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An SEM study of *Acanthogyrus (Acanthosentis) tilapiae* (Acanthocephala: Quadrigyridae) from Africa documenting previously unreported features and host parasite interface

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Abstract. *Acanthogyrus (Acanthopsentis) tilapiae* (Baylis, 1948) is the most widely distributed species of *Acanthogyrus* in many countries associated with the Nile River in Africa. It has been described by various authors but much of its external morphological features remained unknown until recently explored by SEM in our present study from specimens collected from cichlid fishes in Lake Malawi, Africa. Newly observed features include the proboscis armature and sensory pores, epidermal micropores, and trunk spines. Attachment and interface between worm and host intestinal lining are also reported for the first time.

Keywords: *Acanthogyrus (Acanthopsentis) tilapiae*; Acanthocephala; Cichlid fish; Lake Malawi; Africa; Morphology; SEM.

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Introduction

Only 5 of the 38 known species of the subgenus *Acanthosentis* are found in Africa. Thirty-one of the other 33 species are Asian, mostly in the Indian subcontinent and China. In Africa, *Acanthogyrus (Acanthopsentis) tilapiae* (Baylis, 1948) is a widely distributed endemic species mostly in cichlids from countries associated with the Nile River (Amin and Hendrix, 1999). The 4 other African species appear to be of restricted distribution: *Acanthogyrus (Acanthosentis) maroccanus* (Dollfus, 1951) in Morocco, *Acanthogyrus (Acanthosentis) nigeriensis* Dollfus and Golvan, 1956 in the Niger River, *Acanthogyrus (Acanthosentis) papilo* Troncy and Vassiliadis, 1974 in West Africa, and *Acanthogyrus (Acanthosentis) malawiensis* Amin and Hendrix, 1999 in Lake Malawi.

Until 1984, *A. (A.) tilapiae* was reported from at least 10 species of the cichlid genus *Tilapia* in Tanzania (Baylis, 1948), Congo (Prudhoe, 1951; Golvan, 1957), Madagascar (Golvan, 1965), Uganda (Khalil and Thurston, 1973), Chad (Troncy, 1974), Nigeria (Shotter, 1974), Egypt (Amin, 1978), and unidentified locations (Marchand and Mattei, 1976; Marchand, 1984). Amin and Hendrix (1999) added 19 new host records from 18 cichlid and 1 bagrid fish species from southeastern Lake Malawi. Khalil

and Thurston (1973) also reported *A. (A.) tilapiae* from unidentified cichlids in Lake Malawi, Lake Tanganyika, and other lakes in the Congo. In their ecological study of *A. (A.) tilapiae* in Lake Malawi, Amin et al. (2008) examined 9 species of fish from 7 different sites and added 5 new host records.

Since its imperfect description by Baylis (1948), descriptive accounts with various degrees of coverage and detail were reported only by Troncy (1970), Amin (1978), and Amin and Hendrix (1999). The presented SEM study is based on extensive collections of *A. (A.) tilapiae* from cichlid fishes from Lake Malawi, Africa (Amin et al., 2008). The new observations fill an important gap of missing information and enhance our understanding of the morphology of *A. (A.) tilapiae* especially related to the proboscis armature and sensory pores, epidermal micropores, and trunk spines. Attachment and interface between worm and host intestinal lining are also reported.

Materials and methods

About 2,000 specimens of *A. (A.) tilapiae* (Baylis, 1948) were collected from 9 species of cichlid fish hosts (Cichlidae: Perciformes) in Lake Malawi, Africa, during September 2005. Cichlids were captured by gill nets while scuba diving in 7 sites: (1) Chirwa Islands (10°27'48.94"S; 34°16'35.77"E), Chilumba (19 September); (2) Domwe Island (13°58'05.43"S; 34°49'04.01"E) (6 September, 16 September); (3) Luwino Reef (10°26'17.41"S; 34°17'0.16"E) (19 September); (4) Mpanga Rocks (10°25'49.65"S; 34°16'44.64"E) (19 September); (5) Otter Point (14°02'21.33"S; 34°49'23.85"E) (2 September); (6) Thumbi West Island (14°01'22.83"S; 34°49'16.63E) (3 September); and (7) Zimbawee Rock (13°57'40.73"S; 34°48'08.88"E) (10 September). The species of fish included *Labeotropheus trewavasae* (Fryer 1956) (scrapemouth mbuna), *Pseudotropheus emmiltos* (Stauffer et al. 1997) (red top), *Pseudotropheus zebra* (Boulenger 1899) (zebra mbuna), *Melanochromis vermivorus* (Trewavas 1935) (purple mbuna), *Nimbochromis polystigma* (Regan 1922), *Protomelas taeniolatus* (Trewavas 1935) (spindle hap), *Pseudotropheus elongates* (Fryer 1956) (elongate mbuna), *Tropheops microstoma* (Trewavas 1935), and *Rhamphochromis* sp. (Regan 1922) (see Amin et al., 2008).

Fish were kept alive for up to 1 to 3 days before dissection. After the fish were killed with an overdose of MS222, they were weighed, measured, and sexed, and the intestines were removed and transferred to petri dishes containing 0.6% saline. Collected worms were fixed directly in 10% formalin, then later, washed in water and transferred into 70% ethanol to transport to our Arizona facility for processing. Worms were punctured with a fine needle and subsequently stained in Mayer's acid carmine, destained in 4% hydrochloric acid in 70% ethanol, dehydrated in ascending concentrations of ethanol (24 hr each), and cleared in graduated concentrations of terpineol in 100% ethanol to 100% terpineol, then 50% terpineol in 50% Canada balsam (24 hours each). Whole worms were then mounted in Canada balsam.

For SEM studies, 23 specimens from *P. zebra* as well as those affixed to host tissue previously fixed in 70% ethanol were placed in critical-point drying baskets and dehydrated using ethanol series of 95% and 3 N 100% for at least 10 min per soak followed by critical point drying (Lee, 1992). Samples were mounted on SEM sample mounts, gold coated and observed with a scanning electron microscope (XL30 ESEMFEG; FEI, Hillsboro, Oregon). Digital images of the structures were obtained using digital imaging software attached to a computer.

Voucher specimens of *A. tilapiae* from Lake Malawi were deposited at the U.S. National Parasite Collection (USNPC) at Beltsville, Maryland # 99961.

Results and discussion

Morphologically, our specimens were similar to those of the first Lake Malawi survey by Amin and Hendrix (1999), as well as those reported from Egypt by Amin (1978). The Lake Malawi specimens from cichlids from both collections were, however, smaller than those from the Nile River in Egypt from *Tilapia niloticus* (Linnaeus) (up to 60.0 cm SL) and

Tilapia zillii (Gervais) (up to 40.0 cm SL) (Amin, 1978). The Egyptian specimens were, in turn, somewhat smaller than those reported by Baylis (1948) from *Tilapia lidole* (Trewavas) (up to 38.0 cm SL) in Lake Nyasa (Lake Malawi) but of similar size to those reported by Troncy (1970) from the amphibious giant otter shrew *Potamogale velox* du Chaillu (the only known, but accidental, mammalian host of *A. tilapiae)* in Yaoundé (Cameroon). Host factors appear to enhance greater growth in the larger *Tilapia* compared with the smaller cichlids examined (up to 11.1 cm SL) (Amin et al., 2008). Available measurements of other structures such as the proboscis and proboscis hooks were comparable in specimens reported by Baylis (1948), Troncy (1970), Amin (1978) and Amin and Hendrix (1999). There are, however discrepancies in other characters such as the number of sub-cuticular giant nuclei and the number of transverse rings of trunk spines. Baylis (1948) counted 2 dorsal and 3 ventral giant nuclei but we found usually 2 dorsal and 6 ventral nuclei that, however, varied between 2-4 dorsal and 4-6 ventral nuclei in various combinations. Baylis (1948) also counted 14 closely spaced rings of trunk spines in the "forbody" and 18-20 more widely spaced rings in the "body proper". We found no such division of trunk into 2 regions which appeared to be a result of contraction in his specimens, and counted a total of 28-38 and 28-42 rings of spines in males and females, respectively, that appeared to merge closer more posteriorly (figure 8).

New SEM observations

These observations include (1) the ovoid shape of males (figure 1) compared to the elongate shape of females with parallel sides (figure 2). (2) The arrangement of anterior proboscis hooks at alternating levels (figure 3) and not in a straight circle as pictured in Baylis (1948, figure 2) and Golvan (1957, figure 7). (3) The presence of paired sensory pits at the base of the proboscis (figure 4). (4) Dermal micropores with different pore size and spacing distributed in the epidermis of the anterior trunk (figure 5) and posterior trunk (figure 6); see Amin et al. (2009) for implications to differential absorption. (5) The ovoid eggs have corrugated surface (figure 7). (6) The oblong lips of the vulva and the extension of the posterior-most ring of trunk spines to the genital orifice (figure 8). (7) The distribution of trunk spines in the posterior end of the trunk (figure 9). (8) The blunt-ended nipple-like shape of trunk spines was observed to be consistent throughout the body (figure 10). (9) The circular arrangement of posterior trunk spines at the recessed posterior end of a female (figure 11).

Host-parasite interface

This interface is depicted by an attached worm (figure 12), site of attachment within the host intestinal folds after removal of the parasite in figure 12 (figure 13), and the actual site of attachment enlarged to show the holes where proboscis hooks were inserted in a depression caused by the insertion of the proboscis (figure 14).

The description of *A*. (*A*.) *tilapiae* is greatly enhanced by the new information provided and documented for the first time with SEM images. An interesting perspective on the host-parasite interface is also demonstrated showing evidence of the deep penetration of proboscis hooks in host intestinal lining for the first time.

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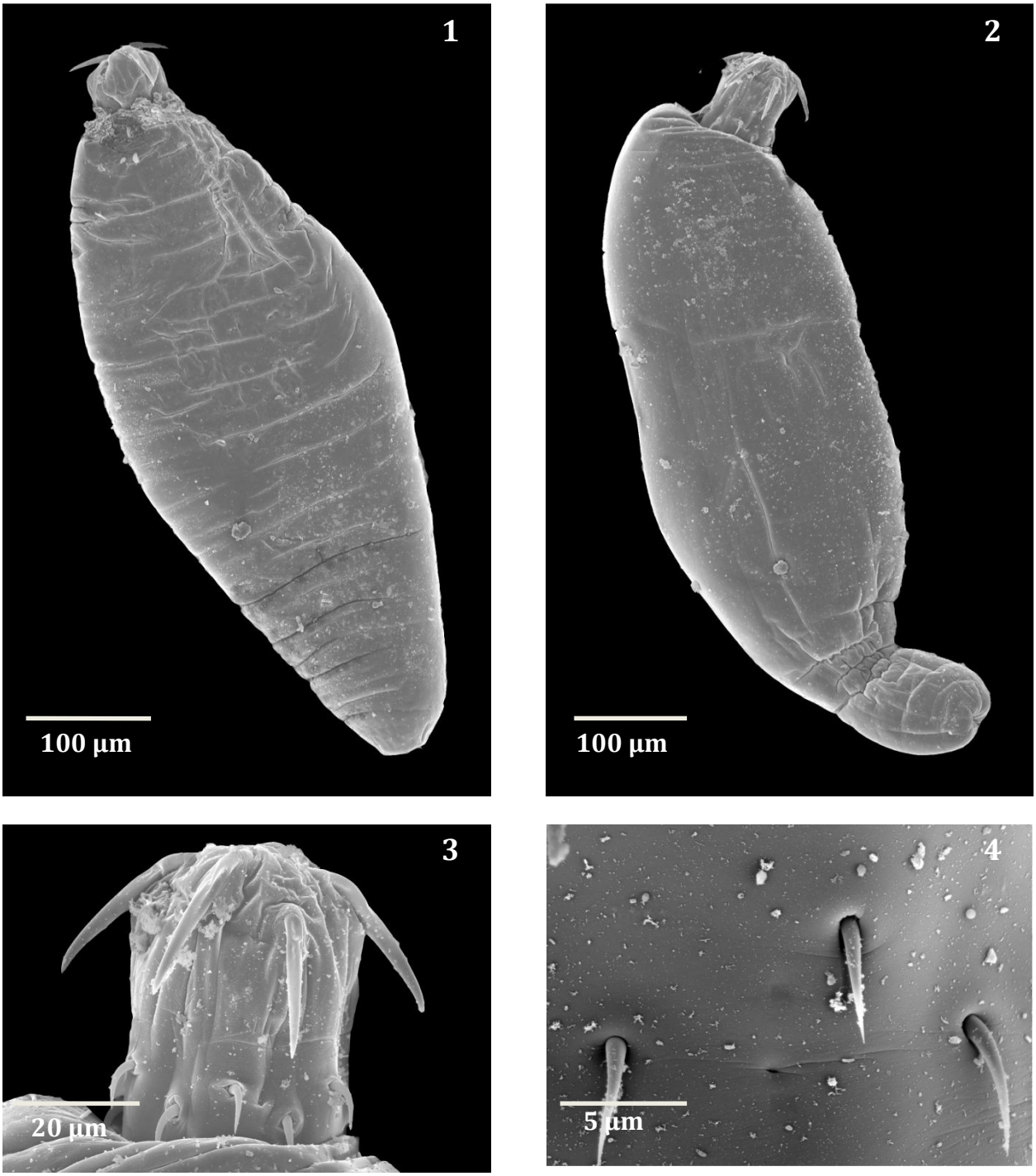
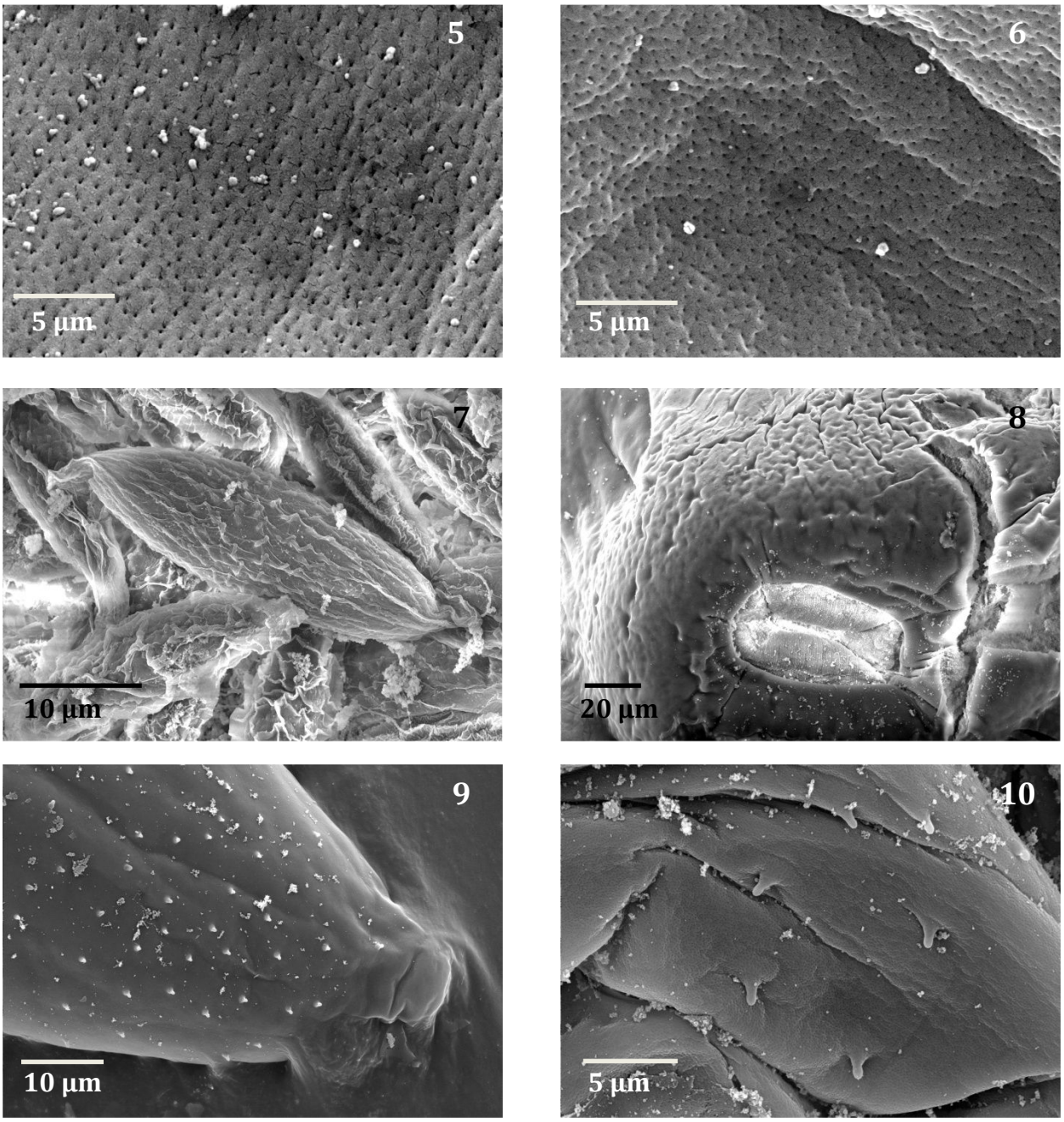
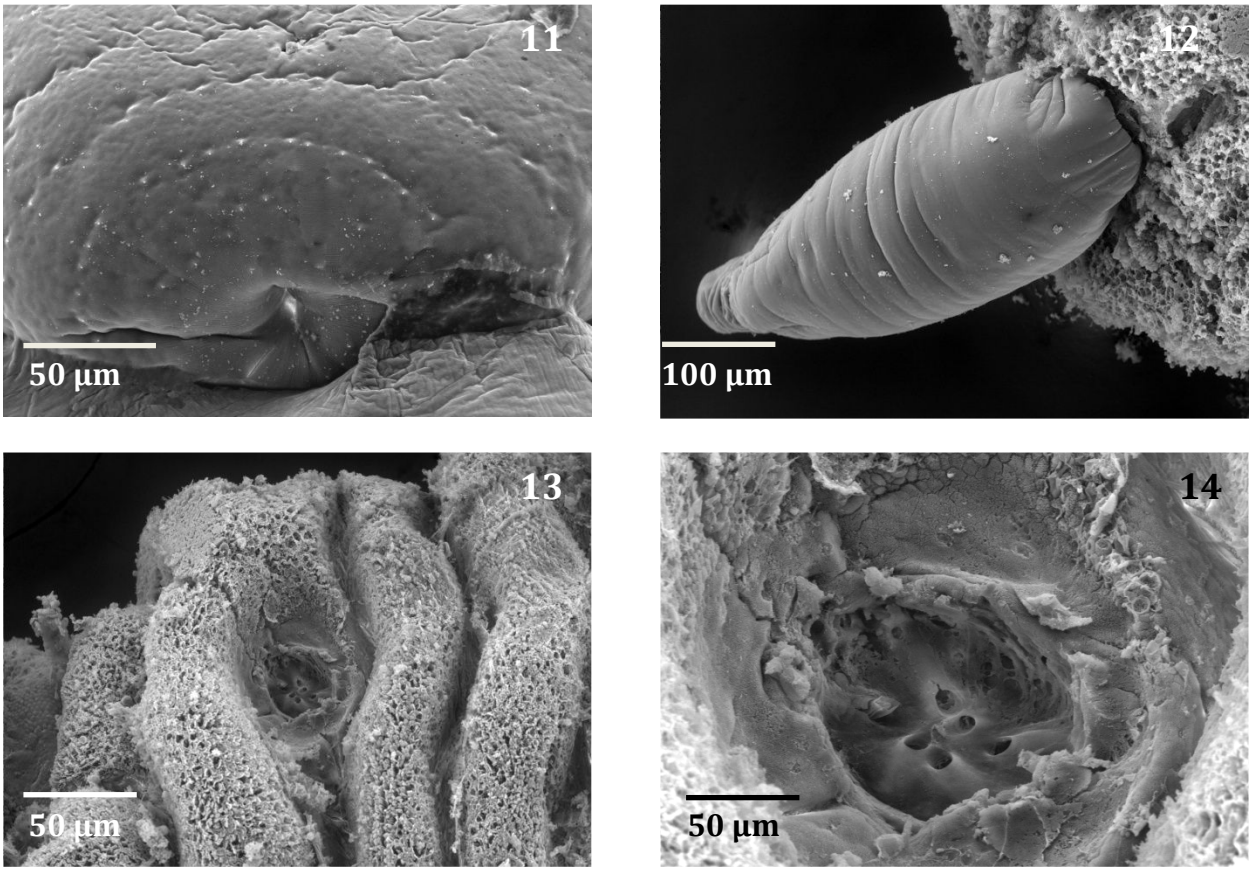


Figures 1-4. SEM of *Acanthogyrus (Acanthosentis) tilapiae* from Lake Malawi cichlids. 1. A male worm. 2. A female worm. 3. The proboscis of a male worm showing the alternate placement of hooks in the anterior ring. 4. Posterior hooks and a sensory pit (lower center) of another proboscis.



Figures 5-10. SEM of *Acanthogyrus* (*Acanthosentis*) *tilapiae* from Lake Malawi cichlids. 5. Micropores in the anterior trunk of a worm. 6. Micropores in the posterior trunk of the same worm in figure 5. 7. Egg. 8. A face view of the posterior end of a female worm showing the lips of the vagina and posterior-most spine ring. 9. Posterior trunk spines of a female specimen. 10. Enlargement of a few trunk spines showing their nipple-like shape.



Figures 11-14. Posterior trunk spines and host-parasite interface of *Acanthogyrus (Acanthosentis) tilapiae* from Lake Malawi cichlids. 11. Perfect rings of trunk spines at the posterior end of the trunk of a female specimen. 12. A worm attached to the intestinal lining of a cichlid host. 13. Intestinal lining of the same fish in figure 12 after removal of the worm. 14. Enlargement of the same attachment site in figure 13 showing the anterior hook attachment marks and depression in the host's mucosal lining

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