Syst Parasitol (2016) 93:173–191
DOI 10.1007/s11230-015-9615-7

Morphological and molecular description of *Tenuisentis niloticus* (Meyer, 1932) (Acanthocephala: Tenuisentidae) from *Heterotis niloticus* (Cuvier) (Actinopterygii: Arapaimidae), in Burkina Faso, with emendation of the family diagnosis and notes on new features, cryptic genetic diversity and histopathology

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Received: 15 June 2015 / Accepted: 2 November 2015

Abstract Specimens described as *Rhadinorhynchus niloticus* Meyer, 1932 (Rhadinorhynchidae) from two male specimens collected from *Heterotis niloticus* (Cuvier) in the Egyptian Nile were later redescribed in the genus *Tenuisentis* Van Cleave, 1936 (Tenuisentidae) based on 12 specimens collected from the same host species in the White Nile. That redescription basically distinguished the two genera based on five traits but did not actually provide a formal description. His account left out information about cerebral ganglion, lemnisci, some reproductive structures, eggs, proboscis hook dissymmetry and roots, size of trunk and a few other structures. We provide (i) the first complete description of this species enhanced by SEM, molecular, and histo-pathological studies; (ii) expand the existing descriptions; (iii) correct questionable accounts advanced by Van Cleave on the cement gland and the hypodermal giant nuclei; and (iv) add descriptions of new features such as the para-receptacle structure which we also report from *Paratenuisentis* Bullock & Samuel, 1975, the only other genus in Tenuisentidae Van Cleave, 1936. The subsequent description of a few more specimens from the same host collected in Mali was more informative yet incomplete and at variance with our specimens from Burkina Faso. Genetic divergence and phylogenetic analyses of mitochondrial (cytochrome oxidase *c* subunit I; COI) and nuclear (18S ribosomal RNA) gene relationships uncovered a cryptic species complex containing two lineages. Based on our studies, the family diagnosis is emended. The acanthocephalan causes damage to the host intestine as depicted in histopathological sections. The invading worm can extend from the mucosal layer to the muscularis externa of the host with subsequent tissue necrosis, villi compression, haemorrhaging and blood loss.

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Introduction

Meyer (1932) described two male specimens of *Rhadinorhynchus niloticus* Meyer, 1932 from the

African arowana, *Heterotis niloticus* (Cuvier) in the Egyptian Nile noting trunk length, proboscis dimensions and armor, length of receptacle, posterior testis and cement gland. Realising that Meyer's specimens did not belong in *Rhadinorhynchus* Lühe, 1911 and examining five males and seven females of the same species from the same host species and waters in the White Nile, Van Cleave (1936) assigned Meyer's material to his new genus *Tenuisentis* Van Cleave, 1936 that he justifiably placed in his new family Tenuisentidae Van Cleave, 1936. Van Cleave's (1936) account was not actually a description but rather an attempt to distinguish *Tenuisentis* from *Rhadinorhynchus* based on five characters: the cement gland pattern, the giant hypodermal nuclei, the proboscis receptacle, the opening of the uterus, and the lemnisci. Van Cleave (1936) further disagreed with Meyer (1932) about certain traits that we disagree with both authors about. Dollfus & Golvan (1956) provided a more informative description of *T. niloticus* from *H. niloticus* in Mali. In their description of *Paratenuisentis* Bullock & Samuel, 1975, the only other genus of Tenuisentidae, Bullok & Samuel (1975) emended the family diagnosis. Dollfus & Golvan (1956) also reported encysted immature *T. niloticus* from the body cavity of the tiger fish, *Hydrocynus brevis* Gunther (Actinopterygii: Alestidae), in the Sudan.

No other taxonomic accounts of *T. niloticus* have been reported since. However, specimens of *T. niloticus* appear to heavily infect *H. niloticus* in the Nile River or waters associated with it in Africa. Meyer's (1932) and Van Cleave's (1936) specimens were from the Nile River in the Sudan. The specimens reported by Dollfus & Golvan (1956) and Khalil (1969) from the same host were collected in Mali and Sudan, respectively. Khalil (1969) only gave a host-parasite record. Akinsanya (2007) and Akinsanya et al. (2007a) reported *T. niloticus* in H. *niloticus* from Lekki Lagoon, Nigeria, and Abowei & Ezekiel (2011) from the Sudan's White and Blue Nile. The distribution of *T. niloticus* in *H. niloticus* does not appear to be homogeneous throughout its range. Fishes examined for parasites in the Great Kwa River, Nigeria (Ekanem et al., 2011) and in the Mare Simenti, Senegal and Kosti, Sudan (Mašová et al., 2010) were not infected with *T. niloticus*. This heterogeneous distribution is not understood and needs to be accounted for.

In the present study, we revise the description of *T. niloticus* and the diagnosis of the family Tenuisentidae, provide new information on anatomical features not previously reported, characterise the DNA profile with special reference to cryptic species diversity and provide the results of a histopathological study of infections in the Arowana intestine.

Materials and methods

Thirty six specimens (13 females, 23 males) *H. niloticus* (standard length 20–54 cm; weight 98–1,249 g) were captured from the River Sourou at Di, Sourou Province (13°04′N, 03°04′W), Burkina Faso in January, 2014. The approximately 120 km long River Sourou flows from Mali into the Black Volta (one of three major Volta basins) in Western Burkina Faso. A dam was built at Léri, south of Di, to control and regulate the flow of water between the Black Volta and the Sourou (Manson & Knight, 2011), in turn affecting the fish population and movement.

Live specimens collected from the dissected host guts were kept in tap water for a few hours until proboscides were everted then fixed in 70% ethanol. Specimens were then shipped to the Arizona facility. For microscopic studies, 40 specimens were stained in Mayer's acid carmine, destained in 4% hydrochloric acid in 70% ethanol, dehydrated in ascending concentrations of ethanol (70%, 80%, 90% twice, 100%), and cleared in 100% xylene, then in 50% Canada balsam and 50% xylene; each step for 24 h. Whole worms were then mounted in Canada balsam. Measurements are in micrometres, unless otherwise noted; the range is followed by the mean values in parentheses. Width measurements represent maximum width; trunk length does not include proboscis, neck, or bursa.

Optical microscopic images from 10 additional specimens were captured using an Olympus BH2 compound light microscope (Olympus Optical Co., Tokyo, Japan) equipped with an AmScope camera MU900 (United Scope, Irvine, California). All images were captured at 400×.

For scanning electron microscopy (SEM) studies, 15 specimens previously fixed in 70% ethanol were placed in critical-point drying baskets and dehydrated using ethanol series of 95% and 100% for at least 10 min per soak followed by critical point drying (Lee, 1992). Samples were gold coated and observed under a scanning electron microscope XL30 ESEMFEG (FEI,

Hillsboro, Oregon, USA). Digital images of the structures were obtained using digital imaging software. For studies of the para-receptacle structure (PRS), specimens were cut with plastic and diamond knives.

For histological studies, both normal and infected tissue (Arowana) from five hosts were fixed in buffered 10% formalin for 24 hours then washed and stored in 70% ethanol. The host fish had been stored in portable ice chests for three days. Following dehydration and paraffin embedding by standard methods (Lillie, 1991; Kiernan, 2002) sections were cut at 4–6 μm and stained with hemotoxylin and eosin. The prepared slides were viewed with a Zeiss Axovert 135 compound light microscope. The sections were cut with a Leica Model RM 2255 microtome. Each slide was viewed and records were obtained on a Pentax K100 Digital Camera attached to the microscope.

DNA was extracted from 54 ethanol preserved (70%) specimens using a Qiagen DNAeasy Blood and Tissue kit (Qiagen Inc., Venlio, Limburg, Netherlands). Entire individuals were soaked in 500 μl of ATL buffer for 10 min prior to DNA digestion. Samples were macerated by scissors and the protocol followed as outlined by the manufacturer.

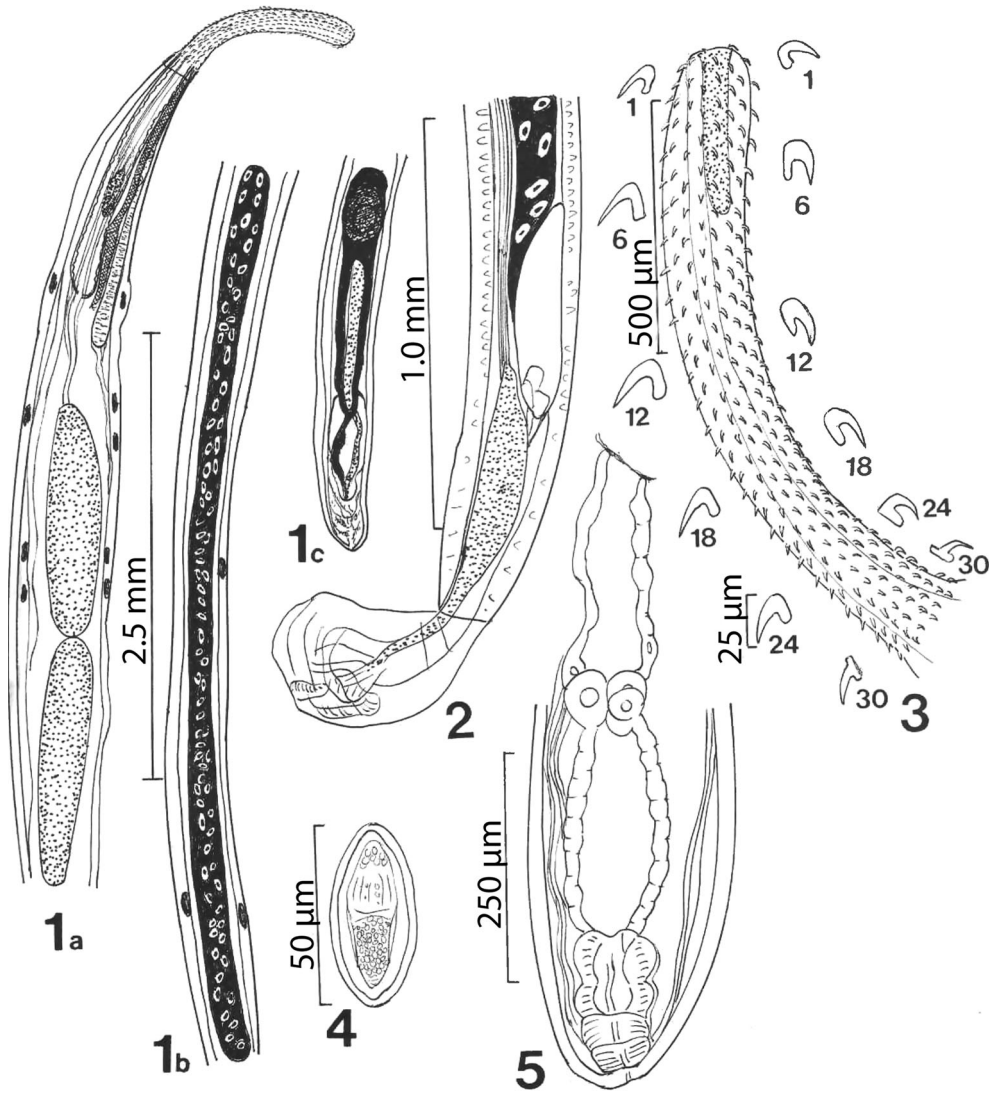
For polymerase chain reaction (PCR) amplification of a 682-bp fragment of the mitochondrial cytochrome oxidase *c* subunit 1 gene (COI), we used the primers 5′-AGT TCT AAT CAT AA(R) GAT AT(Y) GG-3′ and 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Folmer et al., 1994). Primers used for the amplification of a 1,747-bp fragment of the nuclear 18S ribosomal RNA gene (18S) were 5′-AGA TTA AGC CAT GCA TGC GTA AG-3′ and 5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′ (Near et al., 1998). Reaction cocktails were 12.5 μl in volume and included the following reagents: DNA template (*c*.150 ng), nuclease free water (2.25 μl), oligonucleotide primers (10 pmol each), and Promega GoTaq® Green Master Mix (6.25 μl). The thermal profile began with an initial denaturation step of 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 90 s, and concluded by a rapid cool down to 4°C. Successful amplifications were verified qualitatively by viewing PCR products under ultraviolet radiation following electrophoresis through 1.0% agarose gels. Millipore MultiScreen$_{\mu 96}$ filter plates were used to purify PCR products following the manufacturer's protocol.

Cycle sequencing reactions were performed using the ABI Big Dye Terminator protocol (Applied Biosystems, Foster City, CA, USA). Reaction cocktails were 10.5 μl in volume, and were mixed using the following reagent amounts: purified PCR product (*c*.150 ng), nuclease free water (2.75 μl), 5× Tris buffer (1.75 μl), primer (6 pmol), and dye terminator reaction mix (0.5 μl). Both DNA strands were sequenced using the same primers that were used to amplify the genes *via* PCR. The thermal profile for the sequencing reactions consisted of 25 cycles of 96°C for 10 s, 50°C for 5 s, 60°C for 4 min, followed by a rapid cool down to 4°C. All sequencing was carried out on an ABI 3730xl automated sequencer in the DNA Sequencing Center at Brigham Young University. Sequences for COI and 18S generated from this study are available from the GenBank (Accession numbers KT970469–KT970471).

Sequences were initially aligned with Sequencher v. 4.8 (Gene Codes Corp., Ann Arbor, Michigan, USA) and subsequently corrected by eye. Tree reconstructions were carried out using maximum parsimony (MP) and maximum-likelihood (ML) methods. Bootstrapping of MP and distance-based reconstructions entailed 1,000 replications, with random additions of taxa, on informative sites only in MP. The PAUP*4.2a program package (Swofford, 2003) was used for MP reconstructions. The appropriate model of sequence evolution was selected using jModelTest 0.1.1 (Posada, 2008) as implemented in PhyML v. 3.0 (Guindon & Gascuel, 2003). ML phylogenies were generated using TreeFinder (version of October 2008; Jobb, 2008), and nodal support was estimated by performing 1,000 bootstrap replicates. The rotiferan *Brachionus plicatus* Müller, 1786 was used as the outgroup based on the sister taxa status of Rotifera and Acanthocephala (García-Varela et al., 2000). Concatenation of the COI (663 bp) sequence with the 18S (610 bp) sequence for each sample was implemented using Geneious (v7.1.4; Kearse et. al., 2012).

Results

A total of 29 of 36 (81%) examined specimens of *H. niloticus* captured in the River Sourou at Di in January were infected with 957 acanthocephalans: 122 in the stomach, 215 in the caeca, 620 in the intestines (a mean of 33 per infected fish).

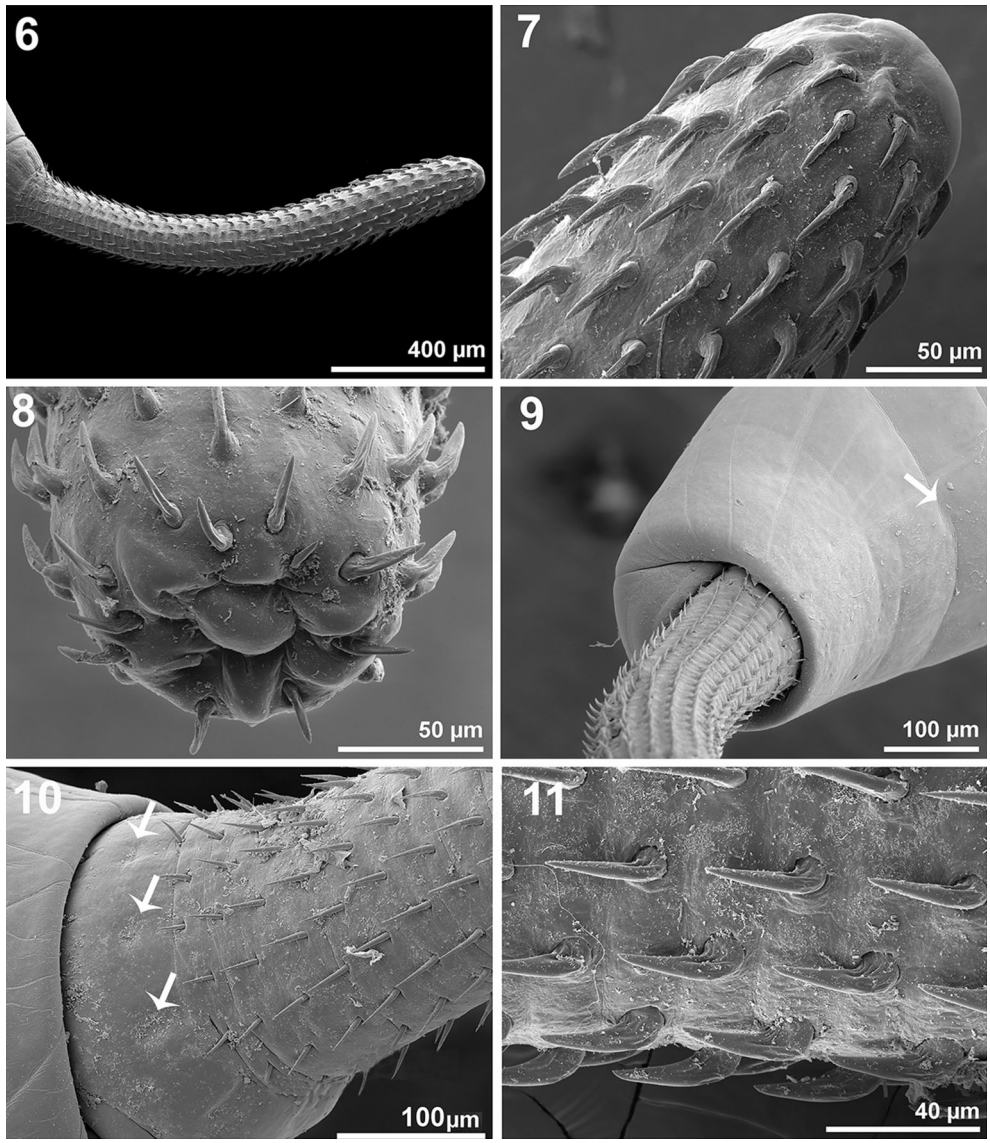


Figs. 1–5 *Tenuisentis niloticus* ex *Heterotis niloticus.* 1, Mature male specimen in three parts (a–c); para-receptacle structure is checkers-shaded next to lemnisci (1a); sperm duct is speckled-shaded between cement gland ducts (1c); 2, Posterior portion of the male reproductive system, lateral view; paired reproductive glands not shown; 3, Proboscis of male in Fig. 1 showing inverted apical organ and magnified selected hooks and roots in assigned positions (dorsal side is to the left, ventral to the right); 4, Egg; 5, Female reproductive system (note lateral reproductive ligaments)

Family Tenuisentidae Van Cleave, 1936
Genus *Tenuisentis* Van Cleave, 1936

***Tenuisentis niloticus* (Meyer, 1932; Van Cleave, 1936)**

Host: African arowana, *Heterotis niloticus* (Cuvier) (Osteoglossiformes: Arapaimidae).
Locality: River Sourou at Di, Sourou Province (13°04′N, 03°04′W), Burkina Faso.
Site in host: Intestine.
Voucher material: Four slides of whole-mounted male and female voucher specimens were deposited in the parasite collection of the Harold W. Manter Laboratory of Parasitology (HWML 49998) at the University of Nebraska State Museum, Lincoln, Nebraska, USA.
Comparative material: We examined 3 male and 3 female (6 slides) specimens of Van Cleave's (1936) type-material of *T. niloticus* for comparison. These

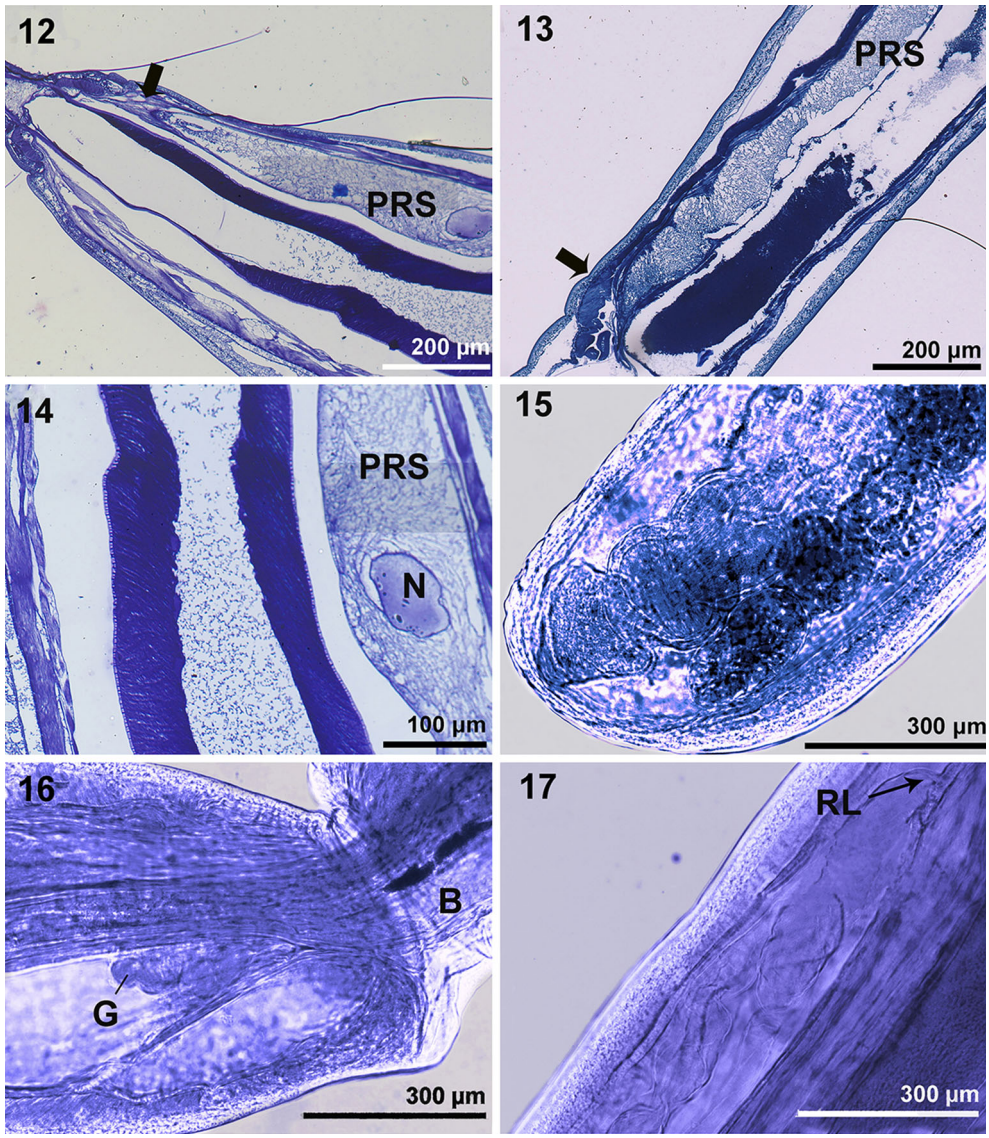


Figs. 6–11 Scanning electron micrographs of *Tenuisentis niloticus* ex *Heterotis niloticus.* 6, Proboscis of male; 7, Anterior end of proboscis in Fig. 6 showing spineless apical end, massive ventral hooks (right) and dorsal hooks (left); 8, Apical end of a partially retracted proboscis showing the apical epidermis cone, ventral hooks (right), and dorsal hooks (left); 9, Posterior end of the proboscis and anterior trunk cone (*arrow* points to margin); 10, Posterior end of the proboscis and conical neck showing depressions similar to sensory structures (*arrows*); 11, Detail of proboscis showing thin latero-dorsal hooks (top) and massive ventral hooks (bottom)

were collected by the Swedish Expedition to the White Nile in 1901, and were available from the U.S. National Parasite Collection, Beltsville, Maryland (USNPC, now housed at the National Museum of Natural History (NMNH) in Washington, D.C) # 064800.00 (SH222: 6–48, 52, 53, 57–59). In order to establish whether specimens of the only other genus of the Tenuisentidae, *Paratenuisentis* Bullock & Samuel, 1975, also have para-receptacle structure like *T. niloticus*, we also examined 9 lots (18 slides) of type- and voucher specimens of *P. ambiguus* Bullock & Samuel, 1975 deposited at the USNPC (USNPC # 006471, 038594–038597, 072905–072907, 100026).

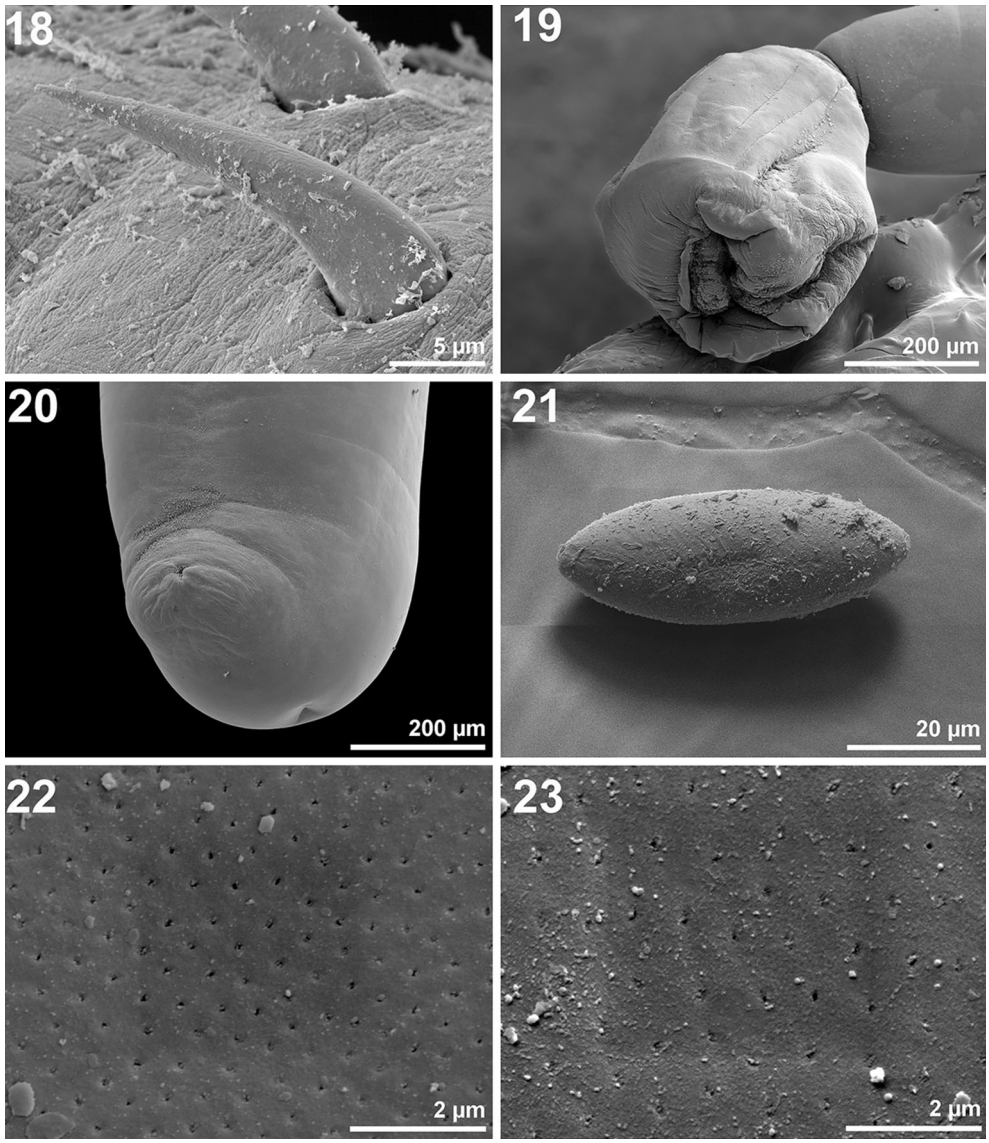
Description (Figs. 1–23)

General. With characters of the genus *Tenuisentis* (Tenuisentidae as emended by Bullock & Samuel, 1975



Figs. 12–17 Photomicrographs of the para-receptacle structure (PRS) and other features of *Tenuisentis niloticus* ex *Heterotis niloticus*. 12, Anterior insertion of PRS in the anterior part of the body wall (*arrow*); 13, Posterior insertion of a PRS at the posterior end of the proboscis receptacle (*arrow*); 14, Detail of specimen shown in Figs. 12, 13 showing detail of the giant nucleus (N); 15, Posterior end of female showing the robust vagina and the subterminal position of the gonopore; 16, Posterior end of male showing the reproductive gland (G) and the bursa (B); 17, Posterior section of male showing the anterior insertion of the reproductive ligaments (RL) to the posterior end of the cement gland

with qualifications). Trunk unspined, cylindrical, elongate (Fig. 1), with unremarkable anterior trunk cone (Fig. 9) and ovoid swelling posterior to proboscis receptacle (Table 1) and variable (3–11) giant hypodermal nuclei per specimen (Fig. 1). Giant nuclei more numerous in males than females, on ventral than dorsal side and in region of trunk swelling than in other regions (Table 2). Body wall with micropores that vary in diameter and distribution in various regions (Figs. 22, 23). Proboscis long, cylindrical, slightly clavate, with long apical epidermal cone (Figs. 3, 8) and apical organ reaching level of 8th hook from anterior and 16 (rarely 15) hook rows with 30–33 hooks each (Figs. 1, 6–11, 18); females with more hooks per row. Proboscis hooks dorso-ventrally differentiated in thickness and curvature but equal in length (Figs. 3, 7, 8, 11). Massive ventral



Figs. 18–23 Scanning electron micrographs of *Tenuisentis niloticus* ex *Heterotis niloticus.* 18, Ventro-lateral view (foreground) and ventral view (background) showing the difference in size between the two hooks; 19, Non-ornate bursa (note the large flat rim); 20, Posterior end of female showing the subterminal position of the gonopore; 21, Egg; 22, 23, Micropores in the mid and posterior trunk (note the difference in the diameter and distribution of pores)

hooks more strongly recurved posteriorly than thin dorsal hooks (Figs. 1, 7, 8, 11). Hooks 27–30 long anteriorly, increase sharply in length to 40–50 in hooks 3–13 from anterior then gradually decrease in length to 20–25 basally. Hooks vary considerably in number and distribution of hooks over 40 in length on proboscis of males and females (Table 3). Total length of all hooks in 1 longitudinal row 891–1,122 (993) in males and 1,069–1,127 (1,105) in females. Hook roots simple, directed posteriorly, slightly shorter than blades throughout length of proboscis except for smallest roots of posterior 2 hooks having prominent anterior manubria. Neck prominent, longer ventrally than dorsally, with depressions around periphery (Fig. 10). Proboscis receptacle markedly longer than proboscis, single, thick-walled except for incomplete posterior end, with large elliptical cephalic ganglion at posterior third hardly visible within retractor muscle fibers. Para-

Table 1 Morphometric data for *Tenuisentis niloticus* from *Heterotis niloticus* in various Nile waters of Africa

Reference Locality	Meyer (1932) Sudan	Van Cleave (1936) Sudan	Dollfus & Golvan (1956) Mali	Present study Burkina Faso
Males	n = 2	n = 5	n = 10	n = 12
Trunk (mm)	9.00–10.00 × –	7.20 (figure 1)	9.00–13.30 × 0.35–0.40	7.87–16.62 × 0.44–0.92 (12.03 × 0.64)
Proboscis (mm)	0.75 × 0.14; 0.11	0.75–1.1 × –	1.20 × 0.15	1.09–1.41 × 0.12–0.15 (1.21 × 0.14)
Hook rows × hooks per row	16 × 23	16 or more × 30–40	16–20 × 32–38	16 × 30–32 (31)
Hook length	–	30 to 47	14–15 to 40	22 to 45 (see Table 2)
Neck	–	–	–	52–104 × 156–250 (78 × 192)
Receptacle (mm)	1.25	–	1.85 × 0.28	1.30–2.44 × 0.15–0.27 (1.86 × 0.22)
Cepahlic ganglion	–	–	–	187–220 × 40–67 (198 × 49)
Lemnisci (mm)	–	–	Not extending past receptacle	1.41–2.03 × 0.06–0.11 (1.61 × 0.08)
Anterior testis (mm)	–	0.80 × – (figure 1)	1.28–1.30 × 0.22	0.77–1.52 × 0.20–0.35 (1.23 × 0.26)
Posterior testis (mm)	0.75 × –	1.00 × – (figure 1)	1.28–1.30 × 0.22	0.90–1.75 × 0.15–0.30 (1.39 × 0.23)
Cement gland (mm)	Reaching 6.0	2.70–4.20 × –	3.80 × 0.16–0.17	3.00–6.92 × 0.13–0.32 (4.93 × 0.19)
Cement gland nuclei	–	*c*.50	–	86–146 (117)
Cement reservoir	–	–	–	468–686 × 125–260 (591 × 184)
Saefftigen's pouch	–	–	–	728–957 × 73–125 (837 × 94)
Common sperm duct	–	–	–	697–1,092 × 83–156 (900 × 112)
Bursa	–	–	–	437–750 × 312–550 (575 × 452)
Females		n = 7	n = 12	n = 10
Trunk (mm)	–	–	12.00–17.00 × 0.40–0.45	10.00–20.25 × 0.30–0.87 (15.16 × 0.65)
Proboscis (mm)	–	0.75–1.10 × –	1.2 × 0.15	1.30–1.42 × 0.13–0.17 (1.34 × 0.15)
Hook rows × hooks per row	–	16 or more × 30–40	16–20 × 32–38	16 (rarely 15) × 31–33 (32)
Hook length	–	30–47	14–15 to 40	20–50 (see Table 2)
Neck	–	–	–	65–87 × 162–230 (79 × 194)
Receptacle (mm)	–	–	1.85 × 0.28	1.09–2.86 × 0.18–0.27 (2.09 × 0.23)

Table 1 continued

Reference Locality	Meyer (1932) Sudan	Van Cleave (1936) Sudan	Dollfus & Golvan (1956) Mali	Present study Burkina Faso
Cephalic ganglion	–	–	–	200–300 × 40–75 (260 ×57)
Lemnisci (mm)	–	–	Not extending past receptacle	1.46–2.60 × 0.04–0.11 (2.00 × 0.09)
Reproductive system	–	500 (figure 2)	600 (figure 1)	624–728 (640) long
Eggs	–	–	–	45–58 × 20–25 (52 × 22)

Table 2 Distribution of the giant hypodermal nuclei in nine male and nine female specimens of *Tenuisentis niloticus*

Region of trunk[a]	Males			Females		
	Dorsal	Ventral	Total	Dorsal	Ventral	Total
A	0	6	6	0	1	1
B	10	7	17	11	17	28
C	15	16	31	12	12	24
D	0	0	0	0	1	1
E	6	10	16	0	3	3
F	0	0	0	0	0	0
All regions	31	39	70	23	34	57
Mean	3.4	4.3	7.8	2.5	3.8	6.3

[a] Regions of trunk in females correspond with the following regions in males: A, Anterior trunk to half proboscis receptacle; B, Receptacle to anterior end of testis; C, Anterior testis to mid-posterior testis (area of trunk swelling); D, Posterior testis to mid-cement gland; E, Balance of cement gland; F, Area posterior to cement gland

receptacle structure (PRS) prominent ventrally corresponding to length of receptacle, anterior limb tubular anteriorly near insertion at anterior body wall and lobulated posteriorly near insertion at posterior receptacle, with posterior limb extending into body cavity (Figs. 12–14). Lemnisci equal, usually extending short distance past posterior end of receptacle but occasionally slightly shorter, and attached posteriorly to body wall with fibrous ligaments. Reproductive ligaments (RL) massive, attach at base of uterine bell and at level of cement reservoir anteriorly, and to body wall posteriorly (Fig. 17).

Male [Based on 12 adults with sperm; measurements in Table 1.] Para-receptacle structure (PRS) 1.13–1.76 (1.37) mm from anterior trunk insertion to posterior receptacle insertion. Testes pre-equatorial, contiguous, short distance behind receptacle. Cement gland syncytial, contiguous with posterior testis, narrowing posteriorly at short interface with cement reservoir, with 86–146 (117) giant nuclei. Cement reservoir with 2 large posterior ducts surrounding ventral common sperm duct. Saefftigen's pouch prominent, dorsal to common sperm duct and contiguous with posterior margin of cement reservoir (Figs. 1, 2). Reproductive ligaments (RL) undulating insert anteriorly at level of posterior tip of cement gland at cement reservoir (Fig. 17). Two lobulated glands (G) insert at posterior end of trunk dorsal to emergence of bursa (Fig. 16). Gonopore terminal (Figs. 1, 2, 16, 19). Bursa bent ventrally, non-ornate, with thick, broad, flat rim (Figs. 2, 19).

Female [Based on 10 mostly gravid specimens; measurements in Table 1]. Para-receptacle structure (PRS) 0.76–2.05 (1.56) mm long from anterior trunk insertion to posterior receptacle insertion. Reproductive system massive, with prominent vaginal sphincter, muscular uterus with few large anterior glands (Figs. 5, 15), uterine bell rim attached to body wall with ligaments, and reproductive ligaments inserted anteriorly at anterior uterus and extending posteriorly. Eggs ovoid, smooth (Figs. 4, 21), without prolongation

Table 3 Hook length in three female (F1–F3) and three male (M1–M3) specimens of *Tenuisentis niloticus* from *Heterotis niloticus* in Burkina Faso

Hook no.	F1	F2	F3	Mean	M1	M2	M3	Mean
1	27	27	29	28	27	30	27	28
2	35	35	35	35	36	37	35	36
3	42	40	40	41	39	40	37	39
4	42	37	40	40	40	40	37	39
5	50	39	40	43	44	40	40	41
6	47	42	40	43	42	39	40	40
7	47	42	42	44	45	39	42	42
8	46	41	45	44	40	37	37	38
9	44	42	45	44	45	35	40	40
10	45	40	42	42	40	32	37	36
11	45	39	40	41	40	32	36	36
12	45	35	40	40	41	30	35	35
13	45	34	37	39	41	27	35	34
14	42	31	37	37	37	27	32	32
15	40	30	40	37	35	30	31	32
16	35	30	35	33	37	30	30	32
17	35	31	34	33	35	27	32	31
18	32	30	32	31	34	27	32	31
19	32	30	35	32	34	27	31	31
20	34	30	34	33	34	25	31	30
21	34	29	29	31	32	27	27	29
22	30	31	30	30	30	25	25	27
23	30	30	30	30	32	25	26	28
24	27	29	27	28	32	22	22	25
25	27	27	27	27	32	25	22	26
26	27	30	27	28	32	22	25	26
27	27	27	25	26	32	25	25	27
28	27	27	29	28	30	25	24	26
29	30	27	29	29	28	22	25	25
30	25	30	30	28	27	22	25	25
31	25	30	30	28	27	–	25	26
32	–	27	27	27	22	–	25	23
33	–	20	25	22	–	–	–	–
Mean	36	32	34		35	30	31	

of fertilisation membrane. Gonopore position variable from near terminal to subterminal (Figs. 5, 20).

Remarks

The following features are reported for the first time:

Apical organ. An unusually long apical organ reaching level of 8th hook from anterior was found in many specimens (Fig. 3). Apical organs have been reported in a number of acanthocephalans (reviewed by Herlyn, 2001). We, however, report for the first time an associated apical epidermal cone in *T. niloticus* (Fig. 8) clearly evident in slightly retracted proboscides similar to that reported in *P. ambiguus* by Herlyn (2001; see his figure 1C). The presence of the apical epidermal cone only in the Eoacanthocephala Van Cleave, 1936 is interpreted as an evolutionary innovation supporting the monophyly of the Eoacanthocephala (see Herlyn, 2001).

Anterior trunk cone. Male and female specimens showed an anterior trunk cone that was not unremarkable in many specimens (Fig. 9).

Neck. The cone-shaped neck had regularly spaced indentations that appear like sensory structures around its parameter (Fig. 10).

Para-receptacle structure. The PRS was clearly prominent in our and Van Cleave's (1936) specimens of *T. niloticus* on the ventral side of the receptacle corresponding to length of proboscis receptacle. The structure in *T. niloticus* was tubular anteriorly near its insertion in anterior body wall and lobulated posteriorly near insertion at posterior receptacle with posterior arm extending into body cavity (Figs. 12–14). Dorsal and ventral PRS were also evident in the specimens of *P. ambiguus* examined by us. It is surprising that neither Van Cleave (1936), Dollfus & Golvan (1956) nor Bullock & Samuel (1975) made any reference to it in the text or illustrations. Herlyn et al. (2001), however, observed the PRS of *P. ambiguus* (their figures 4, 5) but identified it as "receptacle protrusor muscle extending from the body-wall of the neck to its posterior insertion around the posterior end of the receptacle". The receptacle does not protrude and the structure is certainly not a muscle. Herlyn et al. (2001) also reported four nuclei in the anterior limb of the PRS but did not note the posterior limb that extends into the body cavity. The PRS is a tube-like long nucleated structure that runs parallel to the proboscis receptacle in some eoacanthocephalans with single-walled receptacle. It appears to regulate hydrostatic pressure in the receptacle to facilitate the retraction and eversion of the proboscis in some eoacanthocephalans with weak single-walled receptacle (Amin et al., 2007). It has been previously reported in a few species of the Neoechinorhynchidae (Ward, 1917) Van Cleave, 1928 and Quadrigyridae Van Cleave, 1920. It was first described in *Neoechinorhynchus* (*Neoechinorhynchus*) *qatarensis* Amin, Saoud & Alkuwari, 2002 from Qatar and subsequently in *Neoechinorhynchus* (*Neoechinorhynchus*) *golvani*

Salgado-Maldonado, 1978 from Mexico (see Amin et al., 2002, 2011b), *Acanthogyrus* (*Acanthosentis*) *parareceptaclis* Amin, 2005 from Japan, *Neoechinorhynchus ampullata* Amin, Ha & Ha, 2011 from Vietnam, *Neoechinorhynchus* (*Neoechinorhynchus*) *ascus* Amin, Ha & Ha, 2011 from Vietnam, and *Acanthogyrus* (*Acanthosentis*) *barmeshoori* Amin, Gholami, Akhlaghi & Heckmann, 2013 (see Amin, 2005; Amin et al., 2011a, 2013). Two additional species, *Tenuisentis niloticus* and *Paratenuisentis ambiguus* in a third family (Tenuisentidae Van Cleave, 1936) with single-walled receptacle and considerably longer proboscis are now included in the list of acanthocephalans having PRS.

Reproductive ligaments. Prominent undulating ligaments were observed attached to the reproductive system in male and female specimens of *T. niloticus*. In females, they were inserted anteriorly at the distal end of the uterus in females and attached to the body wall posteriorly. In males, they were inserted anteriorly near the posterior end of the cement gland at level of cement reservoir (Fig. 17; marked RL at insertion). No other observers reported these ligaments but Bullock & Samuel (1975) illustrated what appears to be such a structure in a female specimen (their figure 6). We suspect that these ligaments serve to stabilise the position of reproductive structures in these locations.

Male reproductive glands. Two prominent lobulated glands (indicated by G in Fig. 16) insert at the posterior end of the trunk dorsal to emergence of bursa. Their function is not known but may be related to facilitation of conveyance of sperms.

Proboscis hook roots are a routine feature in descriptions and are noted here for the first time. They are invariably simple, directed posteriorly, slightly shorter than blades of all hooks except for smallest roots of the posterior two hooks having prominent anterior manubria (Fig. 3).

Eggs are also reported here for the first time. They are small and ovoid, with concentric shells and no apparent external topographic patterns or folds (Figs. 4, 21) in contrast to those of *P. ambiguus*.

Molecular characterisation

DNA sequences of 682 bp of the mitochondrial COI gene and 1,474 bp of the nuclear 18S rRNA gene were obtained from each of 54 specimens, consisting of 27 male and 27 female individuals. Two mitochondrial haplotypes with COI sequence divergence of 6.4% were unequally distributed with 51/54 individuals harbouring the major haplotype (Lineage 1) and 3/54 individuals harbouring the minor haplotype (Lineage 2). Within this major haplotype (Lineage 1), five nucleotide sequence variants were present differing at a total of 2–6 nucleotides, all which were silent or synonymous mutations. No sequence variants were detected among the three Lineage 2 mtDNA haplotypes. All of the COI sequences were predicted to encode for full length COI polypeptides ruling out the possibility that the COI lineages or variants originated from the sequencing of nucleus located mitochondrial genes (numt). Two 18S rRNA gene sequences, A and B, were identified in the population sample. The sequences differed by 3.4% and the presence of eight insertion/deletions (indels). One individual was homozygous for 18S rDNA sequence A, five individuals were homozygous for 18S rDNA sequence B, and 48/54 individuals were heterozygous for the two (or more) 18S rRNA alleles. Because of multiple indels in the 18S rDNA sequences, the presence of other 18S rRNA alleles in heterozygotes could not be ruled out by phase analysis of sequence data. There was no relationship between the presence of 18S rDNA sequence A and/or sequence B alleles and the presence of Lineage 1 and Lineage 2 mtDNA haplotypes.

Maximum-likelihood (ML) and maximum parsimony (MP) analyses of the 18S, COI, and the combined 18S + COI datasets were congruent with identical nodal structure and topology. The ML tree for the COI data set is shown in Fig. 24. Sequences used in the analysis are as shown in Table 4. The two *Tenuisentis niloticus* lineages grouped with the representatives of the Neoechinorhynchidae, with *Paratenuisentis* as a sister genus. Tenuisentidae was strongly supported as a clade separate from the Neoechinorhynchidae. In every phylogeny reconstruction, *Tenuisentis* was sister to *Paratenuisentis* and Tenuisentidae was sister to Neoechinorhynchidae. The two mitochondrial lineages, 1 and 2, were not linked to the presence of either nuclear 18S rRNA allele, A or B. Although we have a small sample size, it would appear that there in this host ecology, interbreeding of *T. niloticus* individuals was without selection based on haplotype or genotype. Although fixed changes in genes occur in the process of sympatric speciation, an allopatric or geographic isolation model by which fixed

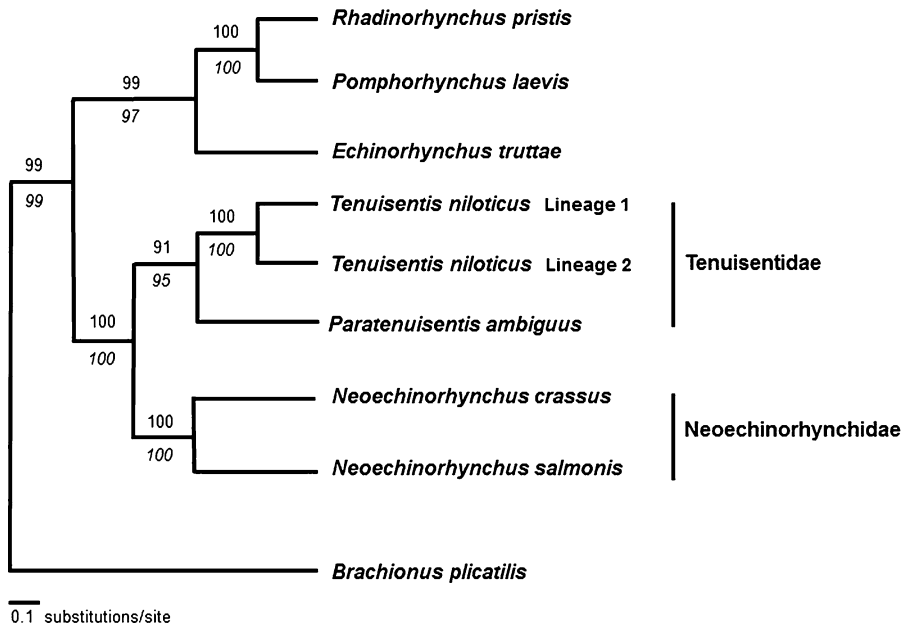


Fig. 24 Phylogeny generated using combined COI and 18S sequence data. Numbers at internal nodes are maximum likelihood (above nodes) and maximum parsimony (below nodes; italicised) support values. The scale-bar indicates the expected number of substitutions per site

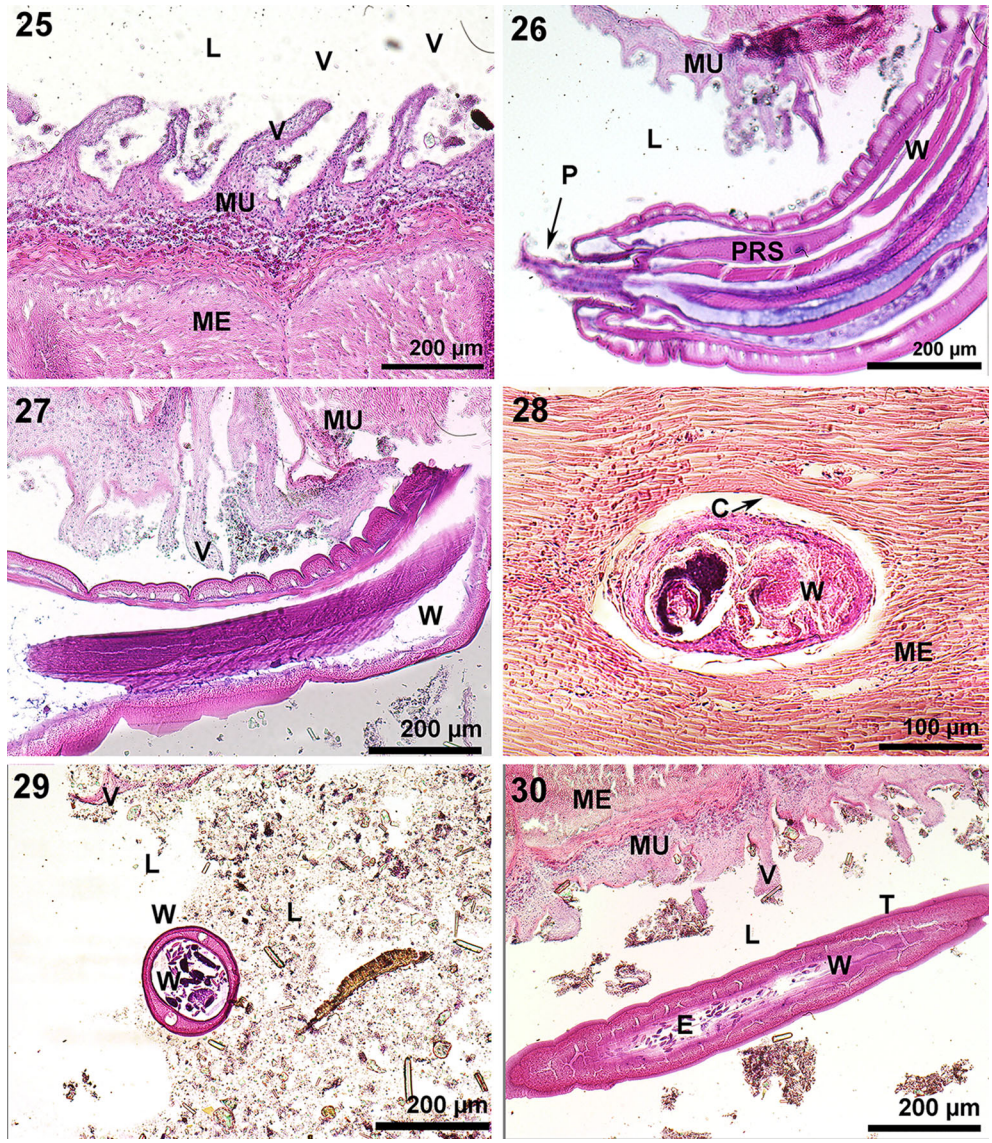
differences accumulate over time is also possible. With the molecular characterisation of *T. niloticus* from a worldwide sample set, allopatric versus sympatric models of accumulated variation can be distinguished. For example, with an allopatric model, we would expect to find locations where only mitochondrial Lineage 1 is found and separate locations where only mitochondrial Lineage 2 is found.

Histopathology

Results of the tissue pathology study of *T. niloticus* infecting *H. niloticus* in Burkina Faso are shown in Figs. 25–30. The uninfected tissue is represented by Fig. 25 with villi (V) and the characteristic layers of the small intestine. The armed proboscis (P) of the acanthocephalan is well displayed by Fig. 26, with hooks (H) and prominent para-receptable structure (PRS). There is pronounced damage to the mucosa of the host with haemorrhaging and tissue loss. Damage to the host tissue is further represented by Fig. 27 where the worm (W) is adjacent to the host intestinal mucosa (MU). The villi (V) are compressed with nucleated blood cells within the lumen. The villi have been damaged, which is characterised by the lack of epithelial cells lining the villi due to worm damage. The lumen (L) contains necrotic host intestinal tissue and blood cells. Worms have migrated into the host muscularis externa (Fig. 28). The worm is surrounded by smooth muscle fibers with limited haemorrhaging near the worm (W). The host has started to generate a collagenous connective tissue capsule (C) around the worm whereby it can isolate the parasite in one area of the host tissue. Cross-sections of the worm are visible in the lumen of the host intestine with surrounding necrotic tissue and blood cells (Fig. 29). Fig. 30 displays the acanthocephalan (W) adjacent to the mucosa of the host. Islands of blood cells and necrotic tissue are visible originating from the intestinal mucosa. The lack of epithelial cells which usually surround the intestinal villi, can be attributed, at least in part, to worm (W) invasion. Eggs are visible within the worm. Both male and female worms were observed in sections of host intestinal tissue. Typical histopathology for an acanthocephalan infection has been displayed by the invading worm.

Discussion

The African arowana is widespread throughout Africa where it is native in watersheds of the Sahelo-Sudanese region, Senegal, Gambia, and parts of



Figs. 25–30 Histopathology of *Tenuisentis niloticus* in the intestine of *Heterotis niloticus*. 25, Normal mucosal lining of the fish host showing loss of surface epithelium due to host tissue preparation; 26, *T. niloticus* (W) within the lumen (L) of the host intestine. Note the para-receptacle structure (PRS), damaged host intestinal mucosa (MU) with free blood cells (haemorrhage) and necrotic tissue; 27, The worm (W) is compressing the villi (V) of the mucosa (MU) of the host intestine and subsequent smooth muscle layers; 28, The worm (W) has extended into the outer smooth muscle coat (ME) of the host intestine, the host is generating a collagenous connective tissue capsule (C, *arrow*) whereby isolating the acanthocephalan; 29, Cross-section of *T. niloticus* (W) in the lumen (L) of the small intestine of the host, showing a section of the displaced villus (V); 30, Worm (W) within the lumen (L) of the host intestine [note the villi (V), part of the mucosa (MU) and eggs (E) within the worm; muscularis externa (ME), the well-formed tegument (T) of the parasite and islands of free blood cells in the lumen (L)]

eastern Africa including the basins of the Corubal, Volta, Ouémé, Niger, Bénoué, and River Nile as well as Lake Chad and Lake Turkana. It has been successfully introduced into many other rivers in the Ivory Coast, Nigeria, Cameroon, Gabon, the Congo and Madagascar (Froese & Pauly, 2015). We do not know the extent of the spread of its parasitic fauna including *T. niloticus*, to introduction streams. Infection of *H. niloticus* with *T. niloticus* appear to also be heavy and prevalent in other reported locations including Khalil

(1969) who reported 14 infected of 15 examined fish (93%) with 5–27 acanthocephalans each in the Sudan. While *P. niloticus* is the typical acanthocephalan parasite of *H. niloticus*, it was recently reported from the electric catfish *Malapterurus electricus* (Gmelin) (Malapteruridae) in the Lekki Lagoon, Lagos, Nigeria (Akinsanya et al., 2007b). The electeric catfish is an opportunistic voracious piscivore that can consume prey up to half their size (Sagua, 2006) and appears to have served as a postcyclic host. Thirty-seven percent of 100 examined catfish were infected with 76 specimens of *T. niloticus* and specimens of one species of nematode and one species of cestode (Akinsanya et al., 2007b).

Meyer (1932) added a sketchy drawing of a male specimen to his very brief description of the species. Van Cleave's (1936) account included good presentations of the internal anatomy of a male specimen of *T. niloticus* (his figure 1), a female reproductive system (his figure 2), and the proboscis (his figures 3, 4). Dollfus & Golvan (1956) provided a sketchy line drawing of a female reproductive system. Based on the above accounts and on our observations of new features not previously reported, it becomes imperative to further emend the diagnosis of the family Tenuisentidae presented by Bullock & Samuel (1975).

Emendation of the Tenuisentidae

Van Cleave (1936) created Tenuisentidae based on his diagnosis of *T. niloticus* which left much to be desired. Bullock & Samuel (1975) emended this diagnosis to include the shorter cement gland and allowing for the posterior position of cephalic ganglion in their specimens *Paratenuisentis ambiguus* while erroneously speculating that the cephalic ganglion of *T. niloticus* "appears to be in the anterior third of the receptacle". Bullock & Samuel (1975) described the hooks of *P. ambiguus* to be "strongly recurved, and similar in size and shape" but their shape in their figure 3 does not appear to be dorsoventrally similar and the roots appear to be similar to the roots in *T. niloticus*. Based on these features and the new ones being reported for *T. niloticus* in this paper, the family diagnosis is herein emended. Additions and changes are shown in italic.

"Tenuisentidae Van Cleave, 1936 emend. Eoacanthocephala. Trunk cylindrical, unspined, with slight anterior enlargement. Longitudinal canals of lacunar system dorsal and ventral. Body wall with few hypodermal nuclei usually paired anteriorly. Proboscis long, with long apical organ and longitudinal rows of many hooks each *usually showing dorsoventral differentiation. Hook roots simple, directed posteriorly, slightly shorter than blades; posteriormost roots may have anterior manubria.* Proboscis receptacle single-walled, with *ganglion in posterior half and para-receptacle structure. Lemnisci equal or subequal, usually extending past receptacle*. Cement gland elongate or tubular, syncytial, multinucleated. *Reproductive ligaments attach anteriorly at base of uterine bell and at level of cement reservoir. Paired sex glands may be present at posterior end of males.* Uterine bell opening in ventral ligament sac. Eggs ovoid, with concentric shells, may be with circular folds. Parasites of fish *in the USA and Africa*."

Meyer (1932) briefly described *T. niloticus* (as *Rhadinorhynchus niloticus*) from two male specimens overlooking the absence of trunk spines and the syncytial nature of the cement gland, among other features. Van Cleave (1936) suggested that "In arranging the species in his monograph, Meyer placed *R. terebra* (Rudolphi, 1819) Lühe, 1911 and *R. niloticus* in juxtaposition, giving evidence that he regarded the spineless males of *R. niloticus* as parallel to the condition encountered in the spineless males of *R. terebra*". Van Cleave's (1936) account was not actually a description but rather an attempt to distinguish *Tenuisentis* from *Rhadinorhynchus* based on five characters: (i) the cement gland pattern being a syncytial mass in *Tenuisentis* rather than separate follicles as in *Rhadinorhynchus*; (ii) the giant hypodermal nuclei being in a distinctive, yet not constant, pattern in *Tenuisentis* (iii) the proboscis receptacle being single-walled in *Tenuisentis* (iv) the opening of the uterus directly into the body cavity in *Tenuisentis*, and (v) the lemnisci being shorter than receptacle in *Tenuisentis* but longer in *Rhadinorhynchus*.

Comparisons

The following is a comparison of the various populations of *T. niloticus* with particular reference to the variables discussed by Van Cleave (1936).

Measurements: Morphometric data for our specimens from Burkina Faso and those from the Sudan and Mali are provided in Table 1. Our specimens reached larger sizes of all reported structures than those from the Sudan and Mali. New structures described and

measured that were not included in the other reports include those of the neck, cephalic ganglion, cement reservoir, Saefftigen's pouch, common sperm duct and eggs (see above).

Proboscis hooks: We found 16 (rarely 15) proboscis hook rows with 30–33 hooks each (Fig. 3). Corresponding figures of 16 × 23 were reported by Meyer (1932), 16 or more × 30–40 by Van Cleave (1936) and 16–20 × 32–38 by Dollfus & Golvan (1956). The 23 hooks per row reported by Meyer (1932) are certainly an underestimate. His figure 31 of a whole male shows a dotted outline of a proboscis, not a solid line like the rest of the figure, suggesting a projection of and not a real proboscis. We assume that his 23 hooks per row was just an estimate. The number of hooks per row reported by Van Cleave (1936) reaching 40 also appears to be just an estimate. Van Cleave noted that "Few of the proboscides were fully everted …" and that "For some partially inverted proboscides as many as 38 or 40 hooks have been calculated for the individual rows". We are not certain that such a pattern of estimation may account for the higher counts of 16–20 × 32–38 reported by Dollfus & Golvan (1956).

Hooks showed marked dorso-ventral dissymmetry in robustness and angle of curvature but were similar in length in the present material. The ventral hooks were markedly more massive and more sharply recurved than the dorsal hooks. Meyer (1932) noted the same using the word "verschieden" (different, distinct, vary, diverse). Van Cleave (1936) did not comment but his figure 3 of a proboscis clearly showed the same dissymmetries that we noted. Surprisingly, Dollfus & Golvan (1956) stated that "… il n'y a pas de dissymétrie dorso-ventrale appréciable".

Van Cleave (1936) stated that "hooks range from 30 to 41 μm in length over the greater part of the proboscis, though a few have a length as great as 47 μm." Hooks in our specimens measuring ≥40 μm started from the third hook from anterior in both males and females and extended to hooks 10, 12 and 15 in females and 5, 9 and 12 in males (Table 3) giving females markedly more anchoring efficiency. Average total hook length in a row was 1,105 μm (range 1,069–1,127 μm) in females and 993 μm (range 891–1,122 μm) in males. We propose that this measurement may be used as a criterion of anchoring efficiency.

Cement glands: Meyer (1932) reported cement glands reaching 6 mm in length in his 9–10 mm long males. Van Cleave (1936) discounted that measurement claiming that Meyer was "unaware of the true nature of the cement gland thinking that he was dealing with a multiple tubular system". We strongly disagree with this statement. Two males from our specimens measuring 13.52 and 14.25 mm in length had 6.62 and 6.92 mm long cement glands. Van Cleave's (1936) specimens were simply not long enough measuring 7.20 mm in length according to his figure 1. Meyer's males were 9–10 mm long. Van Cleave (1936) counted "near 50 (giant) nuclei" in the cement gland. That is vastly an underestimate compared to 86–146 nuclei in our specimens (Table 1; Fig. 1b). We counted 61 nuclei in one of Van Cleave's specimens. The three Van Cleave's male specimens examined by us were so faintly stained that it would have been impossible to take an accurate count of the nuclei. Dollfus & Golvan (1956) did not report on the giant nuclei of the cement gland (Table 1).

The giant hypodermal nuclei: Van Cleave (1936) observed that "the anterior region of the body contains eight subcuticular nuclei disposed in four groups of two each. Four of these are near the posterior tip of the proboscis receptacle, two in the dorsal and two in the ventral body wall. Slightly posterior to this position two nuclei occur in the dorsal wall and two in the ventral … in some males, a single nucleus appears in the ventral wall of the body near the posterior extremity of the cement gland." He then noted the phylogenetic implications of giant hypodermal nuclei. Meyer (1932) made no reference to these nuclei and Dollfus & Golvan (1956) noted that their specimens conformed to Van Cleave's (1936) description. Our specimens from Burkina Faso showed considerable variations from the pattern observed by Van Cleave (1936) (Table 2) even though five of nine male and five of nine female specimens conformed to his pattern with deviations of one or two nuclei. Our results show more nuclei in males, on the ventral side, and in the region of swelling in anterior trunk (Fig. 1). We found six nuclei in three males anterior to level of mid receptacle (Region A) and none at the posterior trunk (Region F) in either sex. Definitely more than a single occasional nucleus is found in the area of posterior cement gland (Region E), especially in males (Table 2). We conclude that the pattern of giant

Table 4 GenBank accession numbers for the taxa used in the phylogenetic analyses

Species	Host	GenBank accession No.		Source
		COI	18S rDNA	
Tenuisentis niloticus (Meyer, 1932) (Lineage 1)	*Heterotis niloticus* (Cuvier)	KT970469	KT970471	Present study
Tenuisentis niloticus (Meyer, 1932) (Lineage 2)	*Heterotis niloticus* (Cuvier)	KT970470	KT970471	Present study
Echinorhynchus truttae Schrank, 1788	*Salmo trutta* (Linnaeus)[a]; *Thymallus thymallus* (Linnaeus)[b]	KP261013	AY830156	Wayland et al. (2015)[a]; Garcia-Varela & Nadler (2005)[b]
Rhadinorhynchus pristis (Rudolphi, 1802)	*Nyctiphanes couchii* (Bell)	JQ061132	JQ061136	Gregory et al. (2013)
Pomphorhynchus laevis (Zoega in Müller, 1776)	*Squalius cephalus* (Linnaeus)[a]; *Gammarus pulex* (Linnaeus)[b]	KF559286	AY423346	Smrzlić et al. (2015)[a]; Perrot-Minnot (2004)[b]
Paratenuisentis ambiguus (Van Cleave, 1921)	*Anguilla anguilla* (Linnaeus)[a]; *Thymallus thymallus* (Linnaeus)[b]	NC019807	AF469414	Weber et al. (2013)[a]; Herlyn et al. (2003)[b]
Neoechinorhynchus crassus Van Cleave, 1919	*Catostomus commersoni* (Lacépède)	–	AF001842	Near et al. (1998)
Neoechinorhynchus salmonis Ching, 1984	*Salvelinus malma* (Walbaum)	KF156889	–	Malyarchuk et al. (2014)
Brachionus plicatilis (Müller, 1786)	–	AY218090	AY218118	Giribet et al. (2004)

[a] Hosts and references for COI sequences; [b] Hosts and references for 18S rDNA sequences

hypodermal nuclei in *T. niloticus* in considerably more variable than advanced by Van Cleave (1936). Dollfus & Golvan (1956) reported that the giant hypodermal nuclei were "rare" and that they have not noted their disposition in groups of two or whether they were in any particular "emplacements".

Proboscis receptacle and cephalic ganglion: The statement by Van Cleave (1936) that the "single heavy muscular wall" in *Tenuisentis* would exclude it from Rhadinorhynchidae was well articulated. He could not, however, observe the cephalic ganglion. "Even under powerful illumination the retinacula and the brain within the receptacle have not been discernible" (Van Cleave, 1936). The unfortunate faint staining of his mounted material also made it impossible for us to discern these structures in his specimens that were readily seen in ours stained with acid carmine. We have determined that the large, elliptical, pointed cephalic ganglion is repeatedly found in the posterior third of the receptacle. Bullock & Samuel (1975) erroneously, however, stated that "the ganglion appears to be in the anterior third of the receptacle in *Tenuisentis*".

Lemnisci: Van Cleave (1936) distinguished Rhadinorhynchidae with tubular lemnisci "exceeding the length of the receptacle" from those in *Tenuisentis* as being "poorly defined in preserved specimens and seem to be shorter than the receptacle." This statement is inaccurate on two counts: (i) the lemnisci in Rhadinorhynchidae are not invariably longer than the receptacle (see Amin et al., 2011b); and (ii) we agree that the lemnisci in Van Cleave's (1936) specimens were not discernible, but in our specimens from Burkina Faso they decidedly extended a short distance past the receptacle; they were, however, somewhat variable and two appeared slightly shorter than the receptacle but it was not clear if they were not completely extended. Dollfus & Golvan (1956) indicated that the lemnisci did not pass past the posterior end of the receptacle.

Body cavities: The anterior margin of the uterine bell appears to open directly into the ventral ligament sac as Van Cleave (1936) noted in *Tenuisentis* and as Bullock & Samuel (1975) noted in *Paratenuisentis*.

Lineages, divergence and cryptic species

The sympatric presence of two lineages and a high percentage of heterozygotes (89%) at the 18S rRNA locus are intriguing and several possible explanations

can be explored. Heterozygotes could provide a selective advantage over either homozygous state. Previously allopatric *T. niloticus* lineages may have been recently reintroduced and the heterozygous individuals represent an F1 generation. Hosts and intermediate host availability may have been disrupted resulting in a collision of the allopatric or sympatric *T. niloticus* lineages in a single host. One possible source of the disrupted separation could be the construction of the Sourou dam at Leri in 1976. Before 1976, the River Sourou, a tributary of the River Mouhoun, would dry out for 1–2 months each year. With the construction of the dam and subsequent canal construction, water from the Mouhoun is directed into the Sourou basin creating a year-long surface water supply in what is now the 55 km long Sourou Reservoir (surface of 22 ha). The increased prevalence of schistosomiasis associated with increases in mollusc prevalence in this same region post-dam construction emphasises the recently occurring ecological changes and habitat disruptions in the study area (Dianou et al., 2003).

A wide range of sequence diversity can exist within species of Acanthocephala. As revealed in this study, *T. niloticus* in this region consists of individuals with multiple molecular trajectories. Both the mitochondrial cytochrome *c* oxidase I (COI) gene and the nuclear 18S rRNA gene have accumulated changes over time and these changes appear as fixed differences. The magnitude of divergence, 6.4% for COI and 3.4% for 18S, is less than seen for other cryptic acanthocephalan species. Two sympatric samples of *Echinorhynchus gadi* (Archiacanthocephala), i.e. species I and species III, collected from the White Sea, harboured COI genes with a 7.2% sequence divergence but had identical 28S rRNA gene sequences (Wayland et al., 2015).

Martínez-Aquino et al. (2009) showed the presence of three lineages in the *Neoechinorhynchus golvani* (Eocanthocephala) cryptic species complex in fresh and brackishwater habitats using 28S rDNA sequences with 19.5–35.3% sequence divergence between the three lineages. Two forms of *Pomphorhynchus laevis* (Palaeacanthocephala), smooth and wrinkled, exhibited high levels of sequence divergence between the intervening transcribed spacer I regions of the rRNA gene (ITS1) and the COI gene, 11% and 20%, respectively (Perrot-Minnot, 2004). In contrast, the acanthocephalan *Profilicollis altmani* (Palaeacanthocephala) showed little nucleotide diversity among Pacific or Atlantic populations (Goulding & Cohen, 2014).

Cryptic species are minimally defined as "… species that cannot be distinguished on the basis of their morphology despite molecular evidence that they are specifically distinct …" (Bray & Cribb, 2015). The sympatric identification of two genetically distinct yet seemingly interbreeding *T. niloticus* lineages deserves future study as to the origin and continued persistence of this genetic diversity.

Acknowledgements Thanks to Michael Standing and Kyrie Carpenter, BYU (Brigham Young University) Microscopy, for their professional help with electron optics equipment and plate preparation and editing. We also thank P.R. Pilitt (USNPC) for making it possible to examine the *Tenuisentis* and *Paratenuisentis* material.

Funding This work was supported by an Institutional grant from the Institute of Parasitic Diseases, Scottsdale, Arizona, USA and by funding from the Department of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah, USA.

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.

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