

Article

Integrated Description of *Paradiplozoon gladysae* n. sp. (Polyopisthocotyla: Diplozoidae), Parasitising the Gills of *Labeo victorianus* (Teleostei: Cyprinidae) in the River Nyando, Lake Victoria Basin, Kenya [†]

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Abstract

Polyopisthocotylans of the Diplozoidae are hematophagous gill parasites of freshwater cyprinoids; however, their diversity in East African river systems remains poorly characterised. In this study, we describe *Paradiplozoon gladysae* n. sp., a novel diplozoid recovered from the gills of an endemic cyprinid *Labeo victorianus* Boulenger in the River Nyando, a tributary of Lake Victoria in western Kenya. An integrated approach combining light and scanning electron microscopy and molecular characterisation of ITS2 rDNA was used to examine the collected species. In general, *P. gladysae* n. sp. is distinguished from congeners by its small clamps and the unique morphology of its median plate. Molecular analysis of the ITS2 region of rDNA confirmed the genetic distinctiveness of the new species and placed the species within the highly supported clade of African species formed by *P. koubkova* Řehulková, Nejat, Benovics, 2023; *P. ichthyoxanthos* Avenant-Oldewage, van Vuren, Mashego, 2013; and *P. vaalense* Dos Santos, van Vuren, Avenant-Oldewage, 2013, with *P. vaalense* being the closest sister taxon to the newly determined species. However, the analysis revealed a clear distinction, with a 39 bp difference and an uncorrected genetic *p*-distance of 6.8% between *P. gladysae* n. sp. and *P. vaalense*. The discovery of *P. gladysae* n. sp. adds to the growing diversity of diplozoid polyopisthocotylans in East Africa and highlights the River Nyando as an important but understudied hotspot for freshwater parasite biodiversity.



Academic Editors: Michael Wink and Simon Blanchet

Received: 16 July 2026

Revised: 7 August 2026

Accepted: 12 August 2026

Published: 19 October 2026

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Keywords: diplozoid; ITS2 rDNA; Ningu; *Paradiplozoon*; Monogenea

1. Introduction

Diplozoids (Polyopisthocotyla: Diplozoidae) are highly specialised blood-feeding ectoparasites of freshwater cyprinid fishes and are characterised by the permanent fusion of two larvae into a single functional adult [1,2]. Despite their remarkable biology and importance as host-specific parasites, their diversity remains poorly documented in many regions of Africa.

Globally, over 70 diplozoid species have been reported [3]. Diplozoidae comprises two subfamilies: Neodiplozoinae Khotenovsky, 1980 and Diplozoinae Palombi, 1949 [2]. The

Neodiplozoinae includes two genera: *Neodiplozoon* Tripathi, 1960 and *Afrodiplozoon* Khotenovsky, 1981 [2]. Five genera are known from Diplozoinae: *Diplozoon* von Nordmann, 1832; *Paradiplozoon* Akhmerov, 1974; *Inustiatius* Khotenovsky, 1978; *Sindiplozoon* Khotenovsky, 1981; and *Eudiplozoon* Khotenovsky, 1984. Of these five genera, *Paradiplozoon* is regarded as the most diverse, with records from Africa, Asia, and Europe [3–6], and continues to expand, with an additional three species described in 2026 [7–9]. To date, seven species have been recorded from various hosts and localities across the African continent [9,10]. *Paradiplozoon aegyptense* (Fischthal et Kuntz, 1963) was originally described from *Barbus macrolepidotus* (Pellegrin) in Uganda, and since then, the species has been reported from several African countries (Egypt, Ghana, Kenya, and Tanzania) and from hosts of various genera (*Enteromius* Cope, *Labeo* Cuvier, and *Raiamas* Jordan) [11–14]. *Paradiplozoon ichthyoxanthos* Avenant-Oldewage, 2013; *Paradiplozoon krugerense* Dos Santos et Avenant-Oldewage, 2016; *Paradiplozoon vaalense* Dos Santos, Jansen van Vuuren, Avenant-Oldewage, 2015; and *Paradiplozoon jordaanae* Truter, Přikrylová, Smit, 2026 are four species described and recorded from cyprinid hosts of *Labeo* and *Labeobarbus* Rüppell, *Cheilobarbus* Smit, and *Sederocypris* Skelton, Swartz, et Vreven in South Africa [9,10,15–17]. The seventh African species, *Paradiplozoon moroccoensis* Koubková, Benovics et Šimková, 2021, from *Luciobarbus pallaryi* (Pellegrin), represents the North African species [5]. *Paradiplozoon aegyptense* is the only African species reported outside the continent (in India) on *Cyprinus* L., *Schizopyge* Heckel, *Schizothorax* Heckel, and *Carassius* Jarocki [18–21]; however, these records are considered questionable [10].

To date, the ITS2 rDNA region remains the most frequently used molecular marker in the systematics and phylogenetics of *Paradiplozoon* species [3,5,6] since the first publication of the specific protocol for diplozoid species [22]. Sequences from other regions, such as *cox1* mtDNA, 18S, and 28S rDNA, have been generated, but the availability of the data for these species remains limited [23–25]. Due to this limitation, a robust and conclusive phylogeny of this group has not been produced; however, consistent results across several studies suggest that *Paradiplozoon* is paraphyletic [3,5,23,25]. Nevertheless, other molecular markers have potential for studying parasites of the Diplozoidae and, when combined, can provide better insight [24,25].

The Lake Victoria Basin supports a rich and partially endemic cyprinid fauna [26], yet its parasite biodiversity remains poorly characterised. This knowledge gap is particularly evident for diplozoids, despite the ecological and fisheries importance of endemic species such as *Labeo victorianus* [27].

In this study, we describe *Paradiplozoon gladysae* n. sp. from the gills of *Labeo victorianus* (Cypriniformes, Cyprinidae) collected in the River Nyando, Lake Victoria Basin, Kenya, using an integrative taxonomic approach combining detailed morphology, scanning electron microscopy, and ITS2 rDNA analyses. Accurate documentation of parasite diversity provides the foundation for understanding host evolution, parasite biogeography, and freshwater ecosystem health, particularly in regions undergoing rapid environmental change.

2. Materials and Methods

2.1. Ethical Approval

Fish collection and laboratory procedures complied with the ethical guidelines of the Kenya Marine and Fisheries Research Institute (KMFRI) and Kisii University. The ethics approval was obtained from the Kisii Teaching and Referral Hospital Institutional Science and Ethical Review Committee (KTRH ISERC), approval reference ISERC/KTRH/025/23 (date 25 January 2024).

2.2. Study Area, Fish Sampling, Parasite Collection, and Fixation

The study was conducted in the River Nyando, located in Kisumu County, western Kenya (see Figure 1 of Rindoria et al. [28]). This river is one of the major tributaries of Lake Victoria, originating from the Nandi Escarpment and flowing through diverse ecological zones before emptying into the Gulf of Winam.

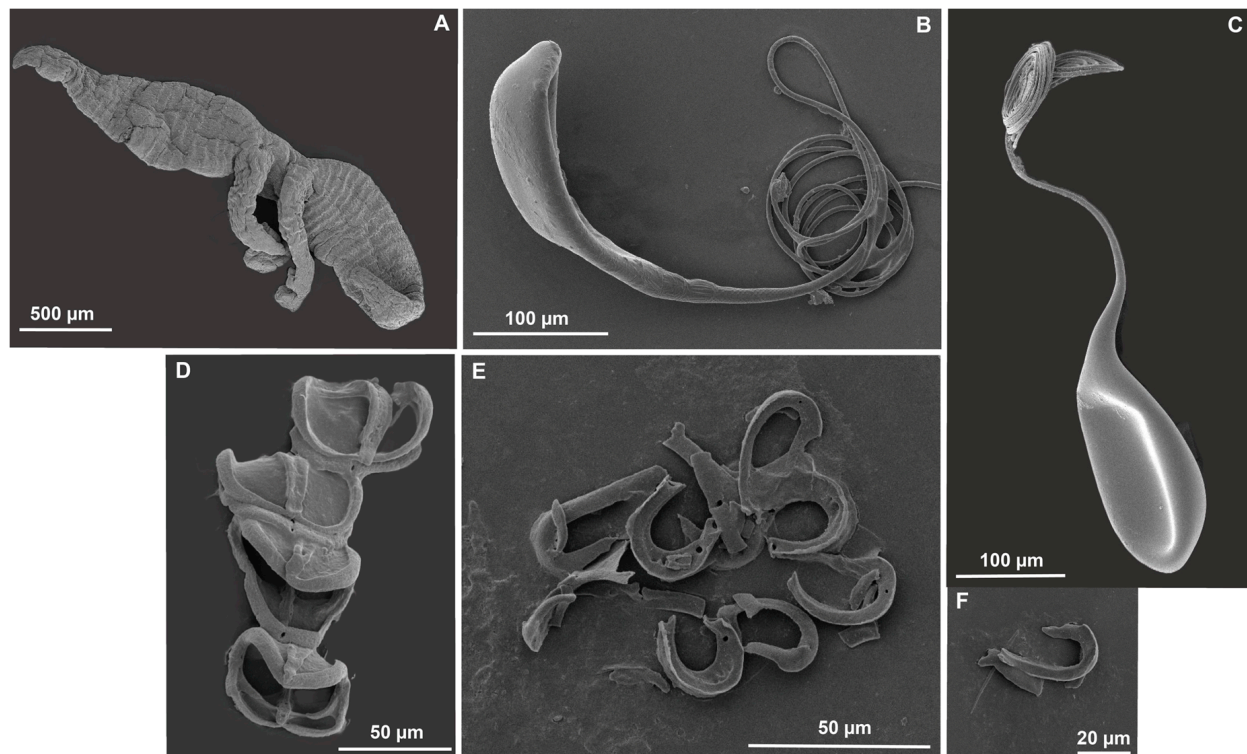


Figure 1. Scanning electron microscopy of *Paradipllozoon gladysae* n. sp. 9: (A) total-body view, (B,C) egg, (D) row of clamps, (E,F) details of the digested sclerites of the attachment clamp.

Sampling was conducted at selected sites to represent the known distribution of *L. victorianus* within the middle and lower reaches of the River Nyando between March and June 2023. These sites were chosen based on known occurrences of *L. victorianus* and accessibility for repeated collection. A total of 47 specimens of *L. victorianus* (total length = 9.5–42.8 cm, body mass = 8.2–286.6 g) were collected by electrofishing (SAMUS 1000, Samus Special Electronics, RX 28371, Shanghai, China). The fish were temporarily kept in aerated plastic tanks, awaiting parasitological examination.

The fish were euthanised by severing the spinal cord, and the gills were excised and placed in Petri dishes containing water for *Paradipllozoon* screening under a stereomicroscope. The recovered parasites were carefully removed using fine needles under a stereo microscope (Leica, Leica Microsystems, Wetzlar, Germany). Some of the worms were cut into anterior (preserved in 96% ethanol for DNA extraction) and posterior parts [mounted on glass slides and fixed with a mixture of glycerin and ammonium picrate (GAP)] for light microscopy (Olympus BX50, Olympus, Tokyo, Japan). The full bodies of some parasites were flattened, stained with acetocarmine, and mounted in Canada balsam on glass slides for morphometric analysis, while others were preserved in 70% ethanol for ultrastructural study by SEM using VEGA3 Tescan (Tescan, Brno, Czechia). Type and voucher specimens, as well as molecular voucher (=hologenophore [29]), were deposited in the parasitological collection in the National Museum, Bloemfontein, South Africa (NMB) and in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, in České Budějovice (IPCAS).

2.3. Light and Scanning Electron Microscopy

An Olympus BX50 compound microscope (Olympus Optical Co. Ltd., Tokyo, Japan) fitted with a digital camera and a drawing attachment was used to view, photograph, and draw the sclerotised structures of the specimens. The illustrations were digitised using Adobe Illustrator© software, version 30.0 (Adobe Inc., San Jose, CA, USA) and a Wacom Intuos Pro drawing tablet (Wacom, Saitama, Japan), following the procedures of Truter et al. [30]. Body measurements followed Khotenovsky [2], whereas clamp and central hook measurements followed Nejat et al. [6]. The metrical measurements were obtained using a digital image analysis system (Motion Stream version 1.9, Olympus, Tokyo, Japan). All measurements are given in micrometres and presented as ranges, with the mean and number of measurements in parentheses. The type material of *P. ghanense* (paratype USNM 1367213) and *P. egyptense* (paratypes 1355450 and 1363700) were examined on loan from the Smithsonian National Museum of Natural History (SNMNH), Washington, DC, USA.

Specimens fixed in 70% ethanol were dehydrated in an ascending series of ethanol concentrations, following the procedures of Rindoria et al. [31], and Bis(trimethylsilyl)amine, as described by Nation [32] and Dos Santos and Avenant-Oldewage [17]. They were then dried in a glass desiccator at room temperature for 6 h and sputter-coated with gold in a Denton Vacuum Desk V sputter coater (Quorum Technologies, Newhaven, UK). Specimens were analysed using a Zeiss Sigma 500VP scanning electron microscope (Jena, Germany) at 6 kV at the University of Limpopo, South Africa.

2.4. DNA Extraction, Amplification, Sequencing, and Phylogenetic Analyses

Three specimens were selected for molecular analysis. Genomic DNA was extracted from individuals with 96% ethanol-preserved polyopisthocotylian using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The ITS2 region of rDNA was amplified using the universal eukaryotic primers D (5'-GGCTYRYGGNGTCGATGAAGAACGCAG-3') and B1 (5'-GCCGGATCCGAATCCTGGT TAGTTTCTTTTCCT-3') [33], targeting ~700 bp, and following the thermal cycling profile of Matějusková et al. [22], namely 95 °C for 10 min; 30 × (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 75 s); and 72 °C for 10 min. The 28S rDNA region was amplified using primers C1 (5'-ACCCGCTGAATTTAAGCA-3') and D2 (5'-TGGTCCGTGTTTCAAGAC-3') [34], targeting ~800 bp, under the thermal cycling profile 94 °C for 2 min; 39 × (94 °C for 20 s, 56 °C for 30 s, and 72 °C for 90 s); and 72 °C for 10 min [25]. The mt *cox1* region was amplified with the use of primers ASmit1 (5'-TTTTTGGGCATCCTGAGGTTTAT-3') and ASmit2 (5'-TAAAGAAAGAACATAATGAAAATG-3') [35], targeting ~500 bp, under the thermal cycling profile 95 °C for 5 min; 39 × (94 °C for 60 s, 50 °C for 60 s, and 72 °C for 60 s); and 72 °C for 7 min [35].

PCR reactions were carried out in 25 µL volumes containing 12.5 µL of DreamTaq Master Mix (Thermo Scientific, Waltham, MA, USA), 0.5 µM of each primer, and ~50 ng of template DNA. PCR products were visualised on a 1% agarose gel using GelRed® (Millipore Sigma, Burlington, MA, USA), and positive PCR amplicons were sent to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa, for purification and sequencing. Contiguous sequences were assembled using Geneious v. 7.1.3 [36]. After generating new sequences, searches were conducted against NCBI's GenBank database to assess sequence similarity to existing *Paradiplozoon* species. Species included in the phylogenetic analysis were those identified during the BLAST (accessed on 22 June 2026) search and following Nejat et al. [6], with *Octomacrum europaeum* Roman & Bychowsky, 1956 (MT441500.1) selected as an outgroup. The full list of species included in the phylogenetic analysis is presented in Table 1. The sequences were aligned using MAFFT with the Fast Fourier Transform algorithm [37], and the L-INS-I approach was applied. The initial alignment

exported from MAFFT was manually adjusted to unify lengths, and the 653 bp-long output was subjected to automated trimming using trimAl v.1.2 [38]. ModelFinder [39] in IQ-TREE v2.2. was used to identify the optimal phylogenetic model based on the Akaike Information Criterion (AIC; [40]). The GTR+F+I+G4 model was used to compute ultrafast Maximum Likelihood (ML) [41] in IQ-TREE v2.2 [42]. The Bayesian Inference (BI) tree was generated in MrBayes v.3.2 [43,44]. Posterior probabilities were calculated for 3×10^6 generations, using the Metropolis-coupled Markov chain Monte Carlo algorithm (MCMC) sampling trees every 10^2 generations. The first 30% of sampled trees were discarded as burn-in after confirming convergence (standard deviation of split frequencies < 0.01). The number of base-pair differences and uncorrected p -distances between the sequences in the alignment were calculated using MEGA X [45]. The resulting trees were visualised in FigTree v.1.3 (<http://tree.bio.ed.ac.uk/software/figtree> accessed on 22 June 2026), and the final tree combining BI and ML results was edited using Adobe Illustrator. Conversion of alignment files was carried out using ALTER v.1.2 [46].

Table 1. List of diplozoid species used for phylogenetic comparison with *Paradiplozoon gladysae* n. sp., in ITS2 sequences, NCBI GenBank accession numbers, host species, collection localities/countries, and references.

Species	Host	Family	Country	Accession No.	Reference
<i>Afrodiplozoon polycotyleus</i>	<i>Labeobarbus marequensis</i>	Cyprinidae	South Africa	LT719088	[47]
<i>Diplozoon kashmirensis</i>	n/a		India	MF460994	unpublished
<i>Diplozoon paradoxum</i>	<i>Abramis brama</i>	Leuciscidae	France	AF369759	[48]
<i>Eudiplozoon kamegaii</i>	<i>Cyprinus carpio</i>	Cyprinidae	Japan	LC517172	[49]
<i>Eudiplozoon nipponicum</i>	<i>Cyprinus carpio</i>	Cyprinidae	France	AF369758	[48]
<i>Inustiatius aristichthysi</i>	<i>Aristichthys nobilis</i>	Xenocyprinididae	China	DQ098894	[50]
<i>Inustiatius inustiatius</i>	<i>Hypophthalmichthys molitrix</i>	Xenocyprinididae	China	DQ098893	[50]
<i>Octomacrum europeum</i>	<i>Alburnoides bipunctatus</i>	Leuciscidae	Poland	MT441500	[5]
<i>Paradiplozoon gladysae</i> n. sp.	<i>Labeo victorianus</i>	Cyprinidae	Kenya	PZ785758	present study
<i>Paradiplozoon barbi</i>	n/a		Malaysia	MN688771	unpublished
<i>Paradiplozoon bingolensis</i>	<i>Cyprinus macrostomum</i>	Cyprinidae	Iraq	OP588754	[6]
<i>Paradiplozoon bliccae</i>	<i>Vimba vimba</i>	Leuciscidae	Turkey	OP588762	[6]
<i>Paradiplozoon cirrhini</i>	<i>Cirrhinus molitorella</i>	Cyprinidae	China	OQ429337	[3]
<i>Paradiplozoon helleni</i>	<i>Tropidophoxinellus hellenicus</i>	Leuciscidae	Greece	MT417730	[5]
<i>Paradiplozoon hemiculteri</i>	<i>Hemiculter leuciscus</i>	Xenocyprinididae	China	KY124654	[51]
<i>Paradiplozoon homoion</i>	<i>Rhodeus amarus</i>	Acheilognathidae	Turkey	MT028131	[52]
<i>Paradiplozoon ibericus</i>	<i>Iberochondrostoma lusitanicum</i>	Leuciscidae	Portugal	MT417727	[5]
<i>Paradiplozoon ichthyoxanthos</i>	<i>Labeobarbus aenus</i>	Cyprinidae	South Africa	HF566124	[15]
<i>Paradiplozoon jalalii</i>	<i>Schizocypris altidorsalis</i>	Cyprinidae	Iran	PP356701	[24]
<i>Paradiplozoon jordaanae</i>	<i>Cheilobarbus serra</i>	Cyprinidae	South Africa	PZ317027	[9]
<i>Paradiplozoon koubkovae</i>	<i>Luciobarbus capito</i>	Cyprinidae	Iran	OP588797	[6]
<i>Paradiplozoon krugerense</i>	<i>Labeo rosae</i>	Cyprinidae	South Africa	LT574865	[17]
<i>Paradiplozoon megan</i>	<i>Leuciscus cephalus</i>	Leuciscidae	Czech Republic	AJ300711	[22]
<i>Paradiplozoon moroccoensis</i>	<i>Barbus lepineyi</i>	Cyprinidae	Morocco	MT417735	[5]
<i>Paradiplozoon nagibinae</i>	<i>Abramis ballerus</i>	Leuciscidae	Czech Republic	AJ563371	[53]
<i>Paradiplozoon opsariichthydis</i>	<i>Opsariichthys bidens</i>	Xenocyprinididae	China	PQ869413	[25]
<i>Paradiplozoon pavlovskii</i>	<i>Leuciscus aspius</i>	Leuciscidae	Czech Republic	AJ300714	[22]
<i>Paradiplozoon sapae</i>	<i>Bellerus sapa</i>	Leuciscidae	Czech Republic	AJ300713	[22]
<i>Paradiplozoon skrabini</i>	<i>Leuciscus leuciscus baicalensis</i>	Leuciscidae	China	KP340974	unpublished
<i>Paradiplozoon vaalense</i>	<i>Labeo umbratus</i>	Cyprinidae	South Africa	HG423142	[16]
<i>Paradiplozoon yarkandense</i>	<i>Schizothorax eurystomus</i>	Cyprinidae	China	MN892639	[54]
<i>Paradiplozoon yunnanensis</i>	<i>Sikukia gudgeri</i>	Cyprinidae	China	MW048388	[55]
<i>Syndiplozoon coreius</i>	<i>Coreius guichenoti</i>	Gobionidae	China	MW992745	[56]
<i>Syndiplozoon ctenopharyngodon</i>	<i>Ctenopharyngodon idella</i>	Xenocyprinididae	China	DQ098898	[49]

3. Results

Six pairs of adult specimens of *Paradiplozoon* were recovered from the gill filaments of four of the 47 examined *Labeo victorianus*. Morphological and molecular analyses demonstrated that these specimens represent a previously undescribed species, herein described as *Paradiplozoon gladysae* n. sp.

Paradiplozoon gladysae n. sp. (Figures 1–3).

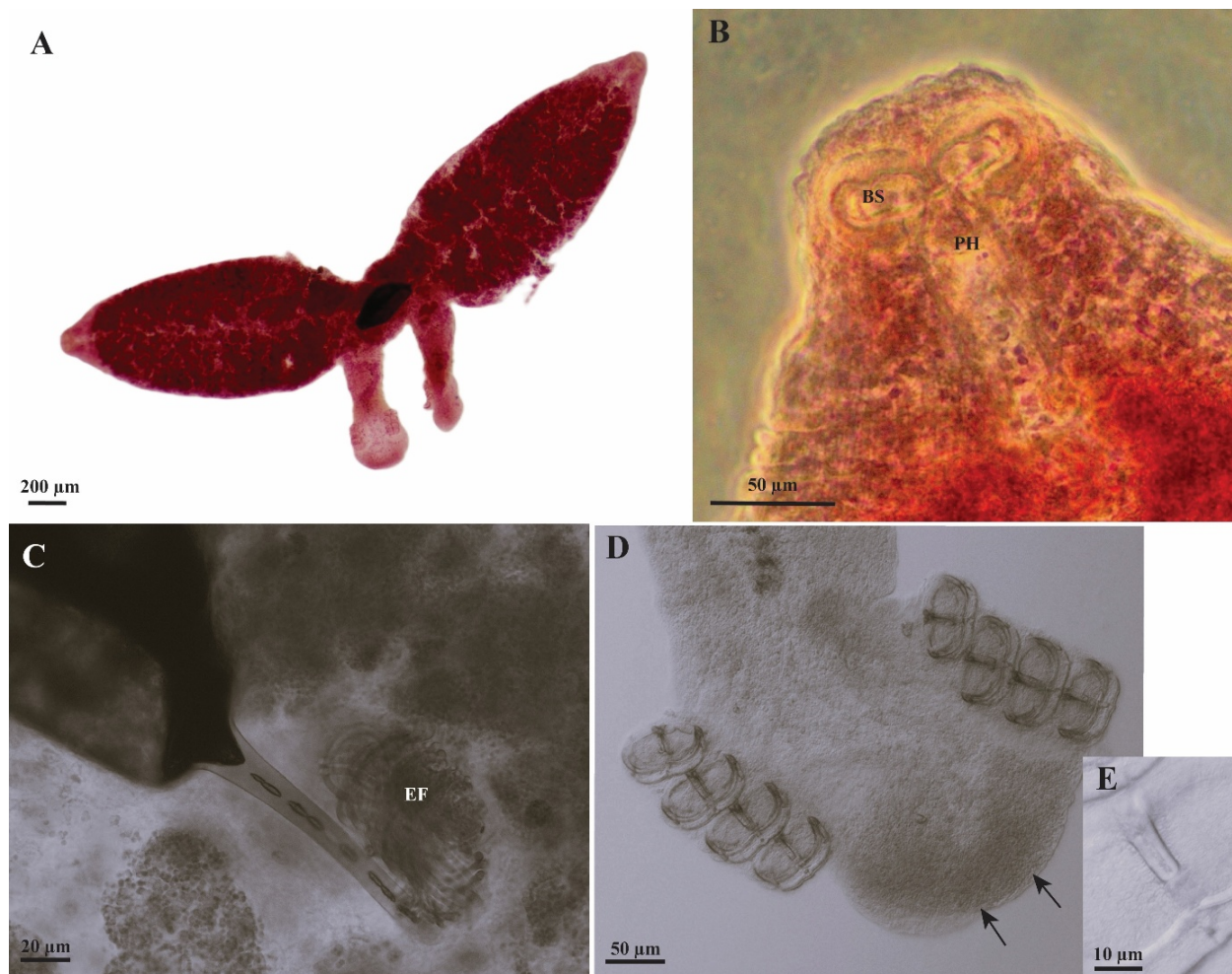


Figure 2. Light-microscope micrographs of *Paradiplazoon gladysae* n. sp.: (A) total body; (B) detail of the distal part of the hind body; (C) egg; (D) haptor with attachment clamps (ventral view), arrows indicating posterior protrusion; (E) anterior end of the median plate. BS = buccal sucker, PH = Pharynx, EF = egg filament.

Type host: *Labeo victorianus* Boulenger, 1901.

Type locality: Nyando River, Lake Victoria Basin, Kisumu County, Kenya (0°9'55.2" S, 34°55'10.8" E).

Site of infection: Gills.

Type material: The Holotype, one paratype, and one hologenophore (IPCAS M-851), one paratype (NMB P 1342).

Genetic material: The unique 719 bp ITS2 rDNA sequence was deposited in GenBank (Accession number PZ785758).

Etymology: This species is named in memory of Gladys Rindoria, the deceased wife of the principal author.

ZooBank registration: The Life Science Identifier (LSID) for this article is urn:lsid:zoobank.org:pub:D802A2A3-7A5F-41C4-B637-FC382A348D44. The LSID for the new name *Paradiplazoon gladysae* n. sp. Rindoria, Luus-Powell et Přikrylová is urn:lsid:zoobank.org:act:7855BD23-26CD-40B3-94DE-2854A2C8E661.

Description: Description based on the four flattened adult pair specimens in GAP and three pair specimens studied by SEM. Specimens of X-shaped bodies, formed by fusion of two mature individuals. Forebody 1678 (1475–1870, $n = 5$) and hind body 857 (683–1039, $n = 6$) long. Tegument with fine transverse folds; present evenly on whole body (Figure 1A). Forebody of narrow leaf shape, replete with vitteline follicles and diges-

tive system (Figure 2A). Mouth positioned subterminally on ventral side. Two muscular buccal suckers, located bilaterally, 46.5×56 ($42.5\text{--}52 \times 50.5\text{--}68$, $n = 6$), pharynx follows buccal cavity, 44 ($n = 1$) long, 36 ($n = 1$) wide, and continues into intestine (Figure 1B). Reproductive organs fill hind body; one egg with long filament reported in body crossing region, 323 ($n = 1$) long and 157 ($n = 1$) wide (Figures 1B,C and 2A,C). Haptors, 310 (275–334, $n = 3$) wide, equipped with two longitudinal rows of four clamps, positioned ventro-laterally (Figures 1D and 2D), and one pair of central hooks (Figure 3). Haptors with developed posterior protrusion (Figure 2D, area pointed with arrows). Size of clamps (based on measurements of three adult specimens/six haptors; width $\times$ length): clamp I = 67 (57–74) $\times$ 43 (36–47); clamp II = 72 (62–84) $\times$ 42 (38–48); clamp III = 70 (60–75) $\times$ 39 (34–45); clamp IV = 68 (56–77) $\times$ 40 (33–44). Clamp sclerites arrangement typical of *Paradiplozoon* species. U-shape median sclerite; anterior half of median plate of uniform width with median groove; anterior end of rectangular shape, no trapezoid spur present (Figures 2E and 3). Median plate anterior end connects to anterior clamp jaw by two anterior joining sclerites (Figures 1 and 3). Two-thirds of posterior half of median plate of more robust appearance compared to anterior half; in distal section, posterior half narrows and continues into wide rounded termination. Posterior joining sclerite connects posterior end of median plate and two arches forming posterior clamp jaw. Posterior clamp jaw comprises two smooth massive arches that connect via two lateral sclerites of slender appearance to anterior jaw. Anterior jaw composed of two slightly robust arches, each has groove perforation medially, in approx. 70% of their length; proximal part turning ventromedially, pointing towards anterior end of median plate. Distal section of anterior jaw sclerite terminating with wind-shaped widening. Pair of central hooks positioned in central haptor area at level of first clamp. Slender straight handle 43 (42–45, $n = 3$) long. Svelte hook, that widens in one-third of its length; hook length 18.5 (18–19, $n = 2$). Hook curved, with a pointed tip, directing slightly upward, point length (from mid of the turn till point tip) 6 (6–7, $n = 2$).

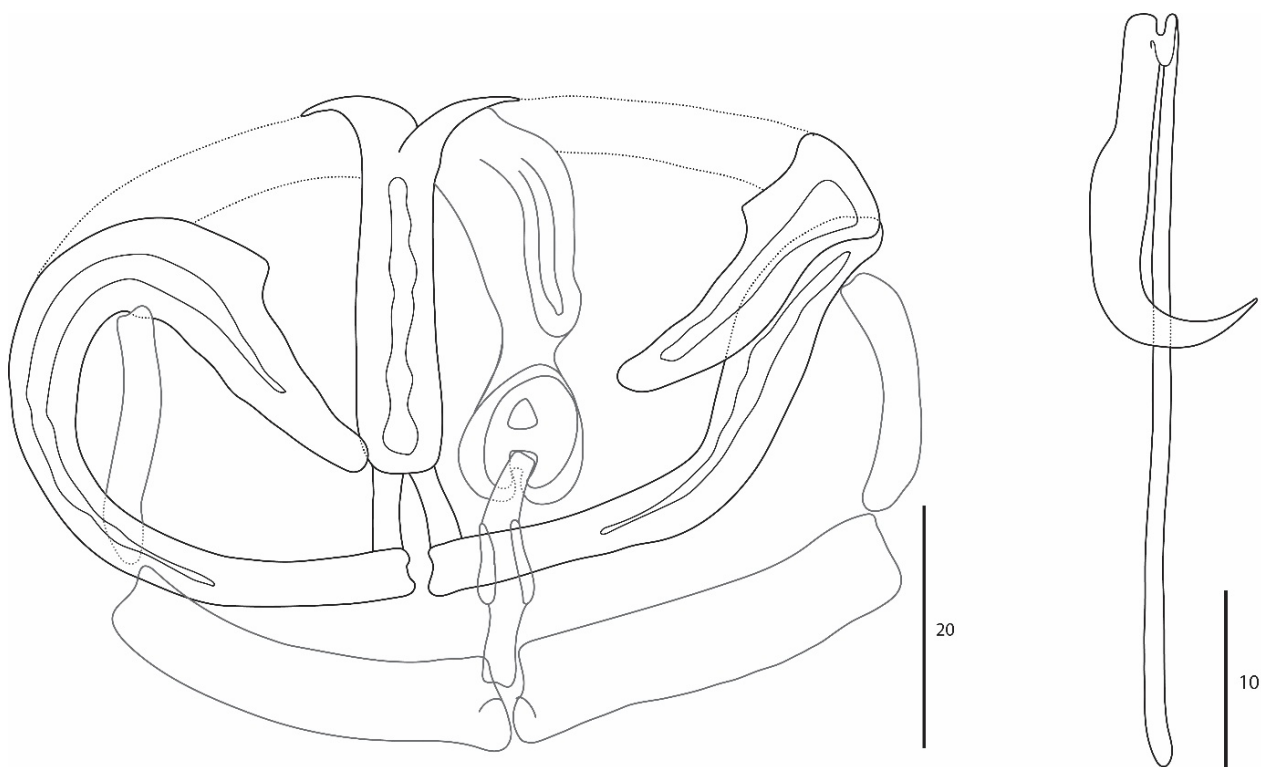


Figure 3. Line drawings of the attachment clamp (III, right row) and the central hook of *Paradiplozoon gladysae* n. sp., ventral view. Anterior sclerites in black, posterior sclerites in grey.

The newly identified *P. gladysae* n. sp. resulted as a sister taxon of *P. vaalense* in a well-supported cluster with *P. ichthyoxanthon* (South Africa) + *P. jordaanae* (South Africa) and *P. koubkova* Řehulková, Nejat and Benovics, 2023 (Iran). The uncorrected genetic *p*-distance between *P. gladysae* n. sp. and the above-mentioned species ranged from 6.8% to 18.4%. The *p*-distances and the number of differences between sequences of the species included in the final alignment are given in Table S1.

3.2. Remarks

The newly identified species, *P. gladysae* n. sp., lacks the hindbody dilatation typical of the genus *Paradiplozoon*, whereas the other genera within the Diplzoinae exhibit variation in the degree of body-section dilatation or enlargement [1]. *Paradiplozoon aegyptense* is the only species previously reported from the host species studied in Kenya. However, an examination of the type material and the detailed metric re-examination presented by Dos-Santos and Avenant-Olgewage [10] shows that *P. gladysae* n. sp. can be distinguished from *P. aegyptense*. Firstly, *P. gladysae* n. sp. is overall smaller in body and clamp dimensions than *P. aegyptense*. Mean anterior body length: 1678 µm for *P. gladysae* n. sp. vs. 3340 µm for *P. aegyptense*; mean posterior body length: 857 µm for *P. gladysae* n. sp. vs. 1410 µm for *P. aegyptense*; the mean clamp length (I–II–III–IV): 43–42–39–40 µm for *P. gladysae* n. sp. vs. 72.3–75.1–73.9–76.9 µm for *P. aegyptense*; the mean clamp width: 67–72–70–68 µm for *P. gladysae* n. sp. vs. 90–95.5–96.3–92.3 µm for *P. aegyptense*. In addition to metric differences, the two species differ in the morphology of the median plate (see Figure S1). The anterior end of the median plate of *P. gladysae* n. sp. lacks a projection or spur, whereas *P. aegyptense* has a well-developed trapezoidal spur extending from the end of the anterior half of the median plate. The wide, rounded termination at the posterior half of the median plate of *P. aegyptense* is more pronounced/more robust than that of *P. gladysae* n. sp. Moreover, in *P. aegyptense*, in the section where the median plate folds, determining the anterior and posterior halves, it forms pronounced wings like extensions/ears, not seen in any other species, nor in *P. gladysae* n. sp. Based on the currently available genetic data, *P. gladysae* n. sp. is the most closely related to *P. vaalense*. Both species share some morphological features, such as the haptor with a prominent cucullate terminal protuberance [see Figure 1D for *P. gladysae* n. sp., Figure 1. in Dos Santos et al. [16] for *P. vaalense*] and the egg with a long operculum filament. However, *P. gladysae* n. sp. can be distinguished from *P. vaalense* by its smaller size. Mean anterior body length: 1678 µm for *P. gladysae* n. sp. vs. 2850 µm for *P. vaalense*; mean posterior body length: 857 µm for *P. gladysae* n. sp. vs. 1410 µm for *P. vaalense*; mean clamp length (I–II–III–IV): 43–42–39–40 µm for *P. gladysae* n. sp. vs. 90–88–89–93 µm for *P. vaalense*; mean clamp width: 67–72–70–68 µm for *P. gladysae* n. sp. vs. 134–145–145–143 µm for *P. vaalense*. The other two African *Paradiplozoon* spp. that resulted as a sister lineage to one formed by *P. gladysae* n. sp. and *P. vaalense*, *P. jordaanae* and *P. ichthyoxanthon* differ from the newly identified species by having eggs with the absence of an operculum filament, and the size of the attachment clamps (See Table 2 in [9]). Additionally, *P. gladysae* n. sp. has a smaller sickle length in the central hooks (18–19 µm) than *P. jordaanae* (24–28 µm).

4. Discussion

The description of *P. gladysae* n. sp. expands the known diversity of African diplozoids and reinforces the view that the parasite fauna of East African freshwater fishes remains substantially underexplored, and highlights the underexplored parasitic diversity associated with *L. victorianus*, a cyprinid of conservation concern in the LVB, and with other cyprinid hosts in Kenyan waters, including a few endemic species. The occurrence of four described *Paradiplozoon* species in South Africa alone [9,10] suggests that the diversity of

African diplozoids remains considerably underestimated, particularly in East African river systems, where parasitological surveys remain limited.

The present findings suggest that the Kenyan material reported by Paperna [13] may have represented *P. gladysae* n. sp. rather than *P. aegyptense*. However, confirmation would require re-examination of voucher material or newly collected specimens from the same host and locality. *Paradiplozoon aegyptense* is the only species reported across the African continent, Egypt, Kenya, Tanzania, and Uganda [10], and has even been documented from India [18–21]; however, non-African localities for this species would require validation by resampling and, in particular, by generating molecular data from newly collected specimens. On the contrary, the majority of African *Paradiplozoon* species have been described and/or reported from a single country and a narrower host range [9,10]. Nevertheless, the possibility of a wider distribution range for a single species cannot be ruled out, as evidenced by the distribution of *Paradiplozoon homoion* (Bychowsky & Nagibina, 1959) in Europe and the Middle East [25].

The integrated approach combining morphometrics and molecular markers in taxonomic studies of the mono- and polyopisthocotylans has proven necessary for distinguishing species with minor differences and providing a solid basis for new species descriptions [57–59]. In addition, the inclusion of an additional technique, such as SEM, in the description of diplozoid species can visualise other morphological features, such as the presence or absence of plicae in the prehaptor area and/or of a cucullate terminal protrusion on the haptor, which can be used for the initial identification of parasites [9,16]. The newly identified *P. gladysae* n. sp. represent a species with the smallest-sized clamps of all African *Paradiplozoon* species. The morphometrics of clamps (pair III) and central hooks are still regarded as very reliable features on which species delineation can be solidly based [58,59]. When this approach is additionally complemented by using the shape of the median sclerite and the anterior joining, it can undoubtedly lead to more precise species differentiation [8,51]. The re-examination of the clamp shape of the type material of *P. aegyptense* did not confirm that the specimens collected during the present study represent a different species from that reported by Paperna [13] (for details, see Figure S1). Clamp morphology remains one of the most informative diagnostic characters in Diplozoidae despite the increasing availability of molecular data.

Species differentiation of marine polyopisthocotylans, for example, those of *Microcotylidae* Taschenberg, 1879, primarily relies on differences in internal morphology [60,61]. Benovics et al. [7] suggest that the characterisation of internal morphology should be incorporated into the classification of diplozoid species; however, very few studies have provided such descriptions. A 3D visualisation of the organisation of the reproductive organs was shown for *P. ichthyoxanthon* [15]; however, this visualisation could not be used for species delineation due to the lack of a key to its application. An effort to obtain more systematic information on internal morphology to delineate diplozoid species should be undertaken, as suggested by Benovics et al. [7].

Molecular evidence corroborates the morphological findings, clearly differentiating *P. gladysae* n. sp. from its closest congeners, with significant divergence in the ITS2 rDNA sequence and phylogenetic position within the well-supported cluster of African *Paradiplozoon* spp. The present study reports uncorrected *p*-distances of 6.8%, 14.5%, and 14.6% between the sequences of *P. gladysae* n. sp. and *P. vaalense*, *P. jordaanae*, and *P. ichthyoxanthon* in ITS2 rDNA fragment, respectively, which, based on the available data, are currently considered the three species most closely related. However, interspecific genetic divergence observed between species of other genera indicates that these species are not close relatives, as much lower interspecific divergence presented as uncorrected *p*-distance was observed for *Eudiplozoon* spp. (0.7%) or *Inustiatius* spp. (3.9%) in the ITS2 rDNA region

(see Table S1, present study). Sequencing of other molecular markers, such as 28S rDNA and mt *cox1*, for diplozoid species should be promoted within the community, as they have proven their utility in phylogenetic studies [23,25]. Future studies combining detailed internal morphology with multilocus molecular datasets are likely to improve species delimitation and provide a more robust framework for diplozoid systematics. The study by Benovics et al. [25] confirms congruence in tree topologies when inferring the phylogeny of diplozoid species based on 18S, 28S, ITS2 rDNA, and *cox1*; only the ITS1 rDNA region failed to resolve relationships among the deeper nodes, resulting in a polytomy. In the present study, attempts to amplify additional molecular markers, namely 28S rDNA *cox1*, failed. However, we strongly support the initiative to use multi-locus sequencing in the characterisation of a new diplozoid species, as demonstrated by Benovics et al. [25] and proposed as a standard approach for the description of these parasites [7]. The phylogeny based on the concatenated alignment of all the above-mentioned molecular markers could yield a well-resolved and well-supported phylogeny of Diplozoidae and provide stronger evidence for the need to revise the paraphyletic genus *Paradiplozoon* [3,5,23,25]. Although multi-locus datasets are desirable, the present study demonstrates that ITS2 remains a highly informative marker for recognising and differentiating closely related African *Paradiplozoon* species.

The phylogenetic position of *P. gladysae* n. sp. is consistent with previous hypotheses, suggesting that African *Paradiplozoon* lineages diversified following historical colonisation events involving cyprinid hosts dispersing through the Middle East. The importance of the region in colonisation events in various directions has been noted for fish [62] and parasites [6]. Additionally, the sister position of *P. koubkova*, a species described and reported from Türkiye and Iran, supports the previously proposed route of *Paradiplozoon* species dispersion together with their host through the Middle East. The close relationship between *P. gladysae* n. sp. and *P. vaalense*, together with their association with *Labeo* species, may reflect host conservatism among African diplozoids. Similar patterns of host specificity have been reported in other monogenean groups and suggest that host phylogeny has played an important role in parasite diversification [63]. Accurate documentation of parasite diversity is fundamental for understanding freshwater ecosystem functioning, host–parasite evolution, and biodiversity conservation. Because many monogeneans exhibit high host specificity, they also provide valuable biological models for investigating historical biogeography and ecosystem change.

5. Conclusions

This study expands the known diversity of African *Paradiplozoon* through the description of *P. gladysae* n. sp. from the endemic cyprinid *L. victorianus* in the Lake Victoria Basin, Kenya. The combined morphological and molecular evidence provides robust support for the recognition of this new species and demonstrates the value of an integrative taxonomic approach in resolving diplozoid diversity. The close phylogenetic relationship of *P. gladysae* n. sp. with southern African congeners further contributes to our understanding of the evolutionary relationships and biogeography of African diplozoids. Importantly, this discovery highlights the Lake Victoria Basin as an underexplored centre of freshwater parasite biodiversity and emphasises the need for continued integrative taxonomic surveys to improve our understanding of parasite diversity, host–parasite associations, and the biodiversity of African freshwater ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d18100641/s1>, Figure S1: Line drawing of the median plates of the attachment clamps, (A) *Paradiplozoon gladysae* n. sp., (B) *Paradiplozoon aegyptense*; Table S1: Uncorrected pairwise genetic *p*-distances in % (lower diagonal) and the number of base-pair differences (upper

diagonal) between the ITS2 rDNA sequences of *Paradiplozoon gladysae* n. sp., and other related diplozoid species included in the phylogenetic analysis (568 bp). *Octomacrum europaeum* Roman et Bychovsky, 1956, was used as an outgroup. Bold values indicate differences between the newly obtained sequence of *Paradiplozoon gladysae* n. sp., and other species in the analysis.

Author Contributions: Conceptualization, N.M.R.; Methodology, N.M.R. and I.P.; Validation, W.J.L.-P.; Investigation, N.M.R. and I.P.; Resources, N.M.R.; Data curation, I.P.; Writing—original draft, N.M.R. and I.P.; Writing—review and editing, W.J.L.-P.; Visualisation, I.P.; Project administration, W.J.L.-P.; Funding acquisition, W.J.L.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This work is based on the research supported in part by the Department of Science, Technology and Innovation (DSTI) and the National Research Foundation (NRF) of South Africa (DSTI-NRF SARCHI Grant Number 101054).

Data Availability Statement: The novel DNA sequences of *P. gladysae* presented in this study are openly available in NCBI GenBank (accession numbers PZ785758).

Acknowledgments: We are grateful to George N Morara and Elijah M Kembenya (KMFRI, Kenya) for the logistical support during fish sampling. Gladys JN Rindoria, Felix O Olonde, and Joan M Maraganaga (Kisii University, Kenya), and Judy K Rindoria (University of Northern British Columbia-UNBC, Canada) for their help with fish processing and parasite collection. R Kuchta (Institute of Parasitology, Biology Centre, Academy of Science Centre, Czech Academy of Science, České Budějovice, Czech Republic) for helping with the type material loan arrangements, and Anna Phillips (Smithsonian Institute, National Museum of Natural History, Washington, USA) for the administration of the type material loan.

Conflicts of Interest: The authors declare no conflicts of interest.

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