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Monogeneans from Catfishes in Lake Tanganyika. I: Two new species of Bagrobdella Paperna, 1969 (Dactylogyridae) from Auchenoglanis occidentalis (Valenciennes, 1840) (Siluriformes: Claroteidae)

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Abstract

In the framework of the study of Siluriform fish monogeneans of Lake Tanganyika, we described two new species of *Bagrobdella* from *Auchenoglanis occidentalis*, *Bagrobdella vanhovei* **sp. nov.** is characterized by the morphology of its MCO which is unique (terminal opening) and *Bagrobdella vansteenbergei* **sp. nov.** characterized by the size of its hooks, which are almost all of the same size, and its male copulating organ with a unique shape: sub-terminal opening and no membrane surrounding.

Keywords

Bagrobdella vanhovei sp. nov., *Bagrobdella vansteenbergei* sp. nov., Congo Basin, East Africa, over dispersion.

INTRODUCTION

Lake Tanganyika is, with 12 million years, the oldest of the East African Great Lakes, (Cohen et al. 1993). It stands out from the rest of the world's lakes by its richly diverse ichthyofauna, which make it an important hotspot for the world's freshwater lacustrine biodiversity (Coulter et al. 1986). The 3200 km^2 of Lake Tanganyika's surface area is unevenly distributed among four countries, including the Democratic Republic of the Congo in the West (14800 km^2 or 45%), Tanzania in the East (13500 km^2 or 41%), Burundi in the North (2600 km^2 or 8%) and Zambia in the South (200 km^2 or 6%) (Cohen et al., 1993; Fermon, 2007) (Fig. 1).

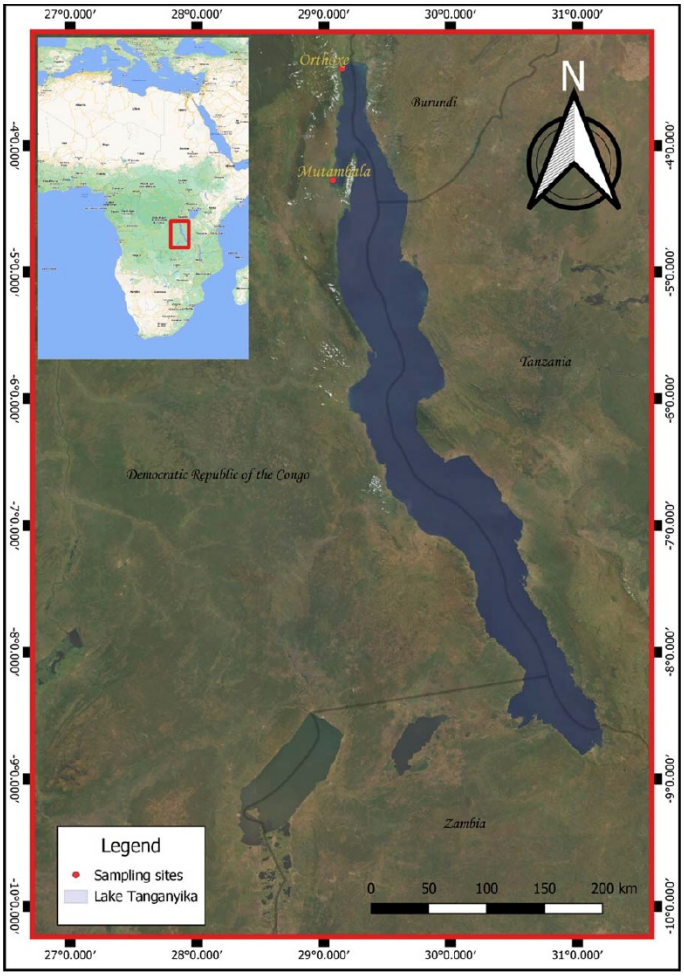

Figure 1. Sampling sites localities

The fish fauna of Lake Tanganyika has received particular attention from evolutionary biologists, mostly because of its phenotypically, ethologically and genetically highly diverse cichlid fauna which consider it as a 'natural laboratory' (Martens 1997, Langenberg et al. 2003, Albrecht & Wilke 2008, Cristescu et al. 2010). Indeed, its cichlid species are phenotypically,

ethically and genetically highly diverse (Nishida 1991, Salzburger et al. 2002, 2005), they show the highest degree of endemism compared to other great lakes in Africa and the Americas (Salzburger et al. 2014), with 239 endemic species out of the 241 described (an endemism rate of 99%) (Ronco et al. 2020, 2021). In addition to evolutionists, Lake Tanganyika Cichlids have also attracted the attention of parasitologists primarily in host biogeography, systematics and parasite evolution (e.g. Bates, 1997, Grégoir et al. 2014, Pariselle et al. 2011, 2015, Raeymaekers et al. 2013, Steenberge et al. 2015, Vanhove et al., 2013, 2015, 2016).

The non-cichlids of Lake Tanganyika, less diversified than the Cichlidae, have attracted very little attention of scientists. However, they are represented by 75 species belonging to 11 families, and have a rate of endemicity of 59 %. It is true that species of some of these families, such as Clupeidae and Latidae, have already been the subject of some ecological (Coulter 1976, Mannini et al. 1999), parasitological (Kmentova et al. 2018, Kmentova et al. 2020) and genomic (De Keyzer et al. 2019) studies. However, the Siluriformes, while representing on their own 5 (Bagridae, Claroteidae, Clariidae, Mochokidae and Malapteruridae) of these 11 families, making Lake Tanganyika the most diverse lake in Siluriformes than any other lake in the world (Fermon 2007, Peart et al. 2014), have been totally left behind. Thus, the present study set out to investigate the Monogenea of the latter group.

The history of the Lake Tanganyika parasitic fauna begins with the description of *Ancyrocephalus limnothrissae* Paperna, 1973 from the gills of *Limnothrissa miodon* (Boulenger 1906). This species was redescribed under the name *Kapentagyrus limnothrissae* by Kmentova et al. (2018). The second description of Lake Tanganyika's monogenean species was one belonging to *Gyrodactylus* by Vanhove et al. (2011). Since then, the number of studies in Lake Tanganyika monogeneans has increased, with particular attention given to *Cichlidogyrus* monogenean of Cichlid fish, which currently has 30 published and 10 other undescribed species (Rahmouni 2017, 2020, 2021).

This is the first study that describes *Bagrobdella* species in Lake Tanganyika. To date, four species are described from Ghana, Uganda, Mali and Cameroon, belonging to this genus: *Bagrobdella auchenoglanii* Paperna, 1969, *B. fraudulenta* Euzet & Le Brun, 1990 and *B. anthopenis* Euzet & Le Brun, 1990 (Euzet & Le Brun 1990) from *Auchenoglanis occidentalis* (Valenciennes, 1840) and one, *B. parauchenoglanis* Akoumba, Pariselle, Tombi & Bilong Bilong, 2017 from *Parauchenoglanis monkei* (Keilhack 1910) (Akoumba et al. 2017).

MATERIALS & METHODS

Sampling

The parasites described in this study were collected from 11 specimens of *Auchenoglanis occidentalis* (Claroteidae, Siluriformes). They were captured in the North-Western part of the lake, a few meters from the orthodox church in Uvira (Northern part) and at the Mouth of River Mutambala (Southern part) (Fig. 1). They were killed by severing their chordal spines, and identified on site as *Auchenoglanis occidentalis* using the Fermon et al. (2012) keys. The gills and a small section of the pectoral fins were stored in 96% ethanol.

Fish DNA isolation was carried out according to the protocol of Aljanabi & Martinez (1997): approximately 50 µg of pectoral fin fragment was sheared into small pieces before being digested at 55 °C overnight with 20 µl of proteinase K (20 mg/ml) in 180 µl of extraction buffer solution (1M Tris, 0.5M Na Cl2, 1 % SDS). The extracted DNA was suspended in 150 µl sterile double distilled water and stored at −20 °C until amplified by PCR (Louizi et al., 2019). The partial mitochondrial Cytochrome Oxidase (COI) gene was amplified using FishF1/F2 and FishR1 universal primers (Ward et al. 2005). Each amplification was performed in a volume of 50 µl containing 0.25 mM of MgCl 2, 0.2 mM of each dNTP, 1 mM of each primer, 5 µl buffer (10×) and 10 units of Taq polymerase. The replication cycle was as follows: 94 °C (3 min), 48 °C (30 s), 72 °C (5 min) for 30 cycles with a final step at 72 °C for 10 min (Louizi et al., 2019). The sequencing step was performed at the genotyping-sequencing platform of the “Institut des Sciences de l'Evolution of Montpellier” (ISE-M) in France. The sequences are obtained with a Genomix sequencer (MGX), following the manufacturing recommendations and, using the same forward and reverse primers as for PCR. Cleaning and alignment of the sequences was done with MEGA X software (Kumar et al., 2018). Vouchers (Fig. 2) were kept in the collection of CRH-Uvira (DRC).

Figure 2. *Auchenoglanis occidentalis* from Lake Tanganyika

The gills were examined under a Wild Heerbrugg® M8 binocular. The monogeneans were recovered using an entomologist needle and some individuals were mounted in a drop of Hoyer's medium (Anderson 1954) on a slide, then covered with a coverslip to 'flatten' the specimen. The slides were left to dry for 24 hours in horizontal position before sealing the coverslip with Glyceel (Bates 1997). Other individuals were mounted using tap water and identified under a Leica® DM 2500 microscope equipped with a digital camera (Leica DMC 4500), after identification, the monogeneans were individually stored in Eppendorf® tubes in 96% ethanol for subsequent genetic studies.

Morphometric analysis

Measurements based on Euzet & Le Brun (1990), were taken using LAS version 4.12.0 software (Fig. 3), and given in µm as average followed by the range in parentheses, number of individuals. Drawings of the sclerotized parts of the Monogeneans were made with CorelDRAW 2019 software using pictures taken from the camera. All analyses were performed using R 4.1.2 software (R core Team 2022).

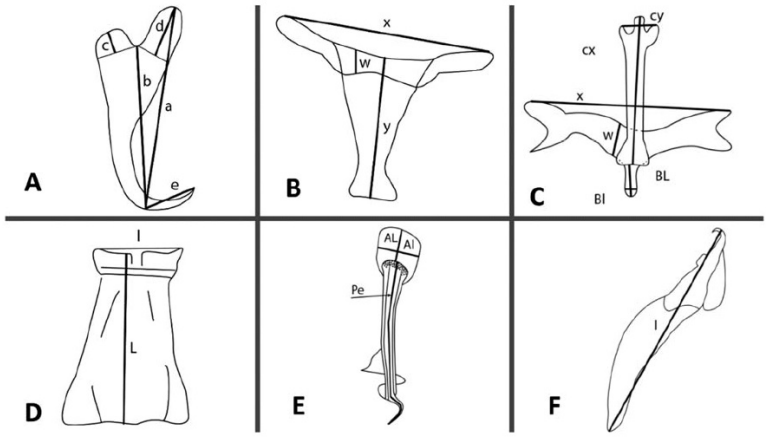

Figure 3. Surveying of sclerotised structures. A. Anchor, a: total length; b: blade length; c: shaft length; d: guard length; e: point length. B. Dorsal bar, x: horizontal length; y: vertical length; w: width. C. ventral bar, x: horizontal length; cx: length of median projection; cy: width of median projection; BL: length of outgrowth of VB; Bl: width of outgrowth of VB. D. Trapezoidal piece, L: length; l: width. E. Male copulatory complex, Pe: length of penis, AL: length of bulb; Al: width of bulb. F. length of hook.

Data from 45 individuals of one new species, 10 individuals of the other and 10 individuals of *Bagrobdella parauchenoglanii*, were subjected to a Principal Component Analysis (PCA). This analysis was done using the FactoMineR (Husson et al. 2020) and factoextra (Kassambara & Mundt 2020) packages in R. *Bagrobdella auchenoglanii*, *B. fraudulenta* and *B. anthopenis*, each one represented by the mean values of the analyzed

parameters obtained in Euzet and Le Brun (1990) were added to the analysis as supplementary individuals.

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 XXXX. For each new species, the Life Science Identifier (LSID) is reported in the taxonomic summary.

Types were deposited in the Helminth collection of the Royal Museum for Central Africa (MRAC, Tervuren, Belgium).

Note that the authors of the new species are different from the authors of this article (International Commission on Zoological Nomenclature 2015).

RESULTS

The identification of host species was confirmed by the molecular analysis, and the sequences were deposited in Genbank (Identification number XXX). Gills of eleven *Auchenoglanis occidentalis* specimens were examined for parasite infection. Seven out of eleven hosts were found be parasitized. The most parasitized fish host is the sole specimen caught at the Mouth of Mutambala River.

TAXONOMY

Bagrobdella vanhovei Mushagalusa Mulega & Pariselle, sp. nov.

Fig. 4 & 5

Type-host. *Auchenoglanis occidentalis* (Valenciennes, 1840).

Site of infection. gills.

Type locality. Mouth of Mutambala River (29°04.4042’E, 04°16.4598’S) & off the Orthodox church of Uvira (29°08’32.6’’E, 03°23’42.0’’S) (DRC) (Fig. 1).

Studied material. 78.

Number of hosts examined. 11.

Prevalence. 6/11 = 54%.

Mean intensity. 318/6 = 53.

Abundance. 318/11 = 2.

Deposited material. holotype deposited at the Royal Museum for Central Africa, Tervuren, Belgium (number…).

Etymology. named after Professor Dr. Maarten P.M. Vanhove (University of Hasselt, Belgium), among others specialist in monogeneans of Tanganyika's Cichlids.

Figure 4. *Bagrobdella vanhovei* n. sp. microphotograph *in toto*.

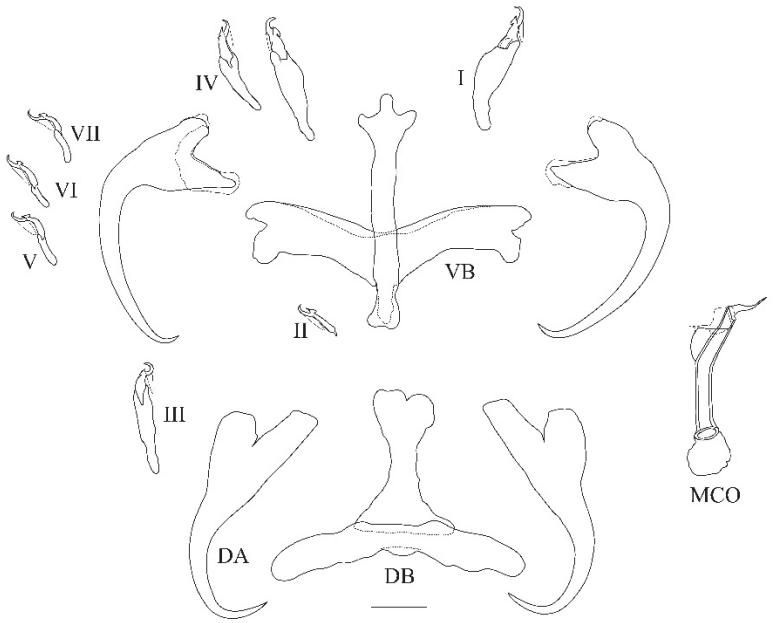

Figure 5. *Bagrobdella vanhovei* n. sp. haptoral structure and MCO. Scale bar = 20µm

Description. The anatomy is that of *Bagrobdella*. Length 681.2 (521.7–964.9) 72; greatest width 200.4(139.8–273.2) 72; pharynx 70.6(49.4–97) 61; Dorsal anchor: a = 81.6(64.7–94.1) 78, b = 66.1 (52.7–75.9) 78, c = 8.9(3.0–17.2) 78, d = 20.9 (12.9–29.6) 78, e = 18.5 (13.3–22.4) 78, no visible filaments. Dorsal bar x = 86.9 (62.2–111.6) 72, w = 14.7 (9–22.6) 71, median projection posteriorly oriented: Y = 59.4 (33.2–81.9) 61. Ventral anchor slightly drilled at the blade beginning: a = 65.3 (38–73.9) 78, b = 70.9 (60.1–79.2) 78, c = 10.5 (4.5–16.3) 78, d = 17.5 (12.6–24.0) 78, e = 6.2 (3.0–9.1) 77, no visible filaments. Ventral bar x = 111.9 (75.6–146) 75, w= 16.3 (9.4–27.4) 74 extends in the form of an outgrowth, BL = 10.6 (4.6–19.8) 60, Bl = 5.8 (3.2–13) 60, median projection posteriorly orientated, cross-shaped, Cx = 72.3 (57.8–91.3) 77, Cy = 20.4 (14.3–28.5) 76. At median projection posterior extremity is attached another sclerotized piece trapeze-shaped. Fourteen hooks arranged in seven symmetrical pairs of different sizes: I (medio-ventral) the largest and longest: 51.6 (34.3–64.2) 77, II (medio-ventral) the smallest hooks: 16.8 (12.9–20) 69, III and IV (latero-dorsal and almost identical in size): 38.6 (26.6–47.5) 74 V to VII (latero-ventral and almost identical in size) 25 (16.5–35.4) 72. A medium-ventral trapezoidal plate (Fig. 6), slightly sclerified, is located between hooks I measures TL: 37.3 (28.4–49.8) 15, Tl: 27.6 (21.7–34.6) 16. The penis (MCO): 60.5 (50.7–67.3) 71 started by a well-developed basal bulb: AL = 15.3 (9.9–22.4) 41, Al = 9.7 (3.4–20.3), followed by a thick-walled tube of constant diameter, folded at 30° at the middle, the distal half is surrounded by a membrane; at the distal extremity of the tube (level of opening) a portion of the wall formed a triangular part tapering at is end, its length being a quarter of that of the tube. Lidded eggs 92.8 (82.7–109.9) 12 are ovoid, slightly arched (Fig. 7), at the pole opposite to the operculum is a filament finished by a small disc, exactly as described by Euzet et Le Brun (1990) in the diagnose of *Bagrobdella*.

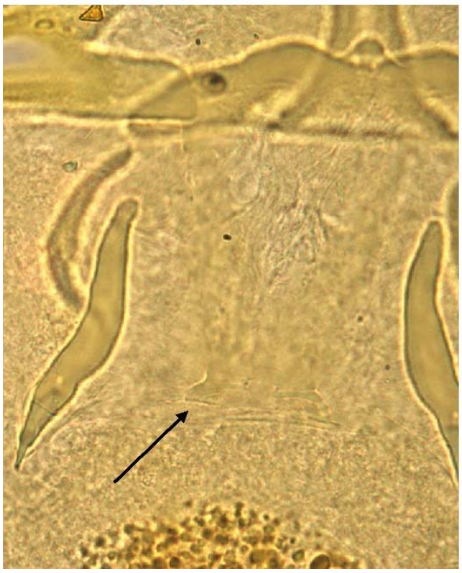

Figure 6. Microphotograph of the trapezoidal plate in the haptor of *Bagrobdella vanhovei*.

Figure 7. Egg (*B. vanhovei*).

Remarks. *Bagrobdella vanhovei* has seven pairs of hooks of different sizes, and has a trapeze-shaped piece associated with the median projection of the ventral bar (Fig. 6, latter visible in 15 of the 78 studied specimens), both characters have been mentioned in the descriptions of the four already known species in this genus. Its dorsal anchors have a long point, while its ventral anchors have a very short one, like in *Bagrobdella auchenoglanii* and *B. fraudulenta* (*B. parauchenoglanii* and *B. anthopenis* having points of the same size for both ventral and dorsal anchors). *Bagrobdella vanhovei* could be easily distinguished from all species already described by the morphology of its MCO which is unique (sub-terminal opening) (Fig. 5).

Bagrobdella vansteenbergei Mushagalusa Mulega & Pariselle, sp. nov.

Fig. 8 & 9

Type-host. *Auchenoglanis occidentalis* (Valenciennes, 1840).

Site of infection. gills.

Type locality. Mouth of Mutambala River (DRC) 29°04.4042'E, 04°16.4598'S.

Studied materiel. 15 individuals mounted in Hoyer's.

Number of hosts examined. 11.

Prevalence. 1/11 = 9%.

Mean intensity. 17/1 = 17.

Abundance. 17/11 = 1.5.

Type-materiel. Holotype deposited at the Royal Museum for Central, Tervuren, Belgium.

Etymology. named after Dr. Maarten Van Steenberge researcher at Royal Belgian Institute of Natural Sciences. Africa freshwater fish specialist.

Figure 8. *Bagrobdella vansteenbergei* n. sp. microphotograph *in toto.*

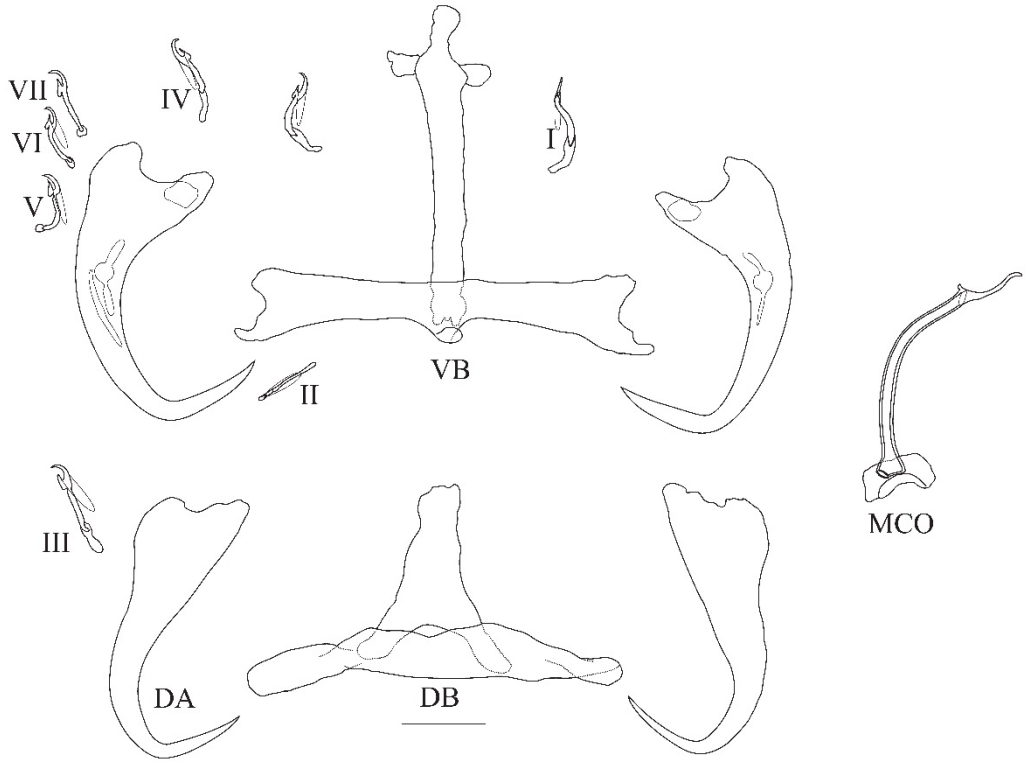

Figure 9. *Bagrobdella vansteenbergei* n. sp., haptoral structure and MCO. Scale bar = 20µm

Description. The anatomy is that of *Bagrobdella*. Total length 621. (542.8–748.7) 12, greatest width 182.2 (112.8–262.1) 13; pharynx 63.8 (42.3–79) 10. Dorsal anchor a = 70.6 (66.7–77.3) 13, b = 64.4 (58.6–70.2) 13, c = 0.7 (0.0–4.9) 13, d = 9.9 (6.8–12.6) 13, e = 22.9 (21.2–26.1) 13. Dorsal bar x = 87.7 (76.9–99.5) 10, w =13.5 (9.5–18.3) 10, median projection posteriorly oriented: Y = 55.1 (50.6–69.5) 6. Ventral anchor slightly drilled at the blade proximal extremity: a = 63 (60.7–67.1) 13, b = 61.9 (59.5–64.3)12, c = 6.0 (3.6–8.1)12, d = 12.2 (9.4–16.6) 12, e = 25.9 (22.3–27.5) 13. Ventral bar: 92.3 (77.5–103.2) 10, w = 13.7 (11.1–17.9) 8, extends in the form of an outgrowth: BL = 7.4 (5.1–10.5) 7, Bl = 4.9 (4.5–6.2) 8. This bar has a median projection posteriorly cross-chapped cx = 76.6 (68.9–84.5) 12, cy = 25.3 (20.8–31.4) 12. All hook pairs have almost the same size: I = 26.1 (23–31.1) 11, II = 16.9 (13.9–19.4)10, III and IV = 22 (18.9–26.7) 10, and V up to VII = 18.8 (17.7–21.5) 12. Penis 66.1 (58.1–71.0) 11, began by an asymmetrical bulb AL = 15.5 (10.7–20) 9, Al = 9.3(5.2–12.1) 9, is a curved tube with thick wall and constant diameter; as for *B. vanhovei* a part of the wall extend the penis extremity of about 25%; contrarily to other species of this genus, no membrane had been seen surrounding the penis.

Remarks. *Bagrobdella vansteenbergei* differs from all other species already described by the size of its hooks which are all of almost the same length (pair I being only slightly longer than the others), when they are of different size in all the previously described species (including *B. vanhovei* sp. nov.) (Fig. 10). More, the trapezoidal piece was not observed in the 17 individuals (15 Hoyer's mounted and 2 preserved in ethanol), it may be thought to be absent in this species, even if it is usually slightly sclerotised and hard to observe. The ventral anchors are slightly holed. Its male copulating organ has a unique shape within *Bagrobdella* spp. (sub-terminal opening and no membrane surrounding) (Fig. 11).

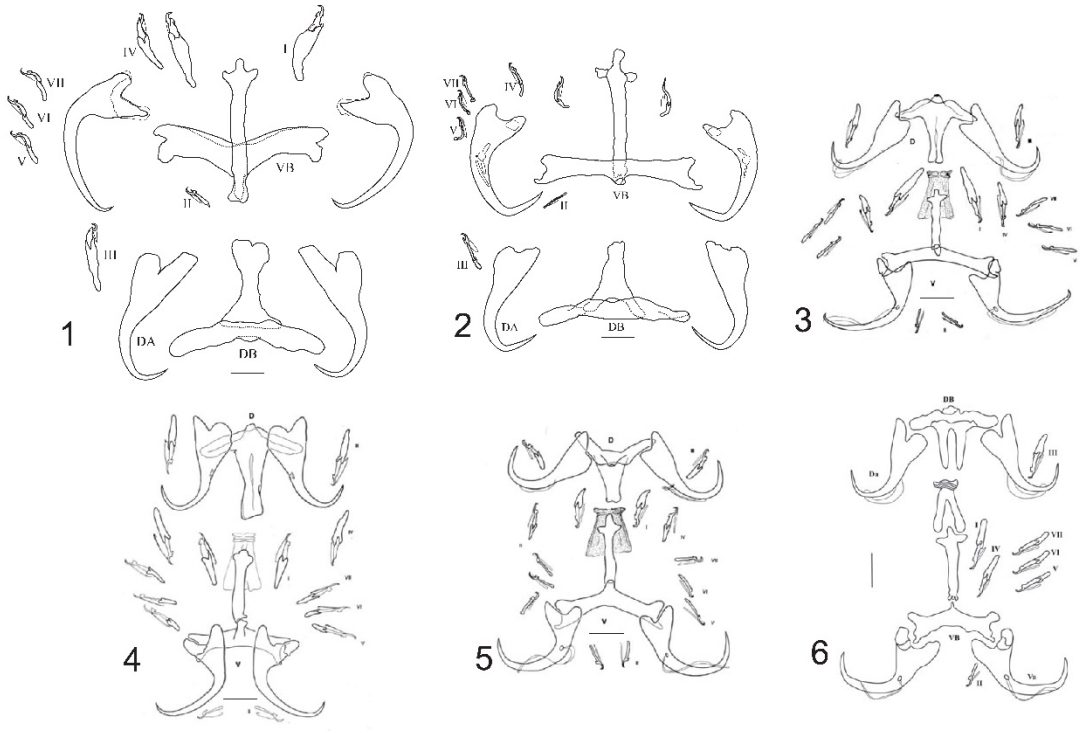

Figure 10. Haptors of the six species belonging to *Bagrobdella*. **1**. *Bagrobdella vanhovei*, **2**. *Bagrobdella vansteenbergei*, **3**. *Bagrobdella auchenoglanii*, **4**. *Bagrobdella fraudulenta*, **5**. *Bagrobdella anthopenis*, **6**. *Bagrobdella parauchenoglanii.* (Akoumba et al 2017; Euzet, Le Brun 1990). Scale bar: 20 µm.

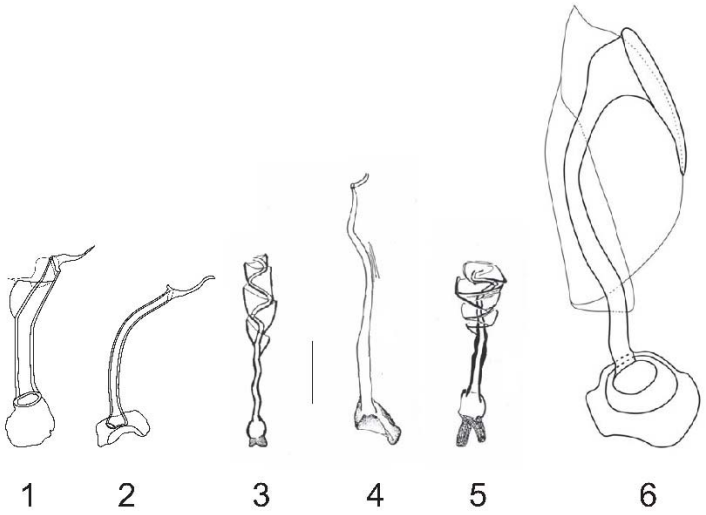

Figure 11. Penis of the six species belonging to *Bagrobdella*. **1**. *Bagrobdella vanhovei*, **2**. *Bagrobdella vansteenbergei*, **3**. *Bagrobdella auchenoglanii*, **4**. *Bagrobdella fraudulenta*, **5**. *Bagrobdella anthopenis*, **6**. *Bagrobdella parauchenoglanii*.(Akoumba et al 2017; Euzet, Le Brun 1990). Scale bar: 20 µm.

Multivariate analyses

Our dataset contained 68 individuals with 21 quantitative variables (Fig. 3). Among the 68 individuals, three were considered as illustrative (#66 corresponds to *B. auchenoglanii*, #67 corresponds to *B. fraudulenta* and #68 corresponds to *B. anthopenis*). The first two dimensions of the PCA represent 71.73% of the total dataset inertia. Individuals are clustered into three groups which correspond to the three species included in the analysis (*Bagrobdella vanhovei, B. vansteenbergei* and *Bagrobdella pauchenoglanii*) (Fig. 12 & 13). The dimension 1 opposes most individuals belonging to the group *B. vanhovei* (to the right of the graph, characterized by a positive coordinate on the axis) to individuals belonging to the groups *B. vansteenbergi* and *B. parauchenoglanii* (from Cameroon), to the left of the graph, characterized by a strongly negative coordinate on the axis).

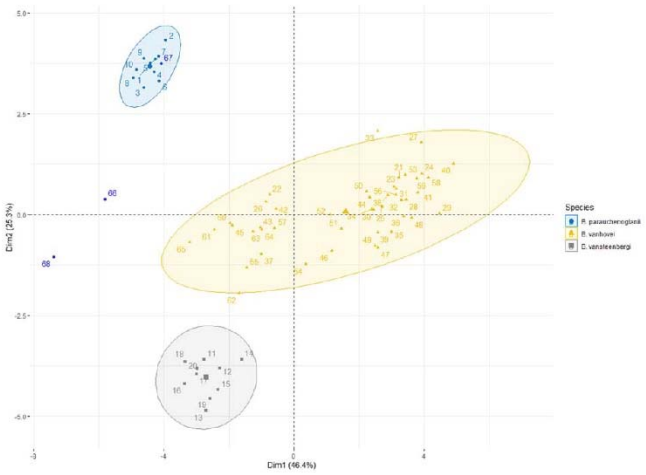

Figure 12. PCA on individuals of species belonging to *Bagrobdella*. 66 = *B. auchenoglanii*, 67= *B. fraudulenta*, 68= *B. anthopenis.*

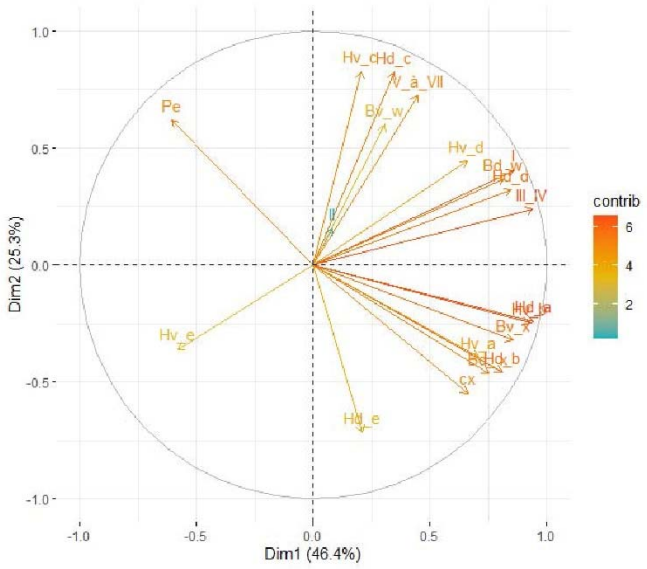

Figure 13. Contribution of the variables in the PCA of species belonging to *Bagrobdella.*

The individuals belonging to the group *B. vanhovei* (characterized by a positive coordinate on the axis) are sharing:

- high values for *III_IV*, *Hd_d*, *Bd_w*, *I*, *Hv_b*, *Hd_a*, *Bv_x*, *Hv_a*, *Hv_d* and *Hd_b* (variables are sorted from the strongest).
- low values for *Hv_e* and *Pe* (variables are sorted from the weakest).

The individuals belonging to *B. parauchenoglanii* (characterized by a negative coordinate on the axis) are sharing:

- high values for the variables *Pe*, *Hv_c*, *Hd_c* and *Bv_w* (variables are sorted from the strongest).
- low values for the variables *Hd_b*, *Bd_.x*, *Bv_x*, *cx*, *Hv_a*, *Hd_a*, *Hv_b*, *Hd_e*, *III_IV* and *Hd_d* (variables are sorted from the weakest).

In the group of *Bagrobdella vansteenbergei* (characterized by a negative coordinate on the axis) individuals share:

- high values for the variables *Hv_e* and *Hd_e* (variables are sorted from the strongest).
- low values for the variables *V_à_VII*, *Hd_c*, *Hv_c*, *I*, *Bd_w*, *Hd_d*, *Hv_d*, *Bv_w* and *III_IV* (variables are sorted from the weakest).

The dimension 2 opposes individuals belonging to *B. parauchenoglanii* (to the top of the graph, characterized by a strongly positive coordinate on the axis) to individuals belonging to *Bagrobdella vansteenbergei*.

On the graph, *Bagrobdella auchenoglanii* (#66) and *B. anthopenis* (#68) are isolated from the other species, when *B. fraudulenta* (#67) grouped with *B. parauchenoglanii*, even so these two latter species being easily distinguishable (at least by their penis morphology (Fig. 11).

DISCUSSION

Among the 11 studied host specimens, the most parasitized was the one caught at the Mouth of Mutambala River. This individual hosted a total of 286 Monogeneans, 269 *Bagrobdella vanhovei* and 17 *B. vansteenbergei*. The other ten individuals that were captured off the Orthodox Church of Uvira possessed only *Bagrobdella vanhovei* individuals. Among them, four had no monogeneans, three had one individual, and the other three hosts had, five, six and 35 individuals respectively. This non-homogeneous distribution is very common in populations of parasite from host fishes (Dold & Holland 2011), for example it has been observed very recently in the *Brachyplatystoma vaillantii* Valenciennes, 1840 (Siluriformes, Pimelodidae) in the Amazon (Brito-Junior & Tavares-Dias 2021).

As there is sometimes a correlation between water quality and helminth infections (e.g. in the Cyprinid fish (Zargar et al. 2012)), overdispersion of *Bagrobdella vanhovei* and the absence of *B. vansteenbergei* in the other six host individuals captured off the Orthodox Church

of Uvira could be due to different water qualities at the level of these two sampling sites. Thus, *Bagrobdella vansteenbergei* could be adapted to the conditions found at the Mouth of Mutambala River, while *Bagrobdella vanhovei* could be more resistant to variations of abiotic conditions.

Bagrobdella vanhovei and *B. vansteenbergei* are both new to science and endemic to Lake Tanganyika. However, the 4 species of *Bagrobdella* already known have been described in different localities: *B. auchenoglanii* has been described in Ghana (with it's redescription done on individuals sampled in Mali and Togo), *B. anthopenis* in Mali, *B. fraudulenta* in Uganda and Mali (see Euzet and Le Brun 1990) and recently *B. parauchenoglani* in Cameroon (Akoumba et al. 2017).

Except for individuals of *Auchenoglanis occidentalis* sampled by Euzet and Le Brun in Bamako (Niger River), which harbor three species of *Bagrobdella* (*B. auchenoglanii*, *B. fraudulenta* and *B. anthopenis*), in other localities: Lake Albert, Uganda (Paperna 1971); Kara River, Togo (Kritsky & Kulo 1999); Oti River (Euzet & Le Brun 1990) and Volta Lake (Paperna 1969), Volta Basin; Sassandra in RCI (Euzet & Le Brun 1990), and on *Parauchenoglanis monkei* in Cameroon (Akoumba *et al*. 2017), it seems that only one species is present (*B. fraudulenta* in Uganda, *B. parauchenoglanii* in Cameroon, *B. auchenoglanii* in the other localities).

So, among the five *Bagrobdella* species described from *A. occidentalis*, three are found in the watershed of the Nilo-Sudanian province, when the two others new one are from the Tanganyika province. As a consequence, either these monogenean species are specific of their ichtyofaunal provinces, or we are in the presence of two different host species (the systematic status of *A. occidentalis* remain debated (see for example Geerinckx & Vreven (2013)).

Bagrobdella vanhovei shows a high variability in its morphometrical characters (Fig. 12), with small, medium and large measurements for similar hard parts. These differences, apart from a great variability of specimens from a single species, may be due to two likely reasons: 1) presence of different species, 2) the use of different medium to prepare the slides (see Fankoua et al. 2017). Knowing that we did not see morphological differences between "large" and "small" specimens, and that both were found together on same slides (in the same medium), we are most probably in the presence of a species with a great variability in the size of its haptoral hard parts.

During the study of these two new species, two small hard parts that were never been reported before in the description of *Bagrobdella* species, were seen. These are: 1) a structure in the shape of a button between the bar and ventral anchors (Fig. 14), 2) a semi-circular structure toping the extremity of the ventral bar median projection (Fig. 15). In order to properly define their fine morphology and function, we need to observe fresh material or to use scanning electron microscopy. For this reason, we did not mention these structures in the description of the two new species.

Figure 14. Button like structure.

Figure 15. Semi-circular structure.

Authors' contributions: AMM and AP designed and supervised this study. FMB and PMM contributed to sampling, the collection and identification of fish, and provided scientific background information on the fish. JFA provide raw data on *Bagrobdella parauchenoglanii*. AMM and AP analysed the data and wrote the paper. All authors critically read and edited the manuscript.

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