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# Integrated morphological and molecular characterization of the fish parasitic nematode *Rhabdochona (Rhabdochona) gendrei* Campana-Rouget, 1961 infecting *Labeobarbus altianalis* (Boulenger, 1900) in Kenya

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## ABSTRACT

During a parasitological survey carried out between May and August 2022 in the River Nyando, Lake Victoria Basin, a single species of *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochoniidae) was recorded from the intestine of the Rippon barbel, *Labeobarbus altianalis* (Boulenger, 1900) (Cyprinidae). Based on light microscopy (LM), scanning electron microscopy (SEM) and DNA analyses the parasite was identified as *Rhabdochona (Rhabdochona) gendrei* Campana-Rouget, 1961. Light microscopy, SEM and DNA studies on this rhabdochoniid resulted in a detailed redescription of the adult male and female. The following additional taxonomic features are described in the male: 14 anterior prostomal teeth; 12 pairs of preanal papillae: 11 subventral and one lateral; six pairs of postanal papillae: five subventral and one lateral, with the latter pair at the level of first subventral pairs when counted from the cloacal aperture. For the female: 14 anterior prostomal teeth and the size and absence of superficial structures on fully mature (larvated) eggs dissected out of the nematode body. Specimens of *R. gendrei* were genetically distinct in the 28S rRNA and cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial gene regions from known species of *Rhabdochona*. This is the first study that provides genetic data for a species of *Rhabdochona* from Africa, the first SEM of *R. gendrei*, and the first report of this parasite from Kenya. The molecular and SEM data reported herein provide a useful point of reference for future studies on *Rhabdochona* in Africa.

## 1. Introduction

*Rhabdochona (Rhabdochona) gendrei* Campana-Rouget (1961) is a parasitic intestinal nematode belonging to the Rhabdochoniidae Travassos, Artigas et Pereira, 1928, the only superfamily of the Thelazioidea Skryabin, 1915 that includes representatives from fishes (Moravec, 2019). Members of this family are characterized by a funnel or barrel-shaped prostom which is an extension of the anterior end of the vestibule/stoma, the presence of teeth (a result of longitudinal thickenings of the internally lined prostom), a muscular oesophagus encircled by a nerve ring, absence of a gubernaculum, and presence of at least 5 pairs of preanal and 5 pairs postanal papillae in males (Moravec, 2010,

2019). Species of *Rhabdochona* Railliet (1916) infect the digestive tract of freshwater fishes, while others accidentally infect other vertebrates (Moravec, 2007, 2010, 2019). It is worth noting that species of *Rhabdochona* occur in all zoogeographical regions (Moravec et al., 2008; Moravec, 2019). To date, only 10 valid species of *Rhabdochona* (two subgenera *Rhabdochona* and *Globochona*) have been recorded in freshwater fishes in Africa (Moravec, 2019). These valid species include: *Rhabdochona (Rhabdochona) centroafricana* Moravec et Jirků, 2014; *Rhabdochona (Rhabdochona) esseniae* Mashego (1990); *Rhabdochona (Rhabdochona) gendrei* Campana-Rouget (1961); *Rhabdochona (Rhabdochona) marcusenii* Moravec et Jirků, 2014; *Rhabdochona (Rhabdochona) moraveci* Puylaert (1973); *Rhabdochona (Rhabdochona) puylaerti*

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in 1% physiological saline and then fixed in hot 10% formalin, 70% ethanol for morphological and 96% ethanol for molecular studies. The samples were transported to the parasitology laboratory in the Department of Biodiversity, University of Limpopo, South Africa for further examination and analysis.

### 2.3. Morphological analyses, microdissection of fully mature eggs and excision of spicules

For LM, 4% formalin and 70% ethanol fixed rhabdochonids were cleared in glycerine (Rindoria et al., 2020), photographed and measured using an Olympus U-DA OC13617 compound microscope with a digital measuring software (model BX50F no. 4C05604 Olympus Optical Co. Ltd, Japan). Measurements were taken and are given in micrometres ( $\mu\text{m}$ ), unless otherwise stated, and reported as a range followed, in parentheses, by the mean values. Soft tissues of the posterior end of the male specimens fixed in 70% ethanol were enzymatically digested as per the guide of Rindoria et al. (2020).

For microdissection of the fully mature (larvated) eggs on the female, the female specimens were observed under a dissecting microscope. The mature females with eggs were microdissected using 2 sharpened dissecting needles, holding the nematode with one needle and cutting with the other. For SEM, the revealed spicule (after digestion), eggs (after microdissection), and whole specimens fixed in 70% ethanol were prepared by dehydrating through a graded ethanol series, followed by a graded series of hexamethyldisilazane (HMDS) (Nation, 1983; Dos Santos and Avenant-Oldewage, 2015). Specimens were dried in a portable glass desiccator, gold coated using an Emscope SC500 sputter coater (Quorum Technologies, Newhaven, U.K.). Specimens were then studied using a Vega 3 LMH scanning electron microscope (Tescan, Brno, Czech Republic) at 6 kV acceleration voltages.

### 2.4. Molecular analyses

Genomic DNA was extracted using a Zymo Research Quick-DNA™ Microprep Plus Kit following the manufacturer's instructions. The 28S rRNA and cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial gene regions were amplified using primer sets 502-F (5'-CAA GTA CCG TGA GGG AAA GTT GC-3') and 536-F (5'-CAG CTA TCC TGA GGG AAA C-3') (Lagunas-Calvo et al., 2019), and COLint-F (5'-TGA TTG GTG GTT TTG GTA A-3') and COLint-R (5'-ATA AGT ACG AGT ATC AAT ATC-3') (Casiraghi et al., 2001), respectively. PCR reactions were performed with a total volume of 25  $\mu\text{l}$  containing: 1  $\mu\text{l}$  of each primer, 12  $\mu\text{l}$  of double distilled water, 7  $\mu\text{l}$  of DreamTaq™ Hot Start Green PCR Master Mix (2X) (ThermoFisher Scientific, Waltham, Massachusetts, USA) and 4  $\mu\text{l}$  of template DNA. The thermal cycling profile had an initial denaturation of 94 °C for 3 min; initial annealing at 94 °C for 30 s and 35 cycles at 54 °C for 30 s, 72 °C for 2 min and final extension at 72 °C for 7 min for the 28S rRNA amplification reaction. The same thermal profile was used for the amplification of the *cox1*, with an adjustment in the annealing temperature to 45 °C for 1 min.

### 2.5. Sequence alignment and molecular analyses

Successful amplification reactions were verified using a 1% agarose gel and sent for cleaning, purification and sequencing to Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa). Sequence data obtained were inspected, aligned and assembled under default parameters of MUSCLE using Geneious Prime v2022.2. (<https://www.genieious.com>). The resulting consensus sequences, 28S rRNA (789bp and 790bp) and *cox1* (484bp and 523bp) were subjected to a Basic Local Alignment Search Tool (BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al., 1990) to identify the closest congeners. Alignments for each gene region were constructed in Geneious and trimming of the resulting alignment was performed in trimAL v.1.2. using the "gappyout" parameter selection under default settings (Capella-Gutiérrez

et al., 2009). The alignments for both gene regions included all available published sequences of *Rhabdochona* since no comparative sequence data is available for African representatives. The final length of the 28S rRNA alignment was 1083bp and that of the *cox1* was 599bp. Details of species included in the phylogenetic inference are presented in Table 1. *Spinitectus mexicanus* Caspeta-Mandujano, Moravec et Salgado Maldonado, 2000 was used as the outgroup for all alignments (Caspeta-Mandujano et al., 2000; Černotíková et al., 2011; Choudhury and Nadler, 2018). Uncorrected pairwise distances (*p*-distances) were estimated in MEGA7 (Kumar et al., 2016) and optimal substitution model selection was done using jModelTest v2.1.3. (Darriba et al., 2012). The HKY + G model was implemented for the 28S rRNA alignment, whereas the HKY + I + G was implemented for the *cox1* alignment. Maximum likelihood (ML) analyses were computed in phyML using the ATGC Montpellier Bioinformatics Platform (<http://www.atgc-montpellier.fr/>) (Guindon and Gascuel, 2003) and 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 1 million 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. Phylogenetic trees generated were visualised in FigTree v1.4.4. (Rambaut, 2018).

## 3. Results

### 3.1. Morphometric and morphological analyses

Family Rhabdochoniidae Travassos, Artigas et Pereira, 1928.

*Rhabdochona* (*Rhabdochona*) *gendrei* Campana-Rouget (1961) (Fig. 2A–F, 4A–H)

**Syns:** *Nec Spiroptera acuminata* Molin, 1859; *Nec Spiroptera acuminata* Von drasche, 1883; *Nec Oxyspirura acuminata* Stossich, 1897; *Nec Rhabdochona acuminata* Travassos, Artigas et Pereira, 1928; *Rhabdochona acuminata sensu Gendre* (1922) and *Nec Rhabdochona acuminata* Vaz et Pereira, 1934.

**General description:** Medium-sized nematodes. Tetragonal oral opening with a pair of adjacent amphids and four submedian cephalic papillae surrounding the opening. Prostom funnel-shaped, armed with 14 anterior teeth. Vestibule straight, relatively long. Deirids average-sized, bifurked/bifurcate, situated approximately mid-length of vestibule. Tail of both sexes conical, with sharply pointed tip.

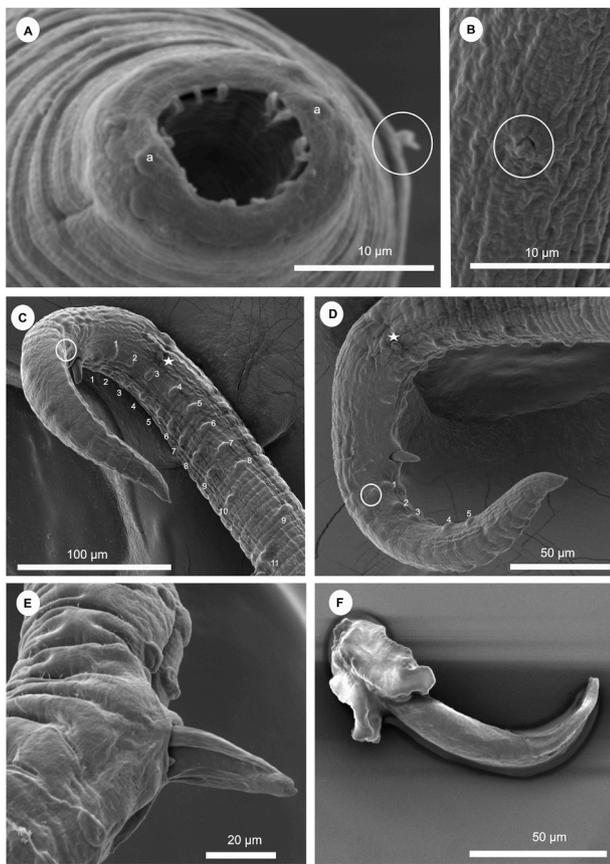
**Male** (9 specimens): Length of body 6.02–9.36 (7.36) mm, maximum width 110.40–169.90 (140.06). Vestibule including prostom 131.04–171.20 (153.50) long; length of prostom 21.36–24.80 (23.11), width 17.22–20.74 (18.86). Length of muscular oesophagus 272.45–430.14 (342.76), of glandular oesophagus 2.65–3.92 (3.16) mm. Deirids, nerve ring, and excretory pore 78.23–85.45 (81.53), 205.56–231.84 (216.28), 278.14–311.21 (298.29) from anterior extremity, respectively. Preanal papillae 10–12 pairs: 9–11 sub-ventral and 1 lateral pairs; latter pair approximately at level of third sub-ventral pair (counted from cloacal aperture). Postanal papillae 6 pairs: 5 subventral and 1 lateral pairs; latter pair approximately at level of first subventral pairs (counted from cloacal aperture). Left spicule 426.79–576.30 (519.01) long. Right spicule 150.26–177.94 (161.29) long, tapering at its distal end, without usual dorsal barb. Length ratio of spicules 1:2.57–3.76 (3.23). Tail 263.71–577.69 (348.26) long, with cuticular spike at tip.

**Female** (5 specimens): Length of body 9.88–20.55 (14.18) mm, maximum width 138.37–348.50 (268.26). Length of vestibule including prostom 148.34–200.35 (163.77); prostom 22.92–24.32 (23.63) long, 15.94–19.11 (17.95) wide, with distinct basal teeth. Deirids, nerve ring and excretory pore 91.56–120.12 (104.20), 221.34–254.63 (236.34), and 311.56–351.02 (328.45), respectively, from anterior extremity. Vulva postequatorial, 4.32–11.36 (8.00) mm from posterior end of body. Size of mature (larvated) eggs 458.75–474.04 (476.66) length,

**Table 1**

Details on the species of *Rhabdochona* Railliet, 1916 and accession numbers of the 28S rRNA and cytochrome c oxidase subunit 1 (cox1) mitochondrial gene regions used in the phylogenetic analyses. Species sequenced in the present study are in bold. \* – Indicate species used as outgroup.

Species	Host		Locality	28S	cox	Reference
<i>Rhabdochona acuminata</i>	<i>Brycon guatemalensis</i>	(Characiformes: Bryconidae)	Mexico	MK341679	MK341634	Santacruz et al. (2020)
			Mexico	MK341680	MK341635	
			Mexico	MK341681	MK341636	
<i>Rhabdochona adentata</i>	<i>Profundulus oxacae</i>	(Cyprinodontiformes: Profundulidae)	Mexico	–	MN927201	Caspeta-Mandujano et al. (2021)
			Mexico	–	MN927199	
<i>Rhabdochona ahuelhuellensis</i>	<i>Ilyodon whitei</i>	(Cyprinodontiformes: Goodeidae)	Mexico	–	MK353475	Lagunas-Calvo et al. (2019)
			Mexico	–	MK353476	
			Mexico	–	MK353477	
<i>Rhabdochona canadensis</i>	<i>Gila conspersa</i>	(Cypriniformes: Leuciscidae)	Mexico	MK341685	MK353485	Santacruz et al. (2020)
			Mexico	MK341686	MK353486	
			Mexico	–	MK341637	
<i>Rhabdochona gendrei</i>	<i>Labeobarbus altianalis</i>	(Cypriniformes: Cyprinidae)	Kenya Kenya	OR096360 OR096361	OR088887 OR088888	Present study Present study
<i>Rhabdochona guerreroensis</i>	<i>Sicydium multipunctatum</i>	(Gobiiformes: Goobiidae)	Mexico	–	MN592669	Caspeta-Mandujano et al. (2021)
<i>Rhabdochona ictaluri</i>	<i>Ictalurus pricei</i>	(Siluriformes: Ictaluridae)	Mexico	MK353492	MK353478	Lagunas-Calvo et al. (2019)
			Mexico	–	MK353479	
			Mexico	–	MK353480	
<i>Rhabdochona juliacarabiasae</i>	<i>Eugerres mexicanus</i>	(Eupercaria: Gerreidae)	Mexico	–	MF405199	Caspeta-Mandujano et al. (2021)
<i>Rhabdochona kidderi</i>	<i>Rhamdia guatemalensis</i>	(Siluriformes: Heptapteridae)	Mexico	MK353490	MK353472	Lagunas-Calvo et al. (2019)
			Mexico	MK353491	MK353473	
			Mexico	–	MK353474	
<i>Rhabdochona lichthenfelsi</i>	<i>Allotoca regalis</i> , <i>Goodea atripinis</i> <i>Goodea atripinis</i>	(Cyprinodontiformes: Goodeidae)	Mexico	MK341682	DQ991009	Santacruz et al. (2020); Mejía-Madrid et al. (2007) Mejía-Madrid et al. (2007) Mejía-Madrid et al. (2007)
			Mexico	–	DQ990982	
			Mexico	–	DQ990984	
<i>Rhabdochona mexicana</i>	<i>Astyanax mexicanus</i>	(Characiformes: Characidae)	Mexico	MK341653	MK341601	Santacruz et al. (2020)
			Mexico	MK341654	MK341603	
			Mexico	MK341660	MK341606	
			Mexico	MK341661	MK341607	
			Mexico	MK341663	MK341612	
			Mexico	MK341664	MK341613	
			Mexico	MK341665	MK341614	
			Mexico	MK341666	MK341615	
			Mexico	MK341655	MK341602	
			Mexico	MK341656	MK341598	
	<i>Astyanax aeneus</i>	(Characiformes: Characidae)	Mexico	MK341657	MK341600	
			Mexico	MK341658	MK341596	
			Mexico	MK341659	MK341611	
			Guatemala	MK341667	MK341617	
			Guatemala	MK341668	MK341619	
			Guatemala	MK341669	MK341616	
			Mexico	MK341670	MK341620	
			Mexico	MK341671	MK341621	
			Mexico	MK341683	MK341632	
<i>Rhabdochona salgadoi</i>	<i>Profundulus</i> sp.	(Cyprinodontiformes: Profundulidae)	Mexico	MK341684	MK341633	
			Mexico	–	MH778492	Caspeta-Mandujano et al. (2021)
<i>Rhabdochona osoroi</i>	<i>Astyanax aeneus</i>	(Characiformes: Characidae)	Mexico	MK341672	MK341622	Santacruz et al. (2020)
			Mexico	MK341677	MK341624	
			Mexico	MK341678	MK341629	
<i>Rhabdochona xiphophori</i>	<i>Poecilia mexicana</i>	(Cyprinodontiformes: Poeciliidae)	Mexico	MK353496	MH778493	Caspeta-Mandujano et al. (2021)
			Mexico	–	MK353483	
	–	–	–	Mexico	–	MN592670
<i>Spinitectus mexicanus</i> *	<i>Pseudoxiphophorus bimaculatus</i>	(Cyprinodontiformes: Poeciliidae)	Mexico	MK341687	MK341638	Santacruz et al. (2020)



**Fig. 2.** *Rhabdochona (Rhabdochona) gendrei* Campana-Rouget (1961) from *Labeobarbus altianalis* (Boulenger, 1900), scanning electron micrographs of the male. A – cephalic end, subapical view; B – deirid; (circled); C, D – posterior end of male, ventrolateral view (white stars and white circles indicate a pair of lateral pre- and postanal papillae, respectively); E – right spicule; F – excised right spicule, following enzymatic digestion. *Abbreviations:* a – amphid; 1–11, and 1–9 – pairs of sub-ventral preanal papillae (in C); 1–5 – pairs of postanal papillae (in D).

302.74–353.63 (324.80) width. Tail conical, 274.54–428.43 (348.64) long, with distinct cuticular spike at end.

*Host:* *Labeobarbus altianalis* (Boulenger, 1900) (Cyprinidae, Cypriniformes).

*Site of infection:* Intestine.

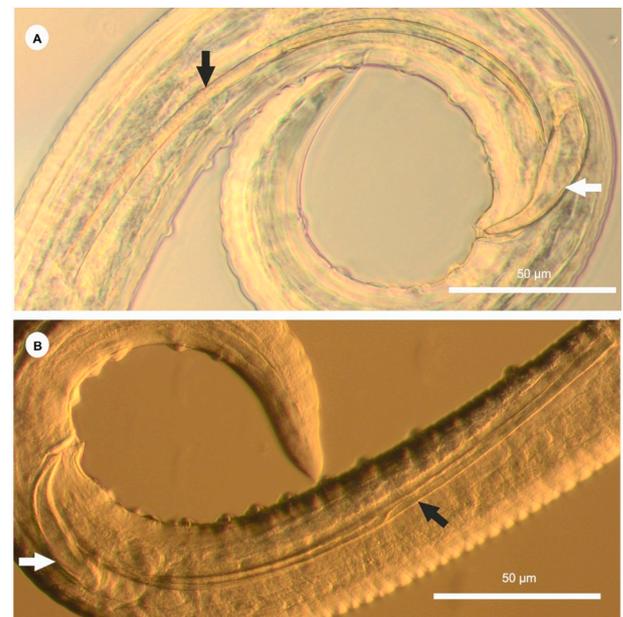
*Locality/collection date:* River Nyando (Lake Victoria Basin) Kisumu County, Kenya (0°0′ 0°22′S, 34°51′E 35°11′E) (see Fig. 1) (collected May 3 and August 3, 2022, by Drs. Nehemiah M. Rindoria and George N. Morara).

*Prevalence and intensity of infection:* 100% (32 fish parasitized of 32 fish examined); 6–11 nematodes.

*Deposition of voucher specimens:* A total of twelve voucher specimens (six adult female and six male) were deposited in the Helminthological Collection of the Institute of Parasitology, the Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS N-1030).

*Deposition of sequences:* Sequence data obtained were deposited in GenBank: 28S rRNA (OR096360–OR096361), *cox1* (OR088887–OR088888).

*Comments.* Reports of Campana-Rouget (1961), Puylaert (1973) and Vassiliades and Troncy (1974) did not record any measurements on this parasite, but the morphology of specimens of the present material is in agreement with their descriptions. Morphometrics of the taxonomic relevant features of *R. gendrei* from the present study is within the ranges recorded by Moravec (1972) who gave a redescription of the same



**Fig. 3.** *Rhabdochona (Rhabdochona) gendrei* Campana-Rouget (1961) from *Labeobarbus altianalis* (Boulenger, 1900), light micrographs of an adult male. A – left (longer) and right (shorter) spicules (dorsolateral view); B – left (longer) and right (shorter – showing the boat-like shape) spicules (ventrolateral view); black and white arrows indicate left and right spicules respectively.

nematode from *B. duchesnii*.

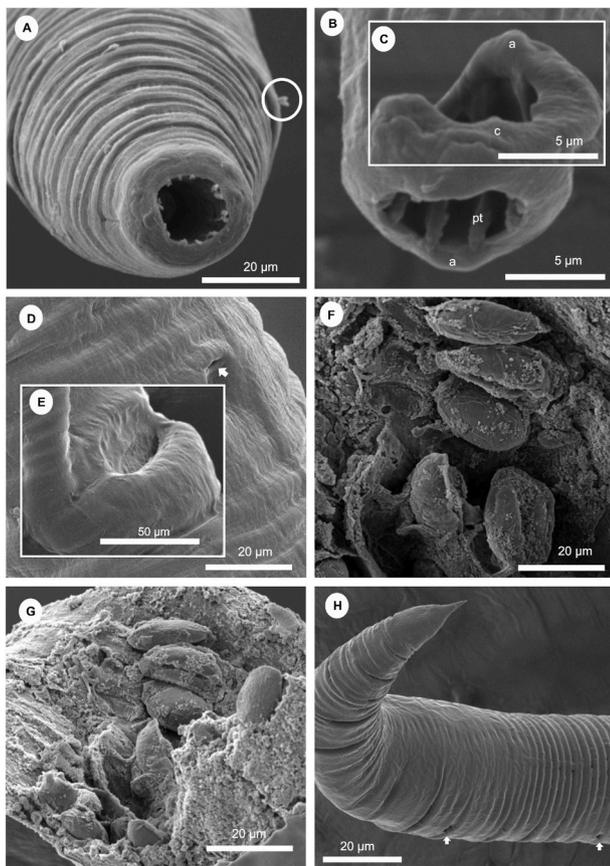
### 3.2. Molecular analyses

Novel sequences of the 28S rRNA and *cox1* were successfully generated for two isolates of *R. gendrei*. The alignment of the 28S rRNA included sequences of nine species of *Rhabdochona* from the Neotropical and Nearctic regions. Tree topologies for the ML and BI analyses of the 28S rRNA region were incongruent. Both tree topologies provide support for the monophyly of the genus. The ML analysis (Fig. 5A) recovered *Rhabdochona acuminata* (Molin, 1860) and *Rhabdochona osorioi* Santacruz Vázquez, Ornelas-García et Pérez-Ponce de León, 2019 as sister group to *Rhabdochona mexicana* Caspeta-Mandujano, Moravec et Salgado-Maldonado, 2000 (Clade A) and *R. gendrei*, from the present study, in the basal position to the remaining species of *Rhabdochona* (Clade B), with low support values. Similarly, the BI analyses (Fig. 5B) recovered *R. gendrei*, with moderate to high posterior probability support, in the basal position of the taxa in Clade B.

The primary difference between the ML and BI topologies is in the sister position of *R. acuminata* + *R. osorioi* in the respective clades. Sequences of thirteen species of *Rhabdochona* were included in the alignment for the *cox1* region. ML and BI analyses recovered different tree topologies. The BI analyses recovered *R. gendrei* as the sister taxon to *R. mexicana* and *R. acuminata* + *R. osorioi* as a sister clade to *R. mexicana* + *R. gendrei*. *Rhabdochona juliacarabiasae* Caspeta-Mandujano, Salinas-Ocampo, Suárez-Rodríguez, Martínez-Ramírez et Matamoros, 2021 as basal to all ingroup taxa with high posterior probability support values (Fig. 6). The ML tree recovered (not shown) provided low resolution with no nodal support. Interspecific divergence of *R. gendrei* to congeners ranged between 7.1 and 10.9% (31–130 base pair differences) and 8.4–13.0% (39–67 base pair differences) for the 28S rRNA (Table S1) and *cox1* (Table S2) regions, respectively.

## 4. Discussion

The morphology of the present specimens, particularly the number of anterior prostomal teeth (Fig. 2A, 4A-C), spicules (left and right)



**Fig. 4.** *Rhabdochona (Rhabdochona) gendrei* Campana-Rouget (1961) from *Labeobarbus altianalis* (Boulenger, 1900), scanning electron micrographs of a female. A (deirids shown by white circle), B, C – cephalic end dorsolateral and anterior views; D – lateral view of the excretory pore (as shown by white arrow); E – detail of vulva sub-ventral view; F, G – fully mature (larvated) eggs dissected out of the nematode body; H – tail tip (white arrows shows openings with a papilla); Abbreviations: a – amphids; c – submedian cephalic papilla; pt – anterior prostomal teeth.

(Fig. 2C–F, 3A–B) are more indistinguishable from the inadequately described *R. gendrei* by Campana-Rouget (1961) who originally described the parasite from *Barbus* sp., *B. altianalis* (now *L. altianalis*), *B. duchesnii* (now *L. intermedius*) and *B. bynni* (now *L. bynni*) (Cyprinidae) from Zaïre (now DRC) in Lakes Albert, Edward and Kivu, and by Moravec (1972) from *B. duchesnii* (now *L. intermedius*) from Lake Kivu in DRC.

Moravec (1972) reported that the number of anterior prostomal teeth is the most important taxonomic feature for species of *Rhabdochona*. This study recorded 14 anterior prostomal teeth on both the male and the female as observed from the apical view using SEM (Fig. 2A and 4A), which agrees with Puylaert (1973) and the redescription by Moravec (1972) who re-examined Campana-Rouget (1961) specimens using light microscopy and recorded same number of teeth. Moravec (2010) argues that the exact number of teeth in *Rhabdochona* species can only be determined in apical view and most ideally by use of SEM. The number of anterior prostomal teeth for this nematode is resolved with SEM as 14. Since the cephalic end of *R. gendrei* had never been examined apically by SEM, the present study findings update the light microscopy observation by Campana-Rouget (1961).

The current study also reports bifurcated/bifurcate deirid that is reduced in adult males (Fig. 2B). Simple deirids were recorded for the female as observed in Fig. 4A. Our findings are in agreement with bifurcate deirids previously reported by Gendre (1922); Campana-Rouget (1961) and Moravec (1972), but the present study emphasises its

reduced nature.

Further, the present study conforms with the findings of Moravec (2010), Campana-Rouget (1961) and Moravec (1972) that the right spicule progressively surrogates the function of the gubernaculum becoming boat-shaped (Fig. 2C–F, 3A, B). This study recorded the presence of a dorsal barb on the distal end of the shorter (right) spicule (Fig. 2F) following enzymatic digestion and examination under SEM. This structure was recorded by Mashego (1990) as being present on *Rhabdochona esseniae* collected from *Barbus* spp. in South Africa. The presence of this structure shows the need to re-examine the type specimens of inadequately described *R. esseniae* to verify the presence of the dorsal barb under SEM.

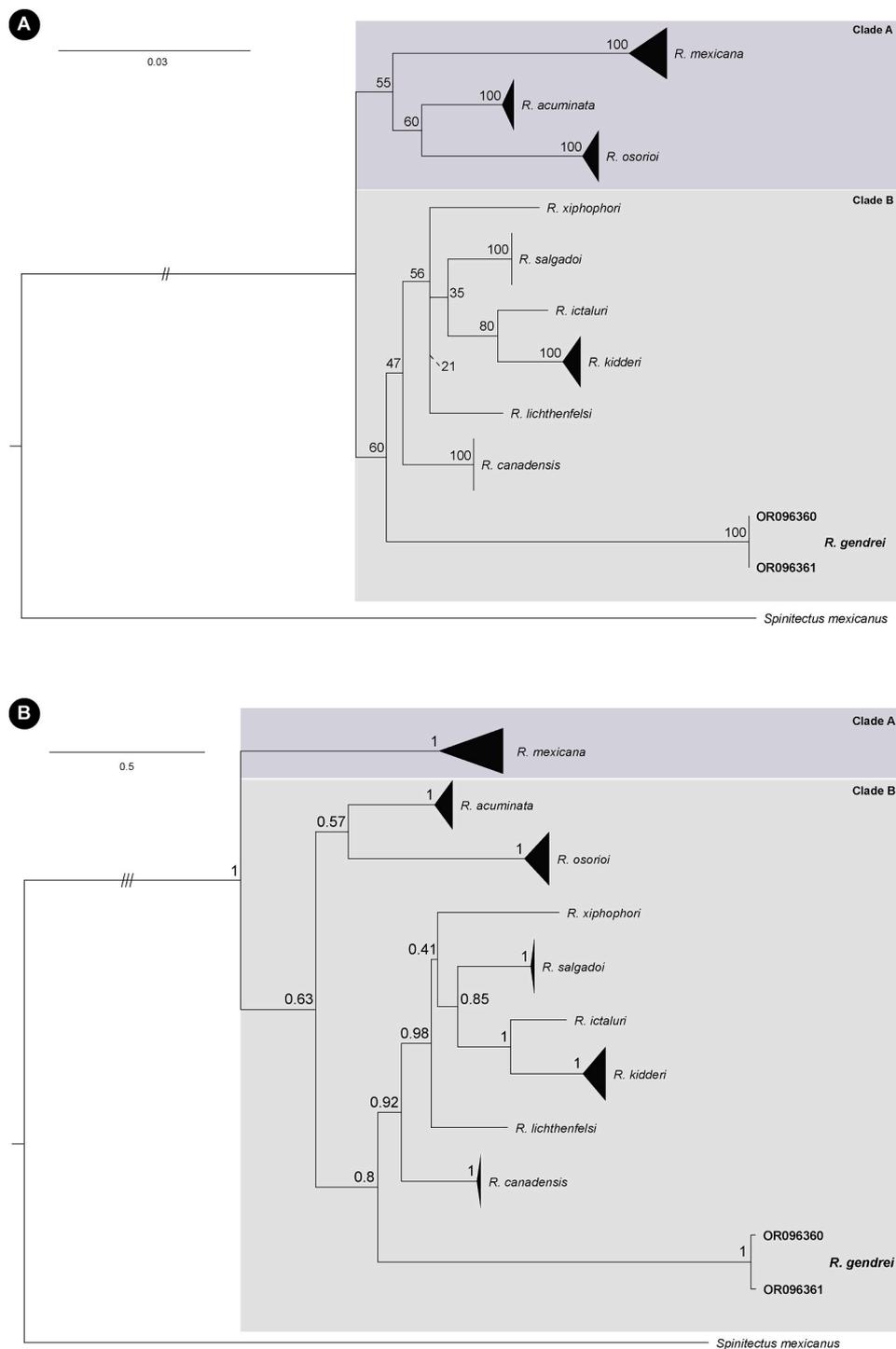
The use of SEM in this study revealed some differences with earlier studies (Campana-Rouget, 1961; Moravec, 1972) in terms of pre and postanal papillae with 10–12 pairs of preanal papillae recorded (Fig. 2C): 11 subventral and one pair lateral (at the level of the third subventral pair when counted from the cloaca opening). A total of six pairs of postanal papillae were revealed: of which five pairs are subventral and one pair is lateral lying along the same side of the first postanal papillae (when counted from the cloaca opening) (see Fig. 2D). This one lateral pair was previously recorded by Moravec (1972) as being the second pair of the postanal papillae, with other remaining five pairs (I, III, IV, V, and VI pairs subventral).

The shape and structure of the male and female tail is a good specific taxonomic character in *Rhabdochona* species. In the present study a conical shape, with a sharp terminal cuticular spike was noted in both sexes of this species (Fig. 2C and D, 4H). This is in agreement with studies of Campana-Rouget (1961) and Moravec (1972).

A detailed study of fully mature (larvated) eggs dissected out of the nematode body (Fig. 4F–G) did not reveal any possible presence of superficial structures on them (e.g., filaments, other gelatinous formations). The shape of the egg looked oval, smooth, and relatively wide. One end of some of the eggs is provided with protuberance (opercula) Fig. 4F–G). This conforms with the previous record of Moravec (1972, 2019).

Through the use of molecular markers, *R. gendrei* could successfully be identified as a separate species among congeners. Tree topologies obtained for the 28S region in the present study yielded similar results to Santacruz et al. (2020) with two main clades. In their analyses, Santacruz et al. (2020) recovered two main clades, with *R. osorioi* as a sister to *R. acuminata* + *R. mexicana*. However, in our analyses, the position of *R. mexicana* was either recovered as a separate paraphyletic clade with *R. acuminata* + *R. osorioi* as the sister clade (Fig. 5A) or as basal to all ingroup taxa (Fig. 5B), and the position of *R. acuminata* + *R. osorioi* could not be resolved. From the analyses of the *cox1* mitochondrial region, *R. gendrei* was recovered with strong posterior probability support as the sister taxon to *R. mexicana* that positioned with *R. acuminata* + *R. osorioi* as a sister clade (Fig. 6). *Rhabdochona juliacarabiasae* was recovered as basal to all ingroup taxa, which is contrasting to Lagunas-Calvo et al. (2019), Santacruz et al. (2020) and Caspeta-Mandujano et al. (2021) who recovered *R. xiphophori*, *R. salgadoi* and *R. mexicana* as the basal taxon to all the ingroup taxa, in their respective analyses.

Apart from Caspeta-Mandujano et al. (2021) that included all species with *cox1* sequence data and recovered a different tree topology than in the present study, it is clear that the phylogenetic relationship between species of *Rhabdochona* is still unresolved. No apparent grouping according to endemism, host preference or zoogeographic region was observed. Moreover, no grouping according to morphological characters is considered to be of taxonomic relevance, such as the number of prostomal teeth, shape of the deirids, presence or absence of superficial formation on eggs, shape of the distal tip of larger spicule and number of post cloacal papillae in males were observed for any of the taxa included in the analyses. Consequently, the phylogenetic relationship of the more than 100 species in the genus from across the globe would need to be supplemented to attempt a more robust and complete analysis. We highly recommend the inclusion of molecular data in future studies on



**Fig. 5.** Phylograms of the 28S rRNA gene region based on the A– maximum likelihood and B – Bayesian Inference analyses. Bootstrap and posterior probability support values are presented along branch nodes. The branch length was reduced to two (//) and three (///) times the scale bar.

all rhabdochonid species, in particular those from Africa, to facilitate robust classical taxonomic and complimentary phylogenetic analyses.

**5. Conclusions**

The use of SEM in this study adds to the available LM reports by giving a concise number of anterior prostomal teeth, preanal and postanal papillae as follows, for the male: 14 anterior prostomal teeth; 12 pairs of preanal papillae: 11 subventral and one lateral; six pairs of postanal papillae: five subventral and one lateral, with the latter pair at

level of first sub-ventral pairs when counted from cloacal aperture and the presence of a dorsal barb on the distal end of the shorter right spicule. For the female: 14 anterior prostomal teeth and absence of superficial structures on fully mature (larvated) eggs dissected out of the nematode body. Both the 28S rRNA and *cox1* gene regions prove to be effective markers in delineation at the species level of rhabdochonids. *Rhabdochona gendrei* could successfully be placed as a separate species from the available sequence data of Neotropical and Nearctic rhabdochonid species. It should be noted that it is imperative that time and resources are allocated to include complementary approaches, such as in

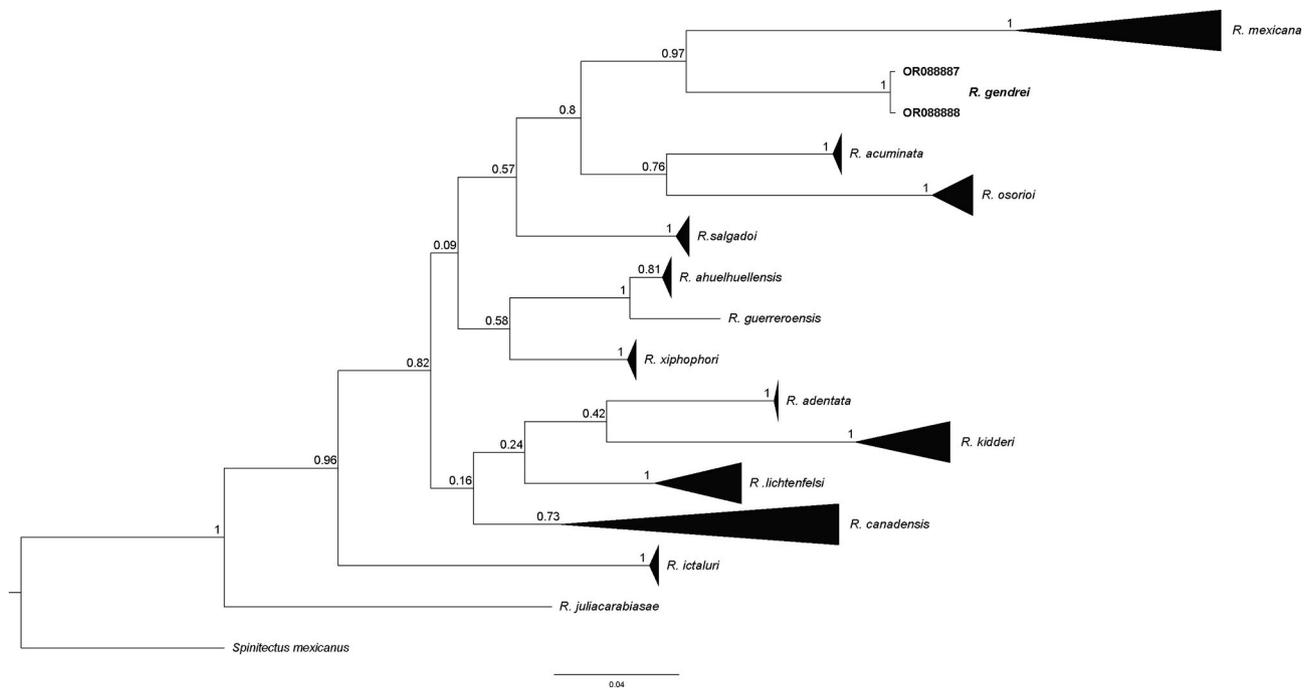


Fig. 6. Bayesian inference phylogram of the *cox1* mitochondrial gene region. Posterior probability support values are presented along branch nodes.

the present study, to define species characters at the material and molecular level to facilitate future studies on this parasitic group and to resolve taxonomic and systematic discrepancies that may arise when restricted data (i.e., geographic, host range) is available to include in analyses.

#### Declaration of competing interest

None.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jpaw.2023.06.002>

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