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Additional data on *Spinitectus petterae* (Nematoda: Rhabditida) from *Clarias gariepinus* (Siluriformes: Clariidae) in the Vaal River system: conserved morphology or high intraspecific genetic variability?

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Abstract: Two species of *Spinitectus* Fourment, 1884 have been recorded from southern Africa, namely *Spinitectus polli* Campana-Rouget, 1961 and *Spinitectus petterae* Boomker, 1993, both from the Limpopo River system. *Spinitectus petterae* was described from North African catfish, *Clarias gariepinus* (Burchell), whereas *S. polli* infects squeakers, *Synodontis* spp. During parasitological surveys in the Vaal River system (Orange River catchment), *Spinitectus* specimens were collected from *C. gariepinus*. These systems are adjacent but not connected. Therefore, this study aimed to identify the specimens collected using morphological and molecular techniques. The morphological study included light and scanning electron microscopy of whole specimens and excised spicules. Specimens were genetically characterised using 18S rDNA, 28S rDNA and *cox*1 mtDNA. Additionally, immature specimens of *S. petterae* were collected near the type locality. Morphological characteristics were most similar to *S. petterae* from *C. gariepinus*, whereas genetic data were dissimilar to all available data for the genus. Additional morphological characteristics noted for *S. petterae* in the present study were the details of the left and right spicule structure and the porous structures on the pseudolabia. Specimens from the Vaal River system differed from those originally described as *S. petterae* by additional spines posterior to the third ring, lacking caudal alae and variable total body and male oesophagus length. Based on 18S rDNA, haplotypes from the type locality varied only slightly from the study material, supporting the morphological identification. However, 28S rDNA and, more conspicuously, *cox*1 mtDNA displayed substantial variation between specimens from these localities, which needs further investigation. Haplotypes generated in the present study were highly dissimilar to those characterised for *S. petterae* from Tanzania and Egypt. Nevertheless, the nematodes collected from *C. gariepinus* in the Vaal River system are considered *S. petterae.* This study expands the geographical distribution and adds additional morphological and genetic information for *S. petterae*, contributing to the limited knowledge of African species of *Spinitectus*.

Keywords: African sharptooth catfish, Rhabdochonidae, Vaal Dam, Crocodile River, South Africa, DNA barcoding.

This article contains supporting tables (Tables S1–S4) online at http://folia.paru.cas.cz/suppl/2023-70-002.pdf

Species of *Spinitectus* Fourment, 1884 are medium-sized nematodes, with transverse annular cuticular rings, infecting the digestive tract of primarily marine and freshwater fishes (Anderson 2000, Anderson et al. 2009). The first anterior ring has large, ordered, posteriorly directed spines embedded in a semi-circular collar, with subsequent rings diminishing towards the posterior and spines becoming sparse and disorganised, attached directly to the cuticle (Boomker 1993, Boomker and Puylaert 1994). Taxonomically significant features include left spicule length, body length of male, number of cuticular spines in the first ring, position of excretory pore in adults, and mature egg features (Boomker 1993, Boomker and Puylaert 1994, Moravec 2019). Khalil and Polling (1997) listed fourteen species of *Spinitectus* from Africa. However, Moravec (2019) reduced the number to nine species based on morphology, distribution and host similarities.

In southern Africa (South Africa, Botswana, Namibia, Lesotho, Kingdom of eSwatini), two species, namely *Spinitectus petterae* Boomker, 1993 and *Spinitectus polli* Campana-Rouget, 1961 (syn. *Spinitectus zambezensis* Boomker, 1993), have been recorded (Boomker 1993, Moravec and Jirků 2017). *Spinitectus polli* was described from *Synodontis schall* (Bloch et Schneider) from the Democratic Republic of the Congo. Thereafter, Moravec and Van As (2015) reported *S. polli* from *Synodontis nigromaculatus* Boulenger from the Shakawe mainstream, Okavango River, Botswana. The junior synonym of *S. polli*, *S. zambezensis*, was described from *Synodontis zambezensis* Peters from the Sabie River in the Kruger National

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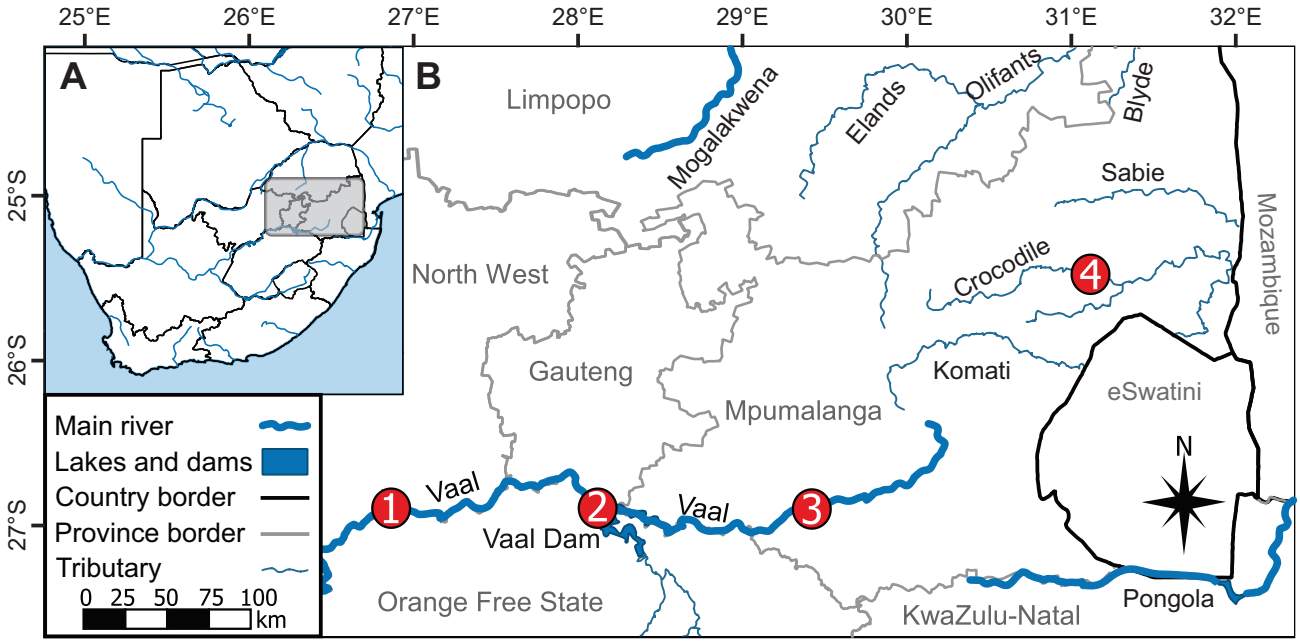

Fig. 1. A – map of South Africa; **B** – map of the river systems in the inlay showing the sampling sites where *Spinitectus petterae* Boomker, 1993 was collected in *Clarias gariepinus* (Burchell). *Abbreviations*: 1 – down-stream of the Vaal River Barrage; 2 – in the Vaal Dam reservoir; 3 – down-stream of the Grootdraai Dam; 4 – Crocodile River.

Park, South Africa (Boomker 1993). *Spinitectus petterae* was described from *Clarias gariepinus* (Burchell) by Boomker (1993) from the Crocodile River, which forms the southern border of the Kruger National Park. *Spinitectus allaeri* Campana-Rouget, 1961 has also been recorded from *C. gariepinus*, but has not been recorded from southern Africa (Moravec et al. 2019)

During parasitological surveys in the Vaal River system (Orange River catchment), *Spinitectus* specimens were collected from *C. gariepinus*, the type host of *S. petterae*. The Orange River catchment is separate, but adjacent to the Limpopo River catchment, where both *S. petterae* and *S. polli* have been recorded. No *Spinitectus* specimens have previously been recorded from the Orange River catchment. Thus, the aim of the present study was to identify and describe the collected specimens using both morphological and molecular approaches.

MATERIALS AND METHODS

Clarias gariepinus were collected from three sites in the Vaal River (Fig. 1) during summer (February 2018 and February–March 2019). These sites were (1) downstream of the Vaal River Barrage (26.7321S, 27.6330E), (2) in the reservoir of the Vaal Dam, close to the University of Johannesburg (UJ) island (26.8662S, 28.1601E), and (3) downstream of the Grootdraai Dam (26.9218S, 29.2815E). During summer (December 2020), additional sampling was done by members of the UJ Parasitology laboratory in the (4) Crocodile River (25.4365S, 30.8693E) close to Kanyamazane (Mbombela), approximately 35 km upstream from the type locality of *Spinitectus petterae*. Only immature specimens were collected from the Crocodile River and were used only for molecular study. All procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals (collection permits CPE2-000125, CPE2-0126, CPE2-0127). The UJ Ethics committee approved this study (number 2019-04-15/Avenant-Oldewage_Austin).

Fish were collected using gill nets and rod and reel. Live fish were kept in shaded and aerated live wells until euthanised on site. The gastrointestinal tract of each fish, from the oesophagus to the rectum, was removed, opened in 0.9 % saline and examined for the presence of helminths using a Zeiss DV4 Stereomicroscope (Carl Zeiss, Göttingen, Germany). Specimens for microscopy were fixed in hot (≈ 40°C) triethanolamine-formalin (TAF, Courtney et al. 1955), or 70% ethanol at room temperature. Specimens for DNA analysis were transferred live to 96% molecular-grade ethanol at room temperature.

For light microscopy (LM), specimens were cleared in glycerine following the method described in Hooper (1970) and mounted following Ryss (2017) using Cobb's hanging slides (Cobb 1917). Photomicrographs and measurements were taken with a Zeiss Axioplan 2 Imaging Light Microscope with Axiovision 4.7.2 software (Carl Zeiss) and darkfield, phase contrast and differential interference contrast (DIC). Minimum and maximum values are given and presented in µm, unless otherwise stated. Drawings of relevant structures were made from photomicrographs with CorelDRAW X6 (2012, Corel Company, Ottawa, Canada).

For scanning electron microscopy (SEM) study, five adult females, two immature females and six adult males (with the left spicules extended) were selected from the samples stored in 70% ethanol. Specimens were dehydrated, transferred through increasing concentrations of hexamethyldisilazane (HMDS) (Merck, Darmstadt, Germany), mounted, and dried in a Sanpla Dry Keeper desiccator cabinet (Kita-Ku, Osaka, Japan). Specimens were sputter-coated with gold using an Emscope SC500 sputter coater (Quorum Technologies, Newhaven, UK) and studied using a VEGA 3 LMH SEM (Tescan, Brno, Czech Republic), at 3–6 kV. To study isolated spicules, the spicules of 12 males (fixed in 70% ethanol) were

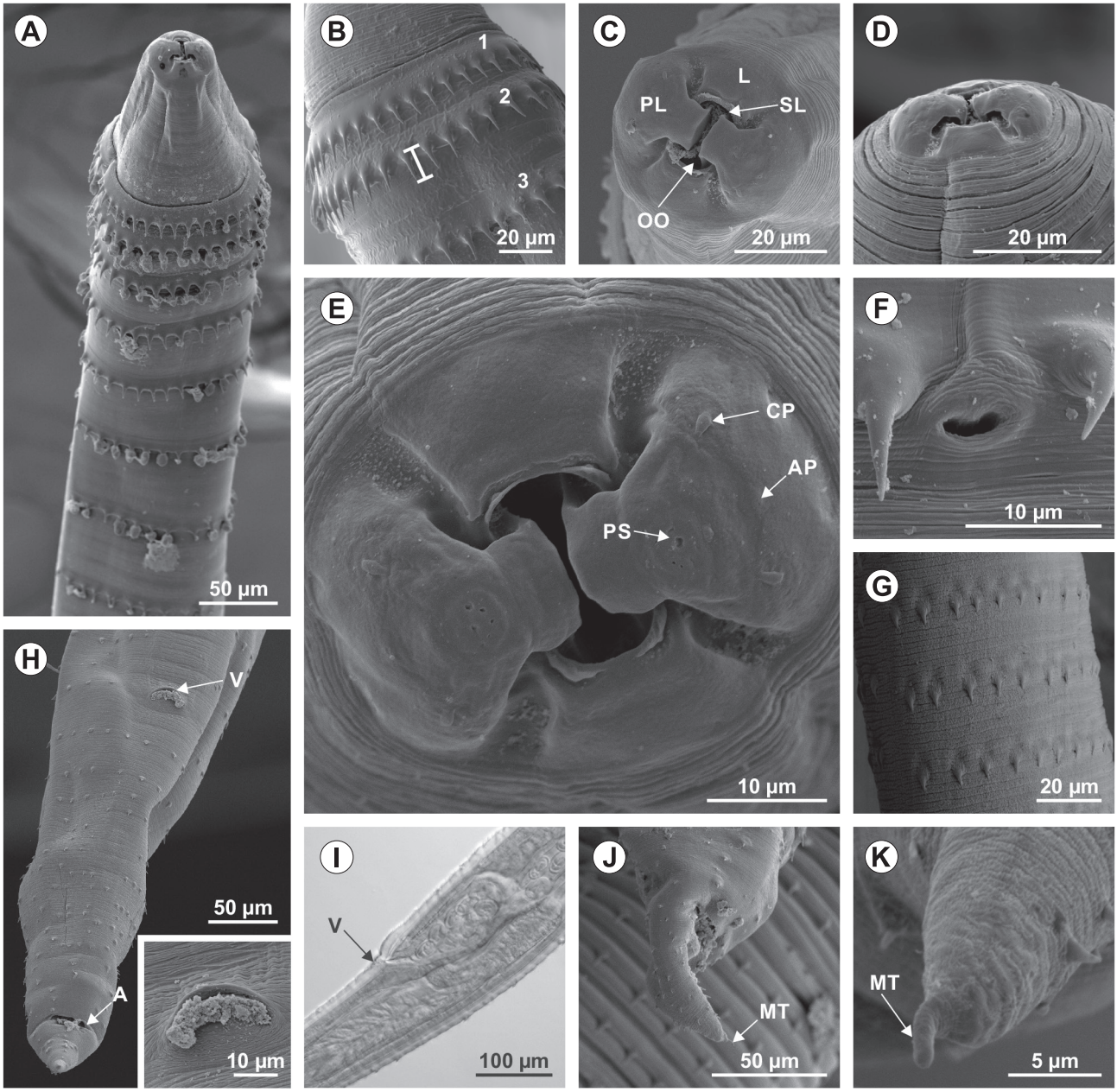

Fig. 2. Light and scanning electron micrographs of adult females of *Spinitectus petterae* Boomker, 1993 collected from *Clarias gariepinus* (Burchell)**. A** – neck showing spines on annular rings; **B** – first three rings on neck, rings indicated numerically and spine length measurement illustrated; **C** – apical view of the cephalic region; **D** – lateral view of cephalic region; **E** – apical view of cephalic structures; **F** – excretory pore; **G** – diminishing spines; **H** – posterior end; inlay gonopore with vulva **I** – posterior end with gonopore, vulva position indicated; **J** – conical tail tip; **K** – conical tail and mucron tip. *Abbreviations*: A – anus; AP – amphid; CP – cephalic papillae; L – labia; MT – mucron tip; PL – pseudolabia; PS – porous structure; OO – oral opening; V – vulva; SL – sublabium.

excised, digested and prepared for SEM following combination of the methods by Rammah and Hirschmann (1987), Dos Santos and Avenant-Oldewage (2015) and Dos Santos et al. (2019).

To identify specimens to genus level, the key provided by Anderson et al. (2009) was used. To identify them to species level, the key of Moravec (2019) was used. The morphology of the specimens was then compared to previous descriptions of both species occurring in southern Africa (Boomker 1993, Moravec and Van As 2015, Moravec and Jirků 2017), as well as other species of *Spinitectus* recorded from *C. gariepinus*. Type material (holotype, allotype and paratypes) of both *S. petterae* and *S. zambezensis* (*S. polli*) was requested on multiple occasions from the Museum National d'Histoire Naturelle (MNHN) for morphological comparison, but to no avail.

Specimens in 96% ethanol collected from the Vaal Dam reservoir (n = 6), downstream of the Vaal River Barrage (n = 2), downstream of Grootdraai Dam (n = 2), and in the Crocodile River (n = 2) were used for genetic characterisation. Specimens were rehydrated, sectioned into proximal, middle and distal sections, with proximal and distal sections returned to 96 % ethanol for morphological study. Genomic DNA was extracted from the middle sections using a DNeasy® Blood & Tissue Kit (QIAGEN®, Inc., Manchester, UK).

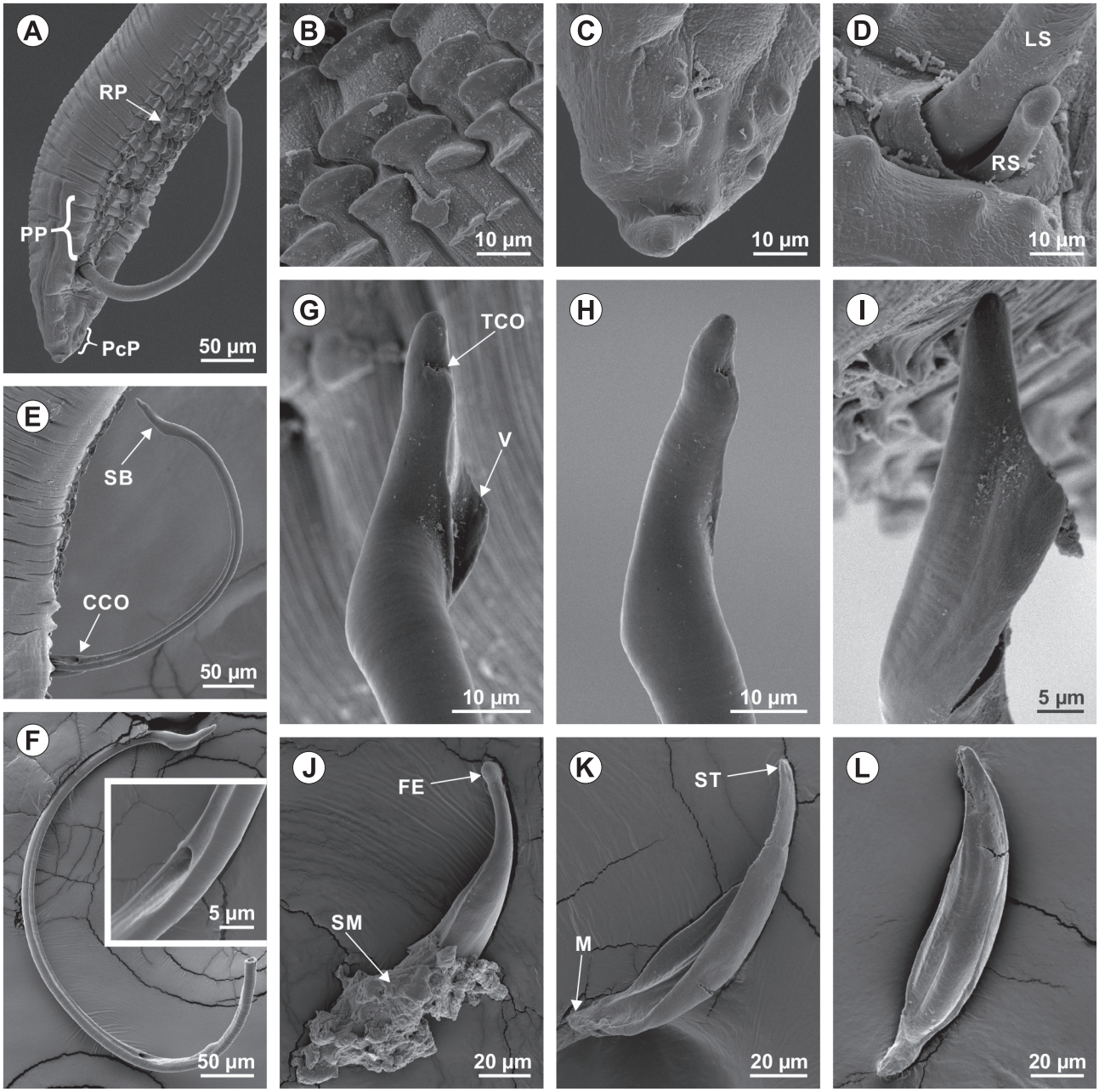

Fig. 3. Scanning electron micrographs of adult males of *Spinitectus petterae* Boomker, 1993 collected from *Clarias gariepinus* (Burchell). **A** – posterior section with extended left spicule; **B** – rugosa plates; **C** – two pairs of three post-cloacal papillae; **D** – base of left spicule and tip of right spicule protruding from cloaca; **E** – left spicule extended to cytoplasmic core opening; **F** – excised left spicule with inlay of cytoplasmic core opening; **G** – spicule blade with V-shaped velum, and terminal cytoplasmic core opening; **H** – spicule blade; **I** – ventral view of velum; **J** – excised and undigested right spicule; **K**, **L** – lateral and ventral aspect of the digested right spicule, respectively. *Abbreviations*: CCO – cytoplasmic core opening; FE – fleshy extension; LS – left spicule; M – manubrium; PcP – postcloacal papillae; PP – precloacal papillae; RP – rugosa plates; RS – right spicule; SB – spicule blade; SM – spicule muscle; ST – spicule tip; TCO – terminal cytoplasmic core opening; V – velum.

A region of the 18S rDNA was amplified with primers NEM18SF (5'– CGC GAA TRG CTC ATT ACA ACA GC–3') (Floyd et al. 2005) and 9r (5'–GAT CCT TCC GCA GGT TCA CCT AC–3') (Giribet et al. 1996). PCR conditions involved 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 54°C, 2 min at 72°C, and a final elongation of 10 min at 72°C. Partial 28S rDNA (D1–D3) was amplified with primers ZX-1 (5'-ACC CGC TGA ATT TAA GCA TAT-3') (Van der Auwera et al. 1994, Waeschenbach et al. 2007) and 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') (Olson et al. 2003, Tkach et al. 2003) following cycling conditions of Scholz et al. (2013). A portion of *cox*1 mtDNA was amplified using primers COIintF (5'–TGA TTG GTG GTT TTG GTA A-3′) and COIintR (5'-ATA AGT ACG AGT ATC AAT ATC-3′) (Casiraghi et al. 2001) following cycling conditions of Lagunas-Calvo et al. (2019), increasing the annealing temperature to 52° C.

To establish if amplification was successful, samples were run on 1% agarose gel containing GelRed® Nucleic Acid Gel Stain (Biotium Inc., Fremont, California) and examined using a UV transilluminator. Amplicons were sequenced following

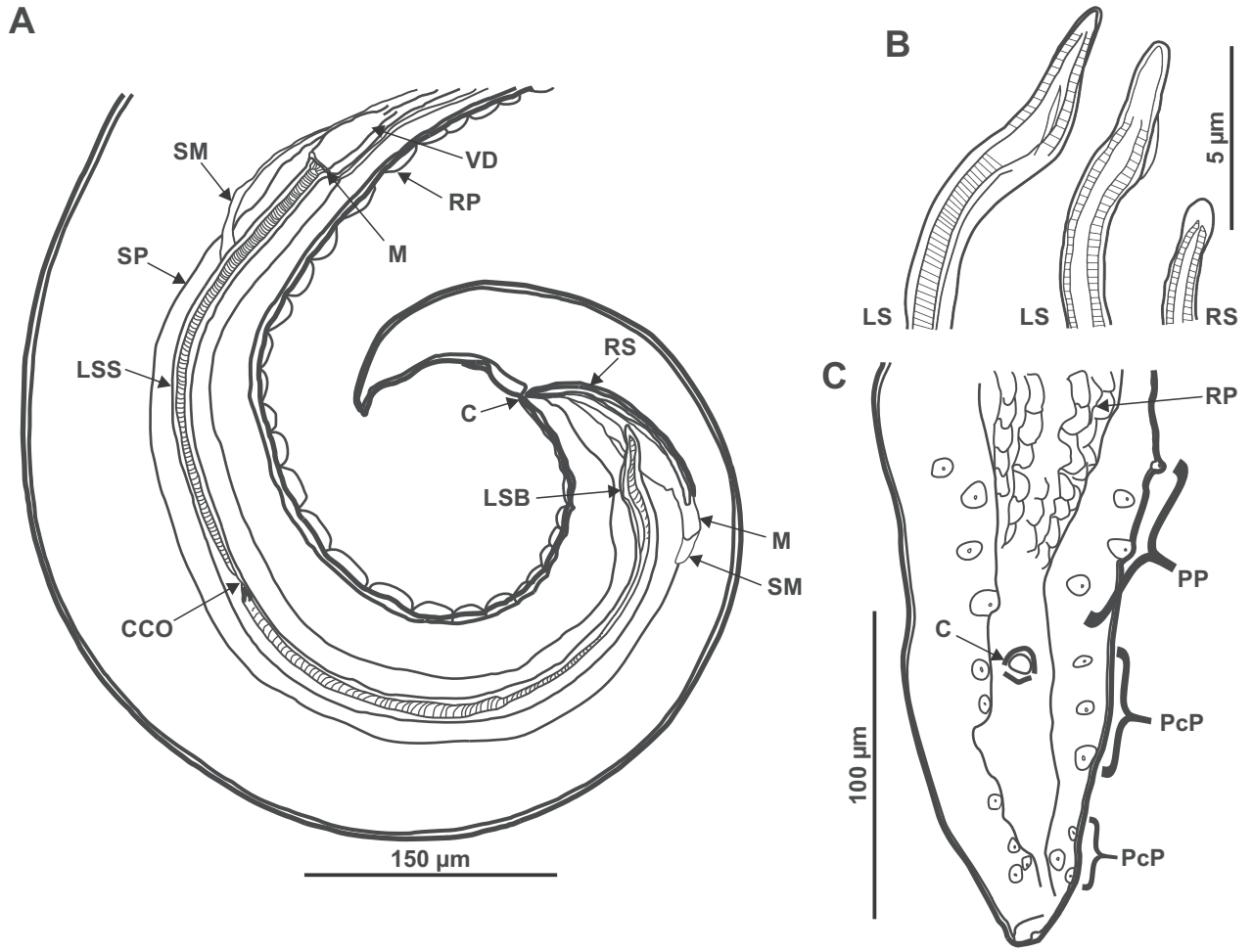

Fig. 4. Illustrations of *Spinitectus petterae* Boomker, 1993 – male, reproductive structures and tail end. **A** – lateral aspect of posterior section with left and right spicules, and associated structures; **B** – tip of left spicule from two views and tip of the right spicule with fleshy extension; **C** – ventral aspect of posterior section with caudal papillae and cloacal opening. *Abbreviation*: C – cloacal opening; CCO – cytoplasmic core opening; LS – left spicule; LSB – left spicule blade; LSS – left spicule shaft; M – manubrium; PcP – postcloacal papillae; PP – precloacal papillae; RP – rugosa plates; RS – right spicule; SM – spicule muscle; SP – spicular pouch; VD – vas deferens.

Avenant-Oldewage et al. (2014) in both directions. Sequencing was done using mostly PCR primers, but alternative or internal primers were used where necessary for 18S and 28S rDNA regions (see Supplementary Table S1). Sequences were analysed, merged, aligned and primers removed in Geneious Prime 2022.1.1 (https://www.geneious.com). BLAST analyses (Altschul et al. 1997) were used to identify similar sequences and all relevant *Spinitectus* spp. data were retrieved from GenBank (Benson et al. 2005) and aligned with data obtained during the current study. Representative data for *Rhabdochona xiphophori* Caspeta-Mandujano, Moravec et Salgado-Maldonado, 2001 were included as outgroup for all analyses, together with *Rhabdochona mazeedi* Prasad et Sahay, 1965 for 18S rDNA only, based on proximity to obtained haplotypes in BLAST analyses.

Using MEGA 6 (Kumar et al. 2016), distance estimation was calculated based on average uncorrected *p*-distance (1,000 bootstrap replicates), including transitions and transversions. Evolutionary histories were only assessed for 18S rDNA and *cox*1 mtDNA due to the lack of available 28S rDNA for *Spinitectus* spp. Both Bayesian inference (BI) and maximum likelihood (Tamura and Nei 1993) approaches were used as implemented in BEAST v2.5.0 (Bouckaert et al 2014) and MEGA 7, respectively. Ten million Markov chain Monte Carlo (MCMC) generations were utilised for BI analyses. The Kimura 2-parameter model (Kimura 1980) with gamma distribution [5 categories (+G, parameter = 0.5)] and invariable sites was used for ML analyses of 18S rDNA, while the *cox*1 mtDNA topology was generated using the Tamura-Nei model (Tamura and Nei 1993) with gamma distribution [5 categories (+G, parameter = 0.19)]. Bootstrap support (1,000 replicates) was used (Felsenstein 1985). A single phylogram based on BI is presented with support for both approaches indicated (BI/ML), with nodes with support lower than 0.5 posterior probability or 50 % bootstrap support not annotated or partially annotated with a hyphen ("-") for values lower than the limit.

RESULTS

Family Rhabdochonidae Skrjabin, 1946

***Spinitectus petterae* Boomker, 1993** Figs. 2–5

Material examined: ten females, ten males and four immature females using LM; five females, six males, 12 sets of spicules and two immature females using SEM.

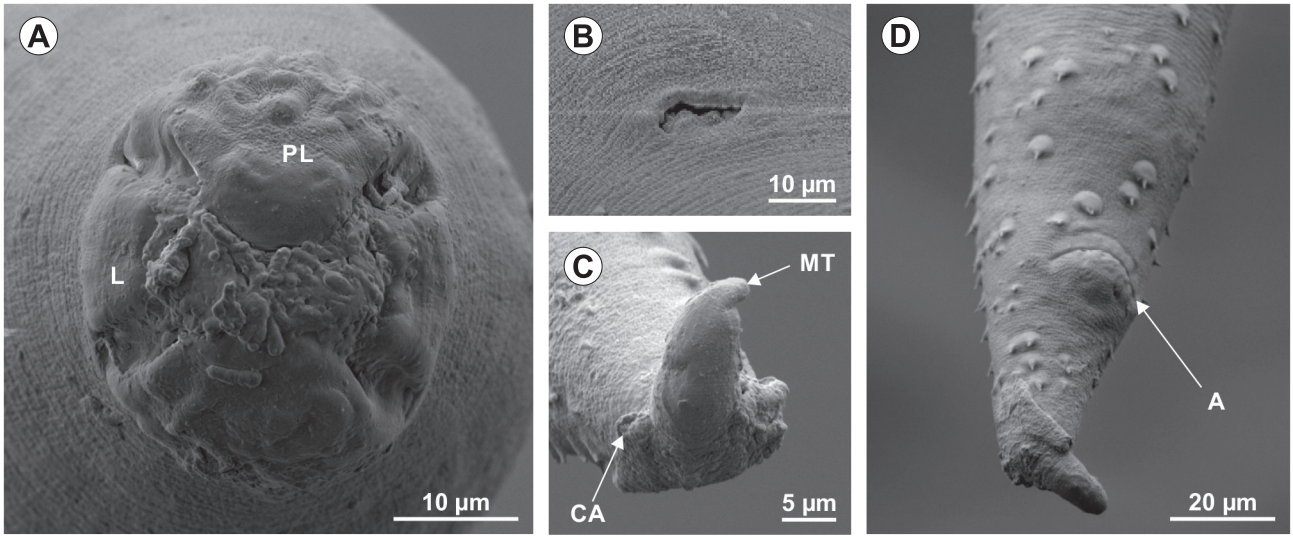

Fig. 5. Scanning electron micrographs of immature female of *Spinitectus petterae* Boomker, 1993 collected from *Clarias gariepinus* (Burchell). **A** – apical view of cephalic region; **B** – vulva; **C** – conical tail end; **D** – conical tail. *Abbrevations*: A – anus; CA – caudal papilla; L – labium; MT – mucron tip; PL – pseudolabium.

Description (for measurements: see Supplementary Tables S2, S3 and S4).

Adult. Specimens small to medium-sized (length 7–12 mm). Intestine brown, remainder of body white and opaque. Gravid females (9.4–12.0 mm) larger than adult males (7.0–9.5 mm). Blunt and rounded cephalic area (Fig. 2A,C,D), elongated body, conical tail at posterior end (Figs. 2H, 3A). Cuticle surface bears annular spines separated into half-circles by lateral line (Fig. 2A,B). First five rings largest, with first two rings closer together. Subsequent spines diminish in size and organisation towards posterior (Fig. 2G,H). Females with spines throughout whole body, males only with spines to their midbody (Fig. 3A). Elongated dorsoventral oral aperture on cephalic area, flanked by two large, flat labia, ventrally and dorsally (Fig. 2C–E). Each labium with broad base, smaller, simple, slightly sclerotised and slender sublabium bordering oral opening. Lateral to oral opening, large, broad, pseudolabia project medially, fold into oral opening. Two elongated submedian cephalic papillae interspersed by two lateral amphids at base of each pseudolabium (Fig. 2E). No deirids observed. Most specimens display thin-walled, distinctly widened vestibule with posterior prostom. Some specimens (three of nine) showed collapsed vestibule. Telostom present, both relaxed and contracted states observed. Alternate states of vestibule and telostom impacted other morphometric characters such as distance of nerve ring to vestibule and anterior extremity. Oesophagus divided into anterior muscular section (more slender and shorter than wide) and long glandular section. Nerve ring location variable, mostly encircling muscular oesophagus. Excretory pore located at fourth annular ring (Fig. 2F).

Male. Ten randomly selected males measured (Supplementary Table S3). Total body length 7.0–9.5 mm, maximum width 100–189. First ring of spines 147–182 from anterior. Number of spines: 39–50 in first, 40–47 in second, 36–48 in third ring. Length of spines 13–16 in first, 15–18 in second, 14–16 in third ring. Two individuals with extra ring of 4 spines between second and third rings. At postoesophageal region, cuticular spines become smaller, sparser, begin to lose annular pattern, absent at posterior (Fig. 3A). Vestibule plus prostom length 47–64, uncollapsed vestibule 11–20 long and 14–23 wide, prostom 30–48 long and 6–9 wide. Relaxed telostom 10–17 long and 7–15. Contracted telostom length 5–12 and width 14–28. Relaxed telostom generally longer, thinner than in contracted state. Muscular oesophagus 408–563 long, glandular oesophagus 1.5–2.7 mm long, ratio of muscular to glandular oesophagus lengths 1 : 3.1–5.1. Vestibule plus total oesophagus length 2.0–3.3 mm, percentage of vestibule plus oesophagus of total body length 24–41%. Nerve ring 117–285 from anterior extremity, 59–339 from vestibule. Excretory pore on fourth ring, 277–379 from anterior extremity, 248–329 from vestibule.

Caudal alae absent. Tail 144–191 long, mucron tip terminal, 2–5 long. Rugosa ridges present on ventral surface, 10 rows (Figs. 3A,B, 4A,C). Pedunculated preanal papillae in four pairs, postanal papillae in three pairs, distal group of three pairs (Figs. 3A,C, 4A,C). Left spicule delicate, with internal supporting rings 0.7–1.0 mm long, shaft length 280–553, left spicule shaft percentage of total spicule 40–53 %. Left spicule covered by spicule pouch; pouch attached to spicular muscle (Fig. 4A). Manubrium at base of spicule, vas deferens connected to manubrium (Figs. 3K, 4A). Lumen of spicule cytoplasmic core opens at anterior third of spicule, and terminates distally (Figs. 3E–H, 4A). Proximal cytoplasmic core opening observed in scanning electron microscopy, resembles arch in LM. Blade of left spicule with V-shaped vellum, twisting around blade, distal cytoplasmic core opening (Figs. 3G–I, 4B). V-shaped vellum resembles spine-like structure in LM. Right spicule small, robust 124–162 long, including distended fleshy membrane 131–170 long (Fig. 3J). Right spicule shallow, boat-like structure with fleshy tip in undigested SEM specimens. Once digested, right spicule structure appears deep-

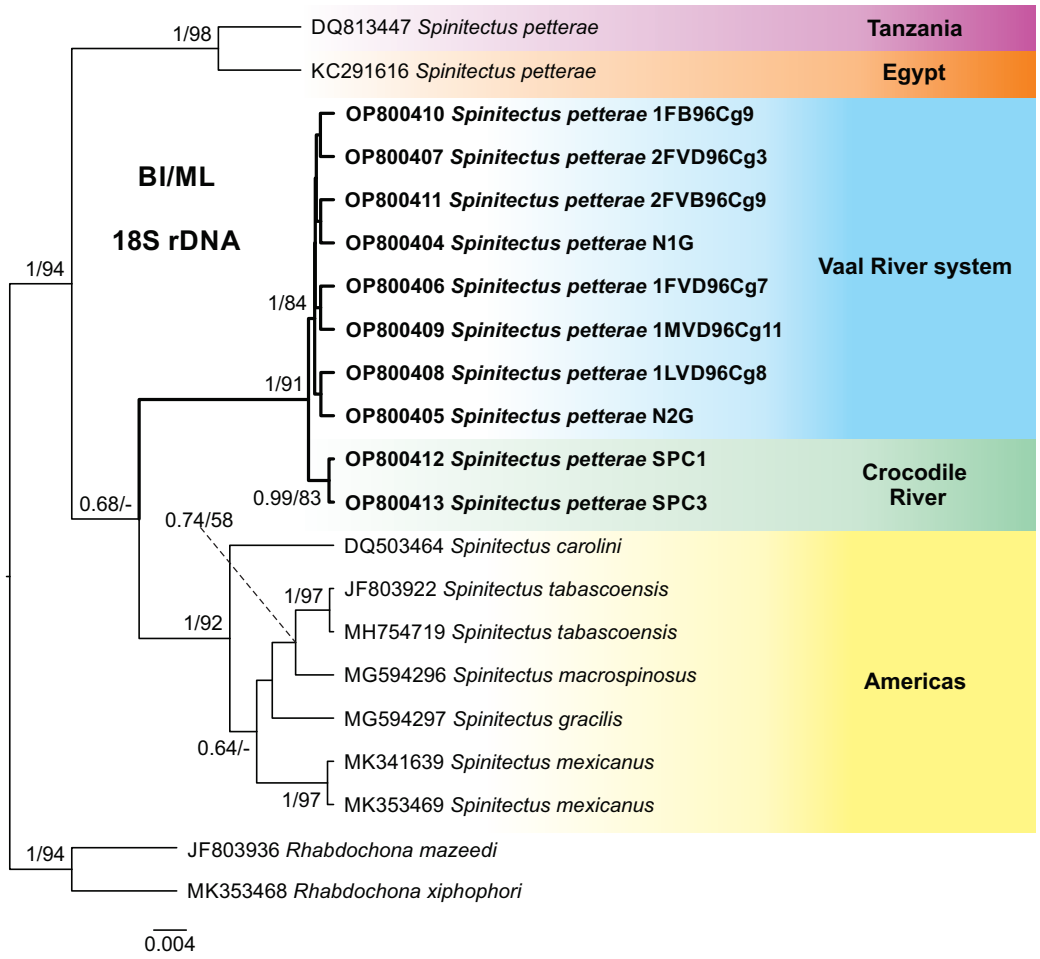

Fig. 6. Phylogenetic relationships of *Spinitectus* spp. based on available 18S rDNA, based on the Bayesian (BI) method, with *Rhabdochona xiphophori* Caspeta-Mandujano, Moravec et Salgado-Maldonado, 2001 and *Rhabdochona mazeedi* Prasad et Sahay, 1965 serving as outgroups. Posterior probability (BI) and 1,000 bootstrap replicate (maximum likelihood (ML)) support indicated (BI/ML), nodes with less than 0.5 (50 %) support not annotated. Data shaded in colour indicate geographical locality or river system.

er with sharp (Fig. 3K,L), fleshy tip, and hollow cytoplasmic core opening visible in LM.

Female. (10 randomly selected gravid females measured – Supplementary Table S2). Total body length 9.4–12.0 mm, maximum width 222–292. First ring of spines 170–192 from anterior extremity. Number of spines: 40–50 in first, 39–52 in second, 37–49 in third ring. Length of spines (measured from spine tip to connection with collar – Fig. 2A,B): 14.0–16.3 in first, 15.5–18.6 in second, 15.5–17.8 in third ring. Three individuals with extra spine ring with 1–9 spines between second and third rings. In post-oesophageal region, cuticular spines become smaller, sparser, begin to lose annular pattern, dissipating towards tail (Fig. 2A,G,H). Vestibule plus prostom 51–80 long, vestibule 15–20 long and 4–22 wide, prostom 36–49 long and 6–9 wide. Relaxed telostom length 11–17 and width 12–22. Contracted telostom length 8–20 and width 16–22. Relaxed telostom generally longer, thinner than contracted one.

Muscular oesophagus length 482–648, glandular oesophagus length 1.4–2.2 mm, ratio of muscular to glandular oesophagus lengths 1 : 2.5–4.7. Vestibule plus total oesophagus length 2 mm, percentage of vestibule plus oesophagus to body length 19–29 %. Nerve ring 73–176 from anterior, 14–115 from vestibule. Excretory pore 363–399 from anterior and 296–338 from vestibule. Vulva not protruding (Fig. 2H,I), in posterior half of body, 9.0–11.4 mm from anterior body, 280–532 from anus, 353–632 from tail tip. Vulva 94–96% from anterior end. Reproductive structure mono-opisthodelphic, ovary with two oviducts posterior to uterus and muscular vagina, all directed anteriorly (Fig. 2I). Fully developed eggs present in uterus of gravid females. Eggs oval, smooth, lacking accessories, thick walled, larvated; 33–36 long and 21–25 wide. Tail 71–99 long, with small spines, terminal mucron tip 1.1–6.0 long (Fig. 2J,K).

Immature female. (four immature females measured Supplementary Table S4). Total body length 8.3–9.1 mm, maximum width 210–257. Cephalic structures underdeveloped (Fig. 5A). First ring of spines 56–70 from anterior. Number of spines generally lower than in adult females: 41–43 in first, 42–47 in second, 36–48 in third ring. Length of spines; 12–13 in first, 15–17 in second, 13–15 in third ring. In postoesophageal region, cuticular spines become smaller, sparse, begin to lose annular pattern. Prostom plus

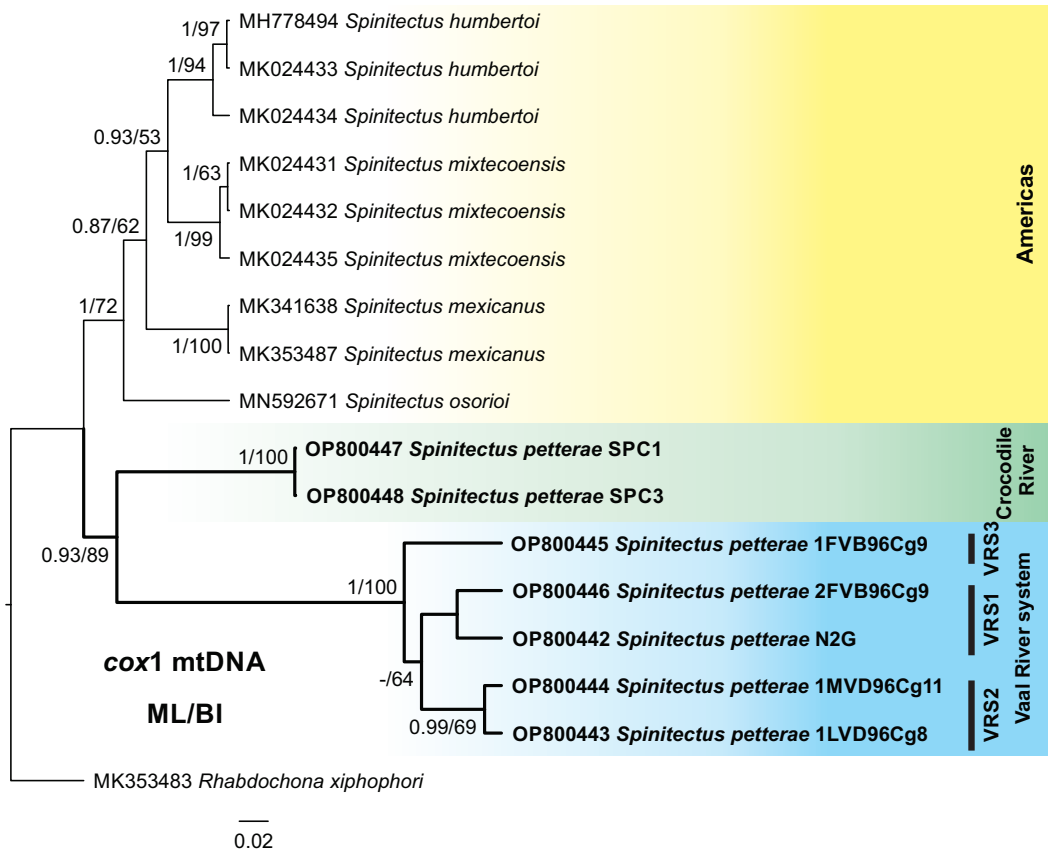

Fig. 7. Phylogenetic relationships of *Spinitectus* spp. based on available *cox*1 mtDNA for *Spinitectus* based on Bayesian inference (BI)), with *Rhabdochona xiphophori* Caspeta-Mandujano, Moravec et Salgado-Maldonado, 2001 as the designated outgroup. Posterior probability (BI) and 1,000 bootstrap replicate (maximum likelihood (ML)) support indicated (BI/ML), nodes with less than 0.5 (50 %) support not annotated. Data shaded in colour from indicated geographical locality or river system, and three haplotypes recorded from the Vaal River system indicated (VRS1–VRS3).

vestibule 54–64 long, prostom 47–50 long, vestibule 8–15 long.

Muscular oesophagus length 304–428, glandular oesophagus 0.7–1.8 mm long, ratio of muscular to glandular 1 : 2.1–5.0. Vestibule plus whole oesophagus length 2.0–2.9 mm; ratio of oesophagus plus vestibule length to total body length 20–30%. Nerve ring not observed. Excretory pore situated between row 1, 3, 4 and 5, 9–214 from anterior extremity. Vulva not protruding, located in posterior-half of body (Fig. 5B), 7.8–8.8 mm from anterior end, 277–429 from anus. Muscular vagina connected to uterus anteriorly. Oval, smooth, unfertilised eggs in uterus, 33–37 long and 20–22 wide. Tail 74–88 long; small, dispersed spines anterior to mucron, mucron length 2–6 (Fig. 5C,D). Two caudal papillae lateral to mucron.

Genetic characterisation

Of the 12 specimens sequenced, all from the Vaal Dam reservoir, the Vaal Barrage, and the Crocodile River produced usable 18S rDNA data, whereas those from the Grootdraai Dam did not. From all samples collected in the Vaal River system (VRS), a single 18S rDNA haplotype was obtained (1,162–1,651 bp depending on primers used), while those from the Crocodile River (CR) displayed a second haplotype (1,617–1,631 bp). The 18S rDNA haplotypes were aligned to available sequence data on *Spinitectus* spp., with *Rhabdochona mazeedi* and *R. xiphophori* serving as outgroups, and trimmed to the primer JLR24 (Campos et al. 1998) due to the short coverage of some published data. The final alignment was 880 bp long, with 806 bp represented by conserved sites, 72 bp variable sites and 51 bp parsimony-informative sites.

For included published 18S rDNA sequence data on species of *Spinitectus*, interspecific distances of 0.41–4.58% (3–29 bp) and intraspecific distances up to 1.79% (9 bp) were calculated (Table 1). The observed intra- and interspecific specific distances overlapped, with a high intraspecific limit observed between the data for *Spinitectus petterae* from Tanzania and Egypt. This was in contrast to other species for which multiple sequences were available because they were identical. *Spinitectus* samples from the VRS were 0.23% (2 bp) distant to the *Spinitectus* samples collected from the CR, less than the calculated interspecific range for the genus, supporting their similarity. However, there was a much greater distance between *S. petterae* data from Egypt (KC291616) to *Spinitectus* specimens from the VRS (3.59%; 18 bp) and the CR (3.39%; 17 bp) than those from Tanzania (DQ813447) compared for the same sites (VRS 1.99%; 10 bp; CR 1.79%; 9 bp), falling within the interspecific range for the family.

Regarding *cox*1 mtDNA, three haplotypes (in total 649 bp long) were observed from specimens collected in the VRS (VRS2 exclusively from the Vaal Dam reservoir, VRS3 exclusively from the Vaal River Barrage, and VRS1 from both localities), while a fourth haplotype was observed for all specimens from the CR (649 bp). The alignment with published data was 649 bp, with 474 bp conserved, 175 bp variable, and 146 bp parsimony-informative sites. From the included *cox*1 mtDNA data on isolates of *Spinitectus*, intraspecific distances of up to 2.56% (11 bp) were observed and an interspecific range of 6.98–11.16% (28–72 bp) was calculated (Table 2). The three haplotypes obtained for specimens from the VRS were 0.15-0.31% (1–2 bp) from each other, confirming their conspecificity. However, the haplotypes from the VRS were 14.02–14.33% (91–93 bp) distant to those in the CR, far exceeding the upper intraspecific limit calculated for the genus.

For 28S rDNA a single haplotype (1,277–1282 bp) was observed in specimens collected in the Vaal River system (one specimen from the Vaal Dam reservoir and two from the Vaal River Barrage), while the second haplotype was observed for all specimens from the Crocodile River (1,245–1,302 bp). The alignment with published data was 1,353 bp, with 973 bp conserved, 333 bp variable and 264 bp parsimony-informative sites. Specimens from the Vaal River system were 3.31% (42 bp) distant to those from the Crocodile River near the type locality of *S. petterae* (Table 3). Unfortunately, 28S rDNA data for only one species of *Spinitectus* are available, namely *Spinitectus mexicanus* Caspeta-Mandujano, Moravec et Salgado-Maldonado, 2000, and is 22.83–22.96% (245 bp) from the haplotypes generated here. Thus, no intra- or intraspecific ranges could be calculated for this marker.

Both phylogenetic approaches produced similar results for 18S rDNA and *cox*1 mtDNA data. Based on 18S rDNA (Fig. 6), species of *Spinitectus* formed three distinct clades, one containing taxa from the Americas, another containing *S. petterae* from Tanzania and Egypt, and the other the data generated in the present study. Sequence data for *Spinitectus* specimens from the Crocodile River and the Vaal River system formed a well-supported monophyletic clade, with specimens from these systems forming well-supported sister groups within this clade. The clade containing *S. petterae* from Tanzania and Egypt is basal to all other isolates of *Spinitectus*, even though the node responsible for this is not well supported. This clade appears distant to the isolates sequenced in the present study. However, in the ML topology, all *S. petterae* isolates formed a very weakly supported monophyletic clade. Within the Americas clade, all taxa formed well-supported monophyletic clades.

The tree based on *cox*1 mtDNA (Fig. 7) provided similar results with two well-supported clades, one with isolates from Africa and the second with isolates from the Americas. Again, specimens of *S. petterae* from southern Africa formed a well-supported monophyletic clade and two well-supported sister groups were present in this clade, suggesting two distinct lineages. Although the three haplotypes recorded for *cox*1 mtDNA for data from the VRS separated in the presented topology, they all grouped together in a well-supported clade.

Taxonomic summary

Type host: African sharptooth catfish, *Clarias gariepinus* (Burchell).

Other host: Werner's catfish, *Clarias werneri* Boulenger.

Type locality: Crocodile River, Kruger National Park, South Africa.

New localities: Downstream of Vaal River Barrage (26.7321S, 27.6330E); Vaal Dam Reservoir (26.8662S, 28.1601E); downstream of Grootdraai Dam (26.9218S, 29.2815E); Crocodile River in Mpumalanga (25.4365S, 30.8693E), all South Africa.

Site of infection: Stomach.

Infection parameters: See Austin et al. (2022).

Voucher material deposited: Three males, three females and one immature female in the Iziko South African Museum, Cape Town, South Africa (SAMC-A094702–8). Three males and three females in the Natural History Museum, London, United Kingdom (NHMUK 2022.10.13.11–16). Three males and three females in the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS N-1274).

Representative DNA sequences: Newly generated sequences are deposited in the GenBank database: 18S rDNA (OP800404–OP800413), 28S rDNA (OP802563–OP802567) and *cox*1 mtDNA (OP800442–OP800448).

Remarks. The specimens studied closely resembled *S. petterae*. However, using the key to *Spinitectus* spp. parasitising freshwater fishes in Africa by Moravec (2019), the specimens could represent *Spinitectus monstrosus* Boomker et Puylaert, 1994. This was disregarded due to morphological discrepancies and the host group of *S. monstrosus* (Mormyridae). The present material was also compared with *Spinitectus allaeri* as this nematode has been recorded from the same clariid host, but the morphology was not compatible. Comparing the morphometrics of the specimens in this study to that of *S. petterae* described by Boomker (1993) and reported by Mwita and Nkwengulila (2004), only a limited number of minor differences were observed (Tables 4, 5). These were considered intraspecific, supported by the morphological variability of other *Spinitectus* species from Africa (Moravec 2019).

DISCUSSION

The nematodes collected from the stomach of *Clarias gariepinus* in the Vaal River system most closely resembled *Spinitectus petterae*, with only minor morphological differences. Differences included male total body length measuring 7.0–9.5 mm (4.3–5.8 mm according to Boomker 1993; 3.0–5.8 mm according to Mwita and Nkwengulila 2004), and female total body length 9.4–12.0 mm (4.4–6.4 mm – Boomker 1993; 3.0–5.8 mm – Mwita and Nkwengulila 2004). Additionally, cephalic structures on the pseudolabia were similar to those recorded by Boomker (1993) when using LM. However, only one pair of cephalic papillae was observed using SEM. Instead, porous structures on the pseudolabia, similar to those of *Spinitectus polli* observed

by Moravec and Jirků (2017), were observed for the first time for *S. petterae* using SEM. As the presumed second pair of "cephalic papillae" was only observed using LM (in the same regions as the porous structures), this alludes to the benefits of using SEM. The first annular ring displayed similar numbers of spines in all studies. However, an extra ring with varying numbers of spines was observed in some specimens posterior to the third ring in the present study. Boomker (1993) also noted an extra ring on the first ring in the population he studied, thus indicating this may be an intraspecific variation.

Length of muscular oesophagus of males (408–563 µm) differed from those reported by Boomker (1993), 230–372 µm, but overlapped with those reported by Mwita and Nkwengulila (2004), i.e., 353–698 µm. Male glandular oesophagus length (1.5–2.7 mm) overlapped with those reported by Boomker (1993), 1.0–1.6 mm, and differed from those described by Mwita and Nkwengulila (2004), i.e., 0.9–1.3 mm. The ratio of the male muscular to glandular oesophagus 1 : 3.1–5.1 overlapped with that reported by Boomker (1993), 1 : 2.7–3.6, but differed substantially from that calculated from the measurements provided by Mwita and Nkwengulila (2004), i.e., 1 : 1.9–2.6. The ratio of left to right spicules 1 : 4.2–7.0 differed compared to 1 : 3.4–3.6 recorded by Boomker (1993). The left spicule blade is similar to what Boomker (1993) reported, except for a fleshy extension not observed in this study. No caudal alae were observed with either LM or SEM, but Boomker (1993) reported only weakly developed caudal alae.

Samples from the Vaal Dam reservoir and Vaal River Barrage produced identical 18S and 28S rDNA haplotypes and highly similar *cox*1 mtDNA haplotypes, thus likely representing the same taxon. Due to the similarity of the 18S rDNA fragment analysed for *Spinitectus* samples collected near the type locality of *S. petterae* in the CR and the samples collected from the VRS, the *Spinitectus* samples from the VRS are likely *S. petterae.* This is based on the interspecific distances calculated for species of *Spinitectus* based on available 18S rDNA data, as well as the produced phylogenies. However, the distance between the data for *S. petterae* from Tanzania and Egypt, and those from southern Africa in the present study are greater than the interspecific distances for species of *Spinitectus* from the Americas. These geographical haplotypes (Egypt, Tanzania and southern Africa) thus likely represent distinct taxa, with those in southern Africa representing *S. petterae* due to the proximity to the type locality.

The number of spines in female nematodes studied by Boomker (1993) and those by Mwita and Nkwengulila (2004) does not overlap, indicating possible morphological variation, but the material studied presently overlaps with both of these. However, the samples studied by Mwita and Nkwengulila (2004) may not represent the same taxon as those genetically characterised by Mwita and Nkwengulila (2010), because the morphologically studied specimens were from *C. gariepinus* while those in the molecular study were from *Clarias werneri*. The GenBank record for both isolates of *S. petterae*, from Tanzania provided by Mwita and Nkwengulila (2010) and the unpublished data from Egypt, have *C. gariepinus* noted as the host, indicating the need for revision of this data. Additionally, while the haplotypes from Tanzania and Egypt are 17.3% (9 bp) different, most base pair differences appear to be due to transversions at genus-conserved regions in the unpublished sequence from Egypt, indicating possible errors in the data. Alternatively, *S. petterae* may display a drastic level of geographic intraspecific variability in 18S rDNA.

The genetic distance between the *cox*1 mtDNA of *Spinitectus* specimens in the VRS and the CR needs further attention. Current *cox*1 mtDNA data would suggest that these populations represent distinct molecular observable taxonomic units (MOTU). However, comparing the morphometric data gathered in the present study to that of Boomker (1993), no robust morphologic characteristic could be identified to distinguish the phenotypes of different populations. The presence of two distinct lineages may indicate the presence of a cryptic species complex, but the results presented here do not support this assumption. As such, extensive population and geographic variability at certain gene regions may be more likely in this case. The monophyly of isolates generated in the current study still supports the relatedness of the *cox*1 mtDNA haplotypes. Limited studies on the genetic attributes of *Spinitectus* spp. are currently available, with most studies generating data for biodiversity and higher phylogeny (Smythe et al. 2006, Mwita and Nkwengulila 2010, Černotíková et al. 2011, Choudhury and Nadler 2018), species descriptions (Barrios-Gutiérrez et al. 2019), and generation of data on *Spinitectus* spp. as an outgroup to study other nematode groups (Santacruz et al. 2020, Caspeta-Mandujano et al. 2021). More thorough morphological examinations, ideally of type material and additional genetic markers, could be beneficial to further investigating species of *Spinitectus* in Africa.

Except for the record by Mwita and Nkwengulila (2004), all records of *S. petterae* have been from *C. gariepinus*. Therefore, the genetic and morphological variation of nematodes from the same host is probably not due to the definitive host. *Clarias gariepinus* is an omnivorous host (Groenewald 1964) with gills and pseudobranchs that allow it to relocate across land between water bodies (Skelton 2001). The intermediate host of *S. petterae* is probably an aquatic insect, as similar insects have been successfully infected with other species of *Spinitectus* in laboratory studies (Keppner 1975, Jilek and Crites 1982). Adult insects may fly between water bodies; it is, therefore, conceivable that the same nematode species can occur in both river catchments due to their proximity in their upper reaches. The intermediate host range and specificity of these nematodes to their intermediate hosts may also help elucidate the high genetic variation observed. Unfortunately, very little is known regarding the life cycle of species of *Spinitectus* in Africa.

In conclusion, this study expands the geographical distribution of *S. petterae* to the Vaal River system and provides additional morphological and genetic data for this species. Additional morphological data included measurements of the prostom, telostom and mucron, more detailed

measurements of the left and right spicules, and the first scanning electron microscopy of *S. petterae,* including excised spicules. The present study is the first to produce 28S rDNA and *cox*1 mtDNA for *S. petterae*, alongside 18S rDNA from specimens collected near the type locality of the species.

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