More than 100 *Dactylogyrus* spp. have been described and recorded in Africa, with the majority parasitising cyprinoids of the genera *Enteromius*, *Luciobarbus* Heckel, *Labeo* Cuvier, and *Labeobarbus* (see Řehulková et al. 2018). Seventeen species of *Dactylogyrus* are reported from cyprinoids in South Africa, from which only two species, *Dactylogyrus spinicirrus* (Paperna et Thurston, 1968) and *Dactylogyrus varicorhini* Bychowsky, 1957, were found in *Labeobarbus* hosts (Price et al. 1969a,b, Mashego et al. 1983, Crafford et al. 2012, Mashego and Matlou 2018, Raphahlelo et al. 2020). Regarding molecular data

Address for correspondence: Aline A. Acosta, Water Research Group, Unit for Environmental Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa. E-mail: alineacosta1@gmail.com

Zoobank number for article: [urn:lsid:zoobank.org:pub:62113318-56B8-4513-9096-9957C7F58D8D](https://zoobank.org/pub:62113318-56B8-4513-9096-9957C7F58D8D)

for *Dactylogyrus* spp. from Africa, only 20 species from Morocco and Senegal have data available for the partial rRNA genes 28S and 18S, and ITS1 region (GenBank <https://www.ncbi.nlm.nih.gov/genbank/>).

Seven species of *Labeobarbus* occur in South Africa (Skelton 2001), from which *Labeobarbus aeneus* (Burchell), known as the smallmouth yellowfish, is endemic to the country. Studies available on *L. aeneus* include its conservation status reviews (de Villiers and Ellender 2007) and behaviour (O'Brien et al. 2013). Since the species is important for recreational fishing, some research on physiological effects of catch and release angling on *L. aeneus* have also been conducted (Smit et al. 2016). However, information on its parasites in general and more specific gill monogeneans is scanty.

Helminths of the classes Monogenea, Digenea and Cestoda have previously been recorded for *L. aeneus* in its endemic area. Avenant-Oldewage et al. (2014) described *Paradiplozoon ichthyoxanthos* Avenant-Oldewage, le Roux, Mashego et van Vuuren, 2013 (Monogenea: Diplozoidae) from the gills, and Crafford et al. (2014) recorded *Dactylogyrus* sp. also from the gills. More recently, Dos Santos et al. (2021) described the allocreadiid digenean *Allocreadium apokryfi* Dos Santos, Gilbert, Avenant-Oldewage et Dumbo, 2021 from the intestine. A few records for the invasive Asian fish tapeworm *Schyzocotyle acheilognathi* (Yamaguti, 1934) can be found in the literature (see Smit et al. 2017 and references therein).

The present study describes a new species of *Dactylogyrus* of the varicorhini-type from the gills of the endemic smallmouth yellowfish *L. aeneus* from the Vaal River, Free State Province, South Africa. Additionally, sequences of the partial 28S and 18S rRNA genes, and entire ITS1 region are presented for the new species.

MATERIAL AND METHODS

Sampling and morphological analyses

As part of a larger fish parasitological survey in May 2019, five individuals of *Labeobarbus aeneus* were collected from an industrial cooling pond associated with the Vaal River using electroshocking and landing nets (permit number NW 8065/03/2019). Fish were killed by percussive stunning and severance of the spinal cord following the NWU protocol for ethical handling for ectothermic vertebrates (SOP No: NWU-00267-17-S5). Subsequently, the gills were removed and screened for parasites under a Nikon SMZ445 Zoom Stereo Microscope (Nikon, Tokyo, Japan).

Monogeneans were removed from the gills using fine needles and flattened on slides with glycerine ammonium picrate (GAP) to study the sclerotised structures (Malmberg 1957). The slides were remounted in Canada Balsam to be kept as permanent, following Ergens (1969). Some specimens were placed in sterilised tubes with 96% ethanol for molecular analyses. The slides were studied under a compound microscope (Nikon Eclipse Ni, Nikon, Tokyo, Japan) equipped with a computerised digital camera system for image analysis with differential interference contrast, and NIS-Elements BR 4.60© software for measurements. Illustrations were made with the aid of a drawing tube attached to the micro-

scope. Plates were created using Photoshop™ and Adobe Illustrator™ software package (Adobe Inc., California, United States of America). Measurements of the dorsal anchor were taken according to Rahmouni et al. (2017), while the measurements followed Mizelle and Klucka (1953) and Kritsky et al. (1985) for the other structures, representing straight lines between extreme points. Measurements are expressed in micrometres as the range followed by mean and number of specimens measured in parentheses. Hook pairs are numbered according to Mizelle (1936). Holotype and paratypes were deposited in the Parasite Collection of the National Museum (NMB), Bloemfontein, South Africa, and paratypes in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czechia (IPCAS).

DNA extraction, amplification and sequencing

Specimens used for DNA extraction were checked under the microscope to confirm their morphology. A conspecific specimen mounted in GAP, followed by preparation as permanent slide in Canada Balsam, was kept as paragenophore (see Pleijel et al. 2008 for terminology). Genomic DNA was extracted using 100 µl of a 5% suspension of Chelex® (Sigma-Aldrich Solutions, Merck KGaA, Darmstadt, Germany) in deionised water and 2 µl of proteinase K, incubated at 56 °C for 6 h, boiled at 90 °C for 8 min and centrifuged at 14,000 rpm for 10 min. Polymerase chain reactions (PCR) had a final volume of 20 µl: 3 µl of DNA extraction supernatant, 10 µl DreamTaq PCR Master Mix (2X) (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 3.8 µl of nuclease free water, and 1.6 µl of each PCR primer.

Partial 28S rRNA was amplified using the forward primer U178 (5'-GCACCCGCTGAAYTTAAG-3') and the reverse primer L1642 (5'-CCAGCGCCATCCATTTC-3') (Lockyer et al. 2003), using the cycling profile: denaturation at 95 °C for 3 min, 35 cycles of amplification (94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min and 30 s), and 4 min extension hold at 72 °C. Partial 18S rRNA and entire ITS1 region were amplified using the forward primer S1 (5'-ATTCCGATAACGAACGAGACT-3') (Sinnappah et al. 2001) and the reverse primer IR8 (5'-GCTAGCTGCGTTCTTCATCGA-3') (Šimková et al. 2003), using the cycling profile: denaturation at 94 °C for 2 min, 40 cycles of amplification (94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min and 30 s), and 10 min extension hold at 72 °C. PCR products were run on 1% agarose gel using GelRed® (MilliporeSigma, Burlington, Massachusetts, USA) and loading buffer. PCR amplicons were purified and sequenced at Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa, using the PCR primers and the internal primer L1200R for 28S (Littlewood et al. 2000). Contiguous sequences were assembled using Geneious v. 7.1.3 (Kearse et al. 2012).

Alignment and phylogenetic analyses

One sequence of the partial 28S rRNA gene (1,519 bp long) and one sequence of the partial 18S gene + ITS1 region (836 bp long) were newly generated in this study, in order to compare the phylogenetic relationship of the new species with some *Dactylogyrus* spp. of the varicorhini-type. Only sequences associated with peer-reviewed publications were included in the analyses (Table 1). The taxa included in the phylogenetic analyses were selected mainly based on the results of Benovics et al. (2021) and Řehulíková et al. (2021). Default parameters of Muscle implemented in Geneious 7.1.3 were used to generate the alignments, which had

Table 1. List of sequences of *Dactylogyrus* spp. included in the phylogenetic analyses. Species in bold was sequenced for the present study. *Dactylogyrus maroccanus* El Gharbi, Birgi et Lambert, 1994 was used as outgroup.

Species	Host	Subfamily	Locality	18S + ITS1	28S	Reference
<i>Dactylogyrus atlasensis</i>	<i>Luciobarbus pallaryi</i>	Barbinae	Morocco	KY629337	KY629356	Šimková et al. (2017)
<i>Dactylogyrus benhoussai</i>	<i>Luciobarbus yahyaoui</i>	Barbinae	Morocco	KX578025	KX553862	Rahmouni et al. (2017)
<i>Dactylogyrus bocagei</i>	<i>Luciobarbus graellsii</i>	Barbinae	Spain	MN365675	MN338210	Benovics et al. (2020)
<i>Dactylogyrus carassobarbi</i>	<i>Carasobarbus luteus</i>	Torinae	Iraq	MZ031071	MZ031060	Benovics et al. (2021)
<i>Dactylogyrus doadrioi</i>	<i>Luciobarbus guiraonis</i>	Barbinae	Spain	MN365682	KY629346	Benovics et al. (2020) Šimková et al. (2017)
<i>Dactylogyrus falsiphallus</i>	<i>Luciobarbus maghrebensis</i>	Barbinae	Morocco	KX578024	KX553861	Rahmouni et al. (2017)
<i>Dactylogyrus fimbriphallus</i>	<i>Luciobarbus pallaryi</i>	Barbinae	Morocco	KY629332	KY629357	Šimková et al. (2017)
<i>Dactylogyrus guadianensi</i>	<i>Luciobarbus comizo</i>	Barbinae	Spain	MN365674	MN338209	Benovics et al. (2020)
<i>Dactylogyrus kulindrii</i>	<i>Carasobarbus fritschii</i>	Torinae	Morocco	KY629336	KY629354	Šimková et al. (2017)
<i>Dactylogyrus lenkorani</i>	<i>Capoeta buhsei</i>	Barbinae	Iraq	MZ031069	MZ031057	Benovics et al. (2021)
<i>Dactylogyrus lenkoranoides</i>	<i>Luciobarbus graellsii</i>	Barbinae	Spain	MN365676	MN338211	Benovics et al. (2020)
<i>Dactylogyrus maroccanus</i>	<i>Carasobarbus fritschii</i>	Torinae	Morocco	KY629333	KY629355	Šimková et al. (2017)
<i>Dactylogyrus mascomai</i>	<i>Luciobarbus graellsii</i>	Barbinae	Spain	MN365680	MN338215	Benovics et al. (2020)
<i>Dactylogyrus scorpius</i>	<i>Luciobarbus rifensis</i>	Barbinae	Morocco	KX578023	KX553860	Rahmouni et al. (2017)
<i>Dactylogyrus varius</i>	<i>Luciobarbus maghrebensis</i>	Barbinae	Morocco	KX578026	KX553863	Rahmouni et al. (2017)
<i>Dactylogyrus volutus</i>	<i>Carasobarbus fritschii</i>	Torinae	Morocco	KY629353	KY629334	Šimková et al. (2017)
<i>Dactylogyrus zatensis</i>	<i>Carasobarbus fritschii</i>	Torinae	Morocco	KY629335	KY629352	Šimková et al. (2017)
<i>Dactylogyrus matlopong</i> sp. n.	<i>Labeobarbus aeneus</i>	Torinae	South Africa	ON391042	ON391043	Present study

their extremities trimmed resulting in one alignment with sequences of the 18S + ITS1 (955 bp long), one alignment with sequences of the 28S (820 bp long), and a concatenated alignment combining the 18S+ITS1 and 28S alignments (1,775 bp long). *Dactylogyrus maroccanus* El Gharbi, Birgi et Lambert, 1994 was used as outgroup for the three alignments, based on the phylogeny of Řehulková et al. (2021). Phylogenetic analyses were run for the concatenated alignment under Maximum Likelihood (ML) and Bayesian Inference (BI) criteria. Maximum Likelihood (ML) trees were generated in RAxML (Guindon and Gascuel 2003), estimating model parameters and bootstrap support values (1,000 repetitions). Bayesian Inference (BI) trees were generated in MrBayes (Ronquist et al. 2012), running two independent MCMC runs of four chains for 10⁷ generations and sampling tree topologies every 10³ generations. Burn-in period was set to the first 25,000 generations. MrBayes and RAxML analyses were performed using the computational resource CIPRES (Miller et al. 2010). Genetic divergences were calculated using the uncorrected p-distances model in MEGA 7 (Kumar et al. 2016) for the 28S alignment. Phylogenetic trees were edited in FigTree v1.4.4 (Rambaut 2009).

RESULTS

Polyonchoinea Bychowsky, 1937

Dactylogyridea Bychowsky, 1937

Dactylogyridae Bychowsky, 1933

Dactylogyrus Diesing, 1850

Dactylogyrus matlopong sp. n. (Fig. 1A–B)

ZooBank number for species:

[urn:lsid:zoobank.org:act:41A8930E-AB3D-46F3-AA8F-582365C1BB04](https://zoobank.org/act:41A8930E-AB3D-46F3-AA8F-582365C1BB04)

Description (based on 15 specimens in GAP). Body 349–664 (475; n = 15) long, maximum width 85–147 (106;

n = 15) at mid-length. Eyespots 4, equidistant; accessory granules small, generally elongate ovate and sometimes sparse in anterior region. Haptor 58–99 (80; n = 15) long, 95–149 (128; n = 15) wide. One pair of anchor of varicorhini-type, base of anchor with a cleft between inner and outer roots, outer root with round base slightly undulated, inner root more conspicuous and elongate, noticeable sharp-stepped narrowing of inner side of anchor shaft before turning into point, point not extending inner root, double filament present (Fig. 1A [DA – dorsal anchor]); anchor total length 45–56 (51; n = 15), length to notch 34–44 (39; n = 15), inner root 14–22 (18; n = 15) long, outer root 5–7 (6; n = 15) long, point length 12–16 (15; n = 15). Transversal bars of varicorhini-type: dorsal bar robust, ‘butterfly-like’ with grooves in middle portion of anterior and posterior margins, tapering towards the ends, rounded ends posteriorly directed, weakly sclerotised patch present on anterior surface (Fig. 1A [DB – dorsal bar]); dorsal bar length 24–34 (30; n = 15), height 8–14 (11; n = 15). Ventral bar not robust, open V-shaped, rounded ends, conspicuous medial projection on anterior margin present, weakly sclerotised patch present on posterior surface (Fig. 1A [VB – ventral bar]); ventral bar length 23–30 (26; n = 15), height 10–18 (13; n = 15). Hooks 7 pairs, similar in shape, delicate point, depressed thumb, robust shank, short hook filament present (Fig. 1A [I–VII]); hook pairs length: I 26–35 (29; n = 12), II 27–35 (30; n = 12), III 30–38 (34; n = 12), IV 25–32 (28; n = 12), V 24–33 (28; n = 12), VI 23–31 (26; n = 12), VII 30–39 (34; n = 12); needles not observed.

Male copulatory complex of varicorhini-type, composed of male copulatory organ (MCO) and accessory piece. MCO a delicate tube with broad base, forming one incomplete ring, 30–43 (37; n = 13) long. Accessory piece articulated to MCO, composed of 5 sclerotised subunits: one conspicuous hook-shaped subunit present at distal portion of the MCO; a rounded subunit weakly sclerotised present at hook tip of the aforementioned subunit; another subunit next to the latter supporting distal portion

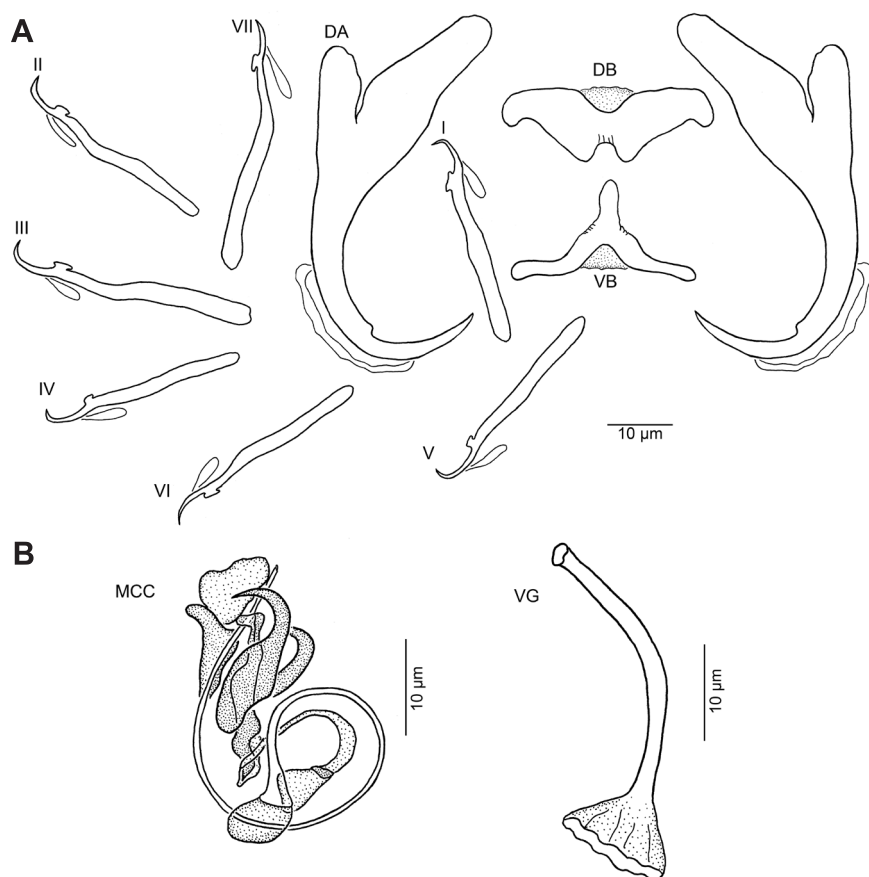


Fig. 1. Sclerotised structures of *Dactylogyrus matlopong* sp. n. from the gills of *Labeobarbus aeneus* (Burchell) from the Vaal River, Free State Province, South Africa. **A** – haptoral structure, scale bar is the same for all structures in A; **B** – reproductive structures. Abbreviations: DA – dorsal anchor; DB – dorsal bar; VB – ventral bar; I–VII – hooks; MCC – male copulatory complex; VG – vaginal tube.

of MCO; one subunit rod-shaped with basal undulations located underneath hook-shaped subunit; and one subunit circumventing side of hook-shaped subunit, articulation to MCO C-shaped with broad basal extremity (Fig. 1B [MCC – male copulatory complex]); accessory piece total length 22–33 (26; $n = 15$). Vaginal tube sclerotised, with weakly sclerotised atrium opening as trapezium with undulated margins, 32–45 (39; $n = 10$) long (Fig. 1B [VG – vagina]).

Type host: *Labeobarbus aeneus* (Burchell) (Cypriniformes: Cyprinidae).

Type locality: Vaal River, Free State Province, South Africa (27.979196, -26.737055)

Site on host: Gills.

Type material: Holotype (NMB P 842); six paratypes (NMB P 843–848), 6 paratypes and 1 paragenophore (IPCAS M-765).

Representative DNA sequences: 1,519 bp long partial sequence of the partial 28S rRNA gene (GenBank ID ON391043); 836 bp long sequence of the partial 18S rRNA gene and entire ITS1 region (GenBank ID ON391042).

Infestation rate: prevalence 100% (5 host specimens); mean intensity 16.8 (6–25).

Etymology: The species epithet *matlopong*, noun in apposition, is a SeSotho word meaning “on the gills”, the attachment site of this monogenean. SeSotho is the main African language spoken in the Free State Province of South Africa.

Remarks. *Dactylogyrus matlopong* sp. n. belongs to the varicorhini-type based on the presence of the following characteristics stated by Paperna (1979), El-Gharbi et al. (1994), and Pugachev et al. (2009): two haptoral connective bars – the dorsal is well-developed with shape resembling a butterfly with open wings, the ventral bar is smaller and V-shaped, anchors shaft with a sharp-stepped narrowing in the inner side before turning into point, base of anchor presents a depression in its middle, MCO as a spiral, and accessory piece presents a gutter and parallel ramifications forming folds (designated as ‘subunits’ in the description of the new species). From the varicorhini-type species, the new species mostly resembles *Dactylogyrus oumiensis* El-Gharbi, Birgi et Lambert, 1994 from *Carasobarbus harterti* (Günther) from Morocco, presenting similar vaginal tube and male copulatory complex. In *D. oumiensis*, the hook-shaped subunit of the accessory piece presents a tapering continuation of the base that extends to the base of the MCO, whereas in the new species, the hook-shaped subunit has a defined round base not reaching the MCO (see Fig. 1B [MCC]; and El-Gharbi et al. 1994). The two species present similar shape of the dorsal bar, but differ in the shape of the ventral bar, which has a conspicuous medial projection on the anterior margin in the new species. Paratypes of *D. oumiensis* are not available at the Muséum National d’Histoire Naturelle, Paris, as stated by the au-

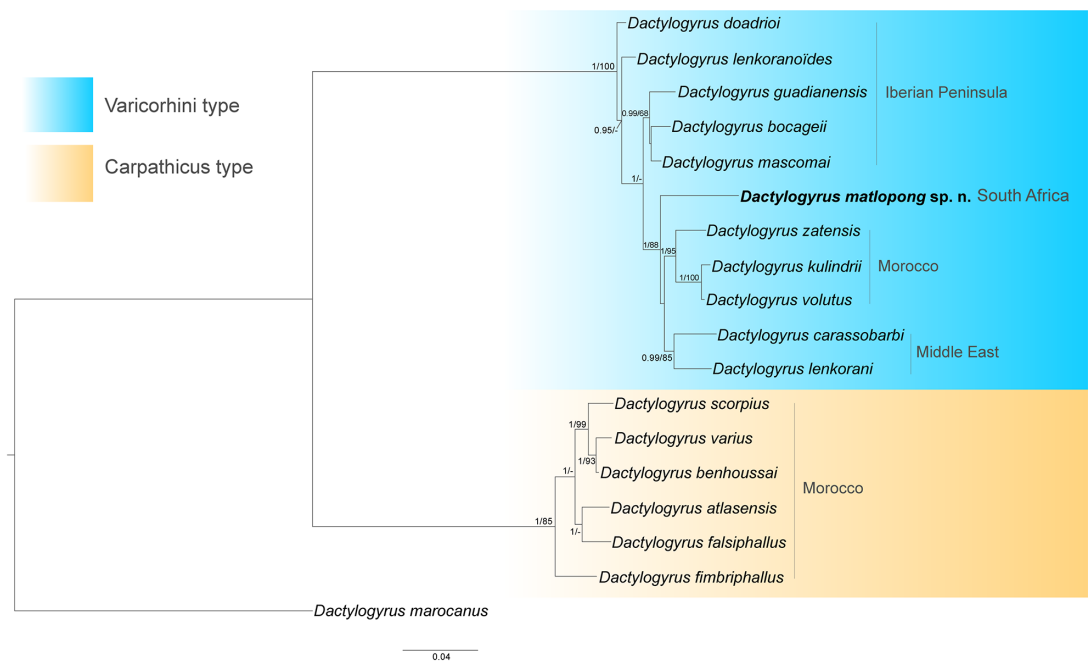


Fig 2. Maximum likelihood phylogram based on a concatenated analysis of sequences of the rRNA genes (partial 28S and partial 18) and entire ITS1 region. Newly sequenced taxon in bold. Posterior probability followed by bootstrap support values are given next to the branches (posterior probability < 0.90 and bootstrap < 60 not shown). *Dactylogyrus maroccanus* El Gharbi, Birgi et Lambert, 1994 was used as outgroup. Branch length scale bar indicates number of substitution per site.

Table 2. Nucleotide genetic divergence values among sequences of the partial 28S rRNA gene of *Dactylogyrus* spp. included in the phylogenetic analyses. The data are based on an 820 base pairs long alignment. Values below the diagonal are expressed in percentage (p-distance) while values above the diagonal represent number of differences in nucleotides. Newly sequenced taxon in bold.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 <i>D. doadrioi</i>		7	13	15	9	24	15	15	17	15	16	131	129	128	128	130	123	162
2 <i>D. lenkoranoides</i>	0.9		14	15	8	25	14	15	17	16	16	133	131	130	131	132	125	165
3 <i>D. guadianensis</i>	1.6	1.8		16	10	30	20	16	18	22	18	137	135	134	132	136	129	168
4 <i>D. bocageii</i>	1.9	1.9	2		9	32	22	18	18	21	21	134	132	131	131	133	126	161
5 <i>D. mascomai</i>	1.1	1	1.3	1.1		27	16	12	12	15	15	134	132	131	131	133	126	165
6 <i>Dactylogyrus matlopong</i> sp. n.	3	3.1	3.8	4	3.4		32	29	30	34	29	141	137	138	139	141	133	170
7 <i>D. zatensis</i>	1.9	1.8	2.5	2.8	2	4.1		17	16	18	19	139	137	136	137	137	131	164
8 <i>D. kulindrii</i>	1.9	1.9	2	2.3	1.5	3.7	2.1		3	23	19	141	139	138	137	138	130	166
9 <i>D. volutus</i>	2.1	2.1	2.3	2.3	1.5	3.8	2	0.4		22	20	140	138	137	137	137	129	167
10 <i>D. carassobarbi</i>	1.9	2	2.8	2.6	1.9	4.3	2.3	2.9	2.8		20	137	135	134	136	136	129	167
11 <i>D. lenkorani</i>	2.2	2.2	2.5	2.9	2.1	4	2.6	2.6	2.8	2.8		135	135	134	137	135	129	159
12 <i>D. scorpius</i>	16.8	17.1	17.6	17.2	17.2	18.1	17.9	18.2	18	17.6	19.1		12	9	15	16	22	161
13 <i>D. varius</i>	16.5	16.8	17.3	16.9	16.9	17.6	17.7	17.9	17.8	17.3	19.1	1.5		3	17	19	22	164
14 <i>D. benhoussai</i>	16.4	16.7	17.2	16.8	16.8	17.8	17.5	17.8	18	17.2	19	1.1	0.4		14	16	19	161
15 <i>D. atlasensis</i>	16.4	16.8	16.9	16.8	16.8	17.9	17.7	17.7	17.7	17.5	19.4	1.9	2.2	1.8		8	19	163
16 <i>D. falsiphallus</i>	16.6	16.9	17.4	17.1	17	18.1	17.7	17.8	17.7	17.5	19.1	2	2.4	2	1		21	163
17 <i>D. fimbriphallus</i>	15.9	16.1	16.6	16.3	16.2	17.2	16.9	16.8	16.6	16.7	18.4	2.8	2.8	2.4	2.4	2.7		158
18 <i>D. maroccanus</i>	21.5	21.5	21.9	21	21.5	22.2	21.5	21.8	21.9	21.8	22.9	20.9	21.3	20.9	21.2	21.2	20.7	

thors in its description (Justine J.-L., personal communication), and no other report of *D. oumiensis* were found in the literature. Therefore, a more thorough comparison of *D. oumiensis* with the new species was not possible.

The accessory piece of *D. matlopong* also resembles that of *Dactylogyrus gabonensis* Price et G  ry, 1968 [in Paperna (1979)] from *Barbus* sp. (*occidentalis* aff.) [*Labeobarbus bynni* (Forssk  l)], *Dactylogyrus doadrioi* El-Gharbi, Renaud et Lambert, 1993 from *Luciobarbus comizo* (Steindachner), and *Dactylogyrus lenkoranoides* El-Gharbi, Renaud et Lambert, 1993 from *Luciobarbus guiraonis* (Steindachner). The three species also present a subunit that is hook-shaped at its distal portion but with prolonged base almost reaching the base of the MCO, whereas the base of the hook-shaped subunit is shorter and rounded in the new species. Moreover, *D. matlopong* differs from the three species by the presence of a conspicuous medial projection on the anterior margin of the ventral bar. *Dactylogyrus lampam* Lim, 1992 from *Barbonymus schwanefeldii* (Bleeker) possesses a ventral bar similar to that of the new species, which is V-shaped with a conspicuous medial projection, but differs from the new species by the dorsal bar

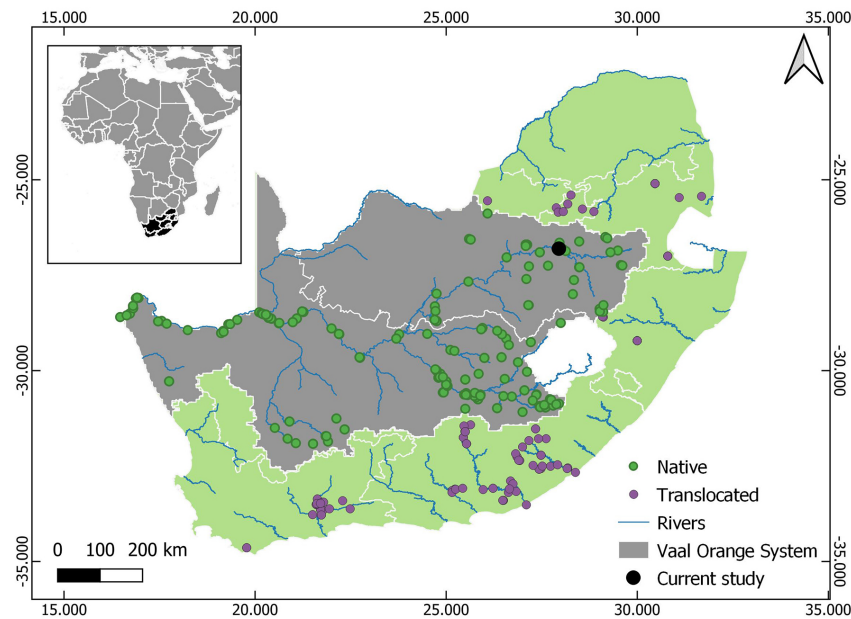


Fig. 3. Distribution of *Labeobarbus aeneus* (Burchell) in South Africa indicating the native distribution in the Vaal-Orange River System as well as the localities where it has been translocated. Sampling locality of the current study are also shown. Distribution data from GBIF (2021).

(groove absent on the posterior margin), accessory piece (J-shaped), MCO (composed of two complete rings), and vaginal tube (65–68 μm [67 μm] long *versus* 32–45 μm [39 μm] long in the new species) (see Lim and Furtado 1986). The presence of a conspicuous medial projection on the anterior margin of the ventral bar is unique to the new species among *Dactylogyrus* spp. of the varicorhini-type described from Africa. *D. matlopong* is the first species of the varicorhini-type described from South Africa.

Phylogenetic analyses

Both ML and BI analyses yielded similar tree topology (Fig. 2). The tree depicted two main well-supported clades: one well-supported clade grouped the species of the varicorhini-type, and another well-supported clade grouped the species of the carpathicus-type. The majority of the varicorhini-type species grouped according to their geographic region. For the species from the Iberian Peninsula, *D. lenkoranoides* and *D. doadrioi* appeared in a basal position to all the varicorhini-type species, while *Dactylogyrus mascomai* El Gharbi, Renaud et Lambert, 1993, *Dactylogyrus bocagei* Alvarez-Pellitero, Simon Vicente et Gonzales Lanza, 1981, and *Dactylogyrus guadianensis* El Gharbi, Renaud et Lambert, 1993 grouped together in a subclade.

Dactylogyrus matlopong sp. n. from South Africa appeared as an independent lineage. The species *Dactylogyrus zatensis* El-Gharb, Birgi et Lambert, 1994, *Dactylogyrus kulindri* El-Gharb, Birgi et Lambert, 1994, and *Dactylogyrus volutus* El-Gharb, Birgi et Lambert, 1994 from North Africa grouped together with strong support. Lastly, *Dactylogyrus carassobarbi* Gussev, Jalai et Molnár, 1993 and *Dactylogyrus lenkorani* Mikailov, 1967 from

the Middle East grouped together with strong support in a subclade. All carpathicus-type species are from Morocco.

The genetic distances were computed for the 28S gene (Table 2), showed as *p*-distance (%) and number of differences in nucleotides (nt). *D. matlopong* showed values of distance among its varicorhini-type African congeners (*D. zatensis*, *D. kulindri* and *D. volutus*) of 3.7–4.1% and 29–34 nt. Values among the new species and the other *Dactylogyrus* spp. used in the phylogenetic analyses are presented in Table 2.

DISCUSSION

This study is the first description of a *Dactylogyrus* species of the varicorhini-type from South Africa, as well as the first monogenean dactylogyrid to be fully described and named from *Labeobarbus aeneus*. This barbel has a natural distribution that includes the Vaal-Orange River system, but due to inter-basin transfers and their use as an angling species, *L. aeneus* has been translocated into the Gouritz, Great Fish, Kei and the Limpopo River systems (Fig. 3) (De Moor and Bruton 1988, Skelton 2001, de Villiers and Ellender 2007). There is evidence in the literature (de Villiers and Ellender 2007, Impson 2020) that *L. aeneus* and *Labeobarbus kimberleyensis* (Gilchrist et Thompson) are genetically indistinct, pointing to potential hybridisation due to anthropogenic impacts.

Since *Dactylogyrus* spp. from endemic fishes are likely to be strictly specific to a single host species (Benovics et al. 2020), *Dactylogyrus matlopong* sp. n. may be a specialist to *L. aeneus*. Hitherto, *Dactylogyrus varicorhini* and *Dactylogyrus* sp. from *L. kimberleyensis*, and *Dactylogyrus spinicirrus* from *Labeobarbus marequensis* (Smith) are the only species of the varicorhini-type recorded in South Africa (Price et al. 1969b, Mashego 1983, Crafford et al.

2014). These dactylogyrids could potentially be used as a biological tag to distinguish between these two closely related and often difficult to distinguish yellowfish species. However, more surveys of monogenean parasites from other populations of *L. aeneus* and *L. kimberleyensis* would be needed to validate this hypothesis. Such research would be of importance to the conservation of hosts and their associated parasitic fauna as *L. kimberleyensis* is currently listed as Near Threatened by the IUCN (Barkhuizen 2017). Furthermore, only 24 species of *Dactylogyrus* have been recorded from 12 of the ~68 species of *Labeobarbus* in Africa, emphasising the necessity of investigating the dactylogyrid communities of these hosts that are endemic to freshwater basins throughout the continent (Skelton 2001, Impson et al. 2008, Řehulková et al. 2018).

The present study provides the first sequences of the partial rRNA genes 28S and 18S, and ITS1 region of a species of *Dactylogyrus* from South Africa. It is very likely that increased sampling of other endemic South African species of *Labeobarbus*, such as *Labeobarbus polylepis* (Boulenger), *L. marequensis* and *Labeobarbus natalensis* (Castelnau), aiming at morphological and molecular

characterisation of their gill monogenean fauna, will result in records and descriptions of more *Dactylogyrus* spp. of the varicorhini-type. Such data could be included into the phylogenetic reconstruction of the genus and provide more insights into the biogeography and evolutionary history of their cyprinid hosts of the Torinae.

Acknowledgements. AAA was funded by a North-West University (NWU) postdoctoral fellowship. We are grateful to Mr. Fortunate Phaka (NWU) for valuable help with the construction of the *SeShoto* species name. We are also thankful for the comments from two anonymous reviewers that improved the manuscript. This work was in part supported by the South African Foundational Biodiversity Information Programme (FBIP) funded through the National Research Foundation (NRF) of South Africa (Grant number 138573). Opinions, findings, conclusions and recommendations expressed in this publication are that of the authors, and the funders accept no liability in this regard. This is contribution No. 705 from the NWU Water Research Group.

Author contributions statement. AAA and MT designed and performed the research and analysed data; WM and NJS performed research and analysed data; all authors contributed to the writing and final editing of the paper.

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Received 21 December 2021

Accepted 6 June 2022

Published online 4 October 2022

Cite this article as: Acosta A.A., Truter M., Malherbe W., Smit N.J. 2022: Morphological description and molecular characterisation of *Dactylogyrus matlopong* sp. n. (Monogenea: Dactylogyridae) from the South African endemic *Labeobarbus aeneus* (Cyprinidae: Torinae). *Folia Parasitol.* 69: 021.