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Tegumental topography and molecular characterisation of two trematodes (Platyhelminthes: Digenea) from *Clarias gariepinus* (Burchell, 1822) in Kenya

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ABSTRACT

The discoveries of new taxonomic features of digenean species through the application of contemporary techniques, such as scanning electron microscopy (SEM) and molecular analysis are still growing. Two species of trematodes *Glossidium pedatum* and *Tylodelphys mashonensis* from the intestine and vitreous humour of *Clarias gariepinus* were recovered from Lake Ol'Bolossat, Kenya. The two endo-helminths were prepared for morphological examination using SEM and molecular characterisation. Additional morphological features were observed for *G. pedatum* such as domed papillae in the anterior extremity and a protruding cirrus which was unarmed, laterally folded and with a blunt tip as the first such observation for the genus and led to additional characteristics of the diagnosis of the genus. *Tylodelphys mashonensis* was characterised by a round oral sucker and tribocytic organ rounded with rows of papillae symmetrically arranged. The molecular analyses using ribosomal marker 28S large subunit (LSU) rDNA and mitochondrial (mtDNA) cytochrome *c* oxidase subunit 1 (*cox*1) for both *G. pedatum* and *T. mashonensis* confirmed the identity of the species and their phylogenetic relationship within the subclass Digenea. This study provides the first mitochondrial (mt)DNA sequence for *G. pedatum* and also extends the geographical record of two parasites to Kenya.

1. Introduction

Globally, studies of fish diseases and parasitic infections are essential for successful and optimal aquaculture and captive fisheries production. This is because parasites are not only a risk factor in captive fish species, but also a threat to the less adoptive endemic fish species, mostly in the wild populations (Smit et al., 2017). The parasites have potential impact in the global production in aquatic ecosystems because of their adaptive behaviour which allows them to switch from introduced to native, or alternative hosts (Adlard et al., 2015). Specifically, the trematodes are among the most prevalent parasitic problems in African ecosystems (Hecht and Endemann, 1998). Nevertheless, the endoparasites are generally underemphasized as a potential threat to their hosts (Smit et al., 2017).

Members of the class Digenea are of significant interest in aquatic parasitology because of their peculiar life cycle characteristics, which include host-specificity and heteroxenous behaviour (requiring more than one host to complete their complex life cycle), where molluscs are mainly the first intermediate hosts (Paperna, 1996). Digeneans are the most abundant and widely found forms of parasitic metazoans with high

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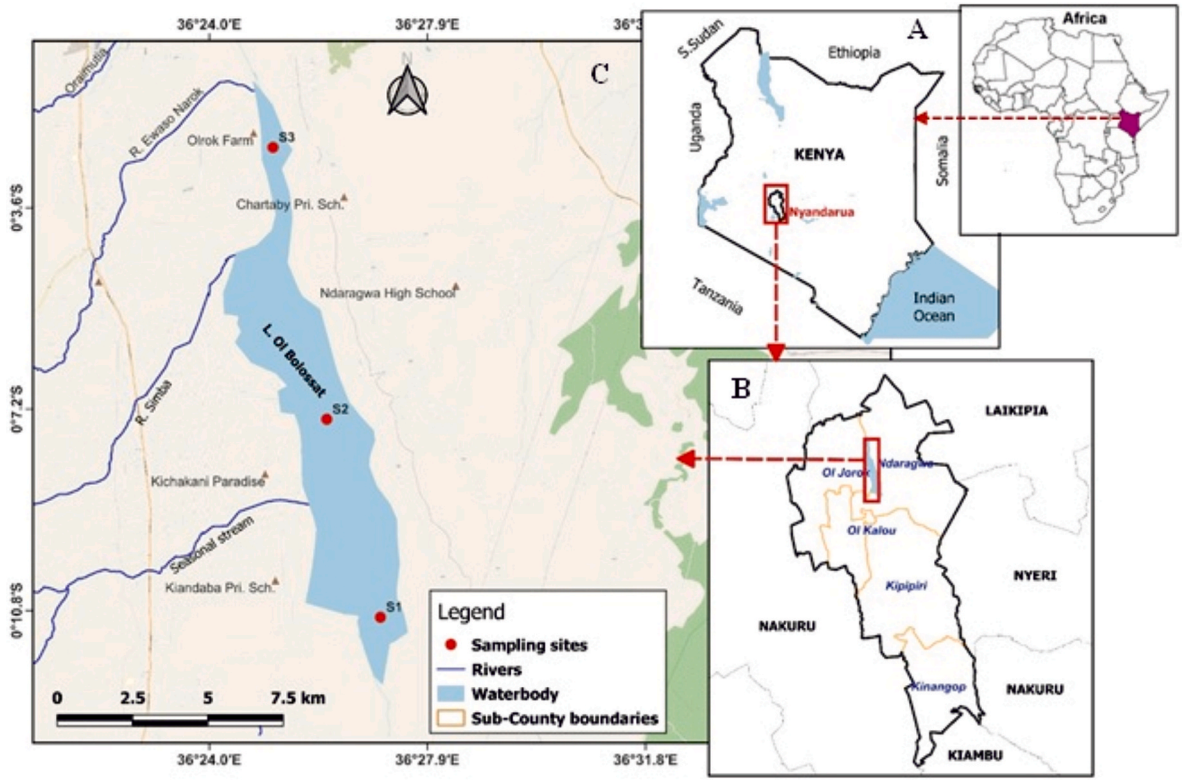

Fig. 1. Geographical location of the study area: **A**– Kenya shaded on the African continent; **B** – shows position of Nyandarua County in Kenya; **C** – indicates the position of the Lake Ol'Bolossat and the sampling sites (S1–S3).

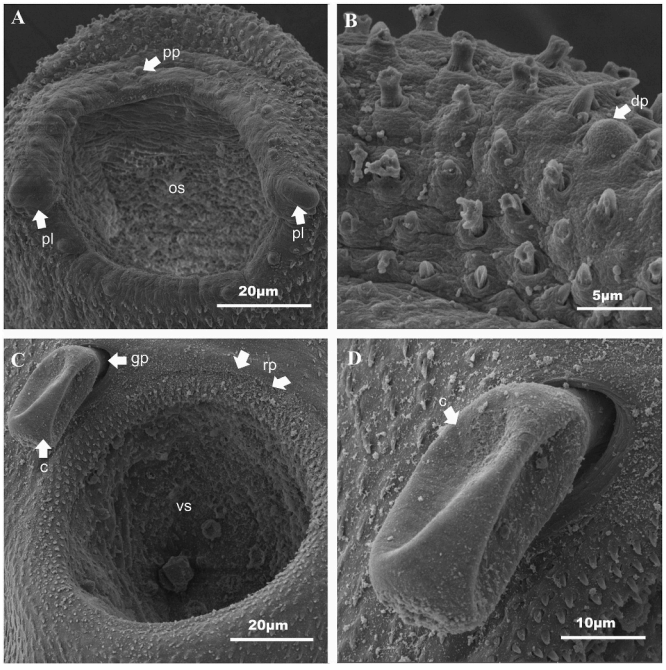

Fig. 2. Scanning electron micrographs of *Glossidium pedatum*. (**A**) Round oral sucker, (**B**) spine surrounding the oral sucker (**C**) location of the genital pore and a protruding cirrus, (**D**) structure of the cirrus sac (*abbreviations*: **pl**-papillae like lappet; **os**-oral sucker; **pp**-papillae; **dp**-dome papillae; **vs**-ventral sucker; **gp**-genital pore; **c** -cirrus; **rp**-rows of small papillae).

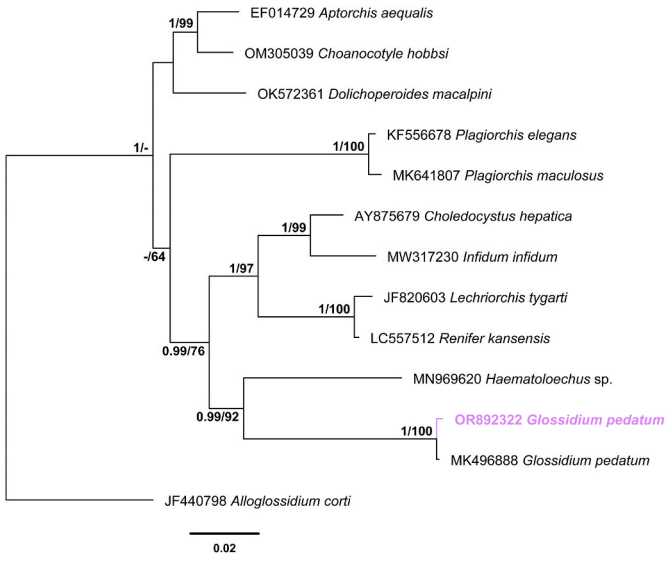

Fig. 3. Phylogenetic relationships of *Glossidium pedatum* Loos, 1899 to other members of Plagiorchioidea based on 28S rDNA. Phylogram was reconstructed using Bayesian Inference (BI) with *Alloglossidium corti* (Lamont, 1921) as an outgroup. Nodal values < 0.90 (BI) are indicated by dashes (sequences of the present study are highlighted in bold).

diversity and more than 18000 nominal species in 2500 genera arranged in 148 recognized families in 25 superfamilies, are recorded (Bray et al., 2008; Bakhoum et al., 2017). Records of digenean parasites from fish species are still growing with discoveries of new taxonomic features and geographical locations (Shimazu, 2013; Alizadeh-Noudeh and Pazooki, 2021).

Various studies, for instance, Waikagul and Thaenkham (2014) critique the reliance on morphological features alone as insufficient to provide detailed descriptions needed for accurate identification of these parasites. The main problem emanates from differences in body features

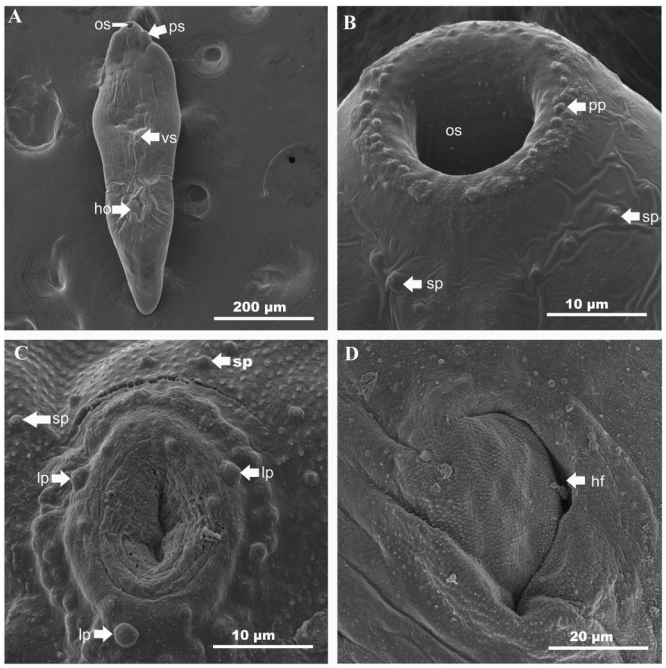

Fig. 4. Scanning electron micrographs of *Tylodelphys mashonensis*. (**A**) ventral surface ultrastructure, (**B**) sensory papillae surrounding the oral sucker (**C**) ventral sucker surrounded by small and large papillae, (**D**) well organized holdfast organ with spines (*abbreviations*: **ps**-pseudo suckers, **os**-oral sucker, **ho**-holdfast organ, **vs**-ventral sucker, **pp**-papillae, **sp**-symmetrical papillae, **lp**-large papillae, **hf**-holdfast fissure).

which could be a result of convergence or reversion during the evolutionary processes of parasites (homoplasy) that lead to the development of identical features shared between or among unrelated groups (Blair, 1996; Nolan and Cribb, 2005; Waikagul and Thaenkham, 2014).

Fortunately, contemporary advances in parasitological analyses, such as SEM and sequencing of deoxyribonucleic acid (DNA) molecules (molecular analysis) have provided additional approaches to taxonomic confirmation of several groups of aquatic parasites (see Caffara et al., 2011; Sereno-Uribe et al., 2018; Waikagul and Thaenkham, 2014; Dumbo et al., 2019a, b; Dos Santos et al., 2021; Rindoria et al., 2020, 2023a, b). Surface ultrastructural through SEM for *Glossidium pedatum* was provided by Ibraheem (2007) and an expanded description was available by Dumbo et al. (2019a), where the definition of its phylogenetic position has led to additional studies on the early stages of the species and detailed structure of the excretory vesicle.

Members of the genus *Tylodelphys* Diesing, 1850 are endoparasites of fish-eating birds, particularly ciconiids, anhingids, and podicipedids across the globe (Sereno-Uribe et al., 2018). While *Tylodelphys* is a widespread diplostomid trematode, its larval stages are important parasitic pathogens that may exert serious impacts in both wild and cultured freshwater fish (Chibwana et al., 2013; Chaudhary et al., 2017; Heneberg and Sitko, 2021). Its morphological identification remains a challenge due to the similarities to other metacercaria, the absence of many adult morphological characteristics and the difficulty in linking life-cycle stages to the respective adults; thus, the use of molecular tools is essential for an effective identification (Kostadinova, 2008).

The present study concerns specimens belonging to two digenetic trematodes found infecting the vitreous humour and intestine of the North African catfish, *Clarias gariepinus* (Burchell, 1822) which were prepared for SEM and phylogenetic analysis. Although *C. gariepinus* is one of the fish species most intensively studied for parasitic infections in the African continent (see Scholz et al., 2018), there have been fascinating discoveries of new features of its parasites that might be of interest in their taxonomic grouping. Therefore, the objective of the current study was to specifically report some morphological details observed through SEM on the specimens and analyze the phylogenetic position through 28S large subunit (LSU) rDNA fragment and cytochrome *c* oxidase subunit 1 (*cox*1).

2. Materials and methods

2.1. Ethical consideration

A permit for this study was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) License No. NACOSTI/P/23/24398. This study was approved by Kisii University under the ethics approval of the Kisii Teaching and Referral Hospital Institutional and Scientific Ethical Review Committee (KTRHISERC), approval number ISERC/KTRH/011/23.

2.2. Study area

This study was conducted in Lake Ol'Bolossat, the only freshwater lake in Central Kenyaat 00° 9′ S and 36° 25′ E. It is situated northwest of the Aberdare Ranges in Nyandarua County where it is bordered by Ol'Kalou, Ol'Joro Orok, and Ndaragwa Divisions, on the north Kinangop Plateau bordering the Satima Escarpment (Fig. 1).

2.3. Parasite collection

A total of 35 *C. gariepinus* specimens were collected and examined for digenean infections in vitreous humour and intestine. Parasites were washed in saline solution (0.85%) and fixed for SEM examination or DNA extraction. Specimens for SEM examination were fixed with 70% ethanol (Dos Santos and Avenant-Oldewage, 2015); and those for DNA extraction were fixed with 96% ethanol (Nagy, 2010).

Table 1
Pairwise distances in % (upper diagonal) and the number of base pair differences (lower diagonal) between *Glossidium pedatum* Loos, 1899, other Plagiorchioidea species, and *Alloglossidium corti* (Lamont, 1921) used an outgroup based on 28S rDNA (present study species are in bold).

	Species	Acc no	1	2	3	4	5	6	7	8	9	10	11	12	13
1	***Glossidium pedatum***	**OR892322**		**0.2**	**8.5**	**9.0**	**7.7**	**8.6**	**9.7**	**8.9**	**9.7**	**8.4**	**8.5**	**8.6**	**12.0**
2	*Glossidium pedatum*	MK496888.1	**2**		8.3	8.8	7.7	8.4	9.7	8.9	9.7	8.2	8.5	8.7	12.0
3	*Haematoloechus* sp.	MN969620.1	**101**	99		7.2	7.2	6.9	9.3	8.6	9.3	7.3	8.1	8.0	11.4
4	*Dolichoperoides macalpini*	OK572361.1	**107**	105	86		6.6	3.6	7.9	7.2	7.7	3.9	6.7	6.9	8.8
5	*Choledocystus hepatica*	AY875679.1	**92**	92	86	78		6.0	7.7	5.0	7.6	6.1	4.7	2.8	10.0
6	*Choanocotyle hobbsi*	OM305039.1	**102**	100	82	43	71		7.2	6.5	7.1	2.1	6.1	5.9	9.1
7	*Plagiorchis maculosus*	MK641807.1	**115**	115	111	94	92	86		8.3	0.5	7.5	7.9	7.8	11.3
8	*Lechriorchis tygarti*	JF820603.1	**106**	106	102	86	59	77	99		8.2	6.8	7.8	5.8	10.9
9	*Plagiorchis elegans*	KF556678.1	**115**	115	111	92	90	84	6	97		7.3	7.7	7.7	11.1
10	*Aptorchis aequalis*	EF014729.1	**100**	98	87	46	72	25	89	81	87		6.3	6.1	9.1
11	*Renifer kansensis*	LC557512.1	**101**	101	96	80	56	73	94	8	92	75		5.5	10.6
12	*Infidum infidum*	MW317230.1	**102**	103	95	82	33	70	93	69	91	73	65		10.5
13	*Alloglossidium corti*	JF440798.1	**142**	142	136	104	119	108	134	130	132	108	126	125	

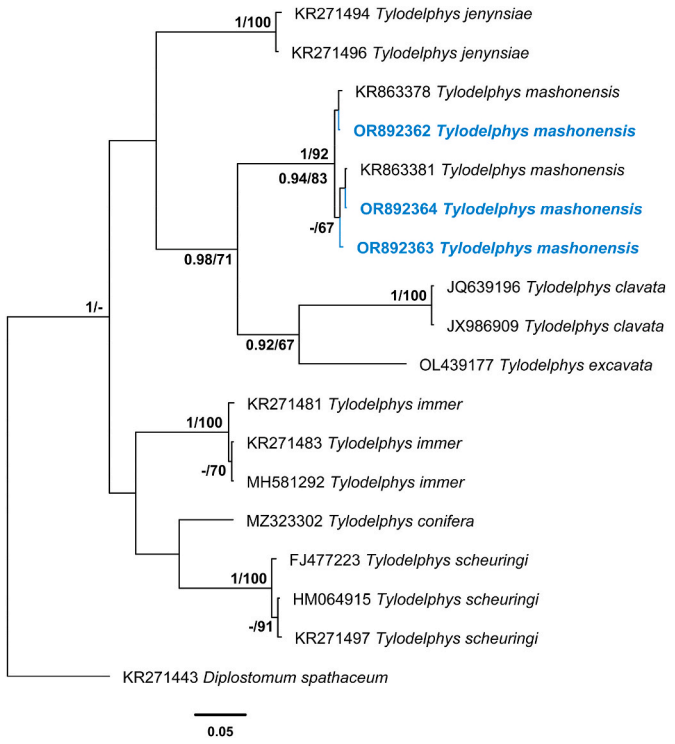

Fig. 5. Phylogenetic relationships of *Tylodelphys mashonensis* (Sudarikov, 1971) to other Diplostomidae based on *cox*1. Phylogram was reconstructed using Bayesian Inference (BI) with *Diplostomum spathaceum* (Rudolphi, 1819) as an outgroup. Nodal values < 0.90 (BI) are indicated by dashes (sequences of the present study are highlighted in bold).

2.4. Scanning electron microscopy

Specimens of trematodes fixed in 70% ethanol were prepared for SEM examination by dehydration through a graded ethanol series as outlined by Rindoria et al. (2023b), followed by a graded series of hexamethyldisilazane (Merck, Darmstadt, Germany) as prescribed in literature (Nation, 1983; Dos Santos and Avenant-Oldewage, 2015). Specimens were dried in a glass desiccator for 24 h at room temperature and gold coated using an Emscope TM Q150T sputter coater (Quorum Technologies Ltd., Newhaven, U.K.). Specimens were analyzed using a Zeiss Sigma 500VP scanning electron microscope (Jena, Germany) at 4 kV at the University of Limpopo.

2.5. DNA extraction, polymerase chain reaction and sequencing

DNA extractions were carried out using the PCRBIO Rapid Extract Lysis Kit (PCRBIOSYSTEMS, London, UK) following the manufacturer's instructions. Partial fragment of the 28S rDNA gene was amplified using the primer combinations Digl2 (5′-AAGCATATCACTAAGCGG-3′) and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′) (Snyder and Tkach, 2001; Tkach et al., 2003) with internal primers 300F (5'-CAAGTACCGT-GAGGGAAAGTTG-3') and ECD2 (5'-CCTTGGTCCGTGTTTCAA-GACGGG-3') for sequencing (Littlewood et al., 1997, 2000). PCR reactions were performed in a total volume of 25 μL containing 1.25 μL of each primer (10 μM), 7 μL of molecular-grade water, 12.5 μL of DreamTaqTM Hot Start Green PCR Master Mix (2X) (ThermoFisher Scientific, Waltham, Massachusetts, USA), and 3 μL of the DNA template, following the thermocycler conditions described by Tkach et al. (2003). PCR reactions for the amplification of the partial fragment of the *cox*1 gene for representatives of *T. mashonensis* were performed in a total volume of 20 μL containing 1.25 μL of each primer (10 μM): Dice1F (5'-ATTAACCCTCACTAAATTWCNTTRGATCATAAG-3') and Dice14R (5'-TAATACGACTCACTATACCHACMRTAAACATATGATG-3') Van

Table 2
Pairwise distances (%, upper diagonal) and the number of base pair differences (lower diagonal) between *Tylodelphys mashonensis* (Sudarikov, 1971) other Diplostomidae species, and *Diplostomum spathaceum* (Rudolphi, 1819) used an outgroup based on *cox*1 (present study species are in bold).

	Species	Acc no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	***Tylodelphys mashonensis***	**OR892362**		**0.6**	**0.6**	**0**	**0.6**	**11.4**	**12.8**	**13.1**	**13.1**	**12.5**	**12.3**	**12.3**	**13.9**	**13.9**	**11.7**	**12.5**	**12.5**	**14.8**
2	***Tylodelphys mashonensis***	**OR892363**	**2**		**0.6**	**0.6**	**0.6**	**12.0**	**12.8**	**13.1**	**13.1**	**13.1**	**12.8**	**12.8**	**13.9**	**13.9**	**12.3**	**12.8**	**12.8**	**15.3**
3	***Tylodelphys mashonensis***	**OR892364**	**2**	**2**		**0.6**	**0**	**12.0**	**12.8**	**13.1**	**13.1**	**13.1**	**12.8**	**12.8**	**13.9**	**13.9**	**12.3**	**13.1**	**13.1**	**15.3**
4	*Tylodelphys mashonensis*	KR863378	**0**	**2**	**2**		0.6	11.4	12.8	13.1	13.1	12.5	12.3	12.3	13.9	13.9	11.7	12.5	12.5	14.8
5	*Tylodelphys mashonensis*	KR863381	**2**	**2**	**0**	2		12.0	12.8	13.1	13.1	13.1	12.8	12.8	13.9	13.9	12.3	13.1	13.1	15.3
6	*Tylodelphys conifera*	MZ323302	**41**	**43**	**43**	41	43		7.8	8.1	8.4	9.2	8.9	8.9	11.4	11.4	12.8	14.2	14.2	13.1
7	*Tylodelphys scheuringi*	FJ477223	**46**	**46**	**46**	46	46	28		0.8	1.1	11.7	11.7	11.7	11.4	11.4	12.8	13.6	13.6	13.6
8	*Tylodelphys scheuringi*	HM064915	**47**	**47**	**47**	47	47	29	3		0.3	12.0	12.0	12.0	11.7	11.7	13.1	13.9	13.9	13.9
9	*Tylodelphys scheuringi*	KR271497	**47**	**47**	**47**	47	47	30	4	1		12.0	12.0	12.0	12.0	12.0	13.4	14.2	14.2	14.2
10	*Tylodelphys immer*	KR271481	**45**	**47**	**47**	45	47	33	42	43	43		0.6	0.6	11.4	11.4	13.1	15.3	15.3	14.5
11	*Tylodelphys immer*	KR271483	**44**	**46**	**46**	44	46	32	42	43	43	2		0	11.1	11.1	13.1	15.0	15.0	14.2
12	*Tylodelphys immer*	MH581292	**44**	**46**	**46**	44	46	32	42	43	43	2	0		11.1	11.1	13.1	15.0	15.0	14.2
13	*Tylodelphys jenynsiae*	KR271494	**50**	**50**	**50**	50	50	41	41	42	43	41	40	40		0	13.1	15.3	15.3	13.6
14	*Tylodelphys jenynsiae*	KR271496	**50**	**50**	**50**	50	50	41	41	42	43	41	40	40	0		13.1	15.3	15.3	13.6
15	*Tylodelphys excavata*	OL439177	**42**	**44**	**44**	42	44	46	46	47	48	47	47	47	47	47		10.9	10.9	12.5
16	*Tylodelphys clavata*	JQ639196	**45**	**46**	**47**	45	47	51	49	50	51	55	54	54	55	55	39		0	14.8
17	*Tylodelphys clavata*	JX986909	**45**	**46**	**47**	45	47	51	49	50	51	55	54	54	55	55	39	0		14.8
18	*Diplostomum spathaceum*	KR271443	**53**	**55**	**55**	53	55	47	49	50	51	52	51	51	49	49	45	53	53	

Steenkiste et al. (2015), 1 μL of molecular-grade water, 12.5 μL of DreamTaqTM Hot Start Green PCR Master Mix (2X) (ThermoFisher Scientific, Waltham, Massachusetts, USA), and 4 μL of the DNA template, following the thermocycler conditions described by Van Steenkiste et al. (2015). For the representative of *G. pedatum*, the partial fragment of the *cox*1 mtDNA was amplified in a PCR reaction volume of 20 μL consisting of 1.25 μL of each primer (10 μM): Dig_cox1Fa (5′-ATGATWTTYTTYTTYYTDATGCC-3′) and Dig_cox1R (5′-TCNGGRTG HCCRAARAAYCAAAA-3′) Wee et al. (2017), 2 μL of molecular-grade water, 12.5 μL of DreamTaqTM Hot Start Green PCR Master Mix (2X) (ThermoFisher Scientific, Waltham, Massachusetts, USA), and 3 μL of the DNA template, following the thermocycler profile of an initial denaturation at 94 °C for 3 min; 40 cycles of 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s; a final extension at 72 °C for 10 min and pause at 4 °C. Successful amplification products were verified using a 1% agarose gel electrophoresis and sent for purification and sequencing at Inqaba Biotechnical Industries (Pty) Ltd in Pretoria, South Africa.

2.6. Phylogenetic analysis

The novel sequence data obtained were assembled using the built-in De Novo Assembly tool followed by manual base calling and editing in Geneious Prime v11.1.4 (https://www.geneious.com). The resulting consensus sequences, 28S rDNA and *cox*1, were subjected to a Basic Local Alignment Search Tool, BLAST (Altschul et al., 1990) to identify the closest congeners. Alignments for each gene fragment were constructed under the default parameters of MAFFT in Geneious v11.1.4. The final alignment lengths were 1269bp for 28S LSU rDNA (*Glossidium*) and for *cox*1 (463 *Tylodelphys* and 489 *Glossidium*). The complete list of species used in the construction of phylogenetic trees is provided in Table S1. *Alloglossidium corti*, and *Diplostomum spathaceum* were selected as outgroups for *Glossidium* and *Tylodelphys*, respectively. The best fitting model was selected for 28S LSU rDNA and *cox*1 alignments according to the Akaike Information Criterion (AIC) from jModelTest v2.1.4. (Darriba et al., 2012) was the GTR + I + G (General Time-Reversible model with Invariant sites and Gamma distribution) model. Bayesian Inference (BI) analyses were performed in MrBayes using the CIPRES (Miller et al., 2010) computational resource. The BI analyses were generated by implementing a data block criterion running two independent Markov Chain Monte Carlo (MCMC) chains of four chains for 1000000 generations. A sampling of the MCMC chain was set at every 1000th generation and a burn-in was set to the first 25% of the sample generations. Maximum likelihood analyses were carried out using PhyML v. 3.0 (Guindon et al., 2010) on the online ATGC platform (http://www.atgc-montpellier.fr/). Phylogenetic trees generated were visualized in FigTree v1.4.4 and combined using Adobe Illustrator (Rambaut, 2012). The uncorrected pairwise distances (*p*-distances) and the number of base pair differences were estimated in MEGA7 (Kumar et al., 2016).

3. Results

A total of 28 digenean specimens were collected from the intestine (*G. pedatum*, n = 20) and vitreous humour (*T. mashonensis*, n = 8).

3.1. Adult: Glossidium pedatum (Fig. 2)

3.1.1. Taxonomic summary

Host: *Clarias gariepinus* (Burchell, 1822) (Siluriformes, Clariidae).

Site of infection: Intestine.

Prevalence and mean intensity: 14%; 4.0.

Locality/collection date: Lake Ol'Bolossat, Kenya, May 2022.

Deposition of sequences: Sequence data obtained were deposited in GenBank: 28S LSU rDNA (OR892322); *cox*1 (OR892362 –OR892364).

Additional SEM morphological characterisation: Tegument covered with spines; sharp pointed spines from ventral sucker decreasing in number and size to posterior extremity but absent at region of excretory pore. Two papillae lappet-like and 10 symmetrically arranged papillae surround the oral sucker aperture (Fig. 2A). Dome-shaped papillate sensory endings occur on the entire surface. Tegument anterior to the oral sucker possesses spines and dome papillae (Fig. 2B). Genital atrium in the anterior margin of ventral sucker, slightly dextral. Genital pore dextral of the median line close to anterior margin of ventral sucker (Fig. 2C-D**)**. Protruding cirrus unarmed and tip blunt; cirrus surface smooth with posterior portion larger, shorter, laterally folded with tip blunt, enclosing bipartite seminal vesicle (Fig. 2D).

Systematic analysis: In the present study, both *cox*1 (mtDNA) and the 28S LSU rDNA were employed to confirm the identity of specimens of *G. pedatum*. There being no *cox*1 sequence of this genus in GenBank comparison could not be made but the sequence generated was deposited in GenBank under accession number OR892362–OR892364. For the 28S LSU rDNA the sequences of *G. pedatum* of the present study formed a well-supported clade with a sequence available with 100% bootstrap support (Fig. 3). Specimens of *G. pedatum* from Mozambique (MK496888.1) and those from Kenya had 2 bp difference (Table 1). Interestingly, the sequence of *G. pedatum* from Kenya also represented a clade closest to *Haematoloechus* sp. (MH285261.1) with 0.99 bootstrap support through BI. Herein, combining the characters reported by Dumbo et al. (2019b) and those observed in the present study, enrich the diagnosis of the genus *Glossidium*.

3.2. Metacercariae: Tylodelphys mashonensis (Fig. 4)

3.2.1. Taxonomic summary

Host: *Clarias gariepinus* (Burchell, 1822) (Siluriformes, Clariidae).

Site of infection: Vitreous humour.

Prevalence and mean intensity: 7%; 3.2.

Locality/collection date: Lake Ol'Bolossat, Kenya, May 2022.

Deposition of sequences: Sequence data obtained were deposited in GenBank: *cox*1 (OR892362 – OR892364).

Morphological characterisation with SEM: Body elongate, wide in anterior region narrowing toward posterior extremity. Tegument without spines, corrugations in entire body. Anterior extremity comprises a subterminal well-developed oral sucker; Oral sucker spherical surrounded by dense row of papillae (Fig. 4A). Three (3) pairs of symmetrical papillae arranged in uniform bilateral pattern underneath rim of oral sucker. Two pseudosuckers close to oral sucker, bilaterally positioned at anterior end of the body. Tegument armed with papillae, sensory structures or non-ciliated receptors (Fig. 4B). Ventral sucker oval to spherical-shaped, smaller in size than oral sucker, placed mid-ventral on body; Its rim exhibits surface corrugations arranged radially and bears 4 large papillae and other 4 small papillae (Fig. 4C). Tegument around ventral sucker contain many non-ciliated receptors and papillae. Holdfast organ partially covered without well recognized shape with fissure placed between ventral sucker and the posterior extremity (Fig. 4D).

Molecular analysis: Sequences of *T. mashonensis* analyzed in the present study are identical to other available sequences in GenBank (KR863378 and KR863381) **(**Fig. 5**)** from the same host. The present sequences and those available in GenBank diverged by a 0–2 base pair difference (Table 2), thus indicating that the sequences are related. The clade comprising *T. mashonensis* and its congeners (*T. clavata* and *T. excavata*) is supported by high bootstrap value meaning that they are evolutionary-related species. Unlikely the present parasite collected in a freshwater host (*C. gariepinus*), *T. clavata* was from an estuarine fish (*Perca fluviatilis*) and *T. excavata* from the anuran *Pelophylax ridibundus*.

4. Discussion

The morphology of specimens of *G. pedatum* in the present study resembles that of Dumbo et al. (2019b), except for the protruded cirrus

and arrangement of the papillae. The observation of the protruded cirrus of *G. pedatum* is the first of its kind for this genus and, it is an additional feature to the diagnosis of this species following the redescription provided by Dumbo et al. (2019b). The authors provided a molecular analysis of the species, herein used to confirm the identity of specimens from Kenya. In that study, the cirrus sac was referred to as cylindrical-shaped based on a light microscope. In the present study, the cirrus appears to be far larger and shorter. Petkevičiūtė et al. (2018) have reported a topography of the cirrus-sac in *Crepidostomum oschmarini* having a smooth surface, similar to that found for *G. pedatum*. Conversely, the protruding cirrus of *G. pedatum* is dissimilar to that reported by Dos Santos et al. (2021) for *Allocreadium apokryfi* which has a lace-like texture surface. Regarding the number and arrangement of the tegumental papillae in *G. pedatum*, they vary according the host location as seen in the present study compared to the record of Ibraheem (2007) and Dumbo et al. (2019b) and it is of less importance for species identification.

The relatedness of *G. pedatum* to *Haematoloechus* sp. (MN969620) is supported by high (0.99 BI) branch support values. Dumbo et al. (2019b) reported the close phylogenetic relationship of *G. pedatum* with *Haematoloechus meridionalis* and *H. abbreviatus* which was well supported by the lowest genetic distance. Notwithstanding the phylogenetic closeness of the genus *Glossidium* to the Haematoloechidae, it could not be placed in that family due to the following morphological dissimilarities: (1) haematoloechids possess the median genital pore at the level of the pharynx or oesophagus, (2) the vitelline follicles usually arranged in rosettes or grape-like clusters; (3) extent of Y-shaped excretory vesicle; and (4) terminal excretory pore. Furthermore, considering the host specificity, species of *Glossidium* are restricted to freshwater fish (*C. gariepinus*), while *Haematoloechus* spp. Are anuran amphibian parasites. Although the diplostomid metacercariae in the present study seem morphologically similar to the metacercariae described by other researchers (Moema et al., 2013; Achatz et al. 2022a, 2022b; Chibwana et al., 2013), morphological studies are not sufficient for species-level identification. The scanning morphology of the specimens *T. mashonensis* from Kenya is similar to the surface topology of other *Tylodelphys* spp. (metacercariae) reported by Blasco-Costa et al. (2017); Sereno-Uribe et al. (2018) and Pelegrini et al. (2019).

5. Conclusion

This study provides additional taxonomic features for *G. pedatum* which enable the diagnosis of this species. Novel sequences for both 28S LSU rDNA and *cox*1 (the first for this genus) are provided. For *T. mashonensis*, additional *cox*1 sequence data is also provided. The two endo-helminth parasites recorded in this study form the first biogeographical records in Kenya.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.100897.

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