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Quadriacanthus (Monogenea, Dactylogyridae) from Catfishes in the Democratic Republic of the Congo: Description of a New Species from *Heterobranchus longifilis* (Teleostei, Clariidae)

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Abstract

Purpose Studies on monogeneans parasitizing representatives of Siluriformes in Africa remain scarce and fragmentary. In order to contribute to fill this gap, we screened *Heterobranchus longifilis* from the Lindi River in the Democratic Republic of the Congo.

Methods *Heterobranchus longifilis* was purchased from fishermen in DR Congo, province of Tshopo, in Kisangani town, the Lindi River. The monogeneans were individually isolated from the gill and transferred directly onto a slide in a drop of Hoyer's medium.

Results *Quadriacanthus lindiensis* n. sp. is morphologically similar to *Quadriacanthus longifilisi.* However, it is distinguished from the latter by its non-sclerotized vagina, its dorsal cunei shape and the size of its bars.

Conclusion Only *Q. lindiensis* n. sp. was found on the gills of the single individual of *H. longifilis* screened. In the future, a representative sampling should enable us to verify if *Q. lindiensis* n. sp. co-occurs with other species known to parasitize *H. longifilis*.

Keywords *Quadriacanthus lindiensis* n. sp. . Lindi . Tshopo . Parasites . Flatworms

Introduction

Catfish is a vernacular name assigned to all fishes belonging to Siluriformes. They are usually identified by their scaleless bodies. They have up to four pairs of barbels, including one nasal, one maxillary, and two mandibular [1]. Catfishes have a wide geographic range and can be found in all continents except Antarctica [2]. Some families are more diverse in Africa than anywhere else in the world, such as Clariidae [3]. Catfishes are primarily freshwater fishes, except for two families (Ariidae and Plotosidae) that (also) contain marine species [2]. Besides the two aforementioned families, Africa shares three catfish families with Asia: Bagridae, Clariidae and Schilbeidae. Additionally, African freshwaters are home to five endemic families: Austroglanididae, Amphilidae, Malapteruridae, Mochokidae, and Claroteidae [1, 4].

Monogenea belongs to Platyhelminthes [5]. Freshwater, brackish, and marine fish are infected by representatives of this group of parasites. The great majority of monogeneans are ectoparasitic (found on surfaces like skin, fins, gills, mouth cavities, and nostrils) and all have a direct life cycle (without intermediate hosts). Although the majority of these flatworms are fish parasites, with a rather high host specificity, other species parasitize cephalopods, amphibians, reptiles, and mammals [6].
Quadriacanthus Paperna, 1961 is a monogenean lineage belonging to Dactylogyridae; its members occur on five families: Clariidae [7], Claroteidae [8], Bagridae [9], Notopteridae [10], and Cichlidae [11]. The type species, *Quadriacanthus clariadis* Paperna, 1961, was described from the gills of *Clarias gariepinus* (Burchell, 1822) [the junior synonym *Clarias lazera* Valenciennes, 1840 was mentioned] from Lake Galilee in Israel [7]. The firstly described African species of this genus is *Quadriacanthus voltaensis* Paperna, 1965, a gill parasite of *C. gariepinus* from Ghana [12]. Other species were subsequently described from various African countries, namely Egypt, Ivory Coast, Kenya, Uganda, Cameroon, and Sudan [13–21]. No representative of this genus is currently known in the DR Congo, where the monogeneans of only two catfishes have been studied: *C. gariepinus* and more recently *Auchenoglanis occidentalis* (Valenciennes, 1840), which respectively revealed the presence of four species, namely *Macrogyrodactylus congolensis* Prudhoe, 1957 and *Gyrodactylus transvaalensis* Prudhoe & Hussey, 1977 for *C. gariepinus*; *Bagrobdella vanhovei* Mushagalusa Mulega & Pariselle and *B. vansteenbergei* Mushagalusa Mulega & Pariselle for *A. occidentalis* [22-24].

As part of a study of monogenean parasites of African siluriform fishes, we examined the gills of *Heterobranchus longifilis* Valenciennes, 1840 from the Lindi River in the Democratic Republic of the Congo (DRC). The Lindi River has its source in Tayna natural reserve (province of North Kivu) and flows in a north-westerly direction through the Maïko National Park. Here, it forms a border between the provinces of North Kivu and Tshopo. To the West of Kisangani, Tshopo River joins the Lindi River, and a few kilometers further, the Lindi River flows into the main channel of the Congo River.

Materials and Methods

The single fish examined in this study, *H. longifilis* (Fig. 1), was opportunistically purchased from fishermen in DR Congo, province of Tshopo, in Kisangani town, the Lindi River (Bac Lindi: 0°33’30.8782’’N, 25°5’27.1866’’E). The sampling site is located in the Congo ichthyofaunal province (Fig. 2) [25]. The new species described in this study was compared morphologically with congeners with strong morphological similarities.

The fish was identified using the keys of Teugels [26] and Lévêque [27]. The gills of the fish were removed in the field and preserved in 98% ethanol. Once in the laboratory, the gills were screened for monogeneans using a Wild Heerbrugg® M8 stereomicroscope. Monogeneans were recovered using an entomological needle and mounted in a drop of Hoyer’s medium [28] on a slide, then covered with a coverslip to flatten the specimens to study the sclerotized structures. The slides were left for 24 hours in a horizontal position before sealing the coverslip with Glyceel [29]. The morphology of monogenean specimens was studied using a Leica® DM 2500 microscope equipped with a digital camera, the Leica DMC 4500. Measurements were based on N’Douba et al. [16] (Fig. 3), and taken using LAS version 4.12.0 software.

Drawings of the sclerotized parts of the monogeneans were made using Inkscape® version 1.2 software from photomicrographs. Maps were created using the free and open-source QGIS version 3.28.5. The type material was deposited in the collection of Research Group Zoology: Biodiversity & Toxicology of Hasselt University (Diepenbeek, Belgium) under accession numbers HU no.886-887 and HU no. 1092-1094. To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN), details of the new species were submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:5A8F8A24-69E5-4825-8737-39CF0A50C399. Note that the authors of the new species are different from the authors of this article; Article 50.1 and Recommendation 50 A of the International Code of Zoological Nomenclature.

Results

A single individual of *H. longifilis* (no voucher kept) was used in this study. All collected flatworms belong to *Quadriacanthus* as the habitus and the sclerotized structures observed show all diagnostic features: body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Two terminal cephalic lobes. Eyes present. Male copulatory organ with an accessory piece, vitellaria well developed. Haptor armed with dorsal and ventral pairs of anchors, dorsal and ventral bars, seven pairs of hooks (Dactylogyridae). Anchors with basal accessory sclerite; ventral bar comprising two components articulating medially, dorsal bar with bilateral arms, expanded at mid-region. Hook pair 4 with elongate dilated proximal shank, pairs 1,3 usually with short proximal dilation of shank; pairs 2,5-7 lacking dilated shank. Parasites of siluriform fishes of Africa and Asia [7, 15]. Collected flatworms belong to a new species of *Quadriacanthus*.

Class Monogenea Van Beneden, 1858
Order Dactylogyridea Bychowsky, 1933
Family Dactylogyridae Bychowsky, 1933
Genus *Quadriacanthus* Paperna, 1961
Description of *Quadriacanthus lindiensis* n. sp. Mushagalusa Mulega & Vanhove (*Fig.* 4; Table 1)
ZooBank registration: urn:lsid:zoobank.org:act:741D5A62-F336-489B-BC48-45276EDEBB37
Type material: holotype HU no.886, paratypes HU no.887, HU no. 1092, HU no. 1093, HU no. 1094
Type host: *Heterobranchus longifilis*
Type locality: Bac Lindi (0°33'30.8782''N, 25°5'27.1866''E; Democratic Republic of the Congo)
Collection date of host: 1st November 2021
Material examined: ten whole-mounted specimens
Infection site: gill filaments
Etymology: the species epithet '*lindiensis*' refers to the site where the host fish was purchased: the Lindi River, city of Kisangani, Tshopo province, Democratic Republic of the Congo.

Description based on ten specimens in Hoyer's medium: Body fusiform, bulging in the middle. Dorsal anchor without roots (outer root, inner root), shaft curved and short point. Dorsal cuneus is almost triangular in shape with sub-equal wings, two sides of which form an angle of approximately 60° and the other side has a sinusoidal shape with an irregular frequency. Dorsal bar with rectangular centre, two lateral expansions, and median process. Ventral anchor with regularly curved shaft ending in a long point. Ventral cuneus Y-shaped. Ventral bar V-shaped, widely open, showing two medially articulated branches. Seven pairs of hooks, pair IV is the longest. Pairs I, III with dilatation comprising about half of the total hook length. Male copulatory organ (MCO), comprising of a straight tubular penis, with small base and tapering. Vagina not observed.
Remarks: *Quadriacanthus lindiensis* n. sp. is similar in morphology and size of the sclerotized structures to another gill parasite of *H. longifilis*: *Q. longifilisi* N'Douba, Lambert & Euzet, 1999. Both species have a dorsal anchor of similar size, without roots, a shaft curved with a short point, a ventral anchor of similar size, a regularly curved shaft ending in a long point and a Y-shaped ventral cuneus. The fourth pair of hooks is the longest and overlaps in size for both species. However, *Q. lindiensis* n. sp. can be distinguished from *Q. longifilisi* by the size of the dorsal bar and ventral bar. The size of the dorsal bar of *Q. longifilisi* (36-43) represents about three quarters of the size of the dorsal bar of *Q. lindiensis* n. sp. (48-72) and the size of the ventral bar of *Q. longifilisi* (50-60) is about three-quarters the size of the ventral bar of *Q. lindiensis* n. sp. (69-83). For *Q. lindiensis* n. sp. the shape of dorsal cunei resembles a triangle with wings of relatively equal length. One pair of wings shows an angle of approximately 60 degrees, while the remaining wing shows a sinusoidal contour with an inconsistent frequency. For *Q. longifilisi*, the dorsal cunei are shaped like an isosceles triangle, with no real sinusoidal contour on either side. The MCO is wide in *Q. longifilisi* but narrow in *Q. lindiensis* n. sp. Unlike *Q. longifilisi*, *Q. lindiensis* n. sp. does not have a sclerotized vagina.

Discussion

In the present study, we describe a new species of *Quadriacanthus* parasitizing *H. longifilis*: *Q. lindiensis* n. sp. This new species brings the number of monogenean species known to infect this fish species to seven. Six of these belong to *Quadriacanthus*: *Q. longifilisi* N'Douba, Lambert & Euzet, 1999, *Quadriacanthus ndoubai* Bahanak, Pariselle & Bilong Bilong, 2017, *Quadriacanthus thysi* N'Douba, Lambert &Euzet, 1999, *Quadriacanthus tricorniculai* Bahanak, Pariselle & Bilong Bilong, 2017, *Quadrianthus triunguisi* Bahanak,

Pariselle & Bilong Bilong, 2017, and *Q. lindiensis* n. sp. Similar to five other *Quadriacanthus* species already known from this fish, the description of *Q. lindiensis* n. sp. is based entirely on the morphology of its sclerotized parts. This species showed a strong resemblance in the shape of some of its sclerotized parts to those of *Q. longifilisi*, but with larger bars. It should be noted that the two studies carried out on *Q. longifilisi* [16,19] used glycerin ammonium picrate (GAP) as the slide-mounting medium. Specimens used to describe *Q. lindiensis* n. sp. herein were ethanol-fixed and Hoyer's medium mounted. According to Fankoua et al. [30] Hoyer's medium softens the sclerotized parts, which thus become more flattened under the coverslip's pressure during the diffusion of the mounting medium. If this were of influence here, there should be a difference in size between all the corresponding sclerotized parts between *Q. lindiensis* n. sp. and *Q. longifilisi*. This was not the case, as the hooks are identical in size.

Our results do not show a co-occurrence of *Q. lindiensis* n. sp. with any other species known to parasitize *H. longifilis*. Only a representative sampling will enable, in the future, to verify this observation based on a single host studied herein. In the Agnéby River in Ivory Coast, this fish is parasitized by two species: *Q. longifilisi* and *Q. thysi* [16]. A study on monogeneans parasitizing the gills of *H. longifilis* in the River Boumba, in Cameroon, revealed a co-occurrence of five species which are *Q. longifilisi*, *Q. thysi*, *Q. tricorniculai*, *Q. tringuisi* and *Q. ndoubai* [19].

Heterobranchus longifilis is present in six ichthyological provinces of Africa: Upper and Lower Guinea, Nilo-Sudan, Congo, Zambezi [30] (Fig. 5) and East Coast [31]. The host has been screened for monogeneans parasitizing its gills in two of the six provinces: Nilo-Sudan and Congo [16, 19].

The Boumba population of *H. longifilis* in the Congo Province shares two parasite species (*Q. longifilisi* & *Q. thysi*) with the population of the Nilo-Sudanian Province, which has only these two species [16, 19]. *Heterobranchus longifilis* from the River Lindi (Kisangani) was infected by a single species of monogenean, *Q. lindiensis* n. sp. and no species known from the River Boumba was found. Based on this observation, we could hypothesize that the population of Boumba was once in contact with the population of Nilo-Sudanian Province, whereas the population of River Lindi (Kisangani) has always been isolated from that of Boumba, despite the fact that they are in the same ichthyofaunal region: Congo Province. It should be noted that the Boumba belongs to the Dja/Sanaga system [32]. In terms of fish fauna, the Dja has a stronger affinity with the Lower Guinea province than the rest of Congo Province as it was only recently captured by the Congo River [33]. It could hence be that *Q. lindiensis* n. sp. may be considered more typical to the Congo Province, than species from the Boumba River. However, this hypothesis can be tested in the future by conducting a representative sampling in various river basins in these provinces. Additionally, incorporating genetic studies is recommended in order to understand the biogeography and evolution of both *H. longifilis*, as was done for another wide-spread clariid species [34], and this effort should also be conducted for its monogeneans.

Conclusion

This study contributes to the knowledge on monogeneans parasitizing representatives of siluriform fishes in Africa. In this work, we examined the gills of a single individual of *H. longifilisi*. A new monogenean species was described as *Q. lindiensis* n. sp.

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Author Contributions Conceptualization AMM, MVS & MPMV; Methodology AMM, MVS, IR & MPMV; Investigation AMM, IR & MPMV; Resources MVS; Data Curation AMM; Writing–Original Draft Preparation AMM; Writing–Review and Editing AMM, MVS, NK, PMM, GKK, IR, AB, MPMV; Supervision MVS & MPMV. All authors have read and agreed to the published version of the manuscript.

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Declarations

Conflicts of Interest The authors declare that they have no conflicts of interest.

Ethical approval This is not applicable in our case, as we bought a dead fish.

Fig. 1 *Heterobranchus longifilis* from Democratic Republic of the Congo, province of Tshopo, in Kisangani town, the Lindi River (Bac Lindi: 0°33’30.8782’’N, 25°5’27.1866’’E); picture by Maarten Van Steenberge

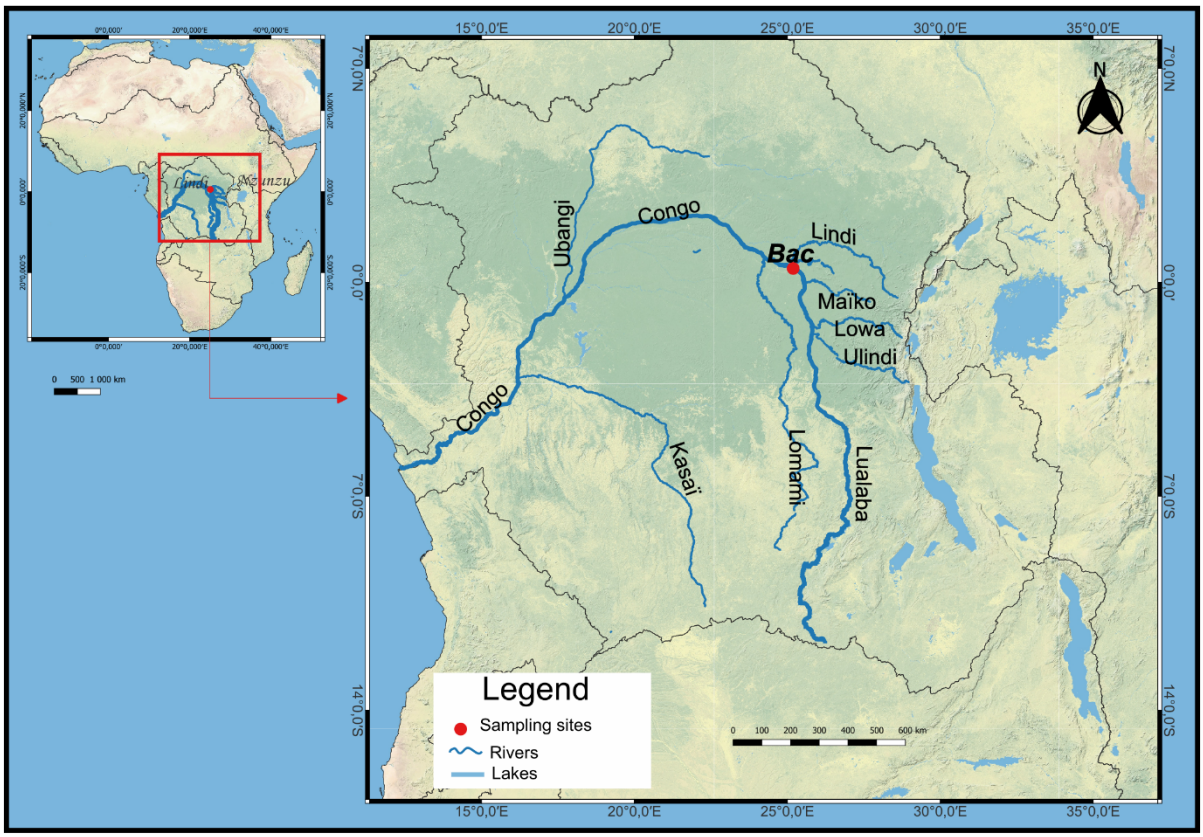

Fig. 2 Sampling site. The boundaries on the map of Africa are modified from those of ichthyofaunal regions of Africa according to Snoeks & Getahun [25]

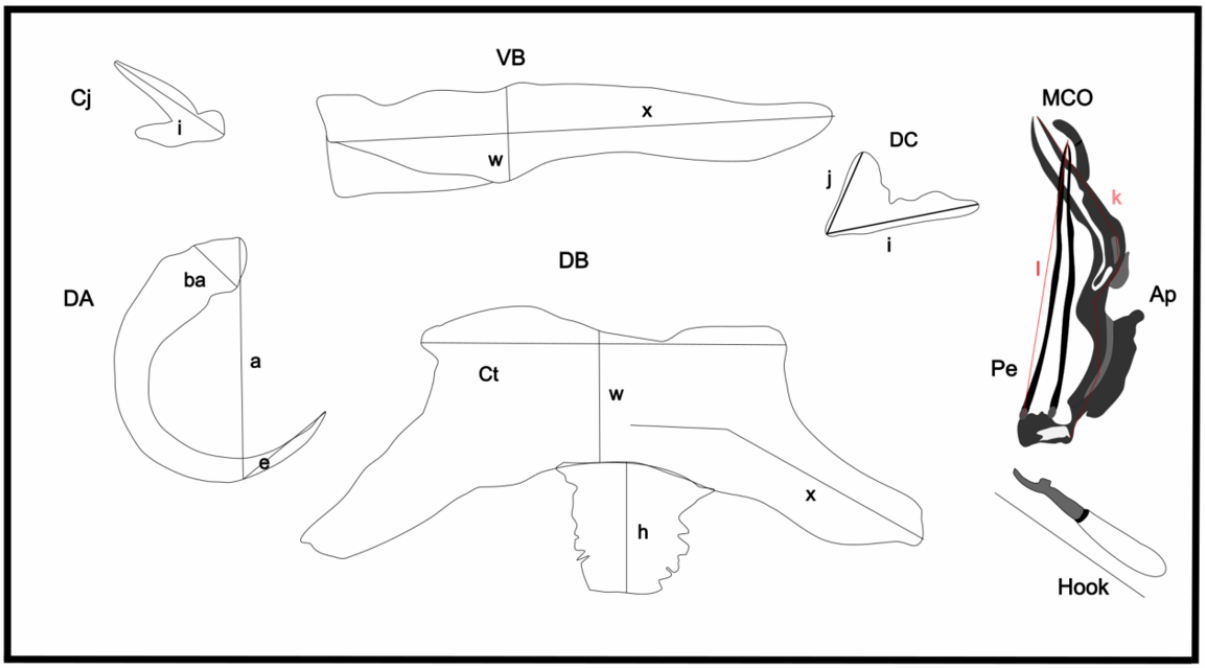

Fig. 3 Scheme of measurements for structures of the new species of *Quadriacanthus* described herein. Cj, ventral cuneus, i= length; VB, ventral bar, x= total length, w= greatest width; DA, dorsal anchor, a= total length, ba= base width, e= point length; DB, dorsal bar, Ct= total length, w= thickness at mid-region, h= process length, x= length of one branch; DC, dorsal cuneus, i= length, j= width; Hook= hook size; MCO, Male copulatory organ, l= total length of penis (Pe), k= total length of accessory piece (Ap)

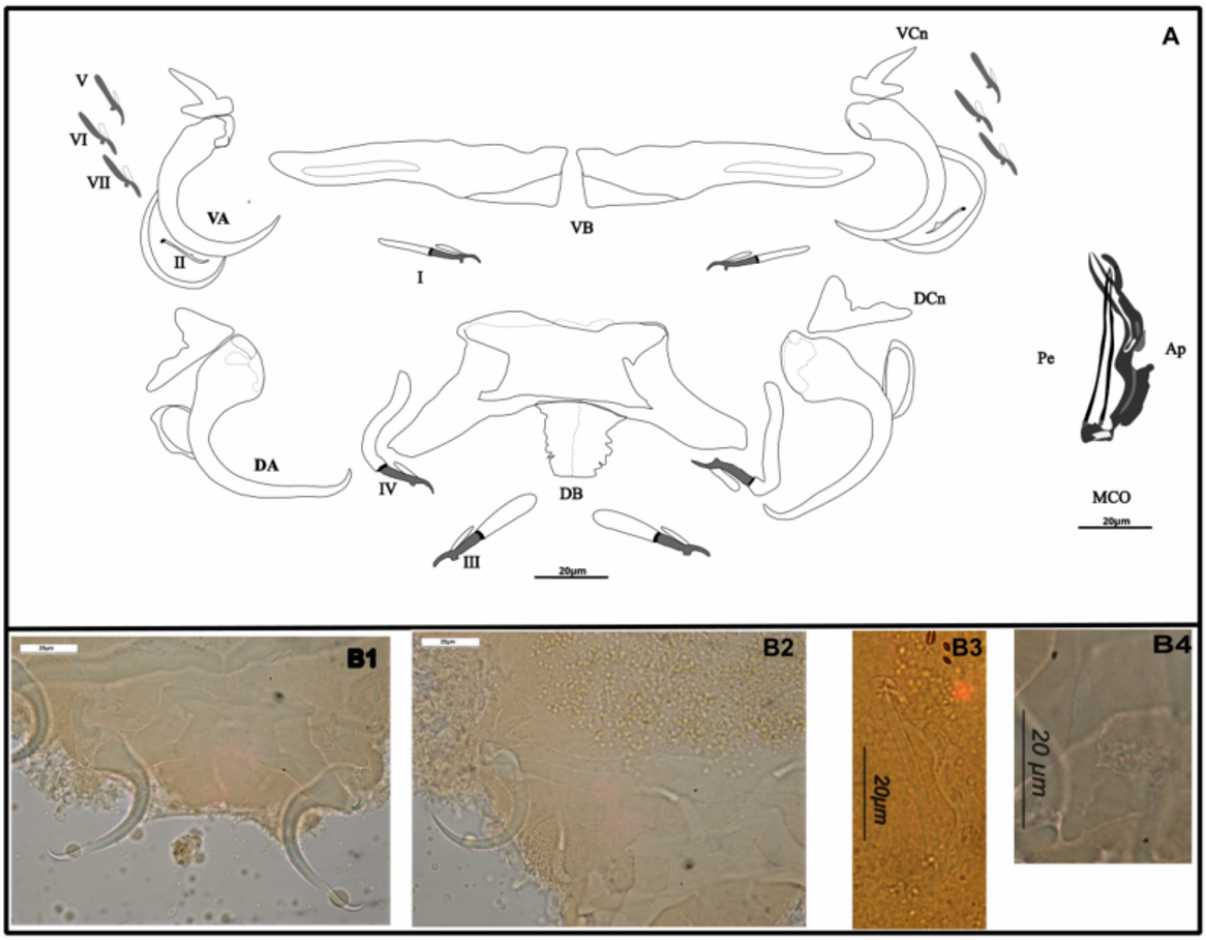

Fig. 4 *Quadriacanthus lindiensis* n. sp. **A**. Drawing (scalebar 20 µm) of sclerotized parts, VB: ventral bar, VA: ventral anchor, VCn: ventral cuneus, I-VII: hooks, DB: dorsal bar, DCn: dorsal cuneus, MCO: male copulatory organ; Pe: penis, Ap: accessory piece. **B**.

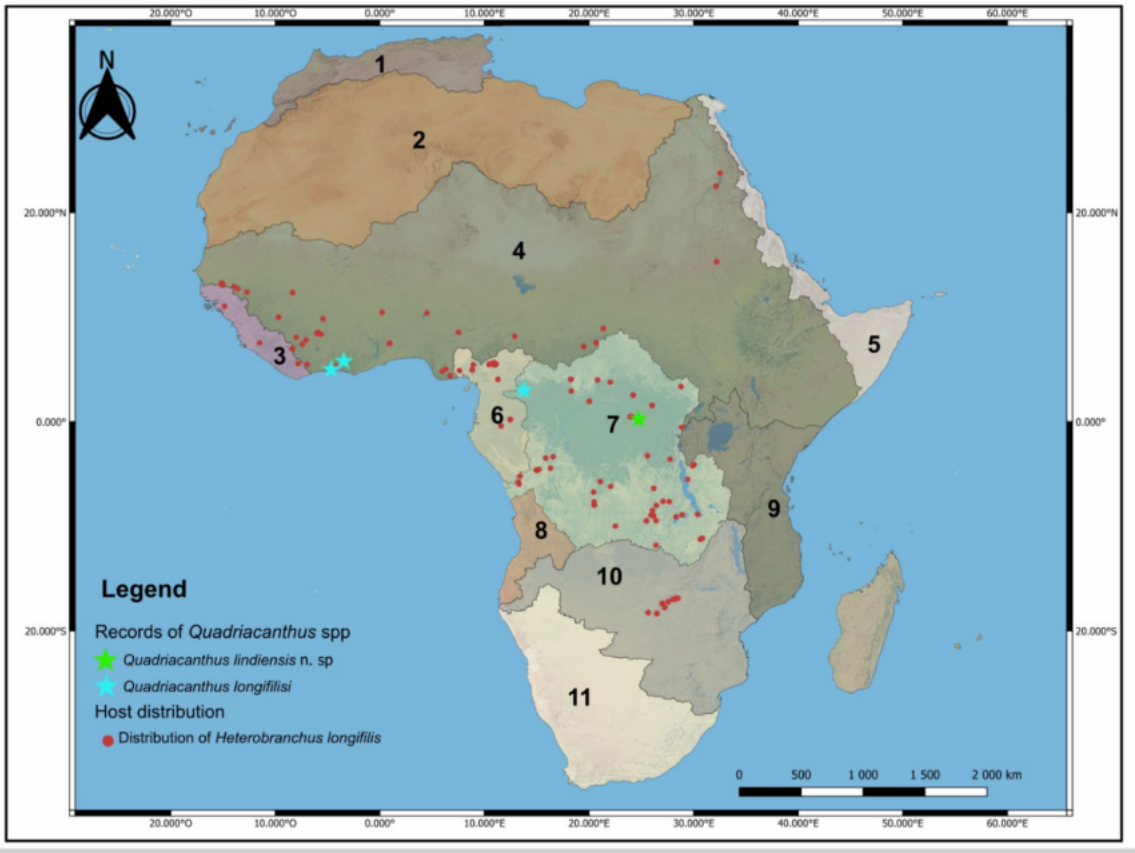

Fig. 5 Records of *Q. lindiensis* n. sp. & *Q. longifilisi* collected from *H. longifilisi* and distribution of the hosts. Records of *H. longifilisi* were downloaded from Fishbase (https://www.fishbase.se/map/OccurrenceMapList.php?genus=Heterobranchus&species=longifilis) Boudandaries of ichthyofaunal regions were modified from Snoeks & Getahun [25]. 1. Maghreb Province; 2. Sahara; 3. Upper Guinea; 4. Nilo-Sudan; 5. Red Sea Coast; 6. Lower Guinea; 7. Congo Province; 8. Quanza Province; 9. East Coast Province; 10. Zambezi Province; 11. Southern (Cape) Province

Table 1 Morphometric data for *Quadriacanthus lindiensis* n. sp. and *Quadriacanthus longifilisi* N'Douba, Lambert & Euzet, 1999 (* from original description [16] and ** from redescription [19]). Measurements were recorded as the average in µm followed by the range in parentheses. Ph: pharynx; L: total length; l: width; VA: ventral anchor. Other measurements are explained in Fig. 3

Photomicrographs of the holotype HU no.886, mounted in Hoyer's medium, taken with a Leica® DM 2500 microscope equipped with a digital camera, the Leica DMC 4500; B1: dorsal anchors and dorsal bar (DA & DB in A), B2: ventral anchor and ventral bar (VA & VB in A), B3: male copulatory organ (MCO in A), B4: dorsal cuneus (Dcn on A)

	Q. lindiensis n. sp.	*Q. longifilisi**	*Q. longifilisi***
Ph	40 (31-48)	40 (30-40)	37 (30-40)
L	514 (441-596)	522 (300-853)	580 (470-795)
l	218 (148-274)	154 (88-176)	138 (100-185)
MCO			
l	47 (41-56)	43 (38-47)	39 (35-42)
k	48 (42-64)	41 (33-44)	40 (38-43)
I	25 (21-32)	20 (16-21)	27 (24-29)
II	13 (12-14)	14 (13-15)	15 (14-17)

III	26 (22-31)	26 (22-28)	27 (24-29)
IV	41 (35-47)	37 (35-41)	37 (33-41)
V	15 (14-17)	14 (13-15)	16 (15-17)
VI	14 (12-16)	14 (13-15)	16 (15-17)
VII	14 (12-16)	13 (12-15)	15 (13-17)
DB X	56 (48-72)	38 (36-40)	39 (33-43)
w	21 (17-27)	16 (14-18)	17 (14-20)
h	17 (13-25)	16 (14-20)	18 (15-21)
ct	52 (46-58)	33 (30-38)	36 (32-42)
DA a	52 (48-56)	50 (48-53)	48 (42-55)
ab	16 (13-18)	15 (13-17)	15 (13-17)
e	5 (4-6)	(4-5)	7 (5-8)
DC j	24 (17-30)	21 (18-24)	20 (13-24)
DC i	14 (10-16)	11 (7-12)	11 (7-17)
VB x	76 (69-83)	52 (50-56)	54 (50-60)
w	11 (9-15)	9 (6-11)	9 (6-11)
VA a	38 (37-40)	38 (36-40)	38 (33-41)
ab	11 (9-12)	10 (7-11)	9 (8-11)
e	13 (10-14)	15 (12-19)	15 (13-17)
VC j	22 (17-29)	15 (13-20)	16 (13-20)

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