



Two New Species of *Annulotrema* (Monogenea: Dactylogyridae) Gill Parasites of *Brycinus macrolepidotus* Valenciennes, 1849 from Nyong River, Cameroon

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Abstract

Purpose Research on gill monogeneans of Characiform has made it possible to find two new species belonging to the genus *Annulotrema*. The purpose of this paper is to carry out their morphological description.

Methods Specimens of *Brycinus macrolepidotus* were captured in the Nyong River at Akonolinga. Each monogenean was mounted between slide and cover slip in a drop of glycerin ammonium picrate mixture. The sclerotized parts of their haptor and reproductive organs were then drawn and measured.

Results Two new species of *Annulotrema* were described. *Annulotrema ngombiensis* n. sp. is morphologically close to *Annulotrema tenuicirra* Paperna, 1973 and *Annulotrema pikei* Price, Peebles and Bamford, 1969. However, the new species differs from these other two mainly by the characteristic well-marked terminal cap and the tubiform prostatic reservoir of the accessory part of its MCO, as well as by its dorsal bar with a characteristic triangular piece in the middle of the fork and its ventral bar without filaments. *Annulotrema nkengfacki* n. sp. is distinguished from all other species previously described as well as from its closest congeners, *Annulotrema helicocirra* Paperna, 1973 and *Annulotrema bouixi* Birgi, 1988 by the structure of its MCO whose accessory part is composed of a trapezoid structure surmounted by another which is forked-shaped and by its arched-shaped ventral bar with an inner lining.

Conclusion *Brycinus macrolepidotus* from Cameroon can harbour two species of *Annulotrema*, both described in the current study. This result contributes to the knowledge of the species diversity of this genus in the Nyong Basin.

Keywords Alestidae · *Annulotrema ngombiensis* n. sp. · *Annulotrema nkengfacki* n. sp. · Platyhelminthes · Nyong basin

Introduction

Monogenea Carus, 1863, is one of the most species-rich classes of fish parasites, which are commonly found on gills and skin [1]. They are generally host specific; one species of parasite occurs in a single species or closely related species of hosts [2]. Although research on monogeneans biodiversity has intensified recently in Africa, there are indices that this taxon remains underestimated. African tetras are known to harbour three genera of monogeneans of which,

Annulotrema Paperna and Thurston, 1969, is the most species-rich, and exclusively parasitizes host belonging to the families Characidae, Hepsetidae and Distichodontidae [3–14]. The first studies relating to the monogeneans of African tetras in Cameroon (Table 1) are those made by Birgi [9, 15]. Since then, systematic research has led to the description of several new species of monogeneans on various fish hosts in Cameroon [16–23]. Despite this intense sampling efforts, no monogeneans were collected from the gills of *Brycinus macrolepidotus* Valenciennes, 1849, which is one of the common fish species, widely distributed in the southern Cameroon waters. However, studies conducted elsewhere on the monogeneans of this fish species have described *Annulotrema alberti* Paperna, 1973, *Annulotrema longipinnis*, Paperna, 1973, *Annulotrema tenuicirra* Paperna, 1973, *Annulotrema elongata* Paperna, 1969 (re described by Kičinjaová *et al.* [13], *Annulotrema alestesnursi* Paperna,

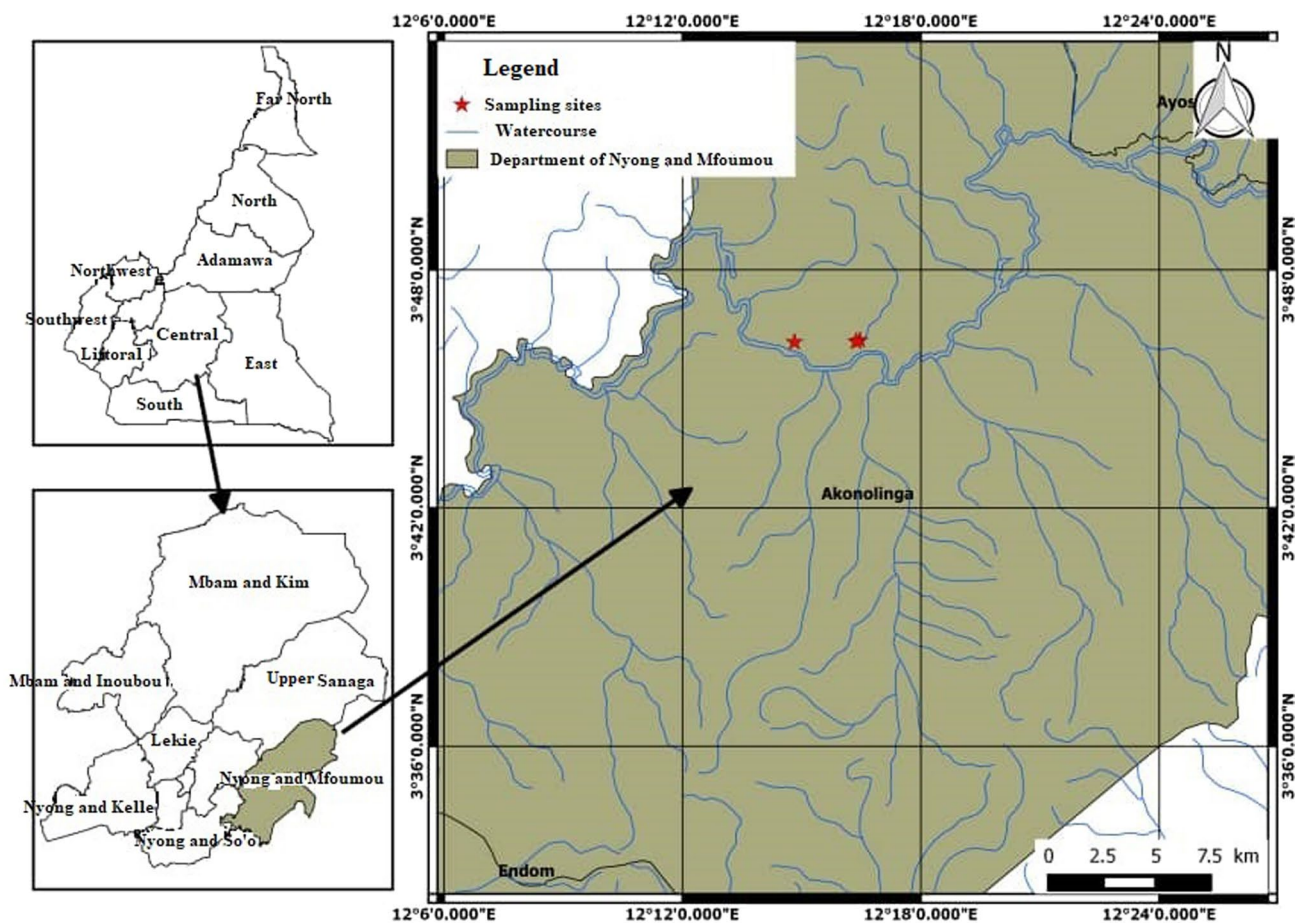
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Table 1 The list of all the *Annulotrema* species (host, locality, authors and year) recorded by Birgi (1987, 1988) in Cameroon

<i>Annulotrema</i> species	Host	Locality	Author and year
<i>A. combesi</i>	<i>Brycinus kingsleyae</i> Günther, 1986	Ebogo: Nyong River	Birgi, 1988
<i>A. nyongensis</i>	<i>Brycinus kingsleyae</i> Günther, 1986	Ebogo: Nyong River	Birgi, 1988
<i>A. gabrioni</i>	<i>Phenacogrammus major</i> Boulenger, 1903 and <i>Hemigrammopetersius pulcher</i> Boulenger, 1909	Sangmelima (Dja Basin) and Yaoundé (Nyong Basin)	Birgi, 1988
<i>A. amieti</i>	<i>Phenacogrammus major</i> Boulenger, 1903 and <i>Hemigrammopetersius pulcher</i> Boulenger, 1909	Sangmelima (Dja Basin) and Yaoundé (Nyong Basin)	Birgi, 1988
<i>A. bouixi</i>	<i>Brycinus kingsleyae</i> Günther, 1986	Ebogo: Nyong River	Birgi, 1988
<i>A. edeensis</i>	<i>Micralestes</i> sp	Somakek: Sanaga	Birgi, 1988
<i>A. kribiensis</i>	<i>Brycinus longipinnis</i> Günther, 1864	Moanko: Sanaga	Birgi, 1988
<i>A. lamberti</i>	<i>Brycinus longipinnis</i> Günther, 1864	Moanko: Sanaga	Birgi, 1988
<i>A. maillardi</i>	<i>Brycinus kingsleyae</i> Günther, 1986	Ebogo: Nyong River	Birgi, 1988
<i>A. moanko</i>	<i>Brycinus longipinnis</i> Günther, 1864	Moanko: Sanaga	Birgi, 1988
<i>A. sangmelinensis</i>	<i>Micralestes humilis</i> Boulenger, 1899	Sangmelima: Dja Basin	Birgi, 1988
<i>A. nannoethiopsis</i>	<i>Nannoethiopsis unitoeniatus</i> Günther, 1872	Nkolya: Nyong River	Birgi, 1988
<i>A. bilongi</i>	<i>Neolebias trewavasae</i> Poll and Goss, 1963	Nkolya: Nyong River	Birgi, 1988
<i>A. endjami</i>	<i>Neolebias trewavasae</i> Poll and Goss, 1963	Nkolya: Nyong River	Birgi, 1988
<i>A. fomenai</i>	<i>Neolebias trewavasae</i> Poll and Goss, 1963	Nkolya: Nyong River	Birgi, 1988
<i>A. hepseti</i>	<i>Hepsetus odoe</i> Block, 1794	Nyong River	Paperna and Thurston, 1969

**Fig. 1** Map of Nyong River with sampling localities

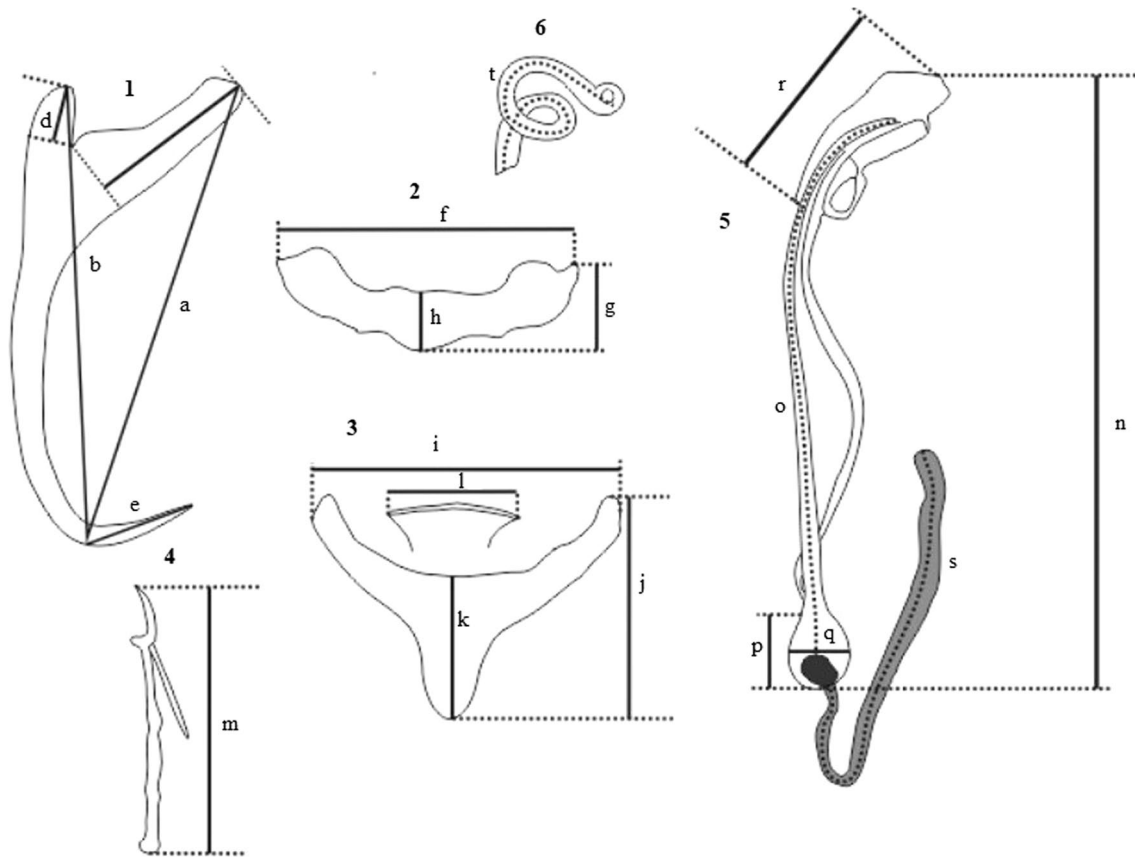


Fig. 2 General scheme of measurements for sclerotized parts of haptor and reproductive organs (1) anchor: **a** inner length, **b** outer length, **c** inner root length, **d** outer root length, **e** point length; (2) ventral bar: **f** total length, **g** total width, **h** median width; (3) dorsal bar: **i** total

length, **j** total width, **k** median width, **l** length of median sclerotized part; (4) marginal hooks: **m** total length; (5) MCO: **n** total length, **o** tube trace length, **p** basal length, **q** basal width, **r** cap length, **s** tube trace length of prostatic reservoir; (6) vagina: **t** tube trace length

1973 (re-described by Kičinjaová *et al.* [13], and *Annulotrema helicocirra* Paperna, 1973.

Research work on the Characiform populations from the Nyong River in Akonolinga has made it possible to undertake an inventory of the monogeneans of the hosts collected. This study led to finding of two species belonging to the genus *Annulotrema* and provides the first evidence for monogenean species on the gills of *B. macrolepidotus* in Cameroon. The purpose of this paper is to carry out the morphological description of both species.

Materials and Methods

A total of 32 specimens of *Brycinus macrolepidotus*, 70–180 mm standard length were caught with gillnets in the Nyong River (Akonolinga, centre Cameroon) on February 2019 at three localities: main bridge (3°46' 10.53''N; 12°14' 49.062 E), Melan (3°46' 11.424''N; 12°16' 22.236''E) and Sombo (3°46' 13.548''N; 12°16' 27.612''E) (Fig. 1). The

captured fish were kept in icebox with ice inside and then, transported to the Laboratory of Parasitology and Ecology of the University of Yaoundé I where they were transferred to the freezer until they were examined for monogeneans.

During examination, the gills from both sides were removed and isolated in Petri dishes containing tap water. Under a stereomicroscope, each monogenean was dislodged from the gill lamellae with fine needle and mounted between slide and coverslip in a drop of glycerin ammonium picrate mixture (GAP) [24]. Two days later, cover-slips were sealed with nail varnish and observations were made using the Yvymen optical microscope with an integrated camera. Identification of monogeneans was based on the morphology and size of the sclerotized parts of the haptor and the copulatory complex. Scheme measurements (Fig. 2) were made according to Kičinjaová *et al.* [13] and Gussev [25]. The nomenclature adopted at ICOPA IV [26] was used to distinguish the seven pairs of marginal hooks (in Roman numerals). Drawings of the sclerotized parts were made using the Corel Draw X4 software (Version 14. 0.0.701;

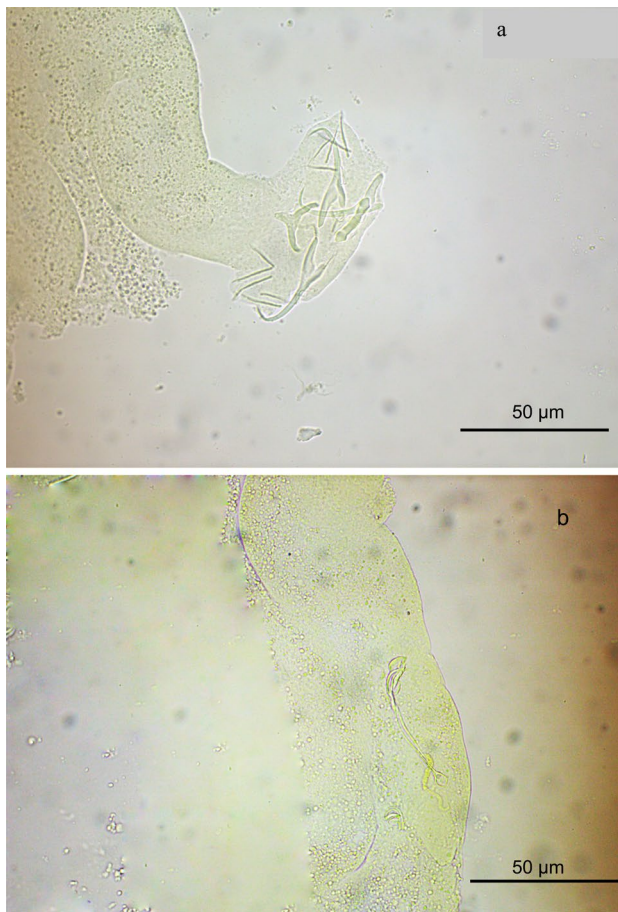


Fig. 3 Photographs of sclerotized parts of *A. ngombiensis* n. sp.: **a** haptor, **b** reproductive organ

Corel Corporation). The various measurements are given in micrometres (μm) and presented as follows: average \pm standard deviation (minimum value–maximum value). Holotypes and paratypes were deposited in the Helminthological collection of the Muséum National d’Histoire Naturelle, Paris, France (MNHN). The remaining specimens have been kept in the collection of Laboratory of Parasitology and Ecology, University of Yaoundé I, Cameroon.

Results

The two monogeneans species collected from the gills of the 32 specimens of *B. macrolepidotus* examined are all comply with the description of members of *Annulotrema* by having the following characteristics: haptor armed with four anchors (the inner and the outer roots generally form a U on the ventral anchors and a V on the dorsal ones) [4, 9], two bars with or without filaments and 14 marginal hooks [3]. Male copulatory organ with an accessory piece and one or two

prostatic reservoirs [9]. Vagina in the lateral position [3, 7]. Gill parasites of African characids (Characiform).

Annulotrema ngombiensis n. sp.

Type Host: *Brycinus macrolepidotus* Valenciennes, 1849.

Infection site: Gills.

Type locality: Akonolinga-Centre near the Nyong Bridge ($3^{\circ}46' 10.53''\text{N}$; $12^{\circ}14' 49.062\text{E}$) Others localities: Sombo ($3^{\circ}46' 13.548''\text{N}$; $12^{\circ}16' 27.612''\text{E}$) and Melan ($3^{\circ}46' 11.424''\text{N}$; $12^{\circ}16' 22.236''\text{E}$).

Specimens studied: 40 individuals mounted in Malmberg’s solution.

Type specimens: Holotype: **MNHN HEL 1646** and Paratypes: **MNHN HEL 1647–1648** deposited at the Muséum National d’Histoire Naturelle (Paris).

Etymology: The name of this species is inspired by that of a small village called Ngombi located in the district of Akonolinga where we had fortuitously captured its host for the first time.

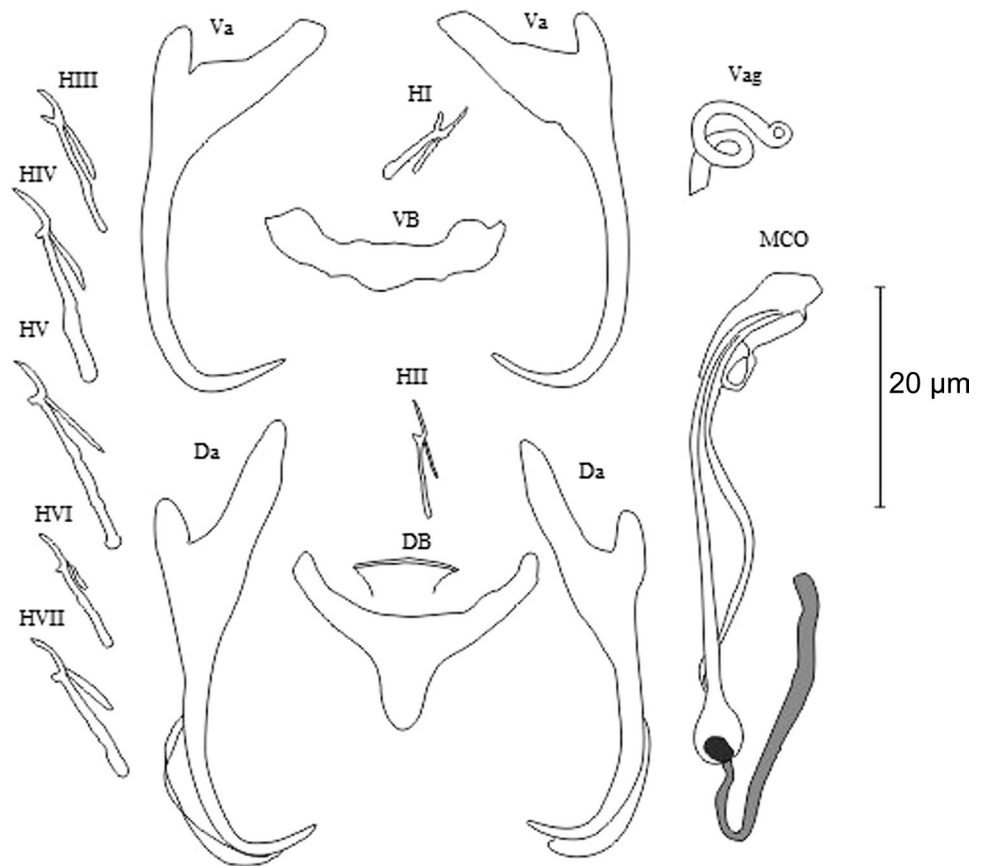
Prevalence: 87.5% (28 infested hosts).

Description (Figs. 3, 4).

Body length haptor included 533 ± 85 (314–790), width 49 ± 9 (24–69) at the level of the pharynx and 66 ± 13.4 (49–86) at the level of the ovaries. Ventral anchor, with inner root robust and approximately three times longer than the outer root, blade straight in its middle part, and ends by a short point; inner length = 37 ± 1.9 (34–41), outer length = 35 ± 2.0 (30–39), inner root length = 12 ± 1.7 (7–16), outer root length = 4 ± 0.9 (3–7) and point length = 8 ± 0.8 (6–10). Dorsal anchor less robust than the ventral one, inner root with a bump at its median part and incurved at its terminal part; outer root reduced, blade small incurved ends with a short point: inner length = 42 ± 2.3 (36–46); outer length = 35 ± 2.3 (30–43); inner root length = 15 ± 2.2 (5–18); outer root length = 5 ± 0.9 (3–7) and point length = 7 ± 0.7 (5–9). Ventral bar arched and slightly bulging at its extremities: total length = 24 ± 1.9 (20–27), total width = 6 ± 0.9 (5–9) and median width = 4 ± 0.8 (3–7). Dorsal bar forked with two branches almost equal and sinuous, small triangular piece in the middle of the fork: total length = 23 ± 1.5 (20–28), total width = 16 ± 2.4 (10–21); median piece length = 9 ± 2.2 (7–11) and median width = 10 ± 1.3 (6–12). Marginal hooks 7 pairs with filament; and dissimilar in size, marginal hook lengths: Pair I = 12 ± 1.5 (9–15); Pair II = 13 ± 1.5 (10–17); Pair III = 16 ± 2.5 (12–24); Pair IV = 18 ± 2.0 (13–22); Pair V = 21 ± 0.6 (17–25); Pair VI = 20 ± 2.1 (15–25); Pair VII = 17 ± 0.4 (13–21).

The male copulatory organ, with a long tubular prostatic reservoir, starts with piriform base following by an elongated accessory piece and ends by a cap which embraces the terminal part of copulatory tube: total length 48 ± 6.7 (34–60); tube trace length 62 ± 6.1 (45–71); base length 7 ± 0.9 (5–8); base width 5 ± 0.6 (4–6); cap length 18 ± 2.8

Fig. 4 Drawings of sclerotized parts of *A. ngombiensis* n. sp.: *Va* ventral anchor, *Da* dorsal anchor, *VB* ventral bar, *DB* dorsal bar, *HI–HVII* marginal hooks (pairs I–VII), *Vag* vagina, *MCO* male copulatory organ



(11–23) and prostatic reservoir tube trace length 35 ± 6.0 (26–49). The vagina tubiform and sinuous forms a number 6 knot to third length and ends with an opening at one extremity; tube trace length 26 ± 3.6 (21–33).

Remarks

Annulotrema ngombiensis n. sp. has some similarities with *A. tenuicirra* drawn by Paperna [5] and redrawn by Christison [27] in having forked dorsal bar, MCO with a visible prostatic reservoir attached to its basal part, elongated copulatory tube, elongated accessory piece whose terminal part forms a spatula or a cap which embraces the distal part of the copulatory tube and same sizes of their ventral and dorsal anchors (34–41 vs 32–35 and 36–46 vs 35–37, respectively). However, *A. ngombiensis* n. sp. has dorsal bar with round base, ventral bar forming a well-marked V, dorsal anchor with a short point, whereas *A. tenuicirra* presents dorsal bar with triangular base, linear ventral bar and dorsal anchor with very curved point. Also, in *A. ngombiensis* n. sp., the terminal part of the copulatory tube remains inside the spatula; the prostatic reservoir is tubiform while in *A. tenuicirra*, the terminal part of the copulatory tube comes out of the spatula and the prostatic reservoir clearly visible forms a small oval mass attached to the base of the MCO.

Annulotrema ngombiensis n. sp. and *A. elongata* have an elongated MCO with an accessory piece that runs up the copulatory tube and ends in a tip. Moreover, the dorsal bar in both species forms a fork with two branches of almost equal length. Despite these similarities, the accessory piece of *A. ngombiensis* n. sp. forms a well-marked cap in its distal part while that of *A. elongata* becomes thinner; the ventral bar of *A. elongata* has posterior extensions and saddle-like while that of *A. ngombiensis* n. sp. does not have any but is thin and U-shaped; the size of ventral and dorsal anchors is higher in *A. elongata* than in *A. ngombiensis* n. sp. (34–41 vs 45–49 and 36–46 vs 48–54, respectively).

Annulotrema ngombiensis n. sp. can be classified in the same morpho-group as *A. pikei* parasite of *Hydrocynus forskahlii* and the two species share the same shape of the elongated MCO with a pear-shaped base and an elongated accessory piece which ends in a cap that surmounts the terminal part, although this cap is wider in *A. ngombiensis* n. sp. In addition, the dorsal bar of each species is forked with a thin median piece and their ventral and dorsal anchors have similar sizes (34–41 vs 36–43 and 36–46 vs 35–43, respectively). Nonetheless, the fork formed by the dorsal bar is less marked in *A. pikei* and the branches less developed than in *A. ngombiensis* n. sp. The ventral bar of *A. pikei* has posterior extensions in the original description [6]

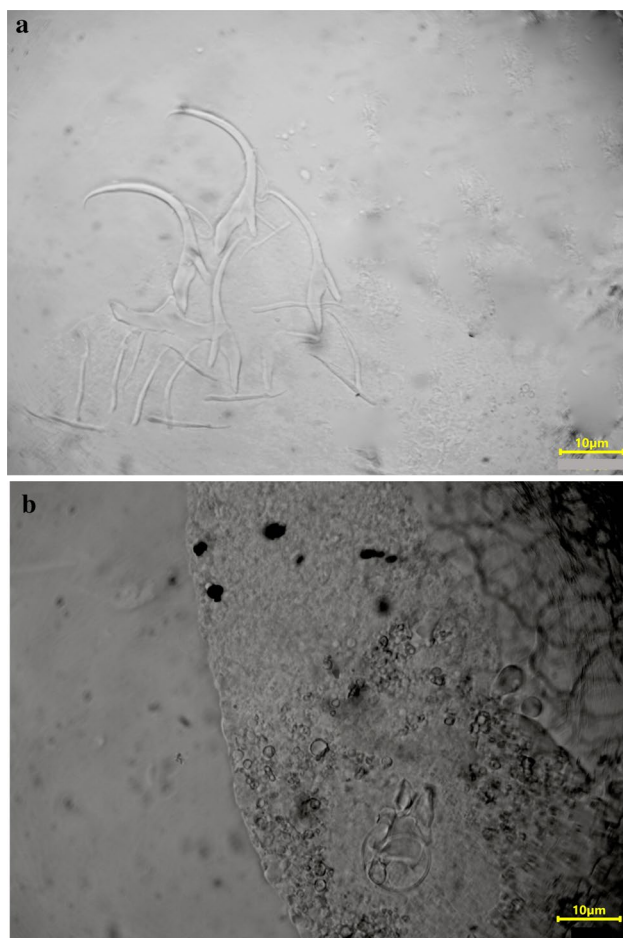


Fig. 5 Photographs of sclerotized parts of *A. nkengfacki* n. sp.: **a** haptor, **b** reproductive organ

and in the re-description of the sclerotized parts made by Řehulková *et al.* [3]. This bar seems to form a saddle composed of several joined pieces in *A. pikei* while it appears single and concave in *A. ngombiensis* n. sp. The accessory piece of *A. pikei* begins at the level of the copulatory tube and extends all along the latter, whereas in *A. ngombiensis* n. sp., this piece starts at the level of the basal part of the male copulatory organ, outlines the copulatory tube before to ascend. It is important to note that the samples provided for the re-description of *A. pikei* are not of good quality and as reported by Řehulková *et al.* [3]; this species requires a re-description on better quality samples. Despite these drawbacks, we cannot consider the species *A. ngombiensis* n. sp as a synonym of *A. pikei*.

Annulotrema nkengfacki n. sp.

Type host: *Brycinus macrolepidotus* Valenciennes, 1849.

Infection site: Gills.

Type locality: Akonolinga-Centre near the Nyong Bridge (3°46' 10.53''N; 12°14' 49.062'E). Others localities: Sombo

(3°46' 13.548''N; 12°16' 27.612''E) and Melan (3°46' 11.424''N; 12°16' 22.236''E).

Specimens studied: 15 individuals mounted in Malmberg's solution.

Type specimens: Holotype: **MNHN HEL 1649** and Paratypes: **MNHN HEL 1650–1651** deposited at the Muséum National d'Histoire Naturelle (Paris).

Etymology: This species was named in honour of Professor Nkengfack Augustin Ephrem, Head of Department of Organic Chemistry of the University of Yaoundé I.

Prevalence: 50% (16 infested hosts).

Description (Figs. 5, 6).

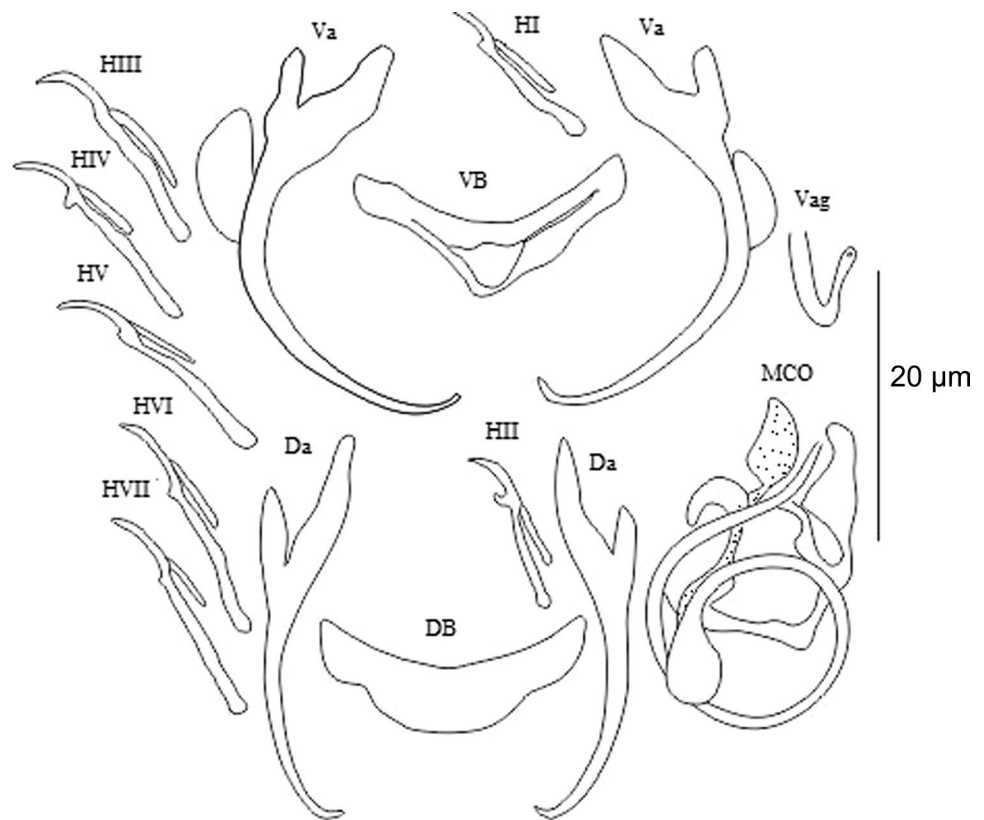
The total body length haptor included 303 ± 50.6 (232–426), width 42 ± 8.6 (27–57) at the level of the pharynx; 68 ± 15.7 (36–96) at the level of the ovaries. Ventral anchor with robust inner root, approximately two times as long as outer root with which it forms an angle of approximately 90°, blade well arched ends with a curved point: inner length = 30 ± 1.6 (28–32); outer length = 29 ± 1.0 (28–31); inner root length = 10 ± 1.0 (8–12); outer root length = 5 ± 1.0 (3–7) and point length = 3 ± 0.6 (2–4). Dorsal anchor little less robust than ventral anchor, inner root twice as long as the outer root with which it forms a more or less acute angle, blade fine and ends with very short point: inner length = 31 ± 1.9 (28–35); outer length = 26 ± 2.2 (24–30), inner root length = 11 ± 1.5 (9–14); outer root length = 5 ± 1.0 (4–7) and point length = 2 ± 0.3 (2–3). Ventral bar V-shaped, with one of the branches more robust and slightly curvy, middle part with trapezoidal structure with an extension on each end of its large base: total length = 17 ± 3.0 (13–20); total width = 9 ± 1.4 (7–12) and median width = 5 ± 1.1 (3–7). Dorsal bar arched in its lower part with ends depressed in a cup: total length = 20 ± 1.2 (18–22); total width = 8 ± 1.9 (6–12) and median width = 4 ± 0.8 (3–5). Marginal hooks 7 pairs with filament and dissimilar in size: Pair I = 12 ± 1.3 (9–14); Pair II = 11 ± 0.8 (10–13); Pair III = 18 ± 0.8 (17–20); Pair IV = 17 ± 1.5 (15–20); Pair V = 17 ± 0.9 (15–19); Pair VI = 16 ± 1.0 (14–18); Pair VII = 15 ± 1.5 (13–18).

Male copulatory organ with ovoid basal piece, following by a tubular penis which forms one turn in clockwise, accessory piece complex with lower part trapezoid and surmounted by a fork in which the copulatory tube slides: total length = 21 ± 2.0 (16–24); tube trace length = 56 ± 10.1 (36–69); base length = 5 ± 0.4 (4–6) and base width = 4 ± 0.5 (3–5). The vagina sclerotized tubiform with a small opening at its terminal end: tube trace length = 6 ± 3.0 (5–7).

Remarks

Annulotrema nkengfacki n. sp., from the morphological point of view presents similarities with *A. helicocirra* in having arched ventral bar, ventral anchor with the blade

Fig. 6 Drawings of sclerotized parts of *A. nkengfacki* n. sp.: *Va* ventral anchor, *Da* dorsal anchor, *VB* ventral bar, *DB* dorsal bar, *HI–HVII* marginal hooks (pairs I–VII), *Vag* vagina, *MCO* male copulatory organ



well-curved and similar sizes (28–32 vs 32–37) spirally coiled MCO. Nonetheless, the dorsal bar which is filiform in *A. helicocirra* with a triangular piece attached to the base is rather curved in *A. nkengfacki* n. sp.; MCO in the species being described presents one turn of spire when there are two in *A. helicocirra*. Although having morphological similarities and exploiting the same host, the differences observed in *A. nkengfacki* n. sp. and *A. helicocirra* lead to separate them clearly.

Annulotrema nkengfacki n. sp. is also similar to *A. bouixi* in sharing the same morphology of the MCO with the tube which describes a loop and an accessory piece that forms a blade into which the end of the copulatory tube penetrates. As with *A. nkengfacki* n. sp., the ventral and dorsal bars of *A. bouixi* form a V which is wider in the dorsal bar. Moreover, the sizes of the ventral and dorsal anchors are similar in both species (28–32 vs 24–32 and 28–35 vs 30–32, respectively). Despite these similarities, the accessory piece of the male copulatory organ in *A. bouixi* starts at the level of the base and forms a characteristic terminal hook while that of *A. nkengfacki* n. sp. begins at the level of the copulatory tube and ends in an opening which frames the terminal part of the copulatory tube of the MCO; the ventral bar forms a V in both species, but in *A. bouixi* the base of the V is distinguished by a characteristic T-shaped expansion which has not been observed in *A. nkengfacki* n. sp. Note that, our work on *B. kingsleyae* monogeneans (In press) allowed us to have

several samples of *A. bouixi* and thus, to confirm the separation with *A. nkengfacki* n. sp.

Discussion

This study supports the presence of two new monogenean species of *Annulotrema* on *B. macrolepidotus* from the Nyong River. They are well distinguished by features of the haptor and genital sclerotized structures especially, the shape of the bars, the size and shape of the anchors, the general shape of the MCO and of the vagina, which are meaningful for discrimination of *Annulotrema* species [9, 10, 13]. These species are different from each other and from all their previously described congeners despite some morphological similarities with some of them.

Several studies have focused on the systematics of monogeneans of Alestidae fish and have contributed based on morphological criteria, to the identification of 48 species of *Annulotrema* [3, 4, 8–10, 13, 14]. Based on the same criteria, the two species described in this article are different from the others and should be considered new. With these species, there are now 50 *Annulotrema* species and this is the first record of monogeneans on *B. macrolepidotus* from Cameroon.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no conflict of interest.

Ethical Approval For this study, fish were sampled using gillnets in accordance with national regulations.

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