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New information on morphology and molecular data of camallanid nematodes parasitising *Xenopus laevis* (Anura: Pipidae) in South Africa

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Abstract: Three species of nematodes from the Camallanidae that are known to infect *Xenopus laevis* Daudin (Anura: Pipidae) were collected from several localities across South Africa. New data on morphology, partial 28S and *cox1* genes, infection levels and distribution are presented herein. The most common species, *Batrachocamallanus slomei* Southwell et Kirshner, 1937, from the stomach and less often oesophagus, was found in eight localities. *Camallanus kaapstaadi* Southwell et Kirshner, 1937, also from the oesophagus, was found in two localities and *C. xenopodis* Jackson et Tinsley, 1995, from the intestine, at a single locality. New localities for both *C. kaapstaadi* and *C. xenopodis* provide a geographical range extension. Males of *C. xenopodis* are described for the first time herein. The existence of a left spicule in the males of both the species of *Camallanus* Railliet and Henry, 1915 is confirmed and measurements are provided. Although *C. xenopodis* is distinguished from *C. mazabukae* Kung, 1948 in the present study, we suggest greater sampling effort in other African amphibians to confirm the species status of the latter taxon. Finally, the new molecular data showed distant relationships between collected species of *Camallanus* and species parasitising fish and freshwater turtles.

Keywords: African clawed frog, parasites, Nematoda, Camallanidae, *Camallanus*, *C. kaapstaadi*, *C. xenopodis*, *Batrachocamallanus*, *B. slomei*, *Procamallanus*

Over 25 parasite genera from seven invertebrate groups have been reported to be associated with the African clawed frog *Xenopus laevis* Daudin (Anura: Pipidae) in its native southern African range (Tinsley 1996). Some of these parasites have followed their invasive hosts out of Africa to North America, as well as to Europe (Lafferty and Page 1997, Kuperman et al. 2004). One group of parasites that, to date, has not been reported from feral populations of *X. laevis* is the nematodes of the family Camallanidae that primarily parasitise marine and freshwater fish and less often amphibians, turtles and snakes (Stromberg and Crites 1974).

In clawed frogs across the southern African subcontinent, representatives of camallanids are widespread – Kenya, Nigeria, Rwanda, Uganda and Zimbabwe (Thurston 1970, Avery 1971, Tinsley et al. 1979). Specifically, three species of camallanids are associated with *X. laevis* in South Africa: *Camallanus kaapstaadi* Southwell et Kirshner, 1937, *C. xenopodis* Jackson et Tinsley, 1995 and *Batrachocamallanus slomei* (Southwell et Kirshner, 1937).

Camallanus kaapstaadi was described from *X. laevis* from Cape Town (South Africa) (Southwell and Kirshner 1937). Subsequently, it was identified from clawed frogs (*X. laevis* and other *Xenopus* spp.) from Cameroon, Ghana, Kenya, Nigeria, Rwanda, northern and south-eastern parts of South Africa (previously known as Transvaal and Transkei), Sudan, Tanzania, Togo, Uganda and Zimbabwe (Avery 1971, Jackson and Tinsley 1995a).

Furthermore, the junior synonym of *C. kaapstaadi*, *C. johni* Yeh, 1960, was described from Tanzania (Yeh 1960) and later also reported from Zimbabwe, Uganda and Nigeria under this name (Thurston 1970). In the meantime, Tinsley et al. (1979) recorded the presence of a species of *Camallanus* Railliet and Henry, 1915 in *X. wittei* Tinsley, Kobel et Fischberg from central Africa, which was later identified as *C. kaapstaadi* (Jackson and Tinsley 1995a). Therefore, *C. kaapstaadi* can be regarded as a widespread parasite of clawed frogs across Africa.

The second camallanid from *X. laevis*, *C. xenopodis*, was described based only on six female specimens from

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the Cape (South Africa) and one female specimen which was recovered in Kenya from *X. borealis* Parker (Jackson and Tinsley 1995a). Neither of these species has been recorded since.

The third species, *B. slomei*, was also described as *Procamallanus slomei* Southwell and Kirschner, 1937 from *X. laevis* from the Cape (South Africa) (Southwell and Kirshner 1937) and was formally recorded for the first time since its original discription as *B. slomei* from clawed frog hosts in South Africa and Zaire (Jackson and Tinsley 1995b). Jackson and Tinsley (1995b) also synonymised *Procamallanus brevis* Kung, 1948 from *X. laevis* in South Africa (Kung 1948) with *B. slomei* and reidentified a *Spirocamallanus xenopodis* Southwell and Kirschner, 1937 in a clawed frog from Kenya (Thurston 1970) as belonging to the same species.

The redescription of *B. slomei* as a member of the genus *Batrachocamallanus* Jackson et Tinsley, 1995 is not without controversy (Jackson and Tinsley 1995b). The genus *Batrachocamallanus* was erected specifically for the four species of *Procamallanus* Baylis, 1923 parasitising African amphibians, based upon the large number of mucrons (more than five) on the female tail, relatively smaller body size and the almost identical cephalic morphology, male caudal structures and female reproductive system that differentiate the amphibian parasites from all other species of *Procamallanus* (see Jackson and Tinsley 1995b).

Moravec et al. (2006) considered differences in female mucron number and affinity to an amphibian host as not sufficient generic characters and advocated for the reduction of *Batrachocamallanus* to a junior synonym of *Procamallanus*. This decision was based on the description of *Procamallanus pacificus* Moravec, Justine, Würtz, Taraschewski et Sasal, 2006 from Pacific eels (*Anguilla* sp.) (Actinopterygii: Anguillidae) bearing four to nine mucrons on the female tail (Moravec et al. 2006) and the discovery of *B. siluranae* Jackson et Tinsley, 1995, previously described from *Xenopus tropicalis* Gray (Anura: Pipidae), from the polypteroid fish *Erpetoichthys calabaricus* Smith (Actynopterigii: Polypteridae) (Řehulková et al. 2005).

However, only one immature female specimen of *B. siluranae* was recovered from the polypteroid fish, which was a specimen imported for the pet trade, leading the authors to believe this to be an accidental infection (Řehulková et al. 2005). Moreover, the small processes on the tail of female *P. pacificus* are 6 μm to 9 μm long whereas the mucrons of *Batrachocamallanus* spp. vary from 10 μm to 15 μm (Jackson and Tinsley 1995b, Moravec et al. 2006). Therefore, the mucrons of *P. pacificus* might not represent the same structures as in *Batrachocamallanus* and probably cannot be used for suppression of the genus. Taken as a whole, these factors cast doubt on the validity of the suppression of *Batrachocamallanus*, leading us to assign our specimens to *B. slomei*.

It is clear that information on the camallanids from *X. laevis* are in need of augmentation, especially on the molecular front. The present studies of three species of camallanids collected from *X. laevis* in different regions of South Africa add information on the infection level, localities, morphology and molecular data of the 28S rRNA and *cox1* genes.

MATERIALS AND METHODS

In total, 97 *Xenopus laevis* were collected in chicken liver baited funnel traps from eight localities across the northern regions of South Africa during November of 2016 and March to May of 2017 and from three localities in the south-western regions during June and July of 2017 (Table 1).

The hosts were anaesthetised in 6% ethyl-3-aminobenzoate methanesulfonate (MS222) (Sigma-Aldrich Co., St. Louis, Missouri, USA) and subsequently euthanised through severing the spine and destroying the brain, according to internationally accepted standard operating procedures. During the total dissection, the alimentary canal was removed and opened in 0.6% amphibian saline. After removal from the oesophagus, stomach and intestine, nematodes were washed in saline, fixed in 70% hot ethanol and subsequently stored in 70% ethanol.

Prior to microscopic examination, the nematodes were cleared in lactophenol. The morphology of the nematodes was studied and photomicrographs were taken using a Nikon E800 and Nikon ECLIPSE *Ni* compound microscopes. Apical and transverse sections were prepared manually.

In total, 601 specimens of *B. slomei*, 39 of *C. kaapstaadi* and 53 of *C. xenopodis* were studied of which 50 (20 males and 30 females), 18 (nine males and nine females) and 28 (four males and 24 females), respectively, were measured. All measurements in the text are given in micrometres, unless otherwise indicated and presented as ranges followed by mean values in parentheses. The representative sample (30 specimens) expressed as mean with standard deviation (SD).

For the molecular studies, the middle fragments of males were used, while taxonomically important anterior and posterior parts were reserved for the morphological identification of species. DNA was extracted using the KAPA Express Extraction Kit DKAPKK7103 (Sigma-Aldrich Co.). *Cox1* amplicons were obtained using the primer pair 'LCO1490' (5'-GGTCAACAAAT-CATAAAGATATTGG-3') and 'HCO2198' (5'-TAAACTTCAG-GGTGACCAAAAAATCA-3'). The thermocycling profile was: 3 min denaturation at 94 °C, 10 cycles of 94 °C for 30 s, 45 °C for 30 s, 72 °C for 60 s and 40 cycles of 94 °C for 30 s, 51 °C for 60 s, 72 °C for 60 s for amplification and 72 °C for 10 min for extension.

The 28S amplicons were amplified using forward primer 'LSU5' (5'-TAGGTCGACCCGCTGAAYTTAAGCA-3') and reverse primer 'n900r' (5'-GGTTCGATTAGTCTTTCGCC-3'). The thermocycling profile was as follows: 5 min denaturation at 95 °C; 40 cycles of 30 s at 95 °C, 30 s at 55 °C, 2 min at 72 °C for amplification; and a final 7 min extension at 72 °C. Sequences were obtained using BigDye® Terminator v3.1 Cycle Sequencing on an ABI3500XL sequencer. DNA products were sequenced in both directions using the pairs of PCR primers; for the nuclear genes the following additional primers were used: internal primers, '300F' (5'-CAAGTACCGTGAGGGAAAGTTG-3') and 'ECD2' (5'-CTTGGTCCGTGTTTCAAGACGGG-3'). Contiguous sequences were assembled, edited using Geneious 9.0 software and submitted to GenBank.

For the phylogenetic analysis, Bayesian inference in the MrBayes program (V. 3.2.2) was used. Prior to analysis, sequences

Table 1. Geographic origin, habitat and level of infection of three nematodes of 97 *Xenopus laevis* Daudin from 11 localities in South Africa. Mean intensity of infection is given with median intensity in square brackets and minimum and maximum values in parentheses. Fields with 'n/a' refer to absent infection level values due to absence of nematodes in the digestive tract.

Nematode	Localities	Coordinates	Habitat	Number of hosts	Intensity	Prevalence	Abundance
Batrachocamallanus slomei Southwell et Kirshner, 1937	Imvubu Lodge, KwaZulu Natal	28°47'34"S; 26°03'02"E	Vegetated pond	15	1	7%	0.1
	Potchefstroom, North-West	26°45'20"S; 27°03'38"E	Shallow vlei (marshland)	9	14 [10] (1–37)	100%	13.7
	Modimolle, Limpopo	24°26'18"S; 28°26'13"E	Ornamental garden pond	6	18 [14] (2–45)	83%	15.2
	Jacana Estate, White River, Mpumalanga	25°20'21"S; 31°01'21"E	Dam in vlei (marshland)	8	9 [8] (4–16)	63%	5.6
	Tasselberry, White River, Mpumalanga	25°19'55"S; 31°02'36"E	Ornamental garden pond	6	24 [26] (9–37)	100%	24.3
	Dullstroom, Mpumalanga	25°23'53"S; 30°02'17"E	Dam in mountain stream	10	13 [12] (1–30)	100%	13.0
	Letsitele, Limpopo	23°47'56"S; 30°11'42"E	Vegetated pond	10	6 [5] (2–12)	100%	5.5
	Hermanus, Western Cape	34°22'13"S; 19°15'25"E	Vegetated pond	7	3 [2] (1–6)	57%	1.6
Camallanus kaapstaadi Southwell et Kirshner, 1937	Dullstroom, Mpumalanga	25°23'53"S; 30°02'17"E	Dam in mountain stream	10	4 [4] (1–8)	80%	3.4
	Site A, Cape Town, Western Cape	33°50'21"S; 18°36'01"E	Urban recreational pond	5	2 [2] (1–2)	60%	1.0
Camallanus xenopodis Jackson et Tinsley, 1995	Dullstroom, Mpumalanga	25°23'53"S; 30°02'17"E	Dam in mountain stream	10	6 [6] (1–13)	90%	5.3
No camallanids present	Site B, Cape Town, Western Cape	33°50'08"S; 18°33'10"E	Urban recreational pond	14	n/a	n/a	n/a
	Rondepan, Limpopo	23°46'10"S; 29°24'17"E	Dam in inundated grass	7	n/a	n/a	n/a

were aligned using the ClustalW tool in Mega (V.7) software and trimmed to the shortest alignment (497 nucleotides of 28S and 312 nucleotides of *cox1* genes). The Bayesian analyses were run with the following nucleotide substitution model settings: lset nst = 6, rates = invgamma, ncat = 4, shape = estimate, inferrates = yes and basefreq = empirical, that correspond to a general time reversible model GTR+G+I. The nucleotide substitution model was determined using JModelTest (V.2.1.7) software. Further analysis was provided using next parameters: mcmc ngen = 200,000 for 28S alignments and ngen = 100,000 for *cox1* alignments, samplefreq = 100, printfreq = 100 and diagnfreq = 1,000. Trees were visualised using the FigTree (V. 1.4.3) software.

RESULTS

Batrachocamallanus slomei Southwell et Kirshner, 1937
Fig. 1

H o s t : *Xenopus laevis* Daudin (Amphibia: Anura: Pipidae).

L o c a l i t i e s : KwaZulu Natal, Mpumalanga, Limpopo, North-West, Western Cape (see Table 1).

S i t e o f i n f e c t i o n : Stomach, oesophagus.

R e p r e s e n t a t i v e D N A s e q u e n c e s : MG948463 (*cox1*), MG947390 (28S).

General description. Body small, usually coiled ventrally, comparatively thick, with maximum width at level of anterior third. Cuticle with prominent transverse striations. Apical (Fig. 1B): oral opening rounded, surrounded by six flat elevations (two larger lateral and four submedian); six labial papillae; four cephalic papillae; two amphids. Buccal capsule with well sclerotised walls, some-what longer than wide with conspicuous basal ring and without internal ridges (Fig. 1A,C). Muscular oesophagus opening surrounded with three tooth-like projections of basal ring (Fig. 1C). Muscular oesophagus club-shaped with elongated posterior bulb. Glandular oesophagus somewhat longer than muscular oesophagus, almost cylindrical but slightly widened posteriorly. Nerve ring encircling muscular oesophagus at level of its proximal third. Excretory pore opening at level of muscular oesophageal mid-length. Minute papilliform deirids situated at level of excretory pore. Intestine straight, narrow. Rectum straight, with thin walls (Fig. 1E). Tail tapering with numerous mucrons in females (Fig. 1E) and rounded tip in males (Fig. 1D).

Male (morphometry based on 20 specimens). Body 1.94–2.80 mm (2.32 mm) long, 96–157 (120) maximum width. Buccal capsule 93–112 (102) long, including basal ring 7–14 (11) long, 53–75 (61) wide with maximum width 67–94 (76) at mid-length and minimum width 26–53 (35) at anterior extremity. Minimum and maximum thickness of buccal capsule walls close to oral opening 2–4 (2.5) and to basal ring 4–10 (6). Muscular oesophagus 188–282 (235) long, 9–12% (10%) of body length; 33–56 (43), 33–43 (38) and 60–97 (75) wide at anterior, mid-length and bulb level, respectively. Glandular oesophagus 208–366 (301) long, 10–15% (13%) of body length; 46–81 (58), 32–68 (52) and 32–88 (65) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 153–200 (177) from anterior extremity. Excretory pore and deirids situated at 127–280 (217), 6–11% (9%) of body length and 213–260 (238), 9–12% (10%) of body length from anterior extremity, respectively. Posterior end coiled ventrally, provided
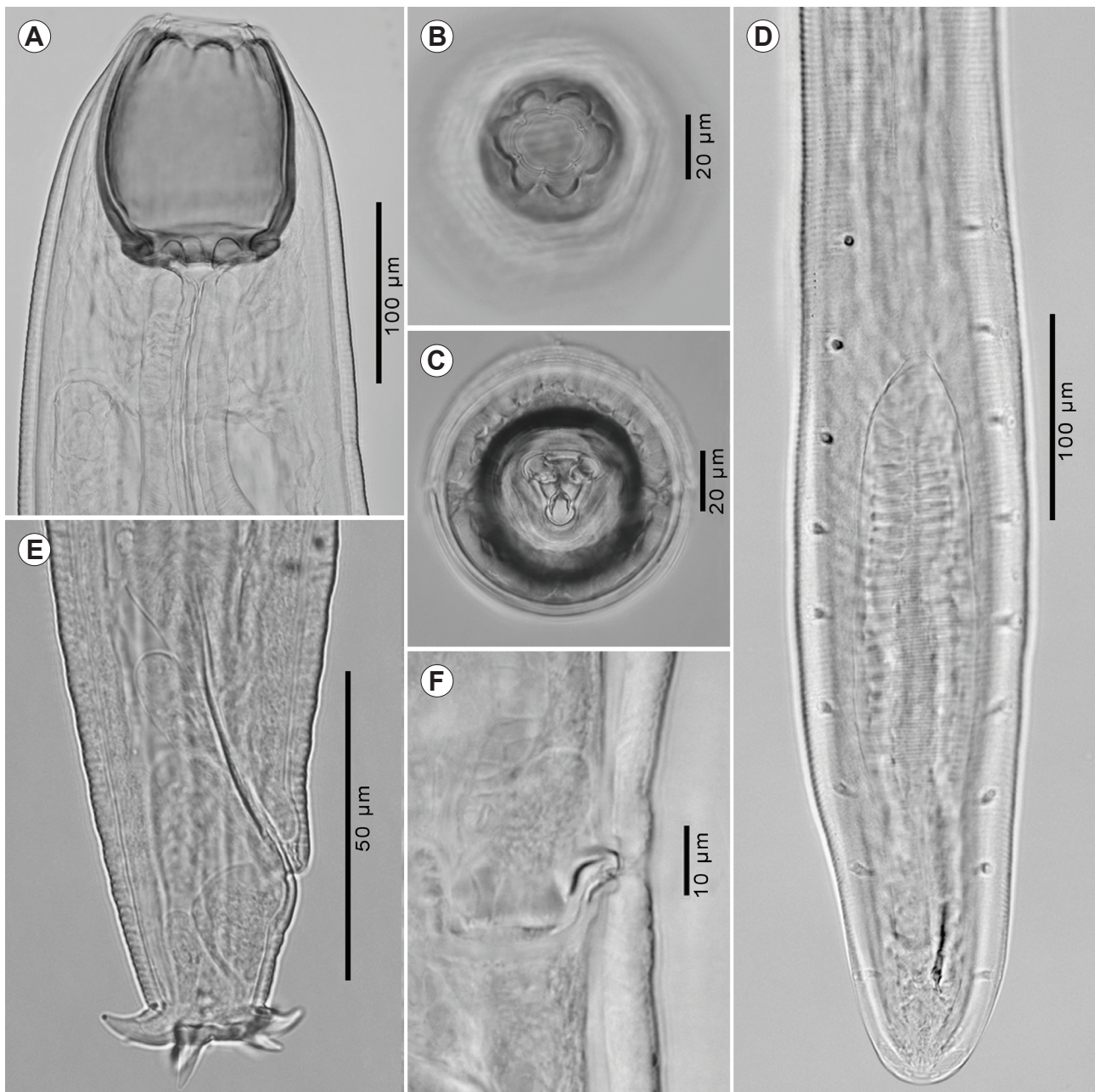
Fig. 1. *Batrachocamallanus slomei* Southwell et Kirshner, 1937 from *Xenopus laevis* Daudin, photomicrographs. **A** – anterior end, female, lateral view; **B** – anterior end, male, apical view; **C** – anterior end, male, optical section at base of buccal capsule level; **D** – caudal end, male, ventral view; **E** – posterior end, female, lateral view; **F** – part of female genital system at vulva level, lateral view.

with well-developed vesicular caudal alae supported by pedunculate papillae near mid-length. Posterior part of alae slightly elevated and joined on ventral surface forming pseudosucker. Tail conical with rounded tip, 37–50 (42) long. Caudal region possessing pedunculate papillae (Fig. 1D): eight pairs of precloacal; one pair of adcloacal and three pairs of postcloacal. Three pairs of sessile papillae surrounding cloaca (one slightly anterior to and two somewhat posterior to). Spicules unequal, simple-shaped with sharply pointed distal ends. Right spicule clearly visible, 90–115 (101) long; left one less sclerotised, 15–37 (25) long. Gubernaculum absent.

Female (morphometry based on 30 specimens). Body 1.67–3.51 mm (2.87 mm ± 0.54 mm) long, 163–284 (225

± 39) maximum width. Buccal capsule 105–138 (122 ± 9) long, including basal ring 10–17 (14 ± 2) long, 63–90 (76 ± 7) wide; maximum width 86–113 (103 ± 6) at mid-length and minimum width 34–71 (48 ± 9) at anterior extremity. Minimum and maximum thickness of buccal capsule walls close to oral opening 2–6 (3 ± 1) and to basal ring 4–11 (8 ± 2). Muscular oesophagus 242–337 (288 ± 22) long, 8–17% (10% ± 2%) of body length; 38–72 (51 ± 8), 40–60 (49 ± 5) and 68–116 (95 ± 12) wide at anterior, mid-length and bulb level, respectively. Glandular oesophagus 248–526 (369 ± 62) long, 9–18% (13% ± 2%) of body length; 48–78 (65 ± 8), 38–76 (60 ± 9) and 57–108 (78 ± 15) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 161–221 (206 ± 14) from anterior extremity. Excretory
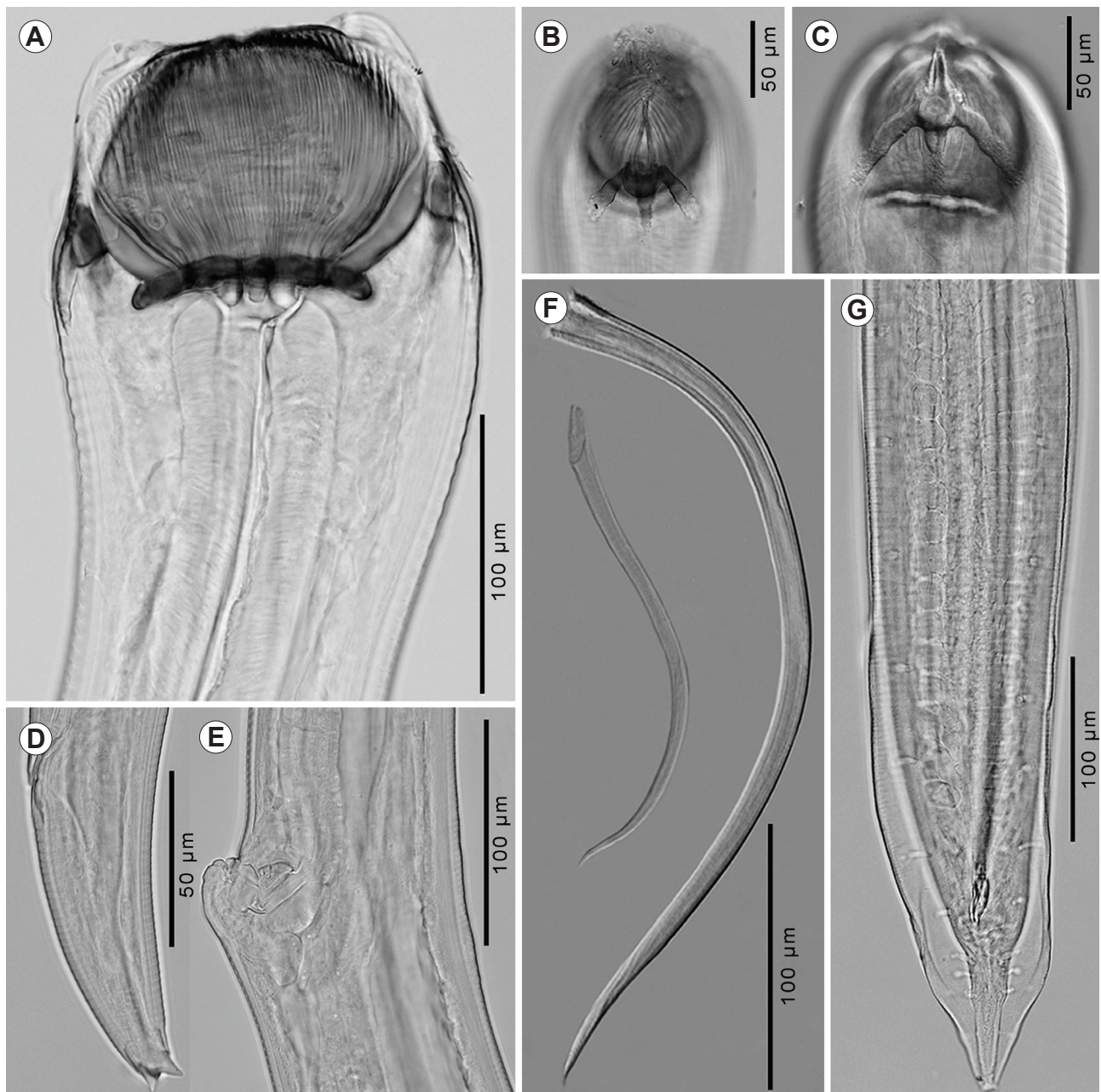
Fig. 2. *Camallanus kaapstaadi* Southwell et Kirshner, 1937 from *Xenopus laevis* Daudin, photomicrographs. **A** – anterior end, male, lateral view; **B** – dorsal trident, male, lateral view; **C** – ventral trident, male, lateral view; **D** – posterior end, female, lateral view; **E** – part of female genital system at vulva level, lateral view; **F** – left and right spicules, lateral view; **G** – caudal end, male, ventral view.

pore and deirids situated at 184–326 (246 ± 27), 7–13%, (9% ± 2%) of body length, and 196–298 (242 ± 27), 6–11% (9% ± 1%) of body length, from anterior extremity, respectively. Vulva (Fig. 1F) situated near body mid-length at 1.13–1.94 mm (1.61 mm ± 0.27 mm) from anterior extremity; 42–60% (54% ± 3%) of body length. Tail 34–59 (48 ± 6) long with five to seven mucrons at its tip (Fig. 1E).

Camallanus kaapstaadi Southwell et Kirshner, 1937

Fig. 2

H o s t : *Xenopus laevis* Daudin (Amphibia: Anura: Pipidae).
L o c a l i t i e s : Mpumalanga, Western Cape (see Table 1).
S i t e o f i n f e c t i o n : Oesophagus, stomach.

R e p r e s e n t a t i v e D N A s e q u e n c e s : MG948461 (*cox1*), MG947391 (28S).

General description. Medium-sized worms, coiled dorsally, body comparatively thick with maximum width at anterior third level. Cuticle with prominent transverse striations along whole body, except at buccal capsule. Apical: oral opening transversely slit; four conspicuous submedian cephalic papillae. Buccal capsule consisting of two valves each supported with numerous primarily completed ridges (Fig. 2A,B). Four sclerotised plates situated on external surface of valves near their anterior margin. Thick sclerotised basal ring present at buccal capsule base. Buccal capsule valves supported by two prominent tridents on

dorsal and ventral side (Fig. 2B,C). Each trident consisted of three posteriorly directed prongs. Central prongs somewhat longer than sublateral ones. Tridents usually unequal, beginning at level of buccal capsule mid-length and ending slightly posterior to level of basal ring. Muscular oesophagus club-shaped with elongated posterior bulb. Glandular oesophagus almost equal in length to muscular oesophagus, cylindrical to ¾ length with slightly widened posterior quarter. Nerve ring encircling muscular oesophagus at level of its anterior quarter. Excretory pore opening on ventral side somewhat posterior to nerve ring level. Deirids minute, papilliform, situated at level of posterior third of muscular oesophagus. Intestine and rectum straight, narrow. Tail tapering.

Male (morphometry based on nine specimens). Body 2.66–3.28 mm (3.04 mm) long, 102–171 (149) wide. Buccal capsule valves 62–100 (83) long, 65–118 (106) wide, supported by 16–31 (26) ridges, of which 7–10 (8) incomplete. Basal ring 7–14 (10) long, 52–89 (77) wide. Dorsal trident 51–72 (66) long and 15–23 (19) wide in lateral projection, ventral one 48–67 (58) long and 17–23 (20) wide.

Muscular oesophagus 261–381 (338.4) long, 9.8–12.3% (11.1%) of body length; 53–63 (58.8), 46–61 (53.9) and 58–95 (86.1) wide at anterior, mid-length and bulb level, respectively. Glandular oesophagus 275–461 (368.5) long, 9.1–14.9% (12.1%) of body length; 35–96 (61.5), 44–82 (59.5) and 42–76 (59.9) wide at anterior, mid-length and posterior level, respectively. Nerve ring, excretory pore and deirids (distance to deirids measured in three specimens) at 150–246 (182.9), 191–277 (216.1) and 226–257 (241.7) from anterior extremity, respectively.

Caudal alae narrow, ventrolateral, supported by papillae: six pairs of pre-cloacal pedunculated papillae (Fig. 2G); one pair of pedunculated papillae at cloaca level; five pairs of post-cloacal pedunculated papillae (three prominent pairs somewhat posterior to cloaca and two pairs of small papillae close to tail end). Additionally, two pairs of sessile ad-cloacal papillae situated slightly anterior and posterior to cloaca. Spicules unequal, simple-shaped with sharpened tips (Fig. 2F). Right spicule prominent, 287–468 (422.5) long; left one less sclerotised (measured in three specimens), 198–220 (211.7) long. Tail conical with two minute spines on tip (Fig. 1G), 74–104 (91.5) long, 2.5–3.2% (3.0%) of body length.

Female (morphometry based on nine specimens). Generally larger than males. Body 2.80–5.13 mm (3.62 mm) long, 201–293 (242) wide. Buccal capsule valves 95–149 (116) long, 81–174 (147) wide, supported by 31–38 (34) ridges, of which 7–10 (9) incomplete. Basal ring 9–20 (13) long, 71–125 (106) wide. Dorsal trident 72–102 (86) long and 21–29 (25) wide in lateral projection, ventral one 63–88 (76) long and 14–35 (24) wide.

Muscular oesophagus 373–451 (414) long, 7–14% (12%) of body length; 58–87 (76), 46–79 (69) and 97–140 (123) wide at anterior, mid-length and bulb level, respectively. Glandular oesophagus 313–531 (390) long, 6–15% (11%) of body length; 50–91 (72), 49–91 (66) and 52–105 (79) wide at anterior, mid-length and posterior level, respectively. Nerve ring, excretory pore and deirids (distance

to deirids measured in five specimens) at 183–242 (220), 229–307 (266) and 261–375 (306) from anterior extremity, respectively.

Vulva post-equatorial, opening posterior to distinct projection of body wall at 1.67–2.64 mm (2.16 mm) from anterior extremity (Fig. 2E), 51–63% (60%) of body length. Tail tapering 124–179 (143) long, 3–5% (4%) of body length, bearing three mucrons 5–11 (8) long at its tip (Fig. 2D).

Camallanus xenopodis Jackson et Tinsley, 1995

Figs. 3, 4

H o s t : *Xenopus laevis* Daudin (Amphibia: Anura: Pipidae).
L o c a l i t y : Western Cape (see Table 1).
S i t e o f i n f e c t i o n : Intestine.
R e p r e s e n t a t i v e D N A s e q u e n c e s : MG948462 (*cox1*), MG947389 (28S).

General description. Medium-sized worms, coiled dorsally, body comparatively thick with maximum width at anterior third level. Cuticle with transverse striations clearly visible from level of basal ring to posterior extremity. Apical: oral opening transversely slit; four conspicuous submedian cephalic papillae. Buccal capsule comparatively small, consisting of two valves, supported by longitudinal ridges, with four thin plates on anterior margin, sclerotised basal ring at base and two tridents (Figs. 3B–D, 4A–C). Tridents prominent, equal in shape, almost equal in size, with central prongs somewhat longer than sublateral ones (Figs. 3C,D, 4B,C). Tridents beginning at level of buccal capsule mid-length, ending somewhat posterior to basal ring. Muscular oesophagus club-shaped with elongated posterior bulb. Glandular oesophagus almost same length as muscular oesophagus, cylindrical along almost entire length with slightly widened or narrowed posterior quarter. Position of nerve ring varying within level of muscular oesophagus anterior third. Excretory pore opening on ventral side somewhat posterior to nerve ring level. Deirids minute, papilliform, situated at level of muscular oesophageal mid-length. Intestine and rectum straight, narrow. Tail tapering.

Male (description based on four specimens). Body 2.88–3.30 mm (3.04 mm) long, 113–127 (118) wide (Fig. 3A). Buccal capsule valves 62–70 (66) long, 72–82 (77) wide, supported by 18–22 (20) ridges, of which 4–6 (5) incomplete. Basal ring 5–11 (9) long, 50–55 (53) wide. Dorsal trident (Figs. 3C, 4B) 57–69 (62) long and 10–18 (15) wide at lateral projection, ventral one (Figs. 3D, 4C) 51–63 (59) long and 9–16 (13) wide.

Muscular oesophagus 257–305 (284) long, 9–11% (9%) of body length; 43–51 (45.5), 38–45 (41) and 59–74 (65) wide at anterior, mid-length and bulb level, respectively. Glandular oesophagus 209–313 (271) long, 7–11% (9%) of body length; 39–61 (47), 38–58 (49) and 34–73 (53) wide at anterior, mid-length and posterior level, respectively. Nerve ring, excretory pore and deirids (distance to deirids measured in one specimen) at 140–159 (149), 184–211 (198) and 213 from anterior extremity, respectively.
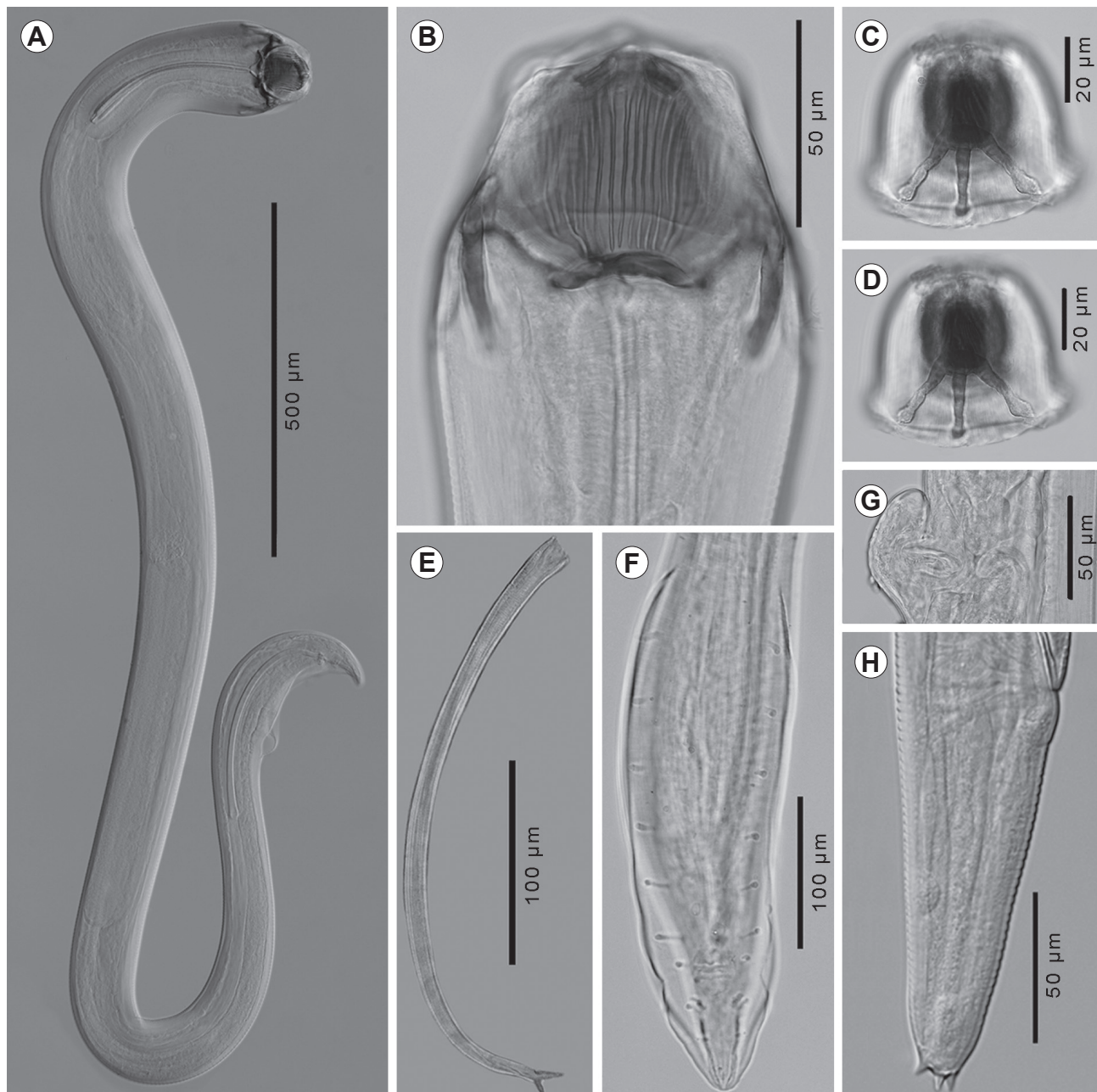
Fig. 3. *Camallanus xenopodis* Jackson et Tinsley, 1995 from *Xenopus laevis* Daudin, photomicrographs. **A** – male, general view; **B** – anterior end, female, lateral view; **C** – dorsal trident, male, lateral view; **D** – ventral trident, male, lateral view; **E** – right spicule, lateral view; **F** – caudal end, male, ventral view; **G** – part of female genital system at vulva level, lateral view; **H** – posterior end, female, lateral view.

Caudal alae comparatively narrow, ventrolateral, supported by papillae (Figs. 3F, 4F): six pairs of pre-cloacal pedunculated papillae; one pair of pedunculated papillae at cloaca level; four pairs of post-cloacal pedunculated papillae (two prominent pairs somewhat posterior to cloaca, one pair at tail mid-length and one pair of small papillae close to tail end). Two pairs of sessile adcloacal papillae situated slightly anterior and posterior to cloaca. Spicules unequal. Right spicule (Figs. 3E, 4E) 323–356 (335) long with short conical process directed dorsally at its tip. Left spicule shorter, 96–98 long (measured in two specimens), poorly sclerotised, with simple-shaped sharpened tip (Fig. 4D). Tail tapering with smoothly rounded tip (Figs. 3F, 4F).

Female (description based on 24 specimens). Generally larger than males. Body 3.33–5.13 mm (4.20 mm) long, 136–241 (177) wide. Buccal capsule valves 73–85 (81) long, 73–104 (93) wide, supported by 14–22 (18) ridges, of which 3–7 (5) incomplete. Basal ring 8–14 (11) long, 62–74 (67) wide. Dorsal trident 53–93 (76) long and 11–25 (16) wide at lateral projection, ventral one 61–85 (75) long and 12–20 (16) wide.

Muscular oesophagus 326–433 (353) long, 7–12% (9%) of body length; 48–68 (58), 39–59 (51) and 76–104 (89) wide at anterior, mid-length and bulb level, respectively. Glandular oesophagus 310–455 (374) long, 7–11% (9%) of body length; 42–84 (61), 51–111 (71) and 48–85 (66)
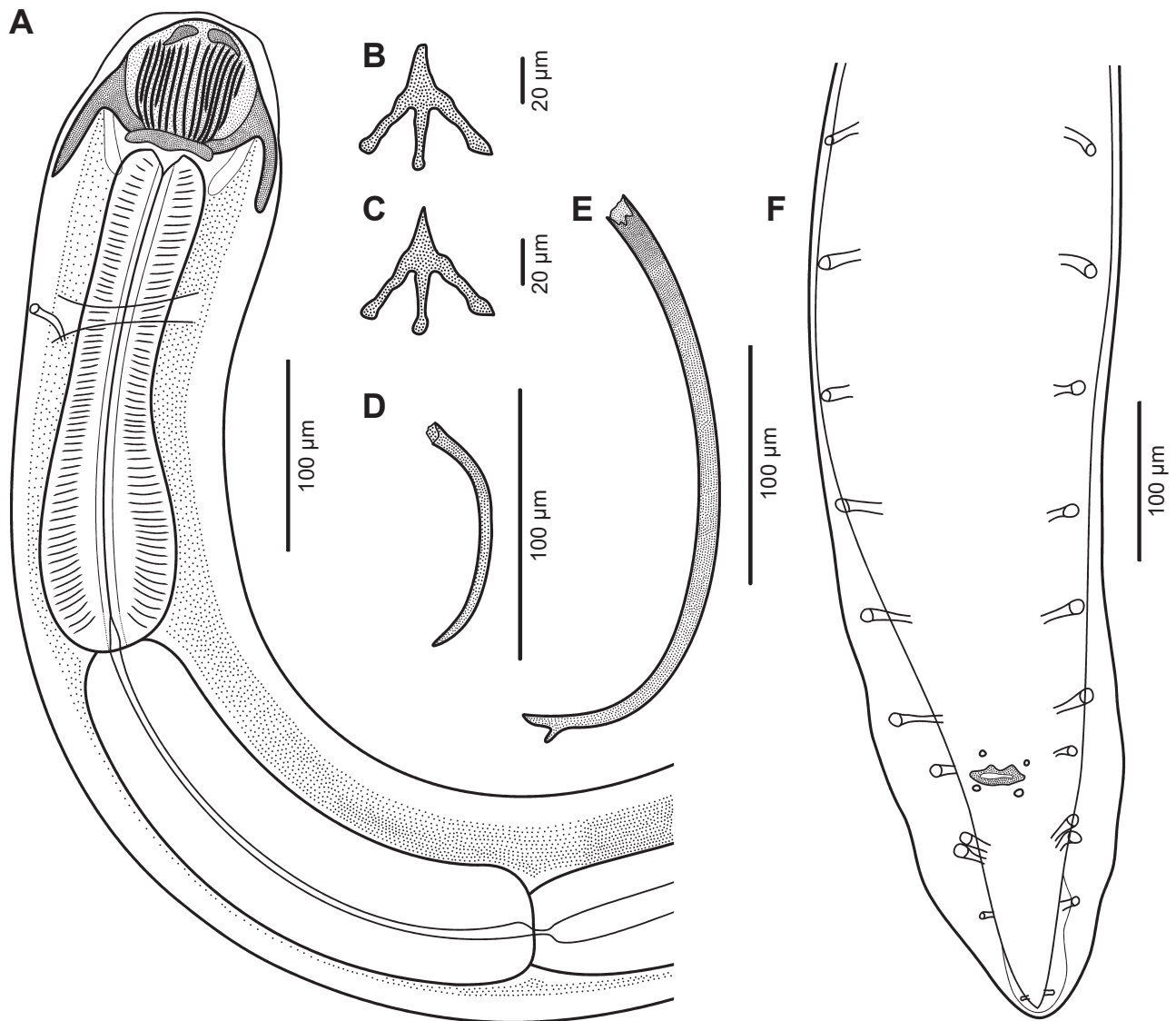
Fig. 4. *Camallanus xenopodis* Jackson et Tinsley, 1995 from *Xenopus laevis* Daudin, male, line-drawings. **A** – anterior end, lateral view; **B** – dorsal trident, lateral view; **C** – ventral trident, lateral view; **D** – left spicule, lateral view; **E** – right spicule, lateral view; **F** – caudal end, ventral view.

wide at anterior, mid-length and posterior level, respectively. Nerve ring, excretory pore and deirids (distance to deirids measured in eight specimens) at 171–212 (186), 210–256 (228) and 180–291 (247) from anterior extremity, respectively.

Vulva opening posterior to distinct projection of body wall almost at level of mid-body at 1.72–2.59 mm (2.14 mm) from anterior extremity (Fig. 3G), 47–54% (51%) of body length. Tail tapering 94–165 (129) long, 3–4% (3%) of body length, bearing three mucrons 5–7 (6) long at its tip (Fig. 3H).

Molecular analysis

For all three species, partial 28S gene and *cox1* fragments were sequenced. Only for five species of *Camallanus* from Australian freshwater turtles partial sequences of the 28S subunit are available in GenBank. These species were used for the phylogenetic analysis and *Serpinema*

octorugatum (Baylis, 1933) was used as outgroup since it represents the most related genus to *Camallanus* (see Kuzmin et al. 2011). The resulting tree (Fig. 5) confirmed the interrelationships among *C. tuckeri* Kuzmin, Tkach, Snyder et Maier, 2009, *C. sprenti* Kuzmin, Tkach, Snyder et Bell, 2011, *C. waelhreow* Rigby, Sharma, Hechinger, Platt et Weaver 2008, *C. nithoggi* Rigby, Sharma, Hechinger, Platt et Weaver 2008 and *C. beveridgei* Kuzmin, Tkach, Snyder et Bell, 2011 showed by Kuzmin et al. (2011) and showed their distant relations to *C. kaapstaadi* and *C. xenopodis*.

The *cox1* sequences are available for only two species of *Camallanus*, namely *C. cotti* Fujita, 1927 and *C. hypophthalmichthys* Dogel et Akhmerov, 1959, originally described from fishes in the Eastern Palaearctic (Moravec et al. 2004, Moravec and Justine 2006). For this analysis, *Spirocamallanus istiblenni* Noble, 1966 was used as outgroup. Phylogenetic tree topology showed distant relation-
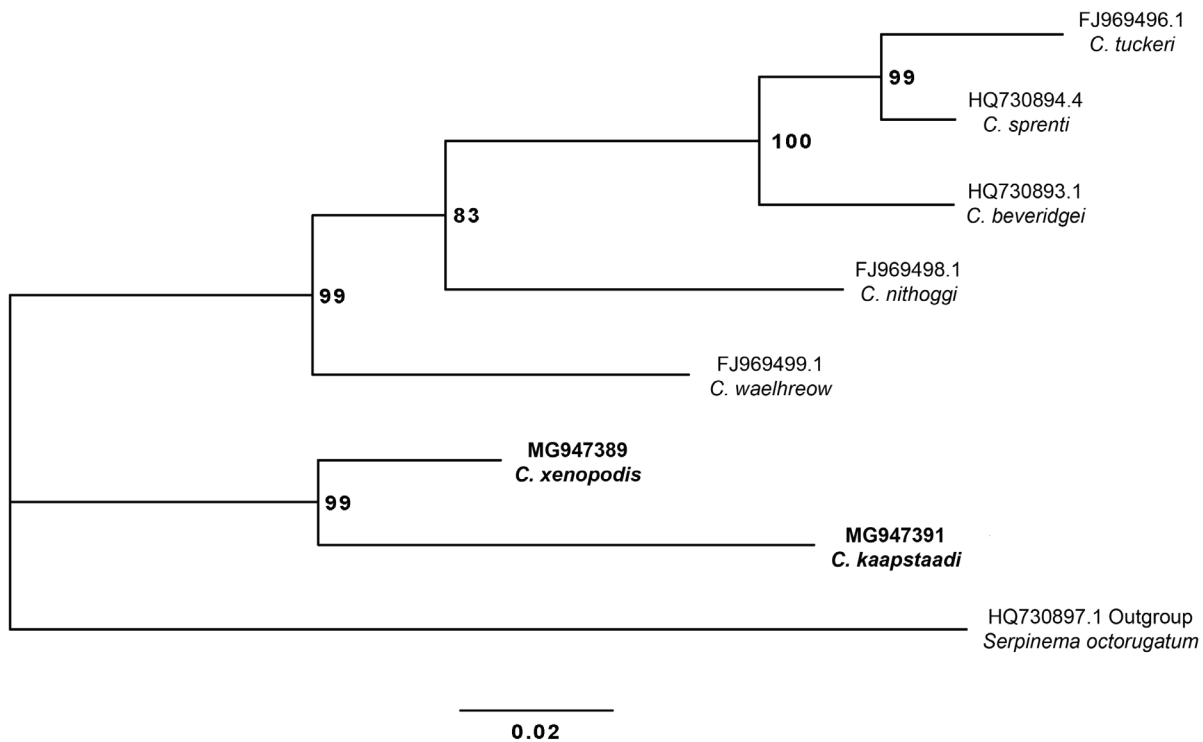
Fig. 5. Phylogenetic tree of seven species of *Camallanus* Railliet and Henry, 1915 based on partial 28S sequences.
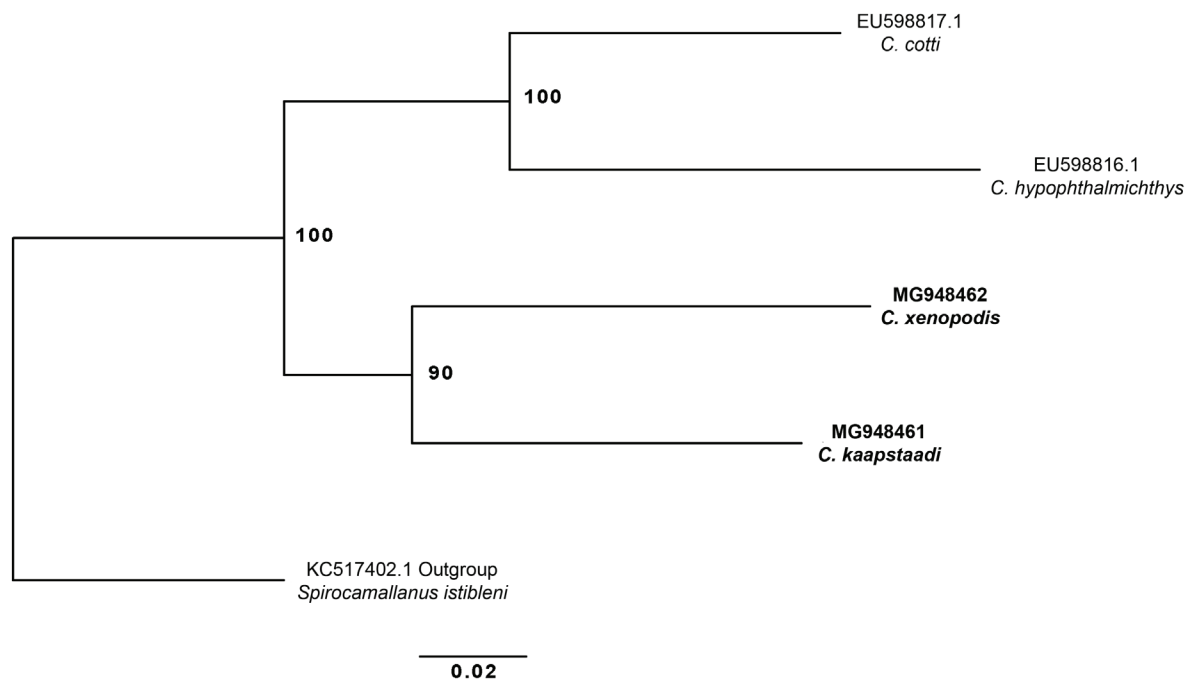

Fig. 6. Phylogenetic tree of four species of *Camallanus* Railliet and Henry, 1915 based on partial *cox1* sequences.

ships between *Camallanus* from African frogs and from Asian fishes which grouped in two strongly supported clades (Fig. 6).

Since neither 28S nor *cox1* sequences of *Batrachocamallanus* and *Procamallanus* are available in GenBank, we are not able to provide phylogenetic analysis for *B. slomei*. Therefore, representative sequences of that species were simply submitted to GenBank.

DISCUSSION

During the course of the present study, all three species of camallanid nematodes that have previously been reported from *X. laevis* were found. The most common nematode encountered in the present study was *B. slomei*, reported from all but three localities with prevalence ranging from 7% to 100% (Table 1). Conversely, the other two species were found only in two localities. Both *C. kaapstaadi* and

C. xenopodis were only found at one locality, namely a series of dams in a mountain stream near Dullstroom (Mpumalanga Province, northern region of South Africa) and only *C. kaapstaadi* was found at one locality in Cape Town (Western Province). The higher level of nematode diversity at this specific locality where both *Camallanus* species occur, corresponds with the higher numbers of other parasites (cestodes, monogeneans, digeneans and parasitic arthropods) also found here in comparison to other localities (unpubl. data). In our opinion, this can be explained by the pristine condition of the habitat and lack of human disturbance allowing a better environment for parasite circulation.

Our results confirm the presence of *C. kaapstaadi* in South Africa, in the light of the fact that many previous records are from more northern regions in Africa such as Cameroon, Ghana, Kenya, Nigeria, Rwanda, Sudan, Tanzania, Togo, Uganda and Zimbabwe (Yeh 1960, Thurston 1970, Avery 1971, Tinsley et al. 1979, Jackson and Tinsley 1995a). We found *C. xenopodis* in the north of South Africa, indicating a less disjunct distribution than the two previous records from the Cape and Kenya suggest (Jackson and Tinsley 1995a). We hypothesise that species of *Camallanus* in clawed frogs are more widespread than previous studies imply, which will be outlined in further studies.

Batrachocamallanus slomei is found primarily in the stomach, but also in some cases in the oesophagus. Our findings suggest no correlation between the number of nematodes in the stomach and their presence in the oesophagus of the same frog. For example, the frog with the highest infection of *B. slomei* harboured 36 nematodes in the stomach and one nematode in the oesophagus, whereas another frog harboured 15 in the stomach and 17 in the oesophagus. Only one specimen of *C. kaapstaadi* was found in the stomach while all other specimens inhabited the oesophagus in four frogs sharing this site with *B. slomei*. *Camallanus xenopodis* is found only in the intestine of *X. laevis* and no other nematode species are known from this part of the digestive tract.

The reported morphometric characters of *B. slomei* vary widely and correspond to the previously known data (Southwell and Kirshner 1937, Jackson and Tinsley 1995b). Our data slightly expand the existing ranges of body length and width, length of male spicules and distance to vulva from anterior part of body in females. Overall, no differences in morphometric characters of *B. slomei* were observed between localities.

Metrical characters of *C. kaapstaadi* and *C. xenopodis* also vary greatly in the studied samples, though most of them fall within the ranges of previously known data (Southwell and Kirshner 1937, Thurston 1970, Jackson and Tinsley 1995a). In the first description of *C. kaapstaadi*, the authors mentioned that the male left spicule is about 200 μm long and less sclerotised than the right one (Southwell and Kirshner 1937). In the later redescription, the authors concurred that the left spicule is poorly visible, but did provide a mean measurement of 155 μm (Jackson and Tinsley 1995a). In our specimens, all males of *C. kaapstaadi* possessed a conspicuous left spicule with a mean

length of about 212 μm. This measurement is longer than that reported for *C. kaapstaadi* from *X. laevis* by Jackson and Tinsley (1995a), probably due to the fact that the specimens in our study were generally bigger (2.25 mm in Jackson and Tinsley (1995a) *vs* 3.04 mm in the present study). This discrepancy is also found in the mean measurement of the right spicule (273 μm *vs* 423 μm). At the same time, the left spicule of *C. xenopodis* is truly inconspicuous under light microscopy, even when viewed under high magnification with differential interference contrast (DIC). Nevertheless, the existence of the left spicule is confirmed based on dissection of the caudal part of male.

In our specimens, more females than males of all the species were found, although only in *C. xenopodis* the females are four times more frequent. Therefore, it is clear why the species was previously described only by females (Jackson and Tinsley 1995a). Males in our samples of *C. xenopodis* possess several characters, such as structure of buccal capsule, number of ridges on its valves, size and shape of tridents and male right spicule, that are similar to *C. mazabukae* Kung, 1948. Despite that, two characters, namely number of mucrons (five or at least four in *C. mazabukae* versus only three in *C. xenopodis*) and the shape of the body (*C. mazabukae* coiled in the ventral direction whilst all found specimens of *C. xenopodis* coiled dorsally) led us to assign the collected specimens to *C. xenopodis*.

However, the image of the female tail of *C. mazabukae* in the original description (Kung 1948) shows that the specimen might really be coiled dorsally. Moreover, the species was described based on only two specimens from the poorly identified host, marked as "bullfrog". Since no *Camallanus* have been found in the giant bullfrog (*Pyxicephalus adsperus* Tschudi) or African bullfrog (*Pyxicephalus edulis* Peters) (Halajian et al. 2013, unpubl. data) before this record or ever since, we suppose that it might represent an opportunistic infection or host misidentification. Given these points, we prefer to identify our specimens as *C. xenopodis* while the taxonomic status of *C. mazabukae* should be illuminated by further studies of *Camallanus* from different amphibians in Africa.

The lack of available DNA alignments in GenBank did not allow us to provide robust phylogenetic analysis of the found species. Both phylogenetic trees (based on partial 28S and *cox1* alignments) show that species of *Camallanus* from South African amphibians form a well-supported clade separate from clades of species from Asian fish or Australian freshwater turtles. Unfortunately, we are not able to consider that group as monophyletic, since no data are available of *Camallanus* from fish (*C. polypteri* Kabre et Petter, 1997) or turtles (*C. chelonius* Baker, 1983) in Africa.

Our study revealed only one species of *Batrachocamallanus*, which limits us in our ability to draw conclusions about the status of the genus. Both the opinions of Jackson and Tinsley (1995b) and Moravec et al. (2006) are based on the morphological characters of nematodes. Recent molecular studies of different groups of nematodes established that morphological characters (especially apomorphies) may appear independently in different lineages

and are often not suitable for phylogenetic studies (Carreno and Nadler 2003, Tkach et al. 2014). Therefore, in our opinion, the real phylogeny and evolutionary relationships between Camallanidae and their hosts will be illuminated in further molecular studies of more species from different hosts around the globe.

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