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ORIGINAL PAPER

# *Annulotrema* (Monogenea: Dactylogyridae) from the gills of African tetras (Characiformes: Alestidae) in Lake Turkana, Kenya, with descriptions of four new species and a redescription of *A. elongata* Paperna and Thurston, 1969

**Maria Lujza Kičinjaová**[1] **· Radim Blažek**[1,2] **· Milan Gelnar**[1] **· Eva Řehulková**[1]

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**Abstract** Four new and four previously described species of *Annulotrema* were collected from the gills of four species (three genera, i.e. *Alestes*, *Hydrocynus* and *Brycinus*) of African tetras from Lake Turkana, Kenya: *Annulotrema alestesnursi* Paperna, 1973 from *Brycinus nurse*; *Annulotrema ansatum* n. sp., *Annulotrema besalis* Řehulková, Musilová and Gelnar, 2014, *Annulotrema bipatens* n. sp., *Annulotrema cucullatum* n. sp., *Annulotrema nili* Paperna, 1973, and *Annulotrema pontile* n. sp. from *Hydrocynus forskahlii*; and *Annulotrema elongata* Paperna and Thurston, 1969 from *Alestes baremoze* and *Alestes dentex*. *A. elongata* is re-described on the basis of new material from *A. baremoze*. The sclerotized structures of the haptor and male copulatory organ of *A. alestesnursi* and *A. elongata* are illustrated from their type material. *H. forskahlii* is a new host record for *A. besalis*. The findings of *A. besalis* and *A. elongata* in Kenya represent a new locality records for these helminths. Three *Annulotrema* spp., namely *A. besalis*, *A. elongata* and *A. pontile* n. sp., share the same type of male copulatory organ, which may indicate a close relationship among these species.

**Keywords** Dactylogyridae · *Annulotrema alestesnursi* · *Annulotrema ansatum* · *Annulotrema besalis* · *Annulotrema bipatens* · *Annulotrema cucullatum* · *Annulotrema elongata* · *Annulotrema nili* · *Annulotrema pontile* · Kenya · Alestidae · *Alestes baremoze* · *Alestes dentex* · *Brycinus nurse* · *Hydrocynus forskahlii*

✉ Eva Řehulková
evar@sci.muni.cz

1 Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

2 Department of Fish Ecology, Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic

## Introduction

Characiformes are one of the largest and most diverse components of African ichthyofauna. African freshwaters harbour more than 200 characiform species currently arrayed in four families, from which the Alestidae (African tetras) is the most speciose (Arroyave and Stiassny 2011). To date, only six (*Alestes*, *Brycinus*, *Hemigrammopetersius*, *Hydrocynus*, *Micralestes* and *Phenacogrammus*) of the 21 valid genera of the Alestidae listed by Eschmeyer and Fong (2014) have been reported as hosts of dactylogyrids. These monogeneans are represented by three genera and 46 species: *Annulotrema* (35 species), *Characidotrema* (10 species), and *Afrocleidodiscus* (1 species) (Khalil and Polling 1997; Řehulková et al. 2014). Among monogeneans collected from Kenya during the past few decades, only two species of dactylogyrids were discovered on the gills of *Alestes jacksonii* (= *Brycinus jacksonii*) caught in the Nzoia River, namely *Characidotrema nzoiae* (Paperna 1979) and *Annulotrema gravis* (Paperna and Thurston 1969).

During investigations into the monogenean fauna of African tetras from Lake Turkana (2008 and 2009), four previously described and four new species of *Annulotrema* were collected from the gills of *Alestes baremoze* (Joaniss, 1835), *Alestes dentex* (Linnaeus, 1758), *Brycinus nurse* (Rüppell, 1832), and *Hydrocynus forskahlii* (Cuvier, 1819). This paper includes both illustrations and measurements of all the species we found, including those described previously.

## Materials and methods

Fish hosts were collected by beach seine net at four sites on Lake Turkana (Kenya): Kalokol (Ferguson Gulf 3°32′38.51″ S, 35°54′26.80″V), Kalokol (Fishing Lodge 3°33′18.26″S, 35°54′56.03″V), Loyingalani (El Molo Bay 2°49′45.55″S, 36°41′55.32″V) and Todonyang (Omo River delta 4°27′ 6.37″S, 35°56′15.44″V). The collections of fish hosts were carried out during two field campaigns in 2008 and 2009.

Monogeneans found on the gills of freshly killed fishes were teased from the gill lamellae with fine needles, put in a drop of water on a slide, and covered by a cover-slip. Specimens used for drawings and measurements of their hard sclerotized structures, haptoral attachment components, vaginal armament and male copulatory organ (abbreviated below as MCO), were flattened under the pressure of the cover-slip and excess water was absorbed with filter paper. The corners of cover-slips were fixed with nail varnish and a drop of a glycerine-ammonium picrate mixture (GAP; Malmberg 1957) was added to the edge of the cover-slip. Specimens subjected to body measurements were fixed in the same way, excluding the flattening step and the removal of excess water with filter paper. Taxonomical evaluation of the mounted specimens was performed using an Olympus BX 61 microscope equipped with phase contrast optics. Drawings of hard sclerotized structures were made with the aid of a drawing attachment. Measurements were taken using Digital Image Analysis (Stream Motion). All measurements are in micrometers and represent means followed by the range and the number (*n*) of specimens measured in parentheses. The scheme of measurement for the sclerotized structures is shown in Fig. 1. The numbering of hook pairs is in Roman numerals and follows the form recommended by Mizelle (1936). After morphometric analysis, the specimens fixed with GAP were remounted into Canada balsam according to Ergens (1969).

Type specimens were deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, in České Budějovice (IPCAS). For comparative purposes, the following specimens of previously described species were examined: *Annulotrema alestesnursi* Paperna, 1973, syntypes M. T. 35. 918 III; *Annulotrema longipenis* Paperna, 1969, holotype M. T. 35. 579, paratype M. T. 35. 906; *Annulotrema elongata* Paperna and Thurston, 1969, holotype M.T. 35. 577, paratypes M. T. 34. 293.

## Results

Species of *Annulotrema* were found on the gills of all four species (100 % prevalence) of tetras examined: *A. baremoze* (16 specimens), *A. dentex* (7 specimens),

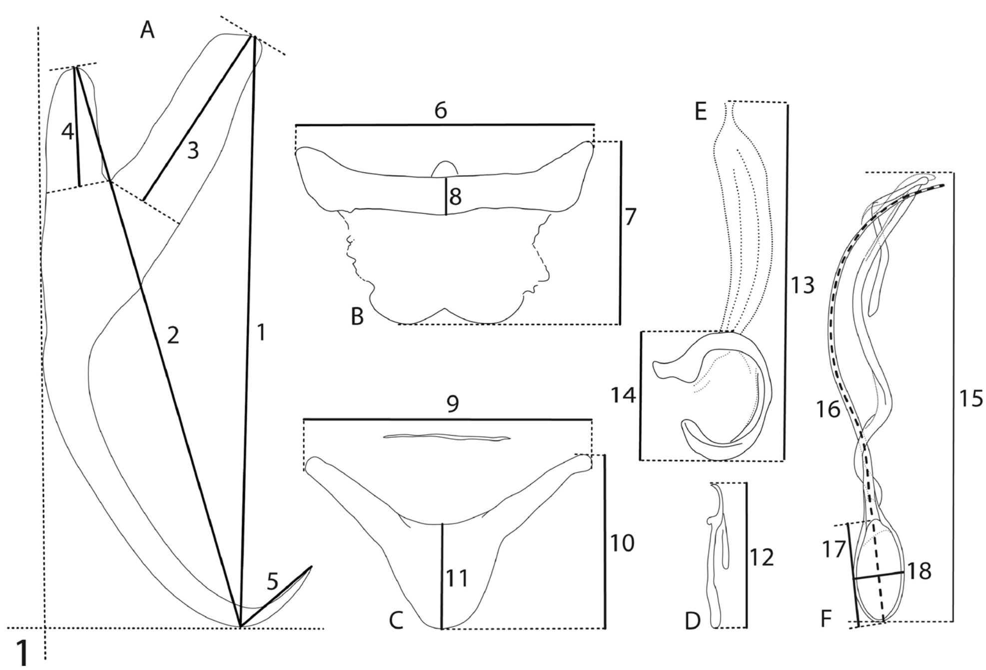


**Fig. 1** Scheme of measurement for sclerotized structures of the haptor and reproductive organs. *A* anchor: *1* = inner length, *2* = outer length, *3* = inner root length, *4* = outer root length, *5* = point length; *B* ventral bar: *6* = total length, *7* = total width, *8* = median width; *C* dorsal bar: *9* = total length, *10* = total width, *11* = median width; *D* hook: *12* = total length; *E* vagina: *13* = total length, *14* = maximal diameter; *F* male copulatory organ: *15* = total length, *16* = tube-trace length, *17* = base length, *18* = base width

*B. nurse* (4 specimens) and *H. forskahlii* (21 specimens). Taxonomical evaluation of the monogeneans found revealed the presence of eight species of *Annulotrema*, of which four species are new to science. Three species of the host fishes were each infected with only one species of *Annulotrema*: *A. baremoze* and *A. dentex* with *A. elongata* Paperna and Thurston, 1969, and *B. nurse* with *A. alestesnursi* Paperna, 1973. *Annulotrema ansatum* n. sp., *Annulotrema besalis* Musilová, Řehulková and Gelnar, 2014, *Annulotrema bipatens* n. sp., *Annulotrema cucullatum* n. sp., *Annulotrema nili* Paperna, 1973, and *Annulotrema pontile* n. sp. were all found on the gills of *H. forskahlii*. All of these species can occur together concurrently, but we have no data on any temporal or spatial distribution patterns on the different gill segments. Four species represent previously described taxa and all of them are reported from Kenya for the first time.

Dactylogyridae Bychowsky, 1933

*Annulotrema* Paperna & Thurston, 1969

*Annulotrema alestesnursi* Paperna, 1973 (Fig. 2)

*Type host and locality*: *Alestes nurse* (= *Brycinus nurse*), Lake Albert, Uganda

*Other records*: *A. nurse* (= *B. nurse*), Ghana (Paperna 1973; Paperna 1979), *A. nurse* (= *B. nurse*), Egypt (Ergens 1988)

*Present record*: *Brycinus nurse*, Lake Turkana: Todonyang (Omo River delta), Kenya

*Site*: Gill lamellae

*Type specimens*: Syntypes M. T. 35. 918 III

*Material examined*: 3 unflattened and 6 flattened specimens in GAP

*Comparative material examined*: 3 syntypes of *A. alestesnursi* Paperna, 1973 (M. T. 35. 918 III) from *A. nurse* (= *B. nurse*) (Uganda).

*Material deposited*: vouchers IPCAS M-325

*Measurements*: Body length 395 (364–424; $n$=3); greatest width 106 (95–118; $n$=3). Dorsal anchors: inner length 37 (32–39; $n$=5); outer length 31 (29–36; $n$=5); inner root length 12 (9–14; $n$=5); outer root length 4 (3–6; $n$=5); point length 4 (3–6; $n$=5). Ventral anchors: inner length 36 (34–37; $n$=5); outer

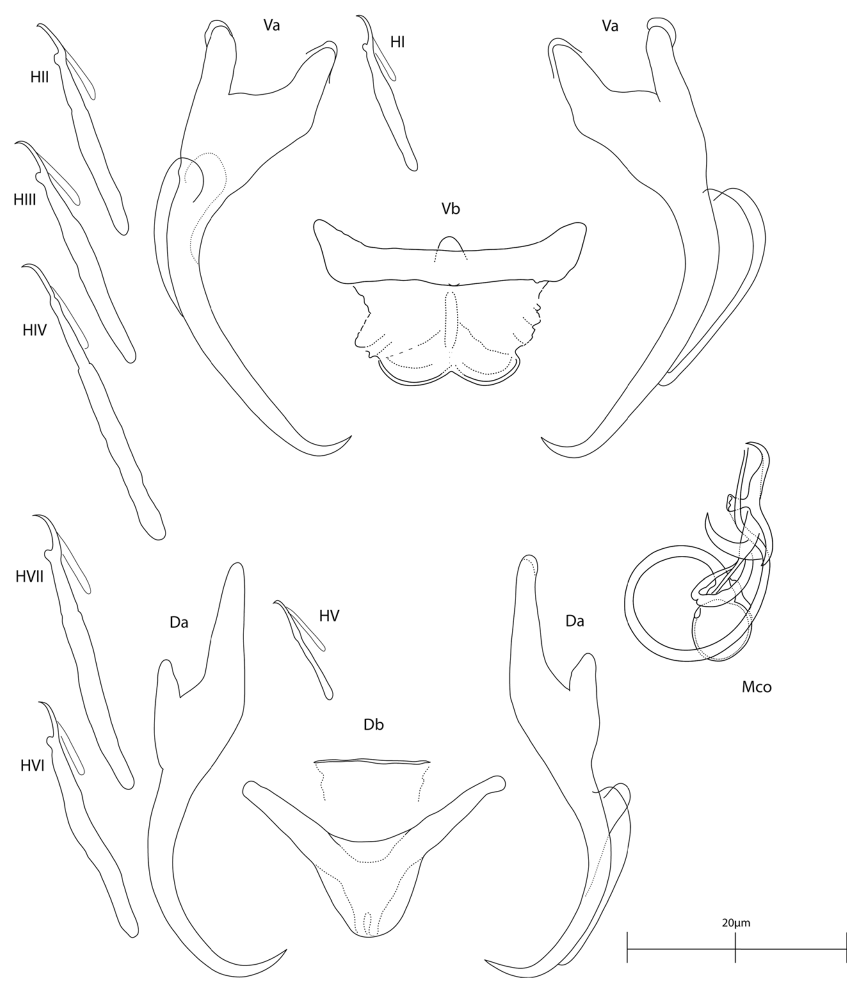


**Fig. 2** *A. alestesnursi* Paperna, 1973. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*=dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Mco*=male copulatory organ

length 37 (27–41; $n$=5); inner root length 10 (9–11; $n$=5); outer root length 5 (2–7; $n$=5); point length 4 (3–4; $n$=5). Dorsal bar: total length 25 (21–27; $n$=5); width 8 (3–10; $n$=4); total width 15 (13–17; $n$=4). Ventral bar: total length 24 (21–27; $n$=5); width 4 (3–5; $n$=5); total width 14 (7–17; $n$=5). Hooks 7 pairs, dissimilar in size; hook lengths ($n$=5): pair I 16 (14–17); pair II 23 (21–26); pair III 22 (19–24); pair IV 28 (26–29); pair V 9 (5–12); pair VI 21 (18–22); pair VII 23 (20–27). Vagina not observed. MCO: total length 21 (19–22; $n$=6); tube-trace length 49 (46–51; $n$=6); base length 7 (5–7; $n$=6); base width 5 (4–5; $n$=6)

*Remarks*:

Although the morphometrics of the investigated specimens differ slightly from those reported in the original description by Paperna (1973, 1979), comparison of the syntypes of *A. alestesnursi* (M. T. 35. 918 III) with those collected during this study confirms their conspecificity (compare Figs. 2 and 3). This species is differentiated from its congeners by possessing a copulatory tube comprising of about one ring and an accessory piece basally articulated to the rounded base of the copulatory tube, medially with an acute bent projection and distally with a flying bird shaped part serving as a guide for the copulatory tube.

*Annulotrema ansatum* Kičinjaová and Řehulková n. sp. (Fig. 4)

*Type host and locality*: *H. forskahlii*, Lake Turkana: Loyingalani (El Molo Bay), Kenya

*Other locality*: Lake Turkana: Kalokol (Fishing Lodge), Kenya.

*Site*: Gill lamellae

*Type specimens*: holotype, paratypes IPCAS M-598

*Material examined*: 3 unflattened and 11 flattened specimens in GAP

*Comparative material examined*: Holotype of *A. nili ruahae* Paperna 1979, M. T. 35. 518.

*Etymology*: The specific name is derived from Latin (*ansa*=handle) and refers to the shape of the base of the copulatory tube, which looks like a ring-pull; treated as an adjective.

*Description*: Body length 714 (646–795; $n$=3); greatest width 105 (94–122; $n$=3). Ventral anchors with inner root recurved at its terminal half, well-developed inner root,

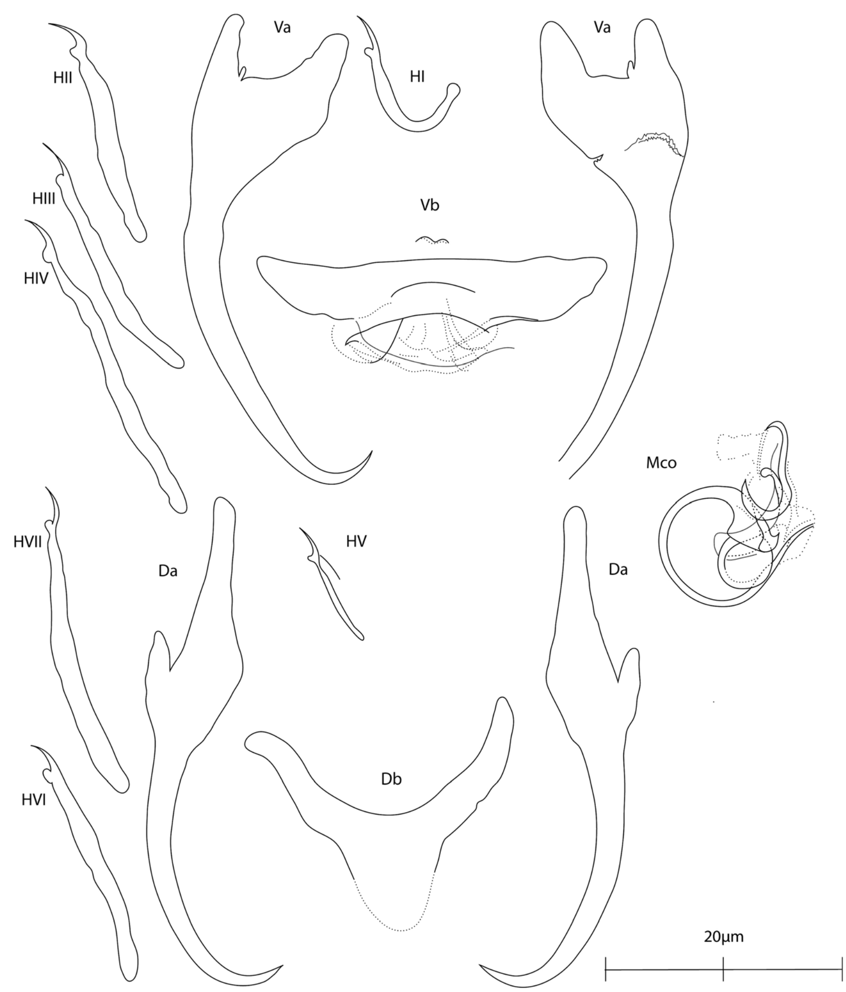


**Fig. 3** *A. alestesnursi* Paperna, 1973. Sclerotized structures from syntypus M. T. 35. 918: *Va*=ventral anchor; *Vb*=ventral bar; *Da*=dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Mco*=male copulatory organ

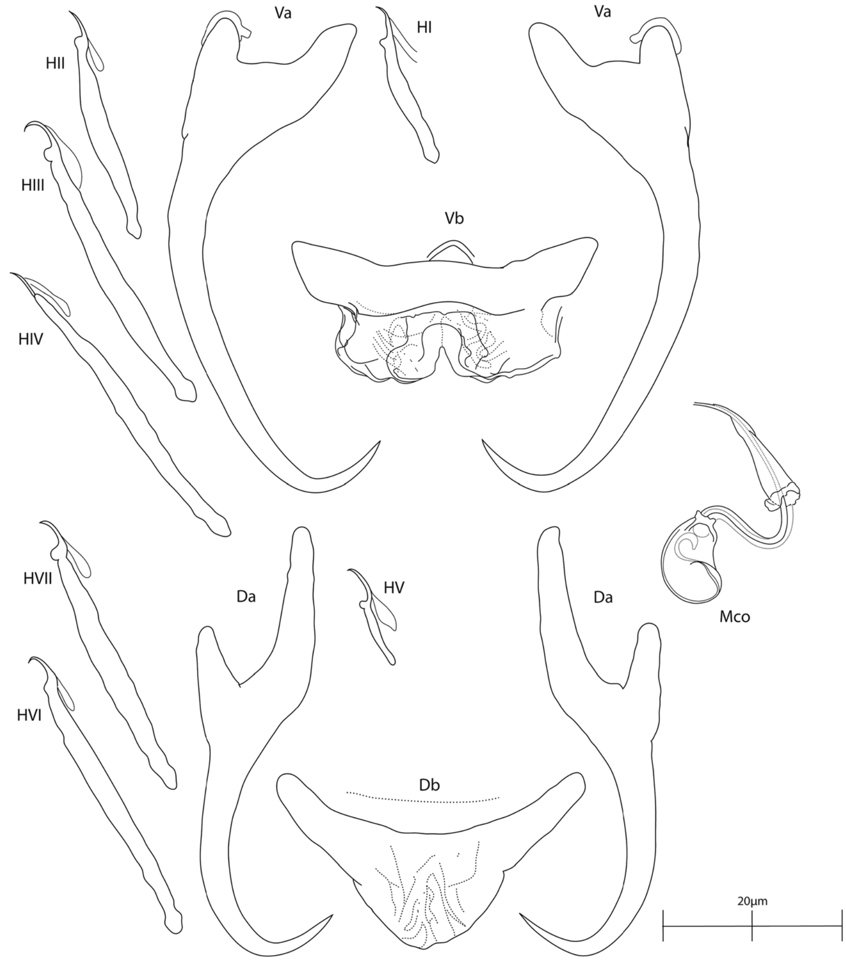


**Fig. 4** *A. ansatum* n. sp. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*= dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Mco*= male copulatory organ

elongated bent shaft, and short point; inner length 52 (49–54; *n*=11); outer length 54 (51–56; *n*=11); inner root length 11 (10–12; *n*=11); outer root length 5 (4–8; *n*=11); point length 9 (8–10; *n*=11). Dorsal anchors with elongated inner root having recurved terminal 2/3, elongated inner root, moderately curved shaft, and short point; inner length 47 (45–48; *n*=11); outer length 36 (34–38; *n*=11); inner root length 19 (18–21; *n*=11); outer root length 6 (5–7; *n*=11); point length 8 (8–9; *n*=11). Ventral bar with triangular process arising from its medial part, supporting membrane well defined; total length 34 (32–43; *n*=11); width 5 (3–7; *n*=11); total width 16 (13–20; *n*=11). Dorsal bar saddle-shaped, with poorly sclerotized membrane; total length 31 (29–34; *n*=11); width 13 (11–13; *n*=11); total width 19 (17–21; *n*=11). Hooks 7 pairs, dissimilar in size; hook lengths (*n*=11): pair I 18 (18–19); pair II 27 (25–33); pair III 34 (32–35); pair IV 36 (34–38); pair V 12 (11–14); pair VI 33 (31–35); pair VII 32 (26–34). Vagina not observed. MCO comprising basally unarticulated copulatory tube, accessory piece; total length 22 (15–26; *n*=11). Copulatory tube S-shaped; tube-trace length 37 (35–41; *n*=11); base length 8 (7–10; *n*=11); base width 6 (4–6; *n*=11). Accessory piece sheath-like, surrounding distal part of the copulatory tube.

*Remarks*:

*A. ansatum* n. sp. resembles *A. nili ruahae* from *Hydrocynus vittatus* from Tanzania (Paperna, 1979) in haptoral morphology and by having an MCO composed of a slender copulatory tube with a thin-walled (pouch-like) base and non-articulated accessory piece supporting the distal part of the tube. It differs from this subspecies by the length of the copulatory tube, which is shorter than in Paperna's original drawings. Because the type material of *A. nili ruahae* M. T. 35. 518 was of very bad quality, which precluded verification of the features of the sclerotized structures, we hesitate to formally synonymize *A. nili ruahae* with the new species.

*Annulotrema besalis* Řehulková, Musilová and Gelnar, 2014 (Fig. 5)

*Type host and locality*: *Hydrocynus brevis*, the Gambia River near Gué de Damantan (13° 02.712′N; 13° 19.266′W), Niokolo-Koba National Park, Senegal.

*Other localities*: Mare de Simenti (13° 01.790′N; 13° 17.608′W), the Niokolo Koba River near Pont Suspendu (13° 01.522′N; 13° 13.220′W), Niokolo-Koba National Park, Senegal (Řehulková et al. 2014).

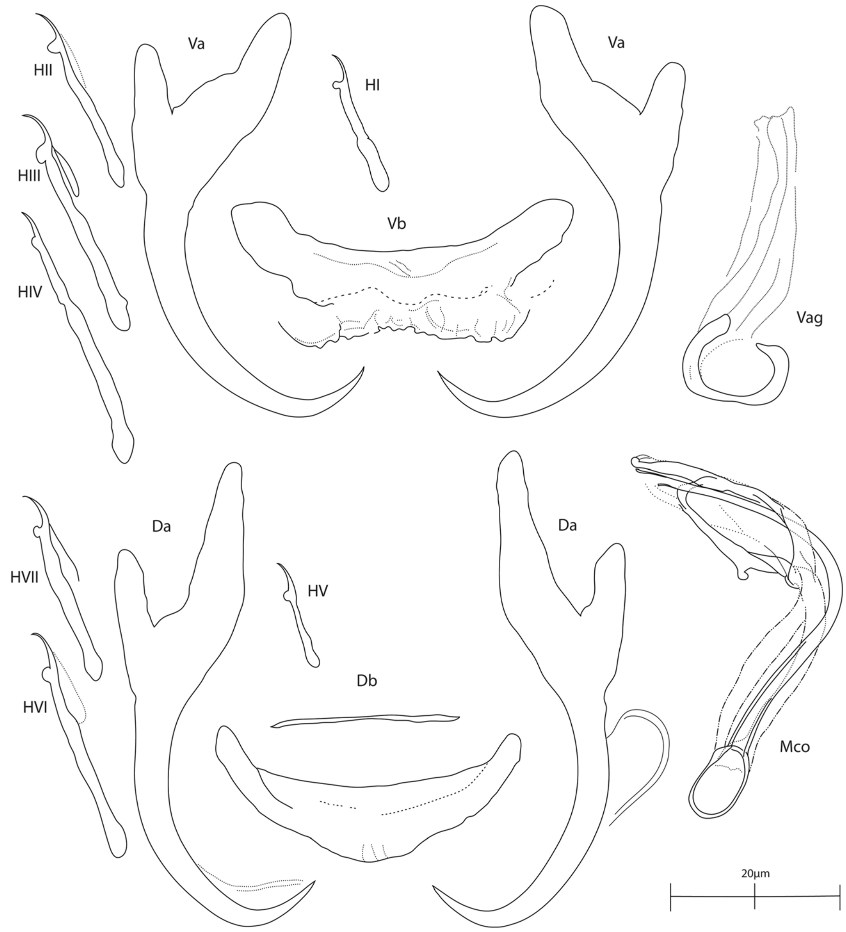


**Fig. 5** *A. besalis* Řehulková, Musilová and Gelnar, 2014. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*=dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Vag*=vagina; *Mco*=male copulatory organ

*Present records*: *H. forskahlii*, Lake Turkana: Kalokol (Fishing Lodge, Ferguson Gulf), Loyingalani (El Molo Bay), Kenya.

*Site*: Gill lamellae

*Type specimens*: holotype, paratypes IPCAS M-551

*Material examined*: 5 unflattened and 10 flattened specimens in GAP

*Material deposited*: vouchers IPCAS M-551

*Measurements*: Body length 1162 (1100–1246; *n*=5); greatest width 138 (119–178; *n*=5). Ventral anchors: inner length 50 (47–54; *n*=10); outer length 50 (46–52; *n*=10); inner root length 17 (14–19; *n*=10); outer root length 7 (5–9; *n*=10); point length 9 (7–10; *n*=10). Dorsal anchors: inner length 56 (54–59; *n*=10); outer length 48 (46–51; *n*=10); inner root length 22 (19–24; *n*=10); outer root length 8 (7–10; *n*=10); point length 9 (7–10; *n*=10). Ventral bar: total length 42 (37–47; *n*=10); width 11 (3–17; *n*=10); total width 19 (16–22; *n*=10). Dorsal bar: total length 38 (35–40; *n*=10); width 10 (9–11; *n*=10); total width 16 (15–17; *n*=10). Hooks 7 pairs, dissimilar in size; hook lengths (*n*=10): pair I 18 (16–20); pair II 24 (22–28); pair III 27 (22–28); pair IV 31 (28–33); pair V 14 (10–19); pair VI 26 (20–29); VII 24 (22–25). Vagina: not or weakly sclerotized, total length 38 (34–40; *n*=10); maximal diameter (12–14; *n*=10). MCO: total length 53 (43–61; *n*=10); tube-trace length 61 (57–65; *n*=10); base length 10 (8–11; *n*=10); base width 5 (5–6; *n*=10).

*Remarks*:

The original description of *A. besalis* is adequate, and the present specimens correspond to it both in size and morphology. Řehulková et al. (2014) reported this species on *H. brevis* in Senegal. Thus, our finding of *A. besalis* on *H. forskahlii* in Kenya represents new host and geographical records.

*Annulotrema bipatens* Kičinjaová, Řehulková and Gelnar n. sp. (Fig. 6)

*Type host and locality*: *H. forskahlii*, Lake Turkana: Loyingalani (El Molo Bay), Kenya

*Site*: Gill lamellae

*Type specimens*: holotype, paratypes IPCAS M-599

*Material examined*: 2 unflattened and 3 flattened specimens in GAP

*Etymology*: The specific name is derived from Latin and refers to the base of the copulatory tube with a double opening (= *bipatens*).

*Description*: Body length 1191 (1158–1223; *n*=2); greatest width 151 (148–154; *n*=2). Ventral anchors with truncated inner root, elongated outer root, proximally bent shaft, and

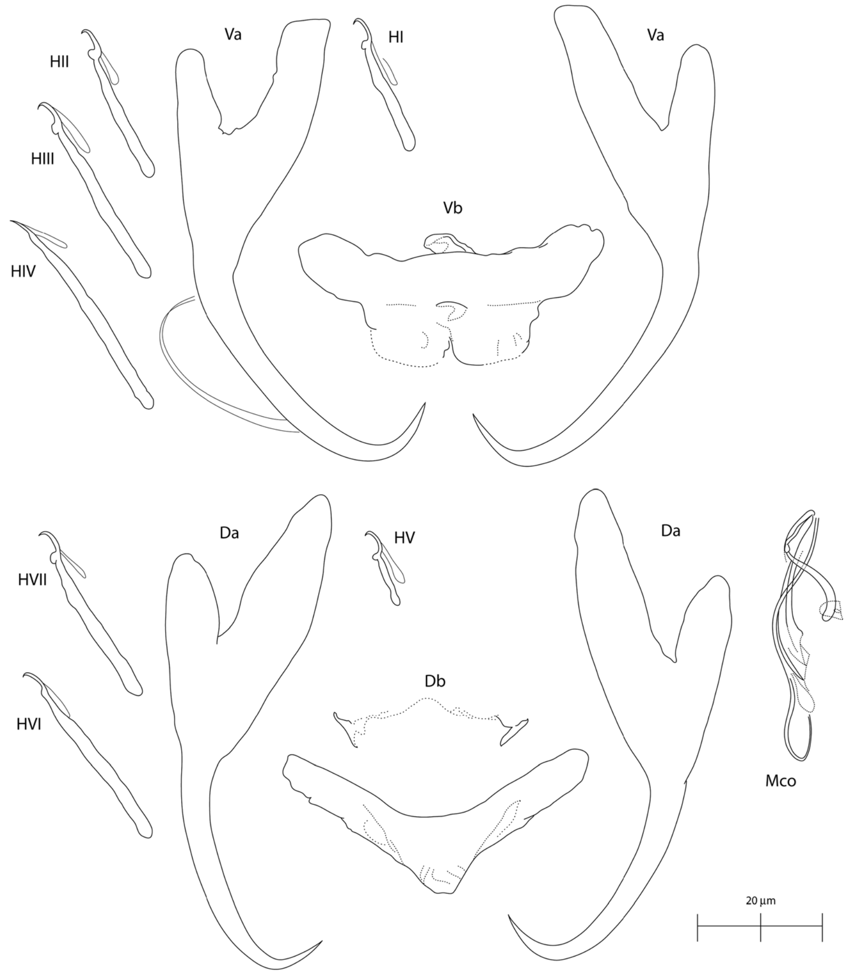


**Fig. 6** *A. bipatens* n. sp. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*= dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Mco*= male copulatory organ

short point; inner length 67 (60–70; *n*=3); outer length 68 (61–72; *n*=3); inner root length 21 (18–23; *n*=3); outer root length 12 (11–13; *n*=3); point length 10 (9–11; *n*= 3). Dorsal anchors with robust elongated roots, slightly curved shaft, and short point; inner length 70 (61–75; *n*= 3); outer length 64 (58–69; *n*=3); inner root length 25 (17–29; *n*=3); outer root length 14 (10–17; *n*=3); point length 9 (*n*=3). Ventral bar with anteromedial process, supporting membrane well defined; total length 46 (36–52; *n*=3); width 17 (11–22; *n*=3); total width 23 (22–24; *n*=3). Dorsal bar broadly V-shaped, supporting membrane weakly sclerotized; total length 43 (35–49; *n*=3); width 13 (11–14; *n*=3); total width 24 (23–25; *n*=3). Hooks 7 pairs, dissimilar in size; hook lengths (*n*=3): pair I 23 (21–26); pair II 29 (27–32); pair III 32 (29–34); pair IV 38 (33–42); pair V 14 (13–14); pair VI 31 (27–37); pair VII 28 (24–34). Vagina not observed. MCO delicate, comprising basally articulated copulatory tube, accessory piece; total length 38 (37–40; *n*=3). Copulatory tube arcuate; tube-trace length 41 (41–42; *n*=3); base length 8 (*n*=3); base width 4 (3–4; *n*=3). Accessory piece with S-shaped filament supporting distal part of the tube.

*Remarks*:

Based on the comparative morphology of the haptoral sclerites, *A. bipatens* n. sp. is most similar to *Annulotrema magnihamula* Paperna, 1973 from *H. forskahlii* (Uganda) (Paperna, 1973, 1979). Both species have robust anchors (with large roots, especially those of the dorsal anchors) and a delicate MCO. This new species differs from *A. magnihamula* by possessing a copulatory tube with an elongated base (rounded base in *A. magnihamula*) and an accessory piece with a filamentous distal part (the accessory piece is plate shaped in *A. magnihamula*).

*Annulotrema cucullatum* Kičinjaová, Řehulková and Blažek n. sp. (Fig. 7)

*Type host and locality*: *H. forskahlii*, Lake Turkana: Loyingalani (El Molo Bay), Kenya

*Other records*: *H. forskahlii*, Lake Turkana: Kalokol (Fishing Lodge, Ferguson Gulf), Kenya

*Site*: Gill lamellae

*Type specimens*: holotype, paratypes IPCAS M-600

*Material examined*: 4 unflattened and 10 flattened specimens in GAP

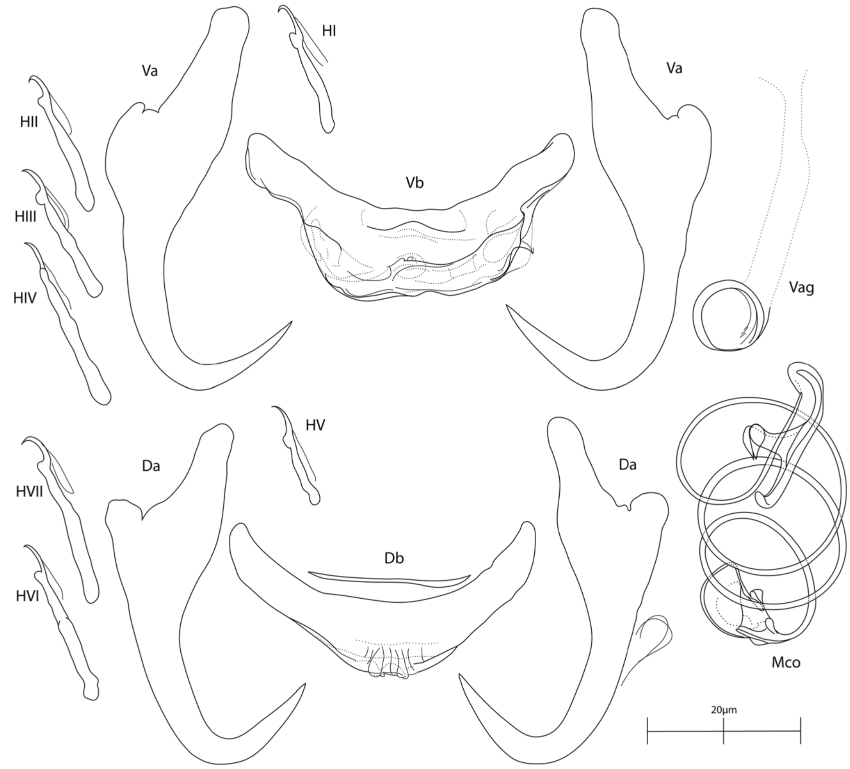


**Fig. 7** *A. cucullatum* n. sp. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*= dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Vag*= vagina; *Mco*=male copulatory organ

*Comparative material examined*: Holotype of *A. longipenis* Paperna, 1969 (M. T. 35. 579) from *Alestes macrolepidotus* (= *Brycinus macrolepidotus*) (Ghana), paratype of *A. longipenis* Paperna, 1969 (M. T. 35. 906) from *Alestes baremose* (Ghana).

*Etymology*: The specific name is derived from Latin (*cucullus* = cone) and refers to the cone-like base of the copulatory tube; treated as an adjective.

*Description*: Body length 730 (643–783; $n$=4); greatest width 141 (115–153; $n$=4). Ventral anchors with elongated inner root, vestigial outer root, slightly curved shaft, and point; inner length 51 (46–54; $n$=10); outer length 41 (38–45; $n$= 10); inner root length 17 (15–18; $n$=10); outer root length 3 (2–5; $n$=10); point length 15 (14–16; $n$=10). Dorsal anchors with well-developed roots, straight shaft, and point; inner length 46 (44–50; $n$=10); outer length 41 (38–45; $n$=10); inner root length 13 (11–16; $n$=10); outer root length 3 (3–4; $n$=10); point length 15 (13–19; $n$=10). Ventral bar with well-defined supporting membrane; total length 45 (41–51; $n$=10); width 7 (5–8; $n$=9); total width 20 (14–22; $n$=10). Dorsal bar with well-defined supporting membrane; total length 42 (40–46; $n$=10); width 10 (9–11; $n$=10); total width 19 (17–22; $n$=10). Hooks 7 pairs, dissimilar in size; hook lengths ($n$=10): pair I 18 (18–20); pair II 21 (19–22); pair III 21 (19–23); pair IV 25 (22–28); pair V 13 (11–17); pair VI 22 (19–23); pair VII 25 (21–27). Vagina not–to weakly sclerotized; vaginal aperture surrounded by lightly sclerotized ring; total length 27 (25–29; $n$=6); maximal diameter 10 (8–11; $n$=6). MCO comprising basally unarticulated copulatory tube, accessory piece; total length 40 (32–50; $n$= 9). Copulatory tube a coil of about three rings, with cone-like base; tube-trace length 191 (181–199; $n$=9); base length 10 (8–11; $n$=9); base width 6 (5–7; $n$=9). Accessory piece smoothing plane-shaped.

*Remarks*:

Based on the drawings of the sclerotized structures, this species markedly resembles *A. longipenis* recorded on the gills of *Hydrocynus forskalii* (= *H. forskahlii*) in Uganda by Paperna (1979). Both species possess similar anchor/bar complexes (robust anchors with a straight and short shaft), size of hooks and a similar MCO comprising a coiled tube and non-articulated hook-shaped accessory piece. However, Paperna's (1979) identification of the specimens determined as *A. longipenis* from *H. forskahlii* is probably wrong. *A. longipenis* was described by Paperna (1969) on the gills of *A. macrolepidotus* (= *B. macrolepidotus*) and *A. baremoze* from Lake Volta (Ghana). Ten years later, Paperna (1979) reported this species on *H. forskahlii* from Lake Albert (Uganda) and pointed out considerable morphometric variations in the sclerotized structures depending on the host species and locality: specimens from Lake Albert are much smaller (total length= 280–320; ventral anchor length=37–39; dorsal anchor length=39–42) than the specimens from the Lake Volta (total length=750; ventral anchor length=75; dorsal anchor length= 70)". Indeed, Paperna's (1979) drawings of the sclerotized structures and our examination of the type specimen of *A.*

*longipenis* show the non-conspecificity of the specimen from *A. macrolepidotus* (holotype M.T.35.579) and specimens from *H. forskahlii* based on the size and morphology of the anchors, bars and hooks. In addition, because *A. longipenis* was originally described from *A. macrolepidotus* in Ghana, the designation of the specimens from *H. forskahlii* (Uganda) on slide M.T. 35.920C as paratypes is obviously erroneous. Unfortunately, these paratypes were not available for study. Thus, although our specimens of *A. cucullatum* n. sp. and those of *A. longipenis* of Paperna (1979) seem to be differentiated only by the number of rings of the copulatory tube (i.e. 3 vs 4, respectively), we do not feel that available information on the two forms (species) justifies a proposal of synonymy at this time. On the other hand, specimens of *A. cucullatum* n. sp. clearly differ from the type specimens (holotype M.T. 35.579, paratype M. T. 35. 906) of *A. longipenis* by having anchors with a short shaft and relatively long point (anchors with a markedly elongated shaft and a short point in *A. longipenis*) and more robust hooks.

*Annulotrema elongata* Paperna and Thurston, 1969 (Fig. 8)

*Type host and locality*: *A. baremoze*, Lake Albert, Uganda

*Other records*: *A. baremoze*, Ghana (Paperna 1969); *A. dentex*, Ghana (Paperna 1979); *A. dentex*, Uganda (Paperna 1979); *A. macrolepidotus* (= *B. macrolepidotus*), Uganda (Thurston 1970)

*Present records*: *A. baremoze*, Lake Turkana: Kalokol (Fishing Lodge), Loyingalani (El Molo Bay); *A. dentex*, Lake Turkana: Loyingalani (El Molo Bay), Kalokol (Fishing Lodge), Kenya

*Site*: Gill lamellae

*Type specimens*: holotype M. T. 35. 577, paratype M.T.34.293 (from *A. baremoze*, Ghana)

*Material examined*: 5 unflattened and 10 flattened specimens in GAP

*Comparative material examined*: holotype of *A. elongata* Paperna and Thurston, 1969 (M. T. 35. 577) from *A. baremose* (Uganda), paratypes of *A. elongata* Paperna and Thurston, 1969 (M. T. 34. 293) from *A. baremose* (Ghana)

*Material deposited*: vouchers IPCAS M-603

*Redescription* (based on five voucher specimens from *A. baremoze* from Kalokol - Fishing Lodge): Body length 847 (751–926; $n$=5); greatest width 141 (115–153; $n$=5). Ventral anchors with inner root recurved at its terminal half, well-developed outer root, slightly curved shaft, relatively long point; inner length 47 (45–49; $n$=10); outer length 43 (41–45; $n$=10); inner root length 15 (14–17; $n$=10); outer root

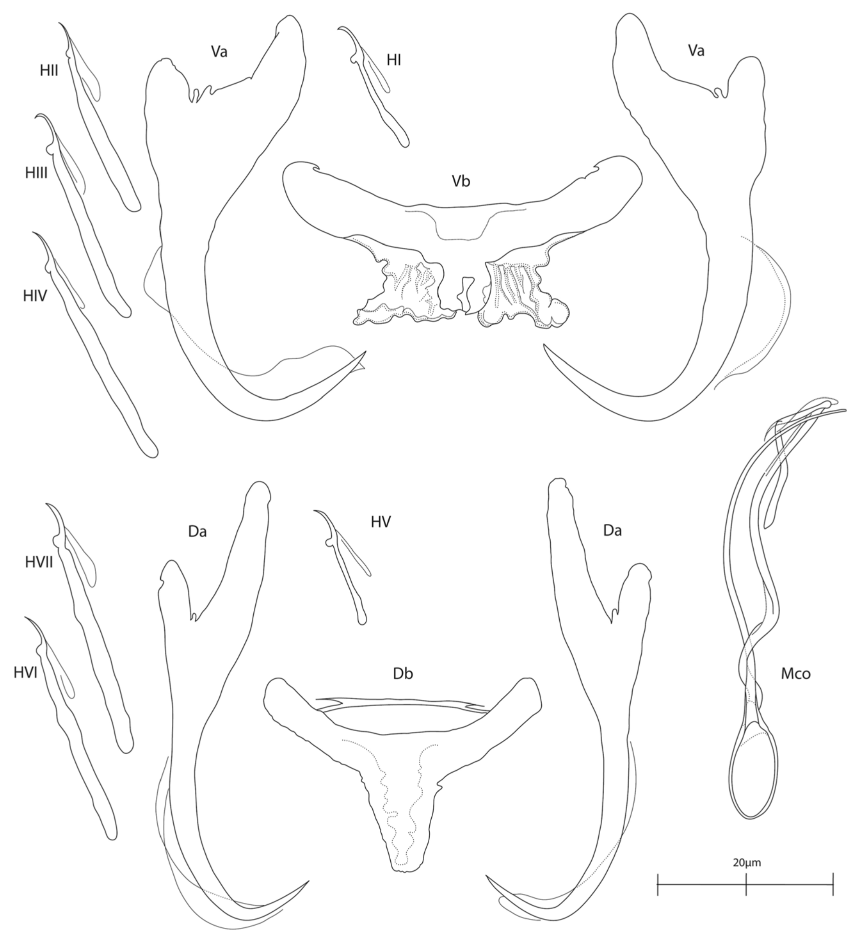


**Fig. 8** *A. elongata* Paperna and Thurston, 1969. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*=dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Mco*=male copulatory organ

length 5 (5–7; $n$=10); point length 12 (7–15; $n$=10). Dorsal anchors with elongated inner root, well-developed outer root, elongated slightly curved shaft, short point; inner length 50 (48–54; $n$=10); outer length 42 (39–46; $n$=10); inner root length 17 (15–18; $n$=10); outer root length 7 (6–8; $n$=10); point length 8 (8–9; $n$=10). Ventral bar with medial perforated protuberance, well developed supporting membrane; total length 40 (35–43; $n$=10); width 6 (5–8; $n$=10); total width 17 (15–19; $n$=10). Dorsal bar saddle-shaped, with posterior expansion, weakly sclerotized supporting membrane; total length 31 (30–32; $n$=10); width 15 (12–17; $n$=10); total width 22 (19–27; $n$=10). Hooks 7 pairs, dissimilar in size; hook lengths ($n$=10): pair I 15 (14–17); pair II 24 (22–31); pair III 26 (24–28); pair IV 28 (26–31); pair V 13 (10–15); pair VI 25 (22–27); pair VII 27 (26–30). Vagina not observed. >MCO comprising basally articulated copulatory tube, accessory piece; total length 43 (35–54; $n$=10). Copulatory tube arcuate, with ovate base; tube-trace length 53 (48–61; $n$=10); base length 10 (7–13; $n$=10); base width 5 (5–6; $n$=10). Accessory piece ribbon-shaped, with terminal loop.

*Remarks*:

The original description of *A. elongata* by Paperna and Thurston (1969) is inadequate for diagnosis because their drawings of the haptoral structures were clearly obtained from two different species (compare Figs. 11 and 12 in the original paper). Indeed, these authors themselves indirectly confirmed our opinion by stating that: "Anchor shafts may, in different specimens, be either short and robust or elongate and delicate". Available type specimens of *A. elongata* were in generally poor condition. Although slide M.T. 35.577 is labelled "holotypus", it was not possible to determine a holotype because a total of four specimens (two of which were without haptors) were present on the slide and none was designated as the holotype. Nevertheless, one specimen from those observed was found in a condition sufficient for specific diagnosis. Comparison of this type specimen of *A. elongata* (Fig. 9) with those collected during this study confirms their conspecificity. Thus, the finding of *A. elongata* on *A. baremoze* and *A. dentex* in Kenya is a new locality record for this monogenean species.

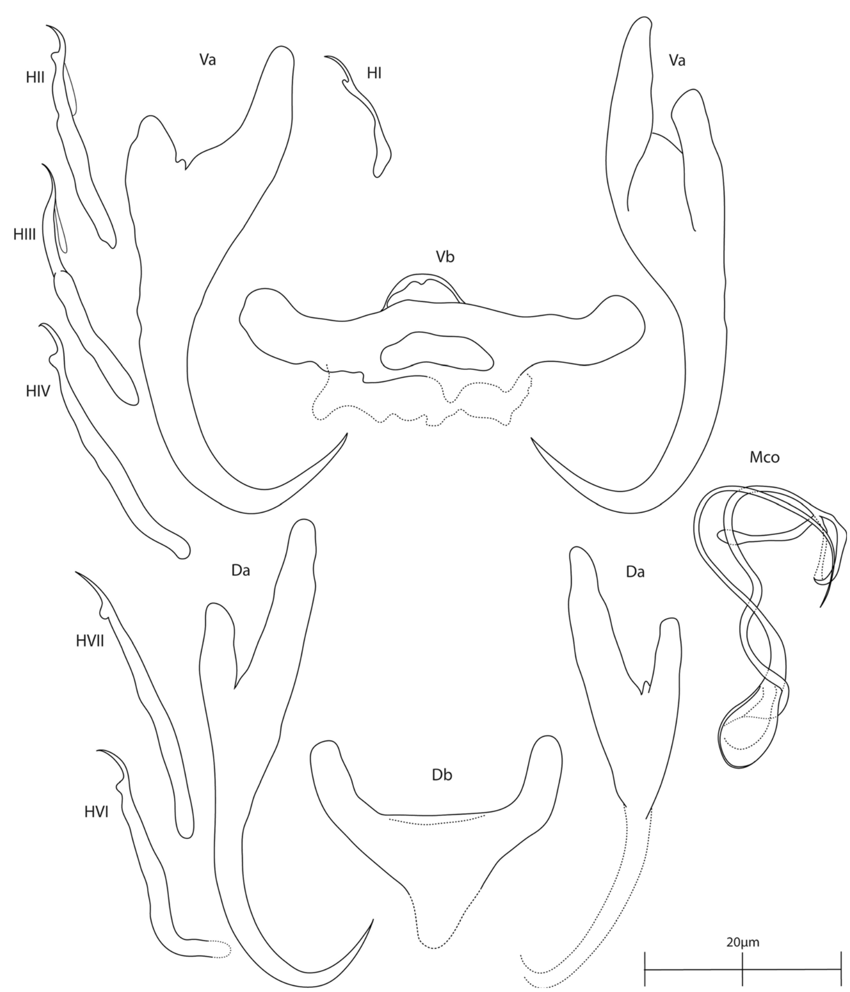


**Fig. 9** *A. elongata* Paperna and Thurston, 1969. Sclerotized structures of holotype M. T. 35. 577: *Va*=ventral anchor; *Vb*=ventral bar; *Da*=dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Mco*=male copulatory organ

*Annulotrema nili* Paperna, 1973 (Fig. 10)

*Type host and locality*: *H. forskalii* (= *H. forskahlii*), Lake Albert, Uganda

*Other records*: *H. forskalii* (= *H. forskahlii*), *H. vittatus*, *H. brevis*, Niger system, West Africa

*Present records*: *H. forskahlii*, Lake Turkana: Loyingalani (El Molo Bay), Kalokol (Fishing Lodge, Ferguson Gulf), Kenya

*Site*: Gill lamellae

*Type specimen*: M. T. 35.717 N, paratype

*Material examined*: 5 unflattened and 10 flattened specimens in GAP

*Material deposited*: vouchers IPCAS M-602

*Measurements*: Body length 820 (709–966; $n=5$); greatest width 157 (82–229; $n=5$). Ventral anchors: inner length 46 (42–52; $n=10$); outer length 52 (46–55; $n=10$); inner root length 15 (13–17; $n=10$); outer root length 8 (6–9; $n=10$); point length 10 (8–10; $n=10$). Dorsal anchors: inner length 61 (56–67; $n=10$); outer length 48 (43–52; $n=10$); inner root length 25 (22–31; $n=10$); outer root length 7 (6–9; $n=10$); point length 9 (7–11; $n=10$). Ventral bar: total length 38 (32–43; $n=10$); width 8 (5–10; $n=10$); total width 24 (21–28; $n=10$). Dorsal bar: total length 36 (30–41; $n=10$); width 16 (14–19; $n=10$); total width 24 (22–28; $n=10$). Hooks 7 pairs, dissimilar in size; hook lengths ($n=10$): pair I 23 (20–25); pair II 29 (27–31); pair III 32 (29–35); pair IV 36 (33–38); pair V 14 (11–16); pair VI 35 (31–38); pair VII 38 (36–41); Vagina not sclerotized, poorly observed. MCO: total length 47 (43–53; $n=10$); tube-trace length 63 (57–69; $n=10$); base length 15 (14–16; $n=10$); base width 12 (10–14; $n=10$); base-trace length 24 (22–30; $n=10$).

*Remarks*:

The morphological features of the specimens found on *H. forskahlii* in Kenya correspond well to the original drawings of *A. nili* from *H. forskalii* (= *H. forskahlii*) in Uganda (Paperna 1973, 1979), especially with respect to the shape of the ventral anchors (exhibiting a sharply proximally bent elongated shaft) and MCO. We do not consider the differences between our measurements and those of Paperna (1973, 1979), which are generally smaller than those reported herein, as sufficient to warrant separate species designation for the Kenyan specimens. *A. nili* is closest morphologically to *A. nili ruahae* (Paperna, 1979) and *A. ansatum* n. sp. in the general morphology of the MCO and dorsal anchor/bar complex. It differs from them by possessing ventral anchors with a sharply proximally bent shaft (*vs* a slightly proximally bent shaft) and an MCO of

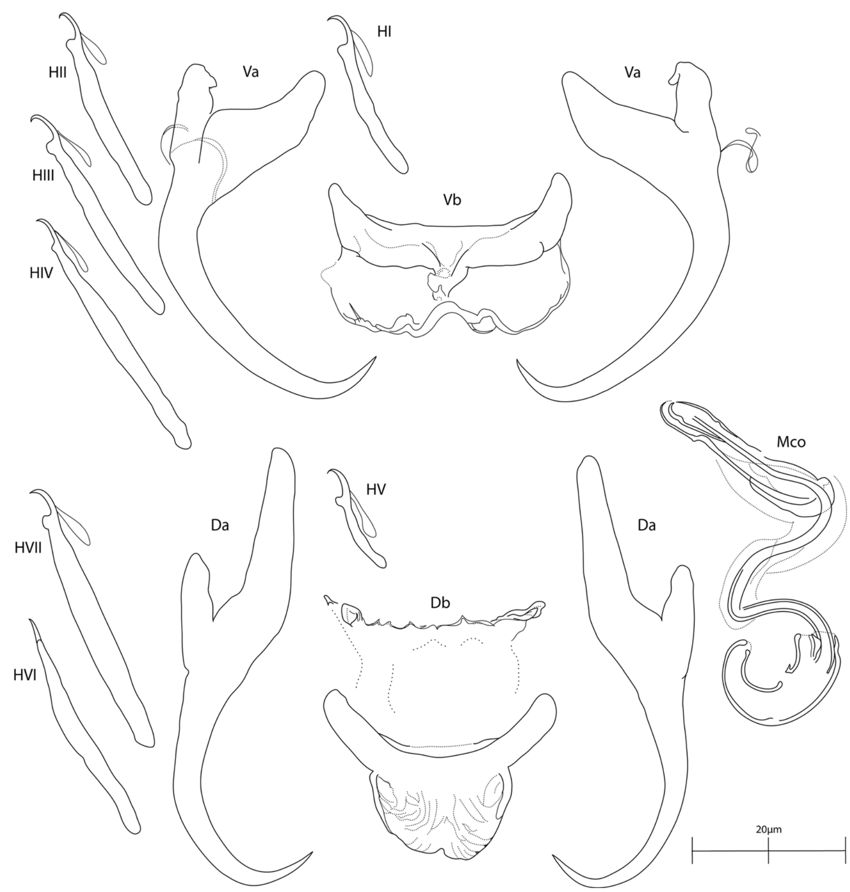


**Fig. 10** *A. nili* Paperna, 1973. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*=dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Mco*=male copulatory organ

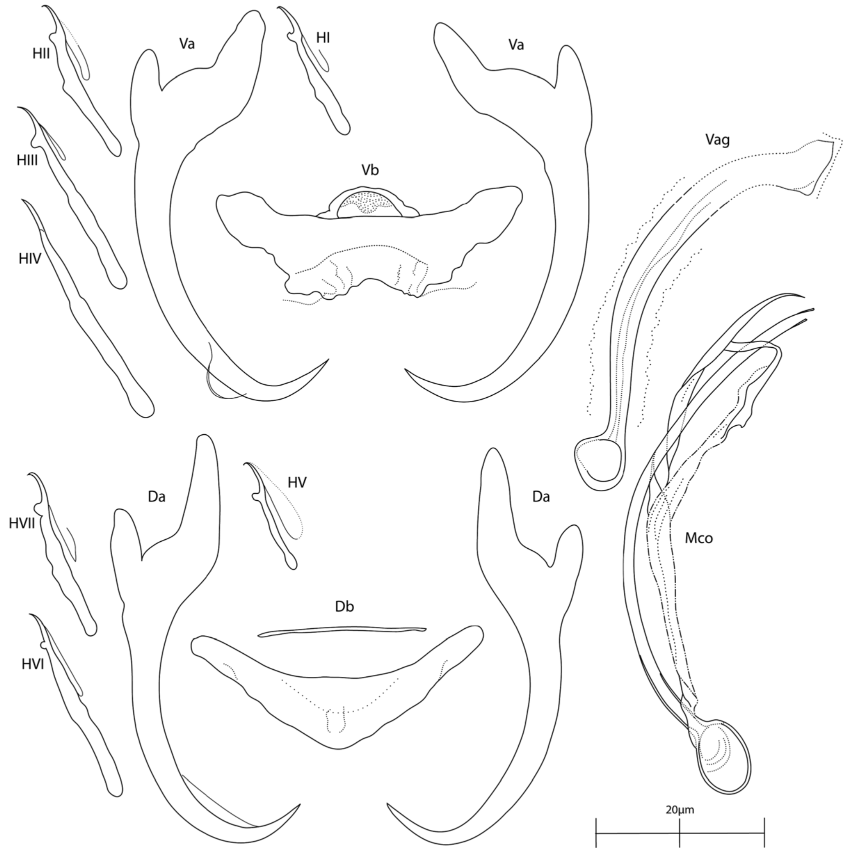


**Fig. 11** *A. pontile* n. sp. Sclerotized structures: *Va*=ventral anchor; *Vb*=ventral bar; *Da*= dorsal anchor; *Db*=dorsal bar; *H*=hooks (pairs I–VII); *Vag*= vagina; *Mco*=male copulatory organ

noticeably larger size in comparison with the size of the haptoral structures. In addition, this species is differentiated from *A. ansatum* n. sp. by the base of its copulatory tube, which has the appearance of a crescent shaped roll (in some of our specimens, the proximal part of the base folds back over the more distal part) with a frayed distal part, and a filamentous accessory piece enveloping the distal portion of the tube like a sheath (in *A. ansatum* n. sp., the accessory piece is a simple sheath with tapered distal end).

*Annulotrema pontile* Kičinjaová and Řehulková n. sp. (Fig. 11)

*Synonym*: *Annulotrema pikei* of Paperna (1979)

*Type host and locality*: *H. forskahlii*, Turkana Lake (Kalokol – Fishing Lodge), Kenya

*Present records*: *H. forskahlii*, Lake Turkana: Kalokol (Ferguson Gulf), Loyingalani (El Molo Bay), Kenya

*Other records*: *H. forskahlii*, Uganda (Paperna, 1979)

*Site*: Gill lamellae

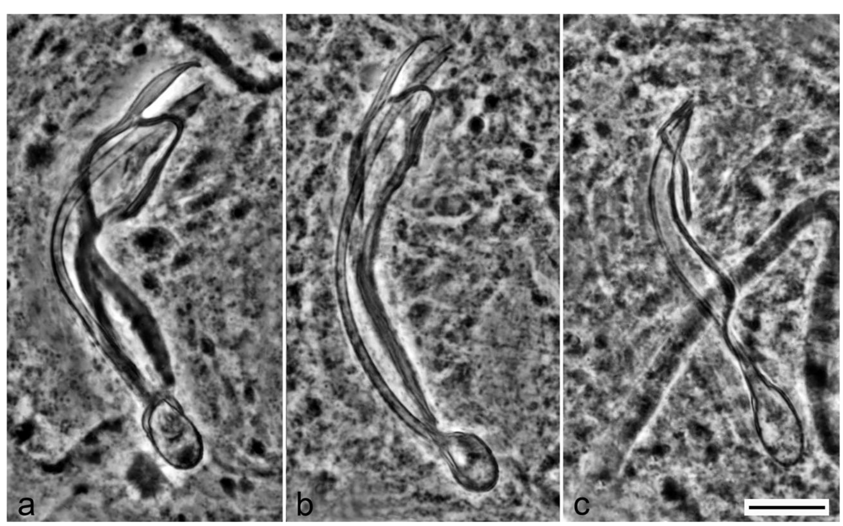


**Fig. 12** Male copulatory organ (phase contrast micrograph) of *A. besalis* (**a**), *A. pontile* n. sp. (**b**), and *A. elongata* (**c**). *Scale bar* 10 µm

*Type specimen*: holotype, paratypes IPCAS M-601

*Material examined*: 4 unflattened and 10 flattened specimens in GAP

*Etymology*: The specific name is derived from Latin (pons = bridge) and refers to the shape of the copulatory tube of the male copulatory organ; treated as an adjective.

*Description*: Body length 747 (718–797; *n*=4); greatest width 107 (99–116; *n*=4). Ventral anchors with inner root recurved at its terminal half, well developed outer root, moderately curved shaft, short point; inner length 46 (44–48; *n*=10); outer length 44 (42–45; *n*=10); inner root length 15 (13–19; *n*=10); outer root length 5 (4–6; *n*=10); point length 8 (7–9; *n*=10). Dorsal anchors with elongated inner root having recurved terminal half, well-developed outer root, moderately curved shaft, and short point; inner length 48 (47–50; *n*=10); outer length 42 (41–43; *n*=10); inner root length 17 (15–18; *n*=10); outer root length 6 (6–7; *n*=10); point length 8 (6–9; *n*=10). Ventral bar with arched process rising from its medial part, supporting membrane well defined; total length 32 (30–36; *n*=10); width 9 (7–11; *n*=10); total width 15 (13–16; *n*=10). Dorsal bar triangular, with poorly sclerotized membrane; total length 31 (29–35; *n*=10); width 9 (7–11; *n*=10); total width 13 (12–14; *n*=10). Hooks 7 pairs, dissimilar in size; hook lengths (*n*=10): pair I 17 (15–18); pair II 22 (20–25); pair III 26 (23–27); pair IV 30 (28–32); pair V 13 (12–14); pair VI 24 (20–27); pair VII 21 (20–22). vagina weakly sclerotized; total length 41 (30–49; *n*=10); maximum diameter 7 (5–9; *n*=10). MCO comprising basally articulated copulatory tube, accessory piece; total length 54 (50–58; *n*=10). Copulatory tube arcuate, with ovate base; tube-trace length 64 (60–68; *n*=10); base length 8 (8–9; *n*=10); base width 5 (4–6; *n*=10). Accessory piece comprising weakly sclerotized rod-shaped proximal half and distal loop with sickle-shaped branch supporting tip of tube.

*Remarks*:

This species was initially identified as *Annulotrema pikei* (Price et al. 1969) on *H. forskalii* (= *H. forskahlii*) in Uganda by Paperna (1979). Recently, Řehulková et al. (2014) described *A. besalis* for specimens collected from *H. brevis* in Senegal and differentiated it from Paperna's voucher of *Annulotrema pikei* (RMC M.T.35.717) from *H. forskahlii* by details of the anchors and MCO. Based on the comparison of our specimens of *A. pontile* n. sp. with drawings provided by these authors (see Fig. 2 in Řehulková et al. 2014), we consider our collection to be conspecific with Paperna's specimen of *Annulotrema*. Thus, we consider *Annulotrema pikei* of Paperna (1979) from *H. forskahlii* to be a synonym of *A. pontile* n. sp. This new species differs from *A. besalis* by having a copulatory tube slightly arcuate along its entire length (copulatory tube is bent at about a 120° angle near its midlength in *An. besalis*), an accessory piece with a uniramous proximal part (biramous in *A. besalis*), and a more slender terminal sickle-shaped claw (compare Fig. 12a with 12b).

## Discussion

Prior to this study, 42 species of *Annulotrema* had been recorded from 14 species of the Alestidae (Khalil & Polling 1997; Řehulková et al. 2014), one species of the Hepsetidae (Paperna and Thurston 1969; Paperna 1969; Paperna 1979; Birgi 1988; N'Douba et al. 1997), and two species of the Distichodontidae (Birgi 1988). The four new species of *Annulotrema* described here raise the number of *Annulotrema* species to 46 and the number of host species to 15.

Kritsky and Boeger (1995) and Řehulková et al. (2014) corrected some initial observations on internal anatomy and haptoral sclerites offered by Paperna and Thurston (1969) in the generic diagnosis of *Annulotrema*. Our present observations add details concerning the structure of bars to their diagnosis. All of the *Annulotrema* spp. presented in this paper possess the same type of ventral and dorsal bars. Both bars are characterized by the presence of lightly sclerotized membranes which probably provide a flat base and firm anchorage for the haptoral musculature. Although Paperna (1979) later provided a more detailed diagnosis of *Annulotrema*, in which he stated that the genus is characterized, among other characteristics, by having a dorsal bar whose convex side usually extends into a median membrane, the generic diagnosis of *Annulotrema* needs to be further updated (emended) to include the presence of a (supporting) membrane also on the ventral bar. In terms of hook size, in all *Annulotrema* species in this paper, the hooks of pair IV were the largest, except for *A. nili*, in which hooks VII were the largest. In the case of the morphology of sclerotized structures, three *Annulotrema* species (*A. besalis*, *A. elongata* and *A. pontile* n. sp.) examined by us share the same type of MCO (see Fig. 12), which may indicate a close relationship among these species. Nevertheless, phylogenetic studies on species of *Annulotrema*, including those eight recorded by us, are necessary to confirm their apparent close relatedness.

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