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

Research Article

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

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Dactylogyrus Diesing, 1850 (Monogenea: Dactylogyridae) is currently the most species-rich genus in Platyhelminthes, with over 900 documented species (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 (anchors, 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...
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/). 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 *Paradiplodozon ichthyoxanthon* 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 allocoreadiid digenean *Allocreadium apokryfi* Dos Santos, Gilbert, Avenant-Oldewage et Dumbo, 2021 from the intestine. A few records for the invasive Asian fish tapeworm *Schyzocotyle aehilognathi* (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 non-thermal vertebrates (SOP No: NWU-00267-17-S5). Subsequent to euthanasia, *L. aeneus* was euthanised using 3.8 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’-GCCACCGCTGAAYTTAG-3’) and the reverse primer L1642 (5’-CCAGCGCCATCCATTITCA-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’-ATTCGGATAACGAGAGACT-3’) (Sinnapah et al. 2001) and the reverse primer IR8 (5’-GCTAGCCTGCGTCTTCTCATCGA-3’) (Simková 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 relationships 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 Řehulková et al. (2021). Default parameters of Muscle implemented in Geneious 7.1.3 were used to generate the alignments, which had
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 marocanus* El Gharbi, Birgi et Lambert, 1994 was used as outgroup for the three alignments, based on the phylogeny of Šimková 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 10^7 generations and sampling tree topologies every 10^4 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

#### Dactylogryidea Bychowsky, 1937

#### Dactylogryidae Bychowsky, 1933

#### Dactylogyrus Diesing, 1850

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

*ZooBank number for species: urn:lsid:zoobank.org:act:41A8930F-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

| Species                  | Host                | Subfamily | Locality | 18S + ITS1 | 28S | Reference              |
|-------------------------|---------------------|-----------|----------|-----------|-----|------------------------|
| *Dactylogyrus atlasensis* | Luciobarbus pallariyi | Barbinae  | Morocco  | KY629337  | KX629356 | Šimková et al. (2017)  |
| *Dactylogyrus benhouassa* | Luciobarbus yahayouni | Barbinae  | Morocco  | KX578025  | KX553862 | Rahmouni et al. (2017) |
| *Dactylogyrus bocagei*   | Luciobarbus graellii | Barbinae  | Spain    | MN365675  | MN338210 | Benovics et al. (2020) |
| *Dactylogyrus carassaobarbi* | Carasobarbus lateus | Torinae   | Torinae  | MZ031071  | MZ031060 | Benovics et al. (2021) |
| *Dactylogyrus doadoroi*  | Luciobarbus guirousis | Barbinae  | Spain    | MN365682  | KX629346 | Benovics et al. (2020) |
| *Dactylogyrus falsiphallus* | Luciobarbus magharebsis | Barbinae  | Morocco  | KX578024  | KX553861 | Šimková et al. (2017)  |
| *Dactylogyrus fimbriiphallus* | Luciobarbus pallariyi | Barbinae  | Morocco  | KX629332  | KX629357 | Šimková et al. (2017)  |
| *Dactylogyrus guadianensis* | Luciobarbus comizo | Barbinae  | Spain    | MN365674  | MN338209 | Benovics et al. (2020) |
| *Dactylogyrus kulindrini* | Carasobarbus fritschii | Torinae   | Torinae  | KY629336  | KX629354 | Šimková et al. (2017)  |
| *Dactylogyrus lenkornani* | Capoeta buhsei | Barbinae  | Barbinae  | MZ031069  | MZ031057 | Benovics et al. (2021) |
| *Dactylogyrus lenkornanoides* | Luciobarbus graellii | Barbinae  | Spain    | MN365676  | MN338210 | Benovics et al. (2020) |
| *Dactylogyrus marocanus*  | Carasobarbus fritschii | Torinae   | Torinae  | KY629333  | KX629355 | Šimková et al. (2017)  |
| *Dactylogyrus mascomai*   | Luciobarbus graellii | Barbinae  | Spain    | MN365680  | MN338215 | Benovics et al. (2020) |
| *Dactylogyrus scorpius*   | Luciobarbus rifissis | Barbinae  | Morocco  | KX578023  | KX553860 | Rahmouni et al. (2017) |
| *Dactylogyrus varius*     | Luciobarbus magharebsis | Barbinae  | Morocco  | KX578026  | KX553863 | Rahmouni et al. (2017) |
| *Dactylogyrus volutus*    | Carasobarbus fritschii | Torinae   | Morocco  | KY629353  | KY629334 | Šimková et al. (2017)  |
| *Dactylogyrus zatensis*   | Carasobarbus fritschii | Torinae   | Torinae  | KY629335  | KY629352 | Šimková et al. (2017)  |

*Dactylogyrus matlopong sp. n.* *Labeobarbus aeneus* | Torinae | South Africa | ON391042 | ON391043 | Present study |
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 18S) 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 marocanus 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 | D. doadrioi | 7  | 13 | 15 | 9  | 24 | 15 | 15 | 17 | 15 | 16 | 131| 129| 128| 128| 130| 123| 162|
| 2 | D. lenkoranoides | 0.9| 14 | 18 | 25 | 14 | 15 | 17 | 16 | 16 | 133| 131| 130| 131| 132| 125| 165|
| 3 | D. guadianensis | 1.6| 1.8| 10 | 20 | 16 | 18 | 22 | 18 | 137| 135| 134| 132| 136| 129| 168|
| 4 | D. bocageii | 1.9| 1.9| 2  | 9  | 32 | 22 | 18 | 18 | 21 | 21 | 134| 132| 131| 133| 126| 161|
| 5 | D. mascomai | 1.1| 1  | 1.1| 27 | 16 | 12 | 12 | 15 | 15 | 134| 132| 131| 133| 126| 165|
| 6 | Dactylogyrus matlopong sp. n. | 3  | 3.1| 3.8| 4  | 3.4| 32 | 29 | 30 | 34 | 29 | 141| 137| 138| 139| 141| 133| 170|
| 7 | D. zatensis | 1.9| 1.8| 2.5| 2.8| 2  | 4.1| 17 | 16 | 18 | 19 | 139| 137| 136| 137| 131| 164|
| 8 | D. kulindrii | 1.9| 1.9| 2  | 2.3| 1.5| 3.7| 2.1| 3  | 23 | 19 | 141| 139| 138| 137| 138| 130| 166|
| 9 | D. volutus | 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 | D. carassobarbi | 1.9| 2  | 2.8| 1.9 | 4.3| 2.3| 2.9| 2.8| 20 | 137| 135| 134| 136| 136| 129| 167|
| 11 | D. lenkorani | 2.2| 2.2| 2.5| 2.9| 2.1| 4  | 2.6| 2.6| 2.8 | 28 | 135| 135| 134| 137| 135| 129| 159|
| 12 | D. scorpina | 16.8| 17.1| 17.6| 17.2| 17.4| 19.1| 18.2 | 18 | 17.6| 19.1 | 12 | 9  | 15 | 16 | 22 | 161|
| 13 | D. varius | 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 | D. benhoussai | 16.4| 16.7| 17.2| 16.8| 16.8| 17.8| 17.5| 17.8| 18 | 17.2| 19 | 11 | 0.4| 14 | 16 | 19 | 161|
| 15 | D. atlantis | 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 | D. falsiphallus | 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 | 2  | 1  | 21 | 163|
| 17 | D. fimbriphallus | 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 | D. marocanus | 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 was found in the literature. Therefore, a more thorough comparison of D. oumiensis with the new species was not possible.

Dactylogyrus matlopong also resembles that of Dactylogyrus gabonensis Price et Géry, 1968 [in Paperna (1979)] from Barbus sp. (occidentalis aff.) [Labeobarbus bynni (Forsskål)], 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 lampan Lim, 1992 from Barbonymus schwanefeldii (Bleecker) 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
(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 varichorini-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 bocageii 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-Gharbi, Birgi et Lambert, 1994, Dactylogyrus kulindri El-Gharbi, Birgi et Lambert, 1994, and Dactylogyrus volutus El-Gharbi, 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. kinibereyensis 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. kinibereyensis 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, Rehulková 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.

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