Nematodes found in Nile crocodiles in the Kruger National Park, South Africa, with redescriptions of *Multicaecum agile* (Wedl, 1861) (Heterocheilidae) and *Camallanus kaapstaadi* Southwell & Kirshner, 1937 (Camallanidae)

Kerstin Junker • Joop Boomker • Danny Govender • Yasen Mutafchiev

K. Junker (✉)

Epidemiology, Parasites and Vectors, ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa; http://orcid.org/0000-0001-6650-1201

e-mail: junkerk@arc.agric.za

J. Boomker

Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort, South Africa

D. Govender

Scientific Services, South African National Parks, Skukuza, 1350, South Africa

D. Govender

Department of Para-clinical Sciences, University of Pretoria, Private Bag X04, Onderstepoort, South Africa

Y. Mutafchiev

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, Sofia, 1113 Bulgaria; http://orcid.org/0000-0002-7901-7205

Abstract

Sixteen Nile crocodiles were collected in the Kruger National Park, South Africa and vicinity during 2010 and 2011. A total of 11 nematode species representing six families were recovered. Heterocheilids were the dominant group, comprising five species, with *Dujardinascaris madagascariensis* (Chabaud & Caballero, 1966) being the most prevalent (75%), followed by *Ingwenascaris sprenti* Junker & Mutafchiev, 2017 (68.8%), which was also the second most numerous nematode. While less prevalent (31.3%), *Typhlophoros kwenae* Junker & Mutafchiev, 2017 was the most abundant species. *Micropleura huchzermeyeri* Junker & Mutafchiev, 2017 (Micropleuridae) was collected from five crocodiles and *Crocodylocapillaria* sp. (Capillariidae) occurred in a single host. Three nematodes, *Camallanus kaapstaadi* Southwell & Kirshner, 1937, *Spirocamallanus* sp. (both Camallanidae) and *Ascarophis* sp. (Cystidicolidae), are considered accidental infections, likely ingested with the hosts' prey. Our findings of *D. dujardini* (Travassos, 1920), *D. madagascariensis* and *Multicaecum agile* (Wedl, 1861) in South Africa constitute new geographic records. *Crocodylocapillaria* sp. represents a new host and geographical record, while *T. kwenae*, *I. sprenti* and *M. huchzermeyeri* have been described as new species during the course of this survey. *Multicaecum agile* is here redescribed based on light and scanning electron microscopy. Previously undescribed morphological characters of *C. kaapstaadi*, typically a parasite of *Xenopus* spp. (Amphibia: Pipidae), but here found in two Nile crocodiles, are also presented.

Introduction

As the largest semi-aquatic freshwater predator in Africa, the Nile crocodile, *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae), is a keystone species of aquatic biodiversity and fulfils an essential ecological role in the wide range of freshwater habitats it uses (Ross, 1998; Leslie & Spotila, 2001; Ashton, 2010). It is widespread in sub-Saharan Africa and is the only crocodilian found in South Africa, where it reaches its southernmost distribution. Targeted eradication programmes in several African countries had reduced the numbers of Nile crocodiles drastically and they were considered endangered or vulnerable for more than three decades (Ashton, 2010). Presently, Nile crocodiles are listed as of least concern on the IUCN Red List of Threatened Species (Crocodile Specialist Group, 1996), due mainly to a large captive breeding population.

In contrast, the South African Red Data Book for Reptiles and Amphibians (Jacobsen, 1988), lists Nile crocodiles in South Africa as vulnerable. Loss of habitat, effects of water

pollution, and human conflict that leads to destruction of nests, have resulted in the decline of crocodile populations in some of the few rivers and lakes that still harbour viable populations (Ashton, 2010; Combrink et al., 2011; Calverley & Downs, 2015). Next to Lake St Lucia and Ndumo Game Reserve, the Kruger National Park (KNP) is believed to be home to one of the largest and possibly secure populations of crocodiles in South Africa (Combrink et al., 2011). However, repeated mortalities in 2008, 2009 and 2010, due to pansteatitis of unknown aetiology (Lane et al., 2013), raised concerns for this population as well.

It is widely accepted that parasites can reduce host fitness and survival and play an important role in regulating the dynamics of host populations (Hudson et al., 2002). Because of their influence on the sustainability of species and populations (Hoberg et al., 2013), knowledge of the composition of parasite assemblages of wildlife and factors that influence patterns of prevalence and abundance is vital for conservation efforts. The helminth fauna of Nile crocodiles has been poorly studied and much of our knowledge is based on taxonomic papers (e.g. Baylis, 1923; Ortlepp, 1932; Sprent, 1977, 1978, 1979; Sprent et al., 1998; Junker & Mutafchiev, 2017a, b, c). Junker et al. (2006) reported on gastric helminths in Nile crocodiles from Botswana, but did not present prevalence or abundance data, as helminths had been incidental collections made during the course of a study on the crocodiles' diet.

The Olifants River mortalities mentioned above, led to the inception of a multidisciplinary team to investigate the disease ecology of pansteatitis in Nile crocodiles in the KNP and to obtain baseline data on their blood chemistry and other physiological parameters. As such, this offered the rare opportunity to study their helminth parasite fauna. In this paper, we provide first-time data on the nematode diversity in the crocodiles, including numerical measures of prevalence and abundance, to aid current and future researchers in the understanding of this host-parasite system. In addition, we redescribe *Multicaecum agile* (Wedl, 1861), a heterocheilid nematode presently known from few specimens only. Our finding of *Camallanus kaapstaadi* Southwell & Kirshner, 1937, a postcyclic camallanid nematode in the Nile crocodiles, but which typically parasitises amphibian hosts, allowed us to expand on the extent of morphological variation in this parasite and to study some of its hitherto undescribed morphological features.

Materials and methods

During 2010 and 2011, a total of 16 Nile crocodiles (eight males and eight females) was collected from different localities in the KNP and vicinity, South Africa (Table 1; see Junker et al., 2016 for additional host data). Collection sites within the KNP represented five perennial rivers (Crocodile, number of crocodiles = 2; Sabie, n = 1; Letaba, n = 2; Olifants, n = 3;

Levuvuh, n = 2), the seasonal Shingwedzi River (n = 1), which does, however, retain a number of permanent and semi-permanent pools along its length, and one impounded internal stream (Silolweni Dam; n = 2). Outside the KNP crocodiles were collected from permanent sedimentation dams of the Phalaborwa Mining Complex (PMC; n = 3); these crocodiles would have likely migrated from the Olifants or Selati Rivers. For the purpose of this paper, each river and the PMC sedimentation dams are considered separate localities, equating to a total of eight localities. Excepting one subadult male and one subadult female, all crocodiles were mature adults. Crocodiles, not specifically selected for signs of disease, were shot in the field and subsequently transported to the post-mortem facilities at Skukuza, where they were examined within 12 hours of death; some animals had to be stored overnight, with temperatures not exceeding 5°C in order to prevent post-mortem migration of parasites.

Table 1 Collection data of 16 Nile crocodiles examined for helminths in the Kruger National Park and vicinity, South Africa

Locality	Date	Geographical coordinates	Sex	TL (cm)	Stomach aliquot[e]	Intestinal aliquot[e]
Crocodile River	12 July 2010	25°27'S, 31°58'E	F	260	1/10	1/10
Crocodile River	12 July 2010	25°25'S, 31°58'E	F	292	1/10	1/1
Olifants River	13 July 2010	24°2'S, 31°13'E	M	373	1/40	1/10
Olifants River	13 July 2010	24°3'S, 31°13' E	M	323	1/1	1/10
Shingwedzi River	13 July 2010	23°7' S, 31°27' E	M	313	1/10	na
Levuvuh River	13 July 2010	22°43'S, 31°30'E	M	323	1/10	1/10
Levuvuh River	13 July 2010	22°25'S, 31°13'E	M	385	1/10	1/10
Silolweni Dam	15 July 2010	24°49'S, 31°50'E	F	304[c]	1/10	1/10
Silolweni Dam	15 July 2010	24°49'S, 31°50'E	M[b]	282	1/10	1/10
Letaba River	15 July 2010	23°50'S, 31°38'E	F	280	1/10	1/10
Letaba River	15 July 2010	23°51'S, 31°35'E	F	280	1/10	1/10
Sabie River	15 July 2010	25°7'S, 31°55'E	M	390[d]	1/10	1/10
PMC[a]	7 February 2011	24°2'S, 31°5'E	F	299	1/10	1/10
Olifants River	8 February 2011	24°4'S, 31°6'E	F	330	1/30	1/20
PMC[a]	8 February 2011	24°0'S, 31°6'E	F[b]	190	1/10	1/10
PMC[a]	9 February 2011	24°1'S, 31°11'E	M	346	1/20	1/10

[a]Phalaborwa Mining Complex: sedimentation dams; [b]subadult; [c]part of the tail missing, estimated length: 340 cm; [d]part of the tail missing, estimated length: 460 cm; [e]total aliquot taken
Abbreviations: F, female; M, male; na, not available; TL, total body length

A full post-mortem examination was done on 15 of the 16 crocodiles, and their overall body condition noted. For helminth recovery, the peritoneal and abdominal cavities were opened and macroscopically examined for parasites. Helminths were fixed in hot 70% ethanol and stored in 70% ethanol. The gastrointestinal tract (GIT) was removed and divided into oesophagus, stomach and small intestine. The oesophagus was opened lengthwise and any helminths present were treated as described above. The contents of the stomach and small intestine were fixed separately in hot saline and stored in 10% formalin until further processing at the ARC-

Onderstepoort Veterinary Institute. Due to the large volume of GIT contents, aliquots had to be taken for some of the crocodiles.

Within ten days of collection, the GIT contents were washed under tap water over a 150 µm sieve and transferred into 70% ethanol. Typically, a further 1/10 aliquot was taken from the field samples; total aliquots taken per crocodile are listed in Table 1. Helminth counts were calculated based on the aliquots taken from the respective hosts; in cases where single worms were collected as well, these were added as single counts to the aliquot calculations. Helminths collected from these aliquots were stored in 70% ethanol.

For morphological observation and identification, specimens were cleared in lactophenol and studied as temporary mounts under a compound light microscope. Specimens used for SEM were dehydrated through a graded ethanol series, immersed in hexamethyldisilazane for 20 min, air-dried, coated with gold in a JEOL JFS 1200 coater and examined using a JEOL JSM 5510 microscope at an accelerating voltage of 10 kV. All measurements are in micrometres unless otherwise indicated. Metrical data are given as the range followed by the mean in parentheses. Specimens were deposited in the National Collection of Animal Helminths, ARC-Onderstepoort Veterinary Institute, South Africa (NCAH), and the helminthological collections of the Museum für Naturkunde, Berlin, Germany (Entozoa: ZMB E) and the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria (IBER-BAS). Accession numbers as well as the authorities of helminth species collected from the Nile crocodiles are listed in Table 2. The terms prevalence, mean abundance and species richness are used as per the definitions of Bush et al. (1997).

Results

A total of 11 nematode species in six families were recovered from 16 Nile crocodiles in the KNP and vicinity (Table 2). All hosts harboured nematode infections, but from one host only larvae were recovered and identified as belonging to the Heterocheilidae. No nematodes were recovered from the oesophagus and the majority of gastrointestinal helminths belonged to the Heterocheilidae. Of these, *Dujardinascaris madagascariensis* had the highest prevalence (75%), followed by *Ingwenascaris sprenti* (68.8%), which was also the second most numerous nematode, with a mean abundance of 402.6 ± 193.7. *Thyphlophoros kwenae*, while less prevalent (31.3%) than *D. madagascariensis* and *I. sprenti*, was by far the most abundant species, with a mean of $2,849.4 \pm 2,771.7$ and a maximum of 44,420 individuals collected from one of the hosts. Specimens of *D. madagascariensis*, *I. sprenti* and *T. kwenae* were typically collected from the stomach, however, in 4 of 12, 9 of 11 and 1 of 5 infected crocodiles, respectively, these

Table 2 Infection parameters of nematode parasites collected from Nile crocodiles (n = 16) from eight localities in the Kruger National Park, South Africa

Species	Accession number of specimens	No. of localities present at	Site in host	No. of infected hosts	Prevalence (%)	Total count	Mean abundance	Abundance, range
Heterocheilidae								
Dujardinascaris dujardini (Travassos, 1920)	S/2018/7[b]	2	Stomach	2	12.5	70	4.4 ± 3.0	0–40
Dujardinascaris madagascariensis (Chabaud & Caballero, 1966)	S/2018/8[b]	7	Stomach, small intestine	12	75.0	2,995	187.4 ± 61.1	0–890
Ingwenascaris sprenti Junker & Mutafchiev, 2017	NCAH.6.3[b]; S/2018/15[b]	6	Stomach, small intestine	11	68.8	6,441	402.6 ± 193.7	0–2,600
Multicaecum agile (Wedl, 1861)	S/2018/9[b]	2	Stomach	3	18.8	280	17.5 ± 13.4	0–210
Typhlophoros kwenae Junker & Mutafchiev, 2017	NCAH.7[b]; S/2018/20-21[b]	4	Stomach, small intestine	5	31.3	45,590	2,849.4 ± 2,771.7	0–44,420
Dujardinascaris sp. larvae	–	1	Stomach	1	6.3	80	5.0 ± 5.0	0–80
Anisakidae								
Contracaecum sp. larvae[a]	S/2018/11[b]	1	Stomach	1	6.3	150	9.4 ± 9.4	0–150
Micropleuridae								
Micropleura huchzermeyeri Junker & Mutafchiev, 2017	ZMB E.7615-17[c]; NCAH.5.1-2[b]	3	Body cavity[d]	5	31.3	35	2.2 ± 1.5	0–25
Camallanidae								
Camallanus kaapstaadi Southwell & Kirshner, 1937[a]	S/2018/12A,B[b]	1	Stomach	2	12.5	100	6.3 ± 4.6	0–70
Spirocamallanus sp.[a]	S/2018/13[b]	1	Stomach	1	6.3	10	0.6 ± 0.6	0–10
Cystidicolidae								
Ascarophis sp.[a]	S/2018/14[b]	1	Stomach	1	6.3	20	1.3 ± 1.3	0–20
Capillariidae								
Crocodylocapillaria sp.	–	1	Stomach	1	6.3	40	2.5 ± 2.5	0–40

[a]These are not considered typical parasites of crocodilians, but have likely been ingested with the host's prey; [b]National Collection of Animal Helminths, ARC-Onderstepoort Veterinary Institute, South Africa; [c]Museum für Naturkunde, Berlin, Germany; [d]some specimens were also recovered from the stomach and small intestine, but see text

nematodes were also found in the small intestine. The capillariid *Crocodylocapillaria* sp. was found in the stomach of a single crocodile, whereas the micropleurid *Micropleura huchzermeyeri* was the only species recovered from the body cavity and had a prevalence of 31.3%, and a mean abundance of 2.2 ± 1.5. Taking into consideration only the seven nematode species considered typical parasites of crocodilians (see Table 2), species richness ranged from 1–4 in 15 infected hosts; of these 13.3% harboured a single species, 40% harboured two, 20% three, and 26.7% were infected with four species. In line with their high prevalence, *D. madagascariensis*, *I. sprenti* and *T. kweanae* were present at the highest number of localities, i.e. at 7, 6 and 4 of the eight localities, respectively. *Dujardinascaris madagascariensis* was only absent from crocodiles collected at the PMC dams, *I. sprenti* was not found in crocodiles from the Sabie and Shingwedzi Rivers, and *T. kwenae* was absent from crocodiles collected from the Crocodile and Letaba Rivers, the Silolweni Dam and the PMC dams.

Superfamily Ascaridoidea Baird, 1853
Family Heterocheilidae Henry & Railliet, 1912
Genus *Multicaecum* Baylis, 1923

***Multicaecum agile* (Wedl, 1861) Baylis, 1923**

Host: *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae).
Locality: Specimens on which the redescription is based were collected from a female crocodile in one of the sedimentation dams of the Phalaborwa Mining Complex, South Africa (24°2'S, 31°5'E; 07.ii.2011); the crocodile would have likely migrated from either the Olifants or Selati Rivers.
Site in host: Stomach.
Prevalence: 3 of 16 crocodiles examined were infected, i.e. 18.8%.
Intensity of infection: One crocodile harboured approximately 10 specimens, another approximately 60 and the third approximately 210 specimens, based on aliquot calculations.
Voucher material: NCAH: S/2018/9 (10 males, 13 mature females, 4 immature females); IBER-BAS N001.126 (SEM stub, fragments of 3 males); IBER-BAS N001.127 (SEM stub, fragments of 3 females).

Description (Figs. 1, 2)

General. Medium-sized heterocheilid nematodes. Body slender, slightly tapering towards both extremities. Cuticle with distinct transverse striations, interrupted along lateral sides of body; distance between striations bearing dense, fine longitudinal striae (Fig. 2C). Organised rows of somatic papillae absent. Anterior extremity with 3 large, rounded lips; lips wider than long, interlabia and postlabial grooves distinct (Figs. 1C, 2B). Anterior half of lips with continuous ridge of rounded denticles around their free margin (Figs. 1B, 2A); denticles approximately uniform in size. Dorsal lip with two large double papillae; subventral lips, each bearing 1 subventral double papilla, 1 smaller lateral papilla and 1 amphid (Figs. 1B, C, 2A, B). Oesophagus, muscular, long and slender, terminating in glandular ventriculus with 2 anterior and 3 posterior appendages (Fig. 1A); median posterior appendage relatively short, anterior and lateral posterior appendages distinctly elongated, with posterior ones being the longest. Intestinal caecum dorsal to oesophagus, with narrow lumen and extending forward for more than half the length of the oesophagus (Fig. 1A). Nerve-ring surrounding oesophagus roughly at first and second sixth of its length. Excretory pore on level of nerve ring or slightly posterior to it. Deirids prominent, dome-shaped, slightly posterior to excretory pore (Figs. 1A, 2C). Anterior width of intestine similar to combined width of caecum and ventriculus.

Male [Based on 11 males; except where otherwise indicated.] Body 19.8–33.3 (24.3) mm long. Maximum width of body 371–610 (459) near midbody; body width just anterior to cloaca 126–230 (158), just posterior to cloaca 77–157 (111). Deirids 11–27 (17; n = 7) in diameter. Postdeirids conical c. 7 high with blunt tip situated in shallow pit (Fig. 1D). Left postdeirid and right postdeirid at 8.9 mm and 14.5 mm, respectively, from posterior extremity of male 26.3 mm long. Oesophagus, including ventriculus, 2,991–5,214 (3,636) long, i.e. 13.1–16.2% (14.9%) of body length. Ventriculus 172–344 (234) long, 145–346 (218) wide; length of shorter and longer anterior appendage 218–388 (290) and 325–578 (423), respectively; length of shorter and longer posterior lateral appendage 410–644 (511) and 448–784 (586), respectively; median posterior appendage 157–282 (223) long. Intestinal caecum 1,875–3,759 (2,542) long, i.e. 61.1–72.8% (69.5%) of total oesophagus length; width at base 160–254 (198). Nerve-ring, excretory pore and deirids at 552–895 (663), 559–895 (694) and 715–1,093 (855), respectively, from anterior extremity. Reflexion of testis situated at 9.8–12.1 (10.7) mm from anterior extremity. Tail curved
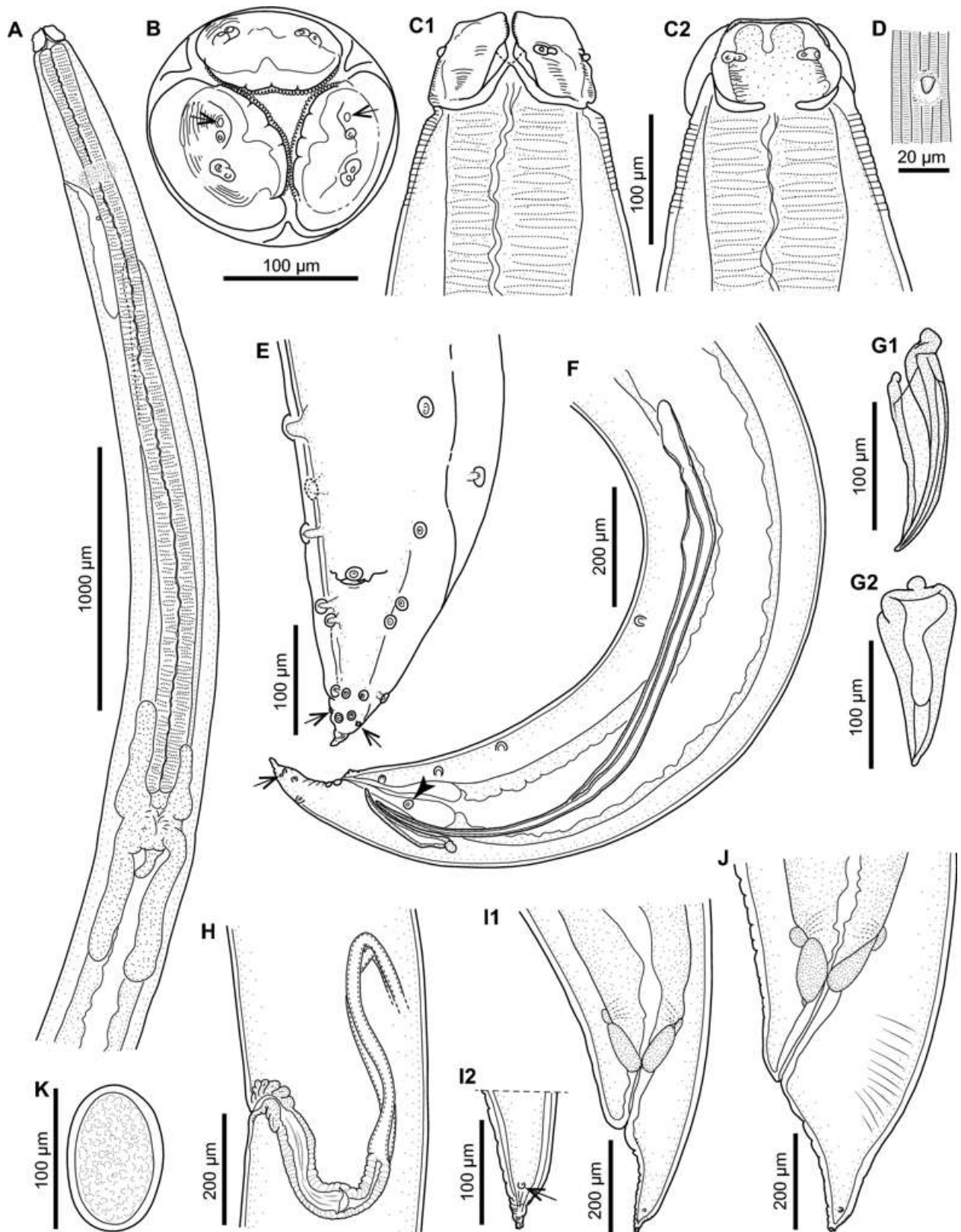
Fig. 1. *Multicaecum agile* from *Crocodylus niloticus*. A, Anterior extremity, male, lateral view, note excretory pore and deirid on level of excretory gland; B, Head region, female, apical view, note amphids (*arrows*); C, Head region, male, dextral view (C1) and dorsal view (C2); D, Postdeirid, male; E, Tail, male, ventral view, note phasmids (*arrows*); F, Posterior extremity male, sinistral view; G, Gubernaculum, sinistral view (G1) and ventral view (G2); H, Vagina and uteri, immature female; I, Tail, immature female, lateral view (I1) and tail tip (I2), note phasmid (*arrow*); J, Tail, mature female, lateral view; K, Egg
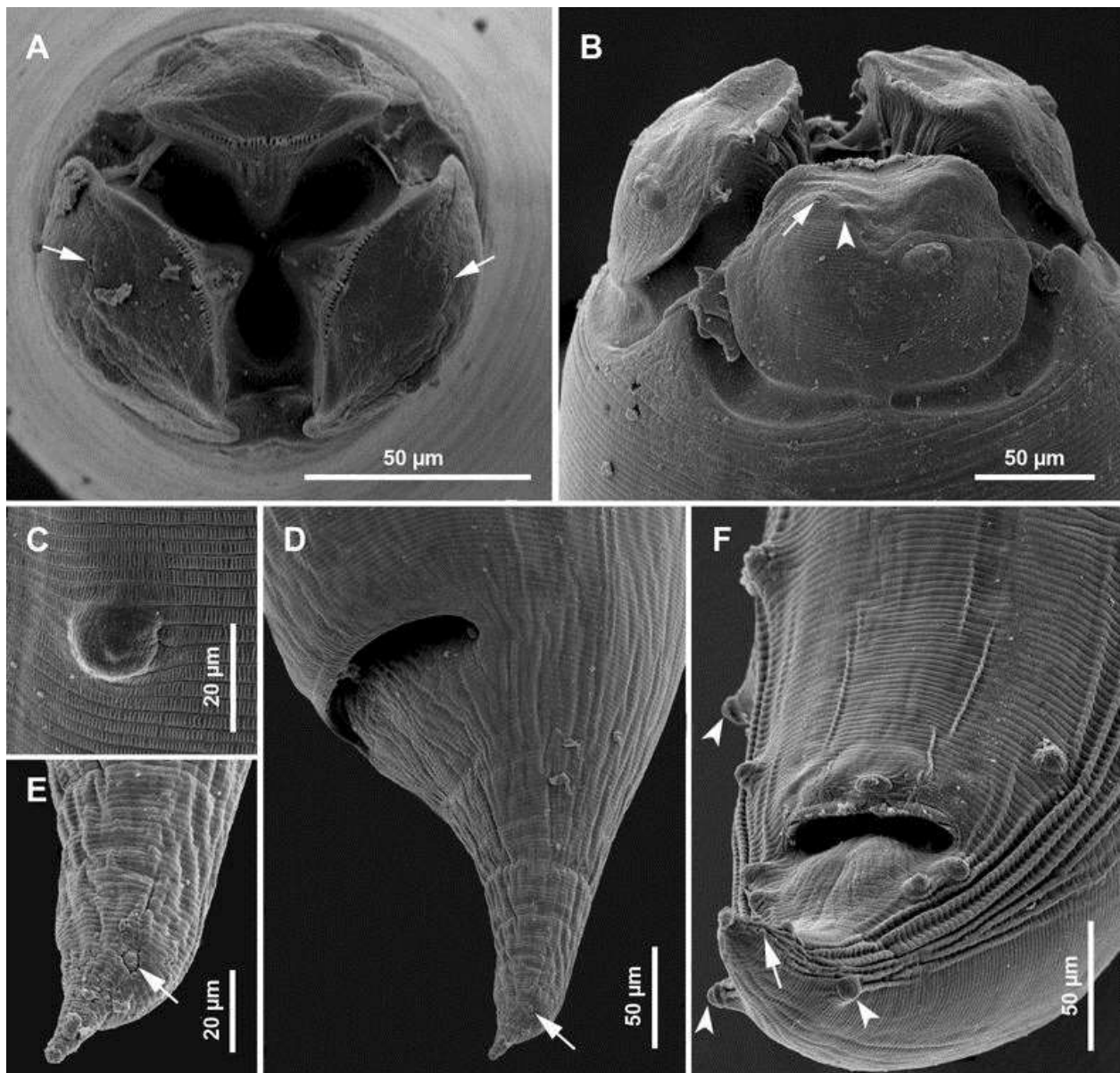
Fig. 2. *Multicaecum agile* from *Crocodylus niloticus*. A, Head region, male, apical view; note amphids (*arrows*); B, Head region, female, dextral view; note amphid (*arrow*) and small single papilla (*arrowhead*); C, Deirid; D, Tail, female, lateral view, note phasmid (*arrow*); E, Tail tip, female, lateral view; note phasmid (*arrow*); F, Posterior extremity, male, ventrolateral view; note phasmid (*arrow*) and lateral caudal papillae (*arrowhead*)

ventrally, with elongated tip (Figs. 1F, 2F); 141–232 (174) long. Caudal alae absent. Caudal papillae 10 pairs, arranged as follows (Figs. 1E, F, 2F): 4 pairs of subventral precloacal papillae, first pair at long distance from second pair, pairs 2 to 4 more evenly spaced; 1 pair of lateral papillae on level between third and fourth precloacal subventral pair; 4 pairs of subventral postcloacal papillae, first 2 pairs grouped at short distance from cloaca, remaining 2 pairs forming subterminal group; 1 pair of lateral postcloacal papillae on level of third postcloacal subventral pair; a single median papilla just anterior to cloacal opening. Phasmids lateral, at level of last subventral pair of postcloacal papillae. Spicules equal, 1,079–1,235 (1,168) long, including handle 168–208 (193; n = 9) long; alate, alae terminating early in posterior third (Fig. 1F). Gubernaculum well-sclerotised, 180–195 (187) long, slender in lateral view, triangular in

10

dorsoventral view, with median knob-like extension at proximal end and tapering towards distal end (Fig. 1G).

Mature female [Based on 11 gravid females; except where otherwise indicated.] Body 30.1–41.8 (37.8) mm long. Maximum body width near vulva, 538–812 (717). Deirids 18–28 (23; n = 8) in diameter. Nerve-ring, excretory pore and deirids at 749–1,072 (937), 766–1,078 (967) and 966–1,362 (1,207), respectively, from anterior extremity. Left postdeirid at 55 posterior to vulva and right postdeirid at 153 anterior to vulva in female 42.8 mm long. Oesophagus, including ventriculus, 4,656–6,376 (5,642) long, or 13.5–16.0% (14.9%) of body length; ventriculus 256–449 (334) long, 251–394 (296) wide; length of shorter and longer anterior appendage 292–571 (394) and 418–662 (539), respectively; length of shorter and longer posterior lateral appendage 554–760 (658) and 592–792 (711), respectively; median posterior appendage 221–370 (296) long. Intestinal caecum 3,433–4,828 (4,102) long, i.e. 68.1–76.9% (72.7%) of total oesophagus length; 192–311 (277) wide at base. Rectum short, conical, with thickened walls (Fig. 1J). Tail short, conical, straight or slightly curved ventrally, 223–373 (299) long; tip of tail with several rounded rugosities (Figs. 1J, 2D), but with three little spikes in one female. Phasmids at 40–58 (52) from tail tip (Fig. 2E). Vulva at 15.3–20.9 (18.4) mm from anterior extremity or 44.4–51.4% (48.7%) of body length. Vagina directed posteriorly, composed of muscular *vagina vera*, 759–1,170 (921; n = 8) long, and *vagina uterina*, 778 and 1,125 long in female 30.1 and 36.1 mm long, respectively. Uteri two, amphidelphic. Eggs at morula-stage, thick-shelled, ovoid to round, measured in uterus of 3 mature females: 92–107 × 74–93 (100 × 79; n = 18), (Fig. 1K).

Immature female [Based on six immature females; see Table 3 for additional measurements.] Deirids 8–14 (12) in diameter. Postdeirids observed at level of vulva in 3 immature females (Fig. 2D); two postdeirid-like structures each observed on right and left side in one immature female 33.8 mm long, on right side 68 anterior and 175 posterior to vulva, and on left side 118 anterior and 68 posterior to vulva. Ventriculus 165–275 (221) long, 142–258 (208) wide; length of shorter and longer anterior appendage 218–345 (275) and 224–470 (381), respectivley; length of shorter and longer posterior lateral appendage 409–545 (502) and 433–577 (534), respectively; median posterior appendage 194–241 (215) long. Intestinal caecum 85–227 (206) wide at base. Tail short, conical, straight or slightly curved ventrally, tip of tail with several rounded rugosities (Fig. 1I1). Phasmids at 39–44 (42) from tail tip (Fig. 1I2). Vagina directed posteriorly, anteriorly in two females; *vagina vera*, 581 and 573 long in 2 specimens 22.4 mm and 25.0 mm long, respectively; *vagina vera* 300 and *vagina uterina* 127 long in specimen 17.6 mm long (Fig. 1H). Uteri two, amphidelphic.

Table 3 Metrical data of *Mulicaecum agile* (Wedl, 1861) from African and Australian crocodiles. Measurements in micrometres unless otherwise indicated

Source	Present study			Baylis (1923)		Sprent (1979)	Sprent (1979)	Sprent (1979)
Host	*Crocodylus niloticus* Laurenti			*Crocodylus niloticus*		African crocodile	African crocodile	*Crocodylus johnstoni* Krefft
Geographical origin	South Africa			White Nile[b]		Africa	Africa	Australia
Specimen sex	Male (n = 11)	Female (n = 11)	Immature female (n = 6)	Male (n = 1)	Female (n = 1)	Male (n = 1)	Female (n = 1)	Female (n = 1)
Total body length (TL) (mm)	19.8–33.3	30.1–41.8	12.4–24.9	34.6	31.6	21.1	26.0	50.0
Maximum body width	371–610	538–812	271–496	650	670	430	480	850
Anterior extremity to nerve-ring	552–895	749–1,072	421–661	820[c]	820[c]	580	710	900
Anterior extremity to excretory pore	559–895	766–1,078	443–687	850–860[c]	850–860[c]	600	710	900
Anterior extremity to deirids	715–1,093	966–1,362	530–855	1,130[c]	1,130[c]	–	–	–
Total length of oesophagus	2,991–5,214	4,656–6,376	2,213–3,816	4,000–4,200[c]	4,000–4,200[c]	3,500	4,000	5,900
Caecum, length	1,875–3,759	3,433–4,828	1,644–2,865			2,500	3,000	3,800
Tail length	141–232	223–373	156–254	220	320	160	220	380
Left spicule length	1,079–1,235	–	–	1,200	–	850	–	–
Right spicule length	1,089–1,226	–	–	1,200	–	850	–	–
Gubernaculum length	180–195	–	–	200	–	190	–	–
Anterior extremity to vulva (mm)	–	15.3–20.9	6.7–12.6[a]	–	15.2	–	12.0	22.7
Oesophagus/TL (%)	13.1–16.2	13.5–16.0	15.0–18.3	11.6–12.1	12.7–13.3	16.6[d]	15.4[d]	11.8[d]
Caecum/Oesophagus length (%)	61.1–72.8	68.1–76.9	68.1–76.5	–	–	71.4[d]	75[a]	64.4[d]
Tail/TL (%)	0.6–0.9	0.7–1.0	0.9–1.3	0.6[d]	1.0[d]	0.8[d]	0.8[d]	0.8[d]
Vulva/TL (%)	–	44.4–51.4	48.6–56.4[a]	–	48.1[d]	–	46.2[d]	45.4[d]

[a]Measurements obtained from five specimens; [b]The White Nile flows through the following countries: Sudan, South Sudan, Rwanda, Tanzania, Uganda, Democratic Republic of the Congo; [c]These measurements were not allocated to male or female specimens; [d]Values calculated from source

Remarks

Presently the genus *Multicaecum* comprises two species, *M. agile* and *M. heterotis* Petter, Vassiliadès & Marchand, 1979. The type-species *M. agile* was described as *Ascaris agilis* from *C. niloticus* (syn. *Crocodilus vulgaris* Cuvier) in Egypt (Wedl, 1861), and has since also been reported from other Old World crocodilians, *Mecistops cataphractus* (Cuvier) (syn. *Crocodylus cataphractus* Cuvier) (Crocodylidae), *Crocodylus palustris* Lesson, *Gavialis gangeticus* (Gmelin in L.) (Gavialidae) and *Crocodylus johnstoni* Krefft, in Africa, India and Australia (summarised by Sprent, 1979; Mašová et al., 2010). Equally an Old World species, *M. heterotis* parasitises *Heterotis niloticus* (Cuvier), an arapaimid fish species in Africa (Mašová et al., 2010).

The present material corresponds well to descriptions of *M. agile* from African crocodiles by Wedl (1861) and Baylis (1923) (see Table 3). The arrangement of caudal papillae in the present specimens conforms to that described by Baylis (1923), with five precloacal and five postcloacal pairs of papillae, of which the lateral precloacal pair is situated between the two subventral precloacal pairs closest to the cloaca, and the lateral postcloacal pair is situated on the level of the third subventral postcloacal pair. The shape of the gubernaculum with "a small solid knob at the proximal end" (Baylis, 1923) and the lateral posterior appendages of the ventriculus being longer than the anterior ones are easily observed features (Baylis, 1923; Sprent 1979; this paper). The present specimens could thus unambiguously be referred to *M. agile*, making it possible to augment the range of morphometric data available for this species, which had so far been limited to measurements and observations from few African specimens (see Table 3).

Sprent (1979) based his detailed description of *M. agile* on specimens from Africa, India and Australia, including illustrations and measurements of specimens from both African and Australian hosts (see Table 3). SEM photographs were obtained from Australian specimens only. The single measured female from Australian hosts is larger in size than females from African hosts (50.0 mm *vs* 26.0–41.8 mm) and has a slightly smaller oesophagus to body length ratio as well as caecum to oesophagus length ratio (11.8 *vs* 12.7–16.0% and 64.4 *vs* 68.1–76.9%, respectively) (see Table 3). The Australian specimen illustrated by Sprent (figure 3 in Sprent, 1979) has lips that are twice wider than long, whereas lips of *M. agile* from African hosts are more rounded (figure 1 in Sprent, 1979; this paper). Another noteworthy difference between *M. agile* from African and Australian hosts is the reduced length of the stout posterior median appendage of the ventriculus in the African specimens (figure 7 in Baylis, 1923; this paper), when compared to the long and slender median appendage in the Australian specimen (fig. 7 in Sprent, 1979). The observed discrepancies emphasise the need for a more detailed description of

additional specimens of *Multicaecum* from Australian crocodiles, in order to establish if these specimens are conspecific with their congeners in Africa or represent a different species.

Superfamily Camallanoidea Travassos, 1920
Family Camallanidae Railliet & Henry, 1915
Genus *Camallanus* Railliet & Henry, 1915

Camallanus kaapstaadi **Southwell & Kirshner, 1937**

Host: *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae).

Locality: Specimens were collected from two crocodiles in the Kruger National Park, South Africa: One adult female and one subadult male, both from the Silolweni Dam (15.vii.2010; 24°49'S, 31°50'E).

Site in host: Stomach.

Prevalence: 2 of 16 crocodiles examined were infected, i.e. 12.5%.

Intensity of infection: The crocodiles harboured approximately 30 and 70 specimens, respectively, based on aliquot calculations.

Voucher material: NCAH: S/2018/12A (4 males, 4 females), S/2018/12B (4 males, 1 female).

Description (Fig. 3)

General. Small-sized camallanid nematodes. Anterior extremity bent ventrally (Fig. 3A); from level of approximately oesophago-intestinal junction posteriorly, body curved dorsally and tapering gradually. Cuticle with prominent external transverse striations along entire body, distance between striations bearing dense, fine longitudinal striae (Fig. 3J). Lateral alae narrow, extending from region of muscular oesophagus almost along entire body (Fig. 3E). Oral opening slit-like, dorsoventrally elongated, surrounded by 4 submedian cephalic papillae and 2 lateral amphids (Fig. 3C). Buccal capsule large, brownish, tilted towards ventral side, consisting of 2 identical valves, each bearing numerous smooth longitudinal ridges; majority of ridges complete, some incomplete and of varying length (Fig. 3B). One pair of sclerotised plates present near anterior margin of each valve. Posterior end of buccal capsule surrounded by narrow sclerotised basal ring; 3 pairs of sclerotised knob-like posterior projections present on each side of basal ring (Fig. 3B). Dorsoventral margins of buccal capsule with posteriorly directed tridents originating at approximately midlevel of buccal capsule and extending slightly beyond level of basal ring; ventral trident somewhat shorter than dorsal one (Fig. 3B, D). Oesophagus distinctly divided into
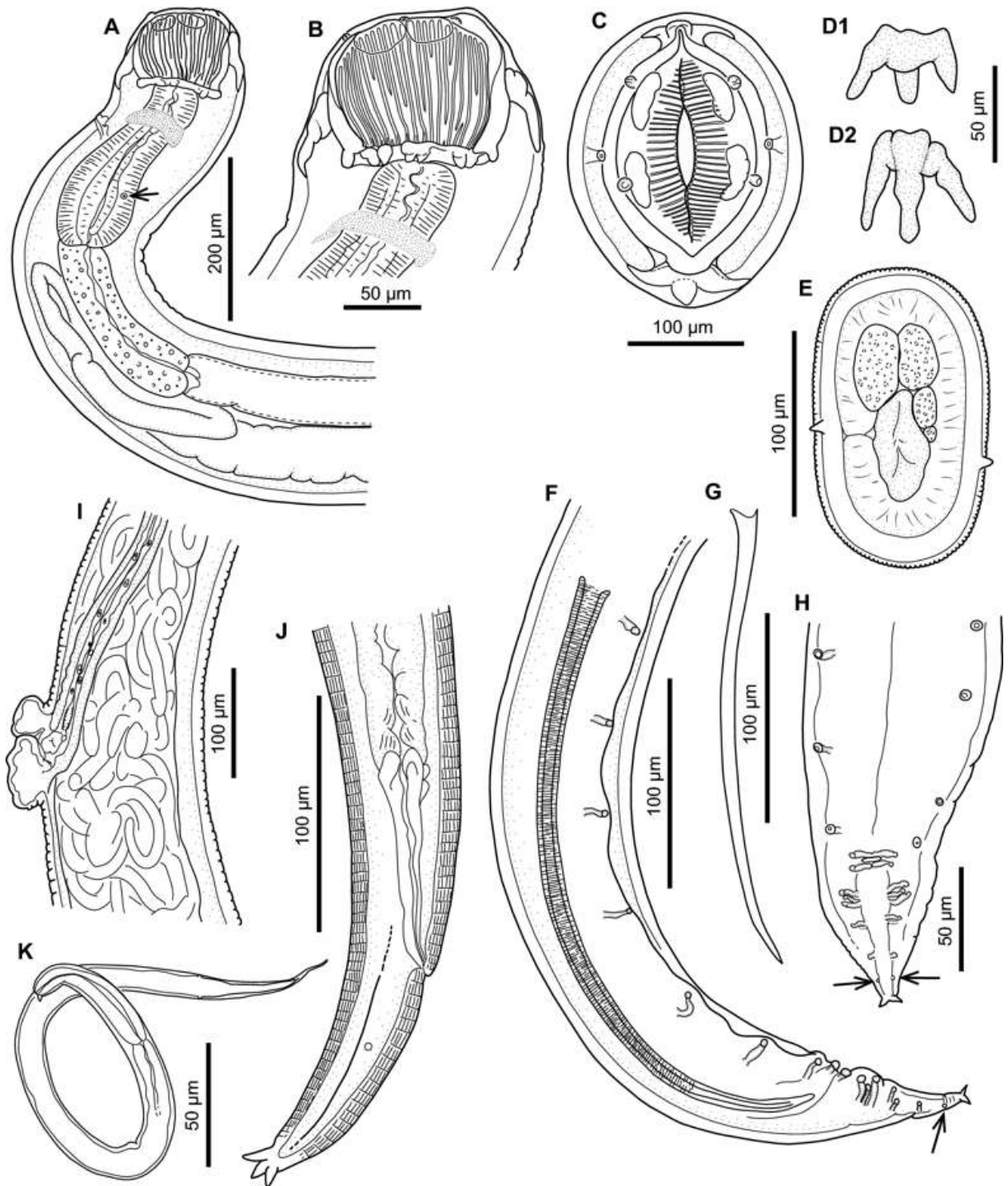
Fig. 3. *Camallanus kaapstaadi* from *Crocodylus niloticus*. A, Anterior extremity, male, lateral view, note deirid (*arrow*); B, Head region, male, lateral view; C, Apical view, female; D, Tridents, male, ventral (D1; seen from the back) and dorsal (D2) view; E, Transverse section at level of glandular oesophagus, male; F, Tail, male, dextral view, note right spicule and phasmid (*arrow*); G, Left spicule; H, Tail, male, ventral view, note phasmids (*arrows*); I, Vulva with prominent anterior and posterior lip; J, Posterior extremity, female, sinistral view; K, First-stage larva, extracted from uterus

club-shaped muscular part with elongate posterior bulb and glandular part of similar length to muscular part, subtly widening towards oesophago-intestinal junction (Fig. 3A). Nerve-ring encircling oesophagus at level of anterior quarter of muscular oesophagus. Excretory pore opening slightly posterior to nerve-ring. Deirids small, papilliform, situated posterior to nerve-

15

ring and excretory pore, on level of bulb of muscular oesophagus (Fig. 3A). Intestine and rectum narrow (Fig. 3A, J). Tail with spine-like processes (Fig. 3F, H, J).

Male [Based on nine males, unless otherwise indicated.] Body 2.24–3.03 (2.58; n = 8) mm long. Maximum width of body posterior to oesophago-intestinal junction, 133–214 (163; n = 8). Buccal capsule, including basal ring, 80–100 (92) long, maximum width 100–120 (110), valves supported by 22–27 (24) ridges, of which 4–10 (7) incomplete. Basal ring 9–14 long (11), 72–103 (87) wide. Dorsal trident 56–68 (62) long, ventral trident 42–53 (47) long. Oesophagus, 459–546 (502) long, i.e. 18.0–22.0% (19.7%) of total body length; muscular part 228–257 (242) long, and 47–61 (54), 46–60 (53) and 70–96 (81) wide at anterior, mid-length and bulb level, respectively; glandular part 220–303 (260) long, and 39–66 (54) and 42–91 (67) wide at anterior and posterior level, respectively; ratio length of muscular part to length of glandular part 1: 0.9–1.3 (1.1). Nerve-ring, excretory pore and deirids at 143–169 (153), 160–195 (176; n = 8) and 220–262 (240; n = 8), respectively, from anterior extremity. Reflexion of testis as far anterior as junction of muscular and glandular oesophagus and as far posterior as level of posterior end of glandular oesophagus, at 305–544 (384; n = 8) from anterior extremity. Lateral alae extending from anterior region of muscular oesophagus to approximately level of first precloacal papillae. Tail curved ventrally, 51–73 (64; n = 8) long, with 2 minute terminal spine-like processes (Fig. 3F, H). Caudal alae narrow, inconspicuous, starting at approximately level of first pair of pedunculate precloacal papillae. Caudal papillae (Fig. 3F, H): 7 pairs of subventral precloacal pedunculate papillae, more or less evenly spaced; 2 pairs of pedunculate adcloacal papillae encasing cloaca, 1 pair anterior, 1 pair posterior to cloaca; 6 pairs of postcloacal pedunculate papillae (a group of 3 subventral pairs at a brief distance from cloaca, 2 pairs at tail midlength, 1 subventral, 1 lateral and a small subventral pair midway between second last pair and tail tip). Phasmids lateral, posterior to last pair of postcloacal papillae. Right spicule well-sclerotised, 295–367 (330); left spicule hardly sclerotised, 230–240 (n = 2) long, could only be observed after dissection; both spicules non-alate, slender with pointed tip (Fig. 3F, G). Gubernaculum absent.

Female [Based on five larvigerous females, unless otherwise indicated.] Body 2.63–3.75 (2.97) mm long. Maximum width of body posterior to oesophago-intestinal junction, 217–338 (252; n = 4). Buccal capsule, including basal ring, 131–161 (147; n = 4) long, 171–195 (182; n = 4) wide, supported by 30–32 (31; n = 4) ridges, of which 6–10 (8; n = 4) incomplete. Basal ring 15–20 long (18), 120–157 (142) wide. Dorsal trident 73–107 (85; n = 4) long, ventral trident 53–67 (58; n = 3) long. Oesophagus, 599–666 (632; n = 4) long, i.e. 17.7–23.2% (21.6%; n = 4) of total

body length; muscular part 296–306 (303; n = 4) long, and 68–74 (72; n = 4), 64–82 (71; n = 4) and 97–124 (110; n = 4) wide at anterior, mid-length and bulb level, respectively; glandular part 282–367 (334; n = 4) long, and 59–66 (64; n = 4) and 73–103 (87; n = 4) wide at anterior and posterior level, respectively; ratio length of muscular part to length of glandular part, 1: 0.9–1.2 (1.1). Nerve-ring and deirids at 204–213 (209; n = 4), and 300–333 (310; n = 4), respectively, from anterior extremity. Tail conical, curved dorsally, 99–138 (114) long, i.e. 3.5–4.2% (3.9%) of total body length, bearing 3 processes 4–7 (6) long (Fig. 1I). Phasmids at 66–81 (74; n = 3) from tail tip (Fig. 3J). Lateral alae extending from posterior region of muscular oesophagus to tip of tail (Fig. 3J). Postdeirids minute, situated just dorsally to lateral alae, slightly posterior to vulva; right postdeirid at 25–58 (n = 2), left postdeirid at 46–183 from vulvar opening (n = 3). Vulva with 2 prominent lips (Fig. 3I), situated at 1,614–2,246 (1,830) from anterior extremity or 59.9–64.3% (61.8%) of total body length. Vagina with short thick-walled distal portion and long muscular, posteriorly directed proximal portion; too convoluted or obscured by larvae for accurate measurements. Monodelphic. Anterior branch of uterus extending up to level of bulb of muscular oesophagus or anterior part of glandular oesophagus, posterior branch of uterus ending blind in posterior fourth of body; uterus filled with numerous first-stage larvae. One larva extracted from uterus (Fig. 3K): anterior extremity bearing single larval tooth; body 334 long, 13 wide at oesophago-intestinal junction; oesophagus 56 long; phasmids situated at 56 from tail tip; tail 91 long. Two larvae measured in utero: 307 and 325 long.

Remarks

The specimens collected from the stomach of *C. niloticus* morphologically correspond to descriptions of *C. kaapstaadi* from *Xenopus laevis* (Daudin) (Amphibia: Pipidae) in South Africa (Southwell & Kirshner, 1937; Svitin et al., 2018), and from *Xenopus laevis laevis* in South Africa as well as from *Xenopus muelleri* (Peters) in Ghana (Jackson & Tinsley, 1995): buccal capsule supported by 16 to 38 ridges; tail in males bearing two, in females three processes; vulva postequatorial; right spicule well sclerotised, 191–468 µm long, and left spicule poorly sclerotised, 148–220 µm long, both with pointed tip.

In the original description of *C. kaapstaadi*, all ridges supporting the buccal capsule are illustrated as being complete (Southwell & Kirshner, 1937). Subsequent authors have, however, commented on the presence of incomplete ridges as found in the present material (Jackson & Tinsley, 1995; Svitin et al., 2018). The basal ring of the buccal capsule in the current specimens bears three pairs of prominent sclerotised projections on either side. No mention of this is made by Jackson & Tinsley (1995) in the description of *C. kaapstaadi*, but the feature is illustrated in

their figure 2 and can also be appreciated on figure 2A in the description by Svitin et al. (2018). Yeh (1960) described this character for *C. johni* Yeh, 1960, which is presently considered a junior synonym of *C. kaapstaadi* (see Svitin et al., 2018). While material studied by Southwell & Kirshner (1937) as well as Svitin et al. (2018) displayed no pronounced tilt of the buccal capsule, both specimens examined by Jackson & Tinsley (1995) and the present specimens are characterised by a distinct tilt of the buccal capsule towards the ventral side.

In none of the 12 species of *Camallanus* previously described from vertebrate hosts in Africa, including *C. kaapstaadi*, mention is made of the presence of lateral alae (Baylis, 1928; Walton, 1932; Kung, 1948; Vassiliadès & Petter, 1972; Moravec, 1973; Durette-Desset & Batchvarov, 1974; Amin, 1978; Jackson & Tinsley, 1995; Kabré & Petter, 1997; Svitin et al., 2018). This is interesting as lateral alae, while being narrow, are nevertheless distinct in the present specimens.

Southwell & Kirshner (1937) and Svitin et al. (2018) recorded only 5 pairs of pedunculate postcloacal papillae, with an initial group of two pairs followed by three single pairs illustrated by Southwell & Kirshner (1937; their figure 11), and three prominent papillae somewhat posterior to the cloaca followed by two small pairs close to the tail tip reported by Svitin et al. (2018). Similar to the present study, Jackson & Tinsley (1995) described in *C. kaapstaadi* six pairs of postcloacal papillae arranged in an initial group of three, followed by a group of two pairs and a single pair closest to the tail tip. Previous authors did not indicate the presence or position of phasmids; we here confirm that phasmids are present and are located posterior to the terminal pair of pedunculate papillae.

The most striking difference between the present material and previously described specimens of *C. kaapstaadi*, and for that matter any of its congeners in Africa, is the presence of two very prominent vulvar lips in the females, whereas in the remaining species lips are either absent or inconspicuous or a distinct anterior lip, but no posterior lip, is present (Baylis, 1928; Walton, 1932; Kung, 1948; Vassiliadès & Petter, 1972; Moravec, 1973; Durette-Desset & Batchvarov, 1974; Amin, 1978; Jackson & Tinsley, 1995; Kabré & Petter, 1997; Svitin et al., 2018). Based on previous descriptions, *C. kaapstaadi* falls in the latter group with a single anterior lip only (Jackson & Tinsley, 1995; Svitin et al., 2018).

Despite the morphological differences between the present specimens and previously described samples of *C. kaapstaadi*, we do not think that these warrant the description of a new species of *Camallanus* from the crocodiles. Future researchers should, however, take note of these differences as well as of the new morphological data such as the position of phasmids, the presence of lateral alae as well as the unique shape of the vulvar opening, which had previously not been available. The observed differences most likely reflect host and/or geographical

differences. While our material contained fully mature males and females and the possibility of host switching cannot be entirely disregarded, we believe *C. kaapstaadi* had most likely been ingested with the crocodiles' prey, a phenomenon known as postcyclic parasitism (see Anderson, 2000). Crocodilians are opportunistic predators and all previously recorded main host groups of *Camallanus*, namely fish, amphibians and turtles, have been found to form part of their diverse prey spectrum (Valentine et al., 1972; Platt et al., 2006, 2013; Wallace & Leslie, 2008).

Discussion

There is a dearth of data on metazoan parasites of crocodilians and this is the first investigation into the nematode assemblages of Nile crocodiles in South Africa. As such, our finding of *D. dujardini*, *D. madagascariensis* and *M. agile*, while having been reported from Nile crocodiles before (see Huchzermeyer, 2003; Junker et al., 2006; Tellez, 2013), represents a new geographical record for these parasites. Similarly, *M. huchzermeyeri*, *T. kwenae* and *I. sprenti* have only recently been described as part of this study as new species and, in case of the latter, new genus, with both *Micropleura* von Linstow, 1906 and *Typhlophoros* von Linstow, 1906 being recorded from the Afrotropics for the first time (Junker & Mutafchiev, 2017a, b, c). The presence of *Crocodylocapillaria* sp. also constitutes a new host and geographical record for this genus, which had previously been recorded from crocodilians in Australia and New Guinea only (Moravec & Spratt, 1998).

Similar to observations made by previous authors (Brooks & O'Grady, 1989; Goldberg et al., 1991), by far the majority of helminths collected from the gastrointestinal tract in the present study belonged to the Heterocheilidae (Ascaridoidea). Of the ten species in seven heterocheilid genera recorded from Nile crocodiles to date (see Huchzermeyer, 2003; Junker et al., 2006; Tellez, 2013; Junker & Mutafchiev, 2017b), five species in four genera were present in South Africa. Representatives of the remaining three genera, *Brevimulticaecum* Mozgovoy, in Skrjabin, Shikobalova & Mozgovoy, 1952, *Ortleppascaris* Sprent, 1978 and *Hartwichia* Chabaud & Bain, 1966, have as yet not been found in South Africa.

Based on their high prevalence as well as abundance, *D. madagascariensis* and *I. sprenti* were the dominant species in the helminth assemblages of Nile crocodiles examined during the present study, whereas *T. kwenae* and *M. huchzermeyeri* took an intermediate position, with *M. agile* and *D. dujardini* being rarer species, followed by *Crocodylocapillaria* sp., which was found in a single host only. Junker et al. (2006) recorded *D. madagascariensis*, *D. dujardini*, *M. agile* and *Brevimulticaecum vandenbrandeni* (Baylis, 1929) [as *Gedoelstascaris vandenbrandeni* (Baylis, 1929)] from Nile crocodiles in Botswana, with *D. madagascariensis* being the most

common parasite there as well. The remaining species were collected from few host individuals only. However, since the latter study had been based on incidental collections made during the course of a study on the Nile crocodiles' diet, it is possible that smaller species such as *T. kwenae* and *I. sprenti* were overlooked; *M. huchzermeyeri*, a parasite of the peritoneal and abdominal cavity, could not have been collected in this manner. Whether the absence of certain parasites in crocodiles from Botswana signifies a true variation in species composition or is an artefact of random sampling combined with low sampling effort in both countries needs further confirmation. Similarly, both *D. dujardini* and *M. agile* were collected at two localities only in the present study. These parasites had a low prevalence (Table 2) and more crocodiles per locality would have to be sampled to establish their true absence from sites at which they were presently not recovered.

Adults of the genera *Camallanus* and *Spirocamallanus* Olsen, 1952 have been recorded as parasites of fishes, amphibians, snakes and turtles (Rigby & Rigby, 2014), whereas many species of *Ascarophis* van Beneden, 1871 have been described from the gastrointestinal tract of marine and estuarine fishes (Moravec & Justine, 2007). The presence of members of these three genera in the Nile crocodiles is thus likely accidental (see above), and we conclude that transmission followed the trophic pathway. The same is likely true for the larvae of *Contracaecum* Railliet & Henry, 1912 found in the stomach of one of the crocodiles. *Contracaecum* spp. use fish as intermediate and piscivorous birds and mammals as definitive hosts (see Anderson, 2000).

A look at the few helminth community studies conducted on other crocodilian hosts shows gastric nematode assemblages of Nile crocodiles to be comparatively species rich. A total of six gastric nematode species for which crocodiles are considered typical definitive hosts were recovered from *C. niloticus* in South Africa. In contrast, studies on *Alligator mississippiensis* (Daudin) (Aligatoridae) at various localities in the United States, typically recorded between two and three species of gastric nematodes, including *Brevimulticaecum baylisi* (Travassos, 1933), *Brevimulticaecum tenuicolle* (Rudolphi, 1819), *Dujardinascaris waltoni* Sprent, 1977 and *Ortleppascaris antipini* (Mozgovoy, 1950) (Hazen et al., 1978; Scott et al., 1997; Waddle et al., 2009; Tellez & Nifong, 2014). Moravec (2001) reported *Dujardinascaris helicina* (Molin, 1860) from *Crocodylus moreletii* Duméril & Bibron in Mexico; *Crocodylus acutus* Cuvier in Mexico harboured *Terranova crocodili* (Taylor, 1924) (Anisakidae) and *D. helicina* (Villegas & González-Solís, 2009); and Goldberg et al. (1991) found four species of gastric nematodes in *Caiman yacare* (Daudin) (Aligatoridae) in Paraguay, namely *B. baylisi*, *Brevicaecum stekhoveni* (Baylis, 1947), *Dujardinascaris paulista* (Travassos, 1933) and *Ortleppascaris alata* (Baylis, 1947). With the exception of *T. crocodili*, all these nematodes belong to the Heterocheilidae.

Members of the Heterocheilidae have typically been reported from the stomach, but a number of species belonging to various genera have also been recorded from the intestine [e.g. *M. agile*, *B. vandenbrandeni*, *D. dujardini*, *Ortleppascaris nigra* (Gedoelst, 1916)] or oesophagus (e.g. *Brevimulticaecum pintoi* Sprent, 1979) of their hosts (Baylis, 1923; Sprent, 1977, 1978, 1979). In the present study, specimens of *I. sprenti*, *T. kwenae* and *D. madagascariensis* were found in both the stomach and small intestine of infected hosts, albeit in smaller numbers in the latter. While we cannot exclude the possibility of post-mortem migration as some hosts could only be processed several hours after their death, we conclude that species of a number of heterocheilid genera have adapted to exploiting both the stomach as well as the small intestine as possible niche within the host.

It is worth mentioning that in two of the five crocodiles infected with *M. huchzermeyeri*, specimens were not collected from their typical predilection site, i.e. the body cavity (Anderson, 2000), but from the gastrointestinal tract; in one crocodile one male and one female were recovered from the stomach and in another three males and one female were recovered from the stomach and small intestine (these specimens were treated as single specimens and not as part of the aliquot counts). While this might be attributable to contamination during post mortem procedures, it is noteworthy that *Micropleura indica* Khera, 1951 has been described from terrapins in India (Khera, 1951). It is thus also possible that terrapins could have been the original host of the specimens collected from the gastrointestinal tract.

Body condition of the crocodiles examined during this study ranged from fair-poor (n = 2), over fair (n = 6) and good (n = 4) to fat (n = 2). A single crocodile was emaciated. None of the nematodes collected during the present study are considered serious pathogens of crocodiles (see Huchzermeyer, 2003 and references therein). Our findings support this, and in none of the examined crocodiles could we determine the helminths present as the cause for serious lesions or poor body condition. While ascaridoids may slow the growth rates of crocodiles, this has only been observed in severe infections and, although often associated with gastric ulcers, it has not been established, if ascaridoids cause these ulcers or simply prefer existing ulcers as attachment sites (Ortlepp, 1932; Ladds & Sims, 1990; Huchzermeyer, 2003). One of the crocodiles examined in this study had multiple large gastric ulcers from which numerous specimens of *I. sprenti* were recovered. However, the crocodile had sustained spinal injuries at some point in the past and was partially paralysed. It is, therefore, likely that the overall poor condition it was found in resulted from these injuries rather than helminth infection. Similarly, digenean trematodes, cestodes and leeches are not considered a health concern in crocodilians (Huchzermeyer, 2003). In addition to the nematode parasites reported on in the present study, we also recovered digenean trematodes from the small intestine of all crocodiles as well as

metacestodes of *Cyclustera magna* (Baer, 1959) (Gryporhynchidae) and specimens of the leech *Placobdelloides multistriata* (Johansson, 1909) (Hirudinea: Glossiphoniidae) in a single crocodile each. As with the nematodes none of these parasites could be associated with obvious lesions and/or the host individual's poor body condition. The two crocodiles harbouring the metacestodes and leeches, respectively, were in fact judged in good condition.

In conclusion, Nile crocodiles, when compared to other crocodilians, have relatively species rich nematode assemblages, with members of the Heterocheilidae being the dominant group. As is typical for predatory hosts, some of the nematodes found in *C. niloticus* are not crocodilian parasites *per se* but were likely ingested with the crocodiles' prey.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

References

Amin, O. M. (1978). Intestinal helminths of some Nile fishes near Cairo, Egypt with redescriptions of *Camallanus kirandensis* Baylis 1928 (Nematoda) and *Bothriocephalus aegyptiacus* Ryšavý and Moravec 1975 (Cestoda). *Journal of Parasitology*, *64*, 93–101.

Anderson, R. C. (2000). *Nematode parasites of vertebrates. Their development and transmission*. 2nd edition. Wallingford: CABI, 650 pp.

Ashton, P. J. (2010). The demise of the Nile crocodile (*Crocodylus niloticus*) as a keystone species for aquatic ecosystem conservation in South Africa: The case of the Olifants River. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *20*, 489–493.

Baylis, H. A. (1923). On the classification of the Ascaridae. III. A revision of the genus *Dujardinia* Gedoelst, with a description of a new genus of Anisakinae from a crocodile. *Parasitology*, *15*, 223–232.

Baylis, H. A. (1928). Some parasitic worms, mainly from fishes, from Lake Tanganyika. *Annals and Magazine of Natural History, Series 10, 1*, 552–562.

Brooks, D. R., & O'Grady, R. T. (1989). Crocodilians and their helminth parasites: macroevolutionary considerations. *American Zoologist*, *29*, 873–883.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, *83*, 575–583.

Calverley, P. M., & Downs, C. T. (2015). Movement and home range of Nile crocodiles in Ndumo Game Reserve, South Africa. *Koedoe*, *57*, 13 pp.

Combrink, X., Korrûbel, J. L., Kyle, R., Taylor, R., & Ross, P. (2011). Evidence of a declining Nile crocodile (*Crocodylus niloticus*) population at Lake Sibaya, South Africa. *South African Journal of Wildlife Research*, *41*, 145–157.

Crocodile Specialist Group (1996). *Crocodylus niloticus*. The IUCN red list of threatened species 1996: e.T46590A11064465. http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T46590A11064465.en, 06/2018.

Durette-Desset, M.-C., & Batchvarov, G. (1974). Deux nematodes parasites d'amphibiens du Togo. *Annales de Parasitologie Humaine et Comparée*, *49*, 567–576.

Goldberg, S. R., Bursey, C. R., & Aquino-Shuster, A. L. (1991). Gastric nematodes of the Paraguayan caiman, *Caiman yacare* (Alligatoridae). *Journal of Parasitology*, 77, 1009–1011.

Hazen, T. C., Aho, J. M., Murphy, T. M., Esch, G. W., & Schmidt, G. D. (1978). The parasite fauna of the American alligator (*Alligator mississippiensis*) in South Carolina. *Journal of Wildlife Diseases*, *14*, 435–439.

Hoberg, E. P., Kutz, S., Cook, J., Galaktionov, K. V., Haukisalmi, V., Henttonen, H., Laaksonen, S., Makarikov, A., & Marcogliese, D. J. (2013). Parasites. In: Meltofte, H. (Ed.), *Arctic Biodiversity Assessment. The Conservation of Arctic Flora and Fauna (CAFF), Arctic Council*, 67–75.

Huchzermeyer, F. W. (2003). *Crocodiles: biology, husbandry and diseases*. Wallingford: CABI, 352 pp.

Hudson, P. J., Rizzoli, A. P., Grenfell, B. T., Heesterbeek, J. A. P., & Dobson, A. P. (2002). *The ecology of wildlife diseases*. Oxford: Oxford Biology, 216 pp.

Jackson, J. A., & Tinsley, R. C. (1995). Evolutionary relationships, host range and geographical distribution of *Camallanus* Railliet & Henry, 1915 species (Nematoda: Camallaninae) from clawed toads of the genus *Xenopus* (Anura: Pipidae). *Systematic Parasitology*, *32*, 1–21.

Jacobsen, N. H. G. (1988). Species account: Nile crocodile (*Crocodylus niloticus*). In: Branch, W. R. (Ed.), South African Red Data Book-Reptiles and amphibians. South African National Scientific Programmes Report No. 151 (pp. 83–85). CSIR, Pretoria.

Junker, K., Wallace, K., Leslie, A. J., & Boomker, J. (2006). Gastric nematodes of Nile crocodiles, *Crocodylus niloticus* Laurenti, 1768, in the Okavango River, Botswana. *Onderstepoort Journal of Veterinary Research*, *73*, 111–114.

Junker, K., Calitz, F., Govender, D., Krasnov, B. R., & Boomker, J. (2016). Pentastome assemblages of the Nile crocodile, *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae), in the Kruger National Park, South Africa. *Folia Parasitologica*, *63*, 040. http://dx.doi.org/10.14411/fp.2016.040.

Junker, K., & Mutafchiev, Y. (2017a). *Micropleura huchzermeyeri* n. sp. (Camallanida: Dracunculoidea: Micropleuridae) from the Nile crocodile, *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae), in South Africa. *Systematic Parasitology*, *94*, 785–795.

Junker, K., & Mutafchiev, Y. (2017b). *Ingwenascaris* n. g. (Nematoda: Ascaridida: Heterocheilidae) established for *I. sprenti* n. sp. and *I. assymmetrica* (Ortlepp, 1932) n. comb., parasites of African crocodiles, and an identification key to the genera of the Heterocheilidae. *Systematic Parasitology*, *94*, 849–859.

Junker, K., & Mutafchiev, Y. (2017c). *Typhlophoros kwenae* n. sp. (Nematoda: Ascaridida: Heterocheilidae), a gastric parasite from the Nile crocodile *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae) in South Africa. *Systematic Parasitology*, *94*, 971–978.

Kabre, G. B., & Petter, A. J. (1997). *Camallanus polypteri* n. sp. (Nematoda: Camallanidae) in freshwater fishes from Burkina Faso. *Onderstepoort Journal of Veterinary Research*, *64*, 33–37.

Khera, S. (1951). A new nematode *Micropleura indica* n. sp. belonging to the family Philometridae Baylis and Daubney, 1926 from the Ganges tortoise, *Trionyx gangeticus* Cuvier. *Indian Journal of Helminthology, 3*, 55–58.

Kung, C. C. (1948). On some new species of spirurids from terrestrial vertebrates, with notes on *Habronema mansioni*, *Physaloptera paradoxa* and *Hartertia zuluensis*. *Journal of Helminthology*, *22*, 141–164.

Lane, E. P., Huchzermeyer, F. W., Govender, D., Bengis, R. G., Buss, P. E., Hofmeyr, M., Myburgh, J. G., Steyl, J. C., Pienaar, D. J., & Kotze, A. (2013). Pansteatitis of unknown etiology associated with large-scale Nile crocodile (*Crocodylus niloticus*) mortality in Kruger National Park, South Africa: Pathologic findings. *Journal of Zoo and Wildlife Medicine*, *44*, 899–910.

Ladds, P. W., & Sims, L. D. (1990). Diseases of young captive crocodiles in Papua New Guinea. *Australian Veterinary Journal*, *67*, 323–330.

Leslie, A. J., & Spotila, J. R. (2001). Alien plant threatens Nile crocodile (*Crocodylus niloticus*) breeding in Lake St. Lucia, South Africa. *Biological Conservation*, *98*, 347–355.

Mašová, Š., Moravec, F., Baruš, V., & Seifertová, M. (2010). Redescription, systematic status and molecular characterisation of *Multicaecum heterotis* Petter, Vassiliadès et Marchand, 1979 (Nematoda: Heterocheilidae), an intestinal parasite of *Heterotis niloticus* (Osteichthyes: Arapaimidae) in Africa. *Folia Parasitologica*, *57*, 280–288.

Moravec, F. (1973). On the nematode *Camallanus longicaudatus* sp. n. from the Nile fish, *Labeo horie* Heck. *Revue de Zoologie et de Botanique Africaines*, *87*, 165–173.

Moravec, F. (2001). Some helminth parasites from Morelet's crocodile, *Crocodylus moreletii*, from Yucatan, Mexico. *Folia Parasitologica*, *48*, 47–62.

Moravec, F., & Justine, J. L. (2007). A new species of *Ascarophis* (Nematoda, Cystidicolidae) from the stomach of the marine scorpaeniform fish *Hoplichthys citrinus* from a seamount off the Chesterfield Islands, New Caledonia. *Acta Parasitologica*, *52*, 238–246.

Moravec, F., & Spratt, D. M. (1998). *Crocodylocapillaria longiovata* n. gen., n. sp. (Nematoda: Capillariidae) from the stomach of crocodiles in Australia and New Guinea. *Journal of Parasitology*, *84*, 426–430.

Ortlepp, R. J. (1932). Two new ascarids from crocodiles. *Journal of the South African Veterinary Medical Association*, *111*, 70–75.

Platt, S. G., Rainwater, T. R., Finger, A. G., Thorbjarnarson, J. B., Anderson, T. A., & McMurry, S. T. (2006). Food habits, ontogenetic dietary partitioning and observations of foraging behaviour of Morelet's crocodile (*Crocodylus moreletii*) in northern Belize. *Herpetological Journal*, *16*, 281–290.

Platt, S. G., Thorbjarnarson, J. B., Rainwater, T. R., & Martin, D. R. (2013). Diet of the American crocodile (*Crocodylus acutus*) in marine environments of coastal Belize. *Journal of Herpetology*, *47*, 1–10.

Rigby, M. C., & Rigby, E. (2014). Order Camallanida: Superfamilies Anguillicolidea and Camallanoidea. In: Schmidt-Rhaesa, A. (Ed.) *Handbook of Zoology. Gastrotricha, Cycloneuralia and Gnathifera. Volume 2. Nematoda*. Berlin: De Gruyter, pp. 637–659.

Ross, C. A. (1989). *Crocodiles and alligators*. London: Merehurst Press, 240 pp.

Scott, T. P., Simcik, S. R., & Craig, T. M. (1997). Endohelminths of American alligators (*Alligator mississippiensis*) from southeast Texas. *Journal of the Helminthological Society of Washington*, *64*, 258–262.

Southwell, T., & Kirshner, A. (1937). On some parasitic worms found in *Xenopus laevis*, the South African clawed toad. *Annals of Tropical Medicine & Parasitology*, *31*, 245–265.

Sprent, J. F. A. (1977). Ascaridoid nematodes of amphibians and reptiles: *Dujardinascaris*. *Journal of Helminthology*, *51*, 253–287.

Sprent, J. F. A. (1978). Ascaridoid nematodes of amphibians and reptiles: *Gedoelstascaris* n. g. and *Ortleppascaris* n. g. *Journal of Helminthology*, *52*, 261–282.

Sprent, J. F. A. (1979). Ascaridoid nematodes of amphibians and reptiles: *Multicaecum* and *Brevimulticaecum*. *Journal of Helminthology*, *53*, 91–116.

Sprent, J. F. A., McKeown, E. A., & Cremin, M. (1998). *Dujardinascaris* spp. (Nematoda: Ascaridoidea) in Old World crocodilians. *Systematic Parasitology*, *39*, 209–222.

Svitin, R., Schoeman, A. L., & du Preez, L. H. (2018). New information on morphology and molecular data of camallanid nematodes parasitising *Xenopus laevis* (Anura: Pipidae) in South Africa. *Folia Parasitologica*, *65*, 1–11.

Tellez, M. (2013). Checklist of host-parasite interactions of the order Crocodylia. University Press California, 376 pp.

Tellez, M. & Nifong, J. (2014). Gastric nematode diversity between estuarine and inland freshwater populations of the American alligator (*Alligator mississippiensis* Daudin, 1802), and the prediction of intermediate hosts. *International Journal of Parasitology: Parasites and Wildlife*, *3*, 227–235.

Valentine Jr, J. M., Walther, J. R., McCartney, K. M., & Ivy, L. M. (1972). Alligator diets on the Sabine National Wildlife Refuge, Louisiana. *Journal of Wildlife Management*, *36*, 809–815.

Vassiliadès, G., & Petter, A. -J. (1972). *Camallanus ctenopomae* n. sp. (Nematoda; Camallanidae), parasite de *Ctenopoma kingsleyae* Gunther (poisson; Anabantidae) au Senegal. *Annales de Parasitologie Humaine et Comparée*, *47*, 383–389.

Villegas, A., & González-Solís, D. (2009). Gastrointestinal helminth parasites of the American crocodile (*Crocodylus acutus*) in Southern Quintana Roo, Mexico. *Herpetological Conservation and Biology*, *4*, 346–351.

Waddle, A. R., Kinsella, J. M., Ross, J. P., Rojas-Flores, E., Percival, H. F., & Forrester, D. J. (2009). Nematodes collected by gastric lavage from live American Alligators, *Alligator mississippiensis*, in Florida. *Journal of Parasitology*, 95, 1237–1238.

Wallace, K. M., & Leslie A. J. (2008) Diet of the Nile crocodile (*Crocodylus niloticus*) in the Okavango Delta, Botswana. *Journal of Herpetology*, *42*, 361–368.

Walton, A. C. (1932). A new nematode (*Camallanus multiruga*, sp. n.) parasitic in a west African frog. *Annals and Magazine of Natural History*, Series 10, *9*, 151–154.

Wedl, K. (1861). Zur Helminthenfauna Ägyptens. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien, Mathematische-Naturwissenschaftliche Classe, 44* (2. Abtheilung), 463–482.

Yeh, L.-S. (1960b). On a reconstruction of the genus *Camallanus* Railliet and Henry, 1915. *Journal of Helminthology*, *34*, 117–124.