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

Archimède Mushagalusa Mulega, Fidel Muterezi Bukinga, John Francis Akoumba, Pascal Masiya Mulungula, Antoine Pariselle

ABSTRACT. In the framework of the study of Siluriform fish monogeneans of Lake Tanganyika, we described two new species of Bagrobdella Paperna, 1969 from Auchenoglanis occidentalis (Valenciennes, 1840), Bagrobdella vanhovei sp. nov. is characterized by the morphology of its MCO which is unique among its congeners, presenting a non-terminal opening, whereas the other species have a 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: a sub-terminal opening and no membrane surrounding. The Multivariate analysis done on morphometrical characters shows that the new and already described species are well individualized, except for Bagrobdella parauchenoglanii Akoumba, Pariselle, Tombi & Bilong Bilong, 2017 and Bagrobdella fraudulenta Euzet & Le Brun, 1990 (but these two species are easily distinguishable by their morphology), and that B. vanhovei sp. nov. has a great intra-specific morphometrical variation.

KEY WORDS. Bagrobdella vanhovei sp. nov., Bagrobdella vansteenbergei sp. nov., Congo Basin, East Africa, intraspecific variation, over dispersion.

INTRODUCTION

Lake Tanganyika is the oldest of the East African Great Lakes (Cohen et al. 1993). Its 3.200 km² of surface area is unevenly distributed among four countries, including the Democratic Republic of the Congo in the West (14.800 km² or 45%), Tanzania in the East (13.500 km² or 41%), Burundi in the North (2600 km² or 8%) and Zambia in the South (200 km² or 6%) (Cohen et al. 1993, Fermon 2007) (Fig. 1).

It stands out from the rest of the world’s lakes by its richly diverse ichthyofauna, which makes it an important hotspot for the world’s freshwater lacustrine biodiversity (Coulter et al. 1986) and a ‘natural laboratory’ for evolutionary biologist (Martens 1997, Langenberg et al. 2003, Albrecht & Wilke 2008, Cristescu et al. 2010).

The cichlid fish of Lake Tanganyika have received particular attention, they are phenotypically, ecologically and genetically highly diverse (Nishida 1991, Salzburger et al. 2002, 2005). With 239 endemic species out of the 241 described (an endemism rate of 99%) (Ronco et al. 2020, 2021), Lake Tanganyika show the highest degree of endemism compared to other great lakes in Africa and the Americas (Salzburger et al. 2014). In addition to calling its importance to the study of biodiversity, the Cichlids of Lake Tanganyika have also attracted the attention of parasitologists, primarily in host biogeography, systematics and parasite evolution (e.g., Bates 1997, Grégoir et al. 2015, Pariselle et al. 2011, 2015, Raeymaekers et al. 2013, Van 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%. Species of some of these families, such as Clupeidae and Latidae, have already been the subject of some ecological (Coulter et al. 1986), parasitological (Kmentová et al. 2018, 2020) and genomic (De Keyzer et al. 2019) studies. Siluriformes represent five (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). However, nothing is known about the parasites of these hosts.

The history of the Lake Tanganyika parasitic fauna began 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 Kmentová et al. (2018). The second description of Lake Tanganyika’s monogenean species was *Gyrodactylus sturmbaueri* Vanhove et al., 2011, *G. thysi* Vanhove et al., 2011 and *G. zimbae* Vanhove et al., 2011 by Vanhove et al. (2011). Since then, the number of studies in Lake Tanganyika has increased, with particular attention given to *Cichlidogyrus* Paperna, 1960 monogenean of Cichlid fish, which currently has 39 published species (Rahmouni et al. 2017, 2018, Rahmouni 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: *Bagrobdella auchenoglanii* Paperna, 1969, *B. fraudulenta* Euzet & Le Brun, 1990 and *B. anthopenis* Euzet & Le Brun, 1990 (Euzet and Le Brun 1990) from *Auchenoglanis occidentalis* (Valenciennes, 1840), and *B. parauchenoglanii* Akoumba, Pariselle, Tombi & Bilong Bilong, 2017 from *Parauchenoglanis monkei* (Keilhack, 1910) (Akoumba et al. 2017).

**MATERIAL AND METHODS**

**Sampling**

The parasites described in this study were collected from 11 specimens of *A. occidentalis*. Fish were captured in the North-Western part of the lake, a few meters from the orthodox church in Uvira (North of this part) and at the Mouth of River Mutambala (South of this part) (Fig. 1). The hosts were euthanized by severing their chordal spines, and identified on site as *A. 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 and Martinez (1997). Vouchers were kept in the collection of CRH-Uvira (DRC).

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 specimens and highlight the sclerotized structures. The slides were left 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).

**Morphometric analysis**

Measurements were based on Euzet and Le Brun (1990), and taken using LAS version 4.12.0 (Figs 2–7), and given in µm by the range in parentheses, and number of individuals. Drawings of the sclerotized parts of the Monogeneans were made with Corel Draw® 2019 software using pictures taken from the camera. All analyses were performed using R 4.1.2 software (R Core Team 2022).

Data from 45 individuals of one new species, 10 individuals of the other, and 10 individuals of *B. parauchenoglanii*, were subjected to a Principal Component Analysis (PCA). This analysis was done using the FactoMineR (Husson et al. 2020) and factoextra (Kassambara and 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. For each new species, the Life Science Identifier (LSID) is reported in the taxonomic summary.

Types were deposited in the Helminth collections of the Royal Museum for Central Africa (MRAC, Tervuren, Belgium), of the Muséum national d'Histoire naturelle (MnHn, Paris, France) and of the Iziko South African Museum, Cape Town, Republic of South Africa (SAMC).

RESULTS

The identification of host species was confirmed by the molecular analysis, and the sequences were deposited in Genbank (Identification number ON682514). Gills of eleven A. occidentalis specimens were examined for parasite infection. Seven out of eleven hosts were found to be parasitized. The most parasitized fish host is the sole specimen caught at the Mouth of Mutambala River. This individual hosted a total of 286 Monogeneans, 269 Bagrobdella vanhovei sp. nov. and 17 B. vansteenbergei sp. nov. The other ten individuals that were captured off the Orthodox Church of Uvira possessed only Bagrobdella vanhovei sp. nov. individuals. Among them, four had no monogeneans, three had one individual, and the other three hosts had, five, six and 35 individuals respectively.

The two new species of Bagrobdella which were found are described herein.

TAXONOMY

Bagrobdella vanhovei Mushagalusa Mulega & Pariselle, sp. nov.

Fig. 8

https://zoobank.org/A7869408-CD17-4AB3-BFDC-9362C5DA9F13

Type-host. Auchenoglanis occidentalis (Valenciennes, 1840). Site of infection. Gills.

Type locality. Mouth of Mutambala River (29°04.402′E, 04°16.459′S) and off the Orthodox church of Uvira (29°08'32.6"E, 03°23'42.0"S) (DRC) (Fig. 1).

Studied material. 78 specimens mounted in Hoyer’s medium.

Number of hosts examined. 11.

Prevalence. 6/11 = 54%.

Mean intensity. 318/6 = 53.

Abundance. 318/11 = 28.9.
Deposited material. Holotype deposited at the Royal Museum for Central Africa, Tervuren, Belgium (RMCA_VERMES_43660), paratypes deposited at the MRAC (number RMCA_VERMES_43661), the MnHn, Paris, France (MNHN HEL1824) and the Iziko South African Museum, Cape Town, Republic of South Africa (SAMC) (SAMC-A094638).

Description. Length 681 (522–965; 72); greatest width 200 (140–273; 72); pharynx 71 (49–97; 61); Dorsal anchor: a = 82 (65–94; 78), b = 66 (53–76; 78), c = 9 (3–17; 78), d = 21 (13–30; 78), e = 19 (13–22; 78), no visible filaments. Dorsal bar x = 87 (62–112; 72), w = 15 (9–23; 71), median projection posteriorly oriented: Y = 59 (33–82; 61). Ventral anchor slightly drilled at the blade beginning: a = 65 (38–74; 78), b = 71 (60–79; 78), c = 11 (5–16; 78), d = 18 (13–24; 78), e = 6 (3–9; 77), no visible filaments. Ventral bar x = 112 (76–146; 75), w = 16 (9–27; 74) extends in the form of an outgrowth, BL = 11 (5–20; 60), Bl = 6 (3–13; 60), median projection posteriorly oriented, cross-shaped, Cx = 72 (58–91; 77), Cy = 20 (14–29; 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: 52 (34–64; 77), II (medio-ventral) the smallest hooks: 17 (13–20; 69), III and IV (latero-dorsal and almost identical in size): 39 (27–48; 74) V to VII (latero-ventral and almost identical in size) 25 (17–35; 72). A medium-ventral trapezoidal plate, slightly sclerotized, is located between hooks I, L: 37 (28–50; 15), L: 28 (22–35; 16). Male copulatory organ (MCO): 61 (51–67; 71) with a well-developed basal bulb: AL = 15 (10–22; 41), Al = 10 (3–20; 41), followed by a thick-walled tube of constant diameter, folded at 30° at the middle, distal half 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 its end, length a quarter of that of the tube. Lidded eggs 93 (83–110; 12) are ovoid, at the pole opposite to the operculum is a filament finished by a small
Bagrobdella from Lake Tanganyika

Bagrobdella vansteenbergei Mushagalusa Mulega & Pariselle, sp. nov.

Fig. 27

https://zoobank.org/47A4EA89-ED69-4878-BFD7-A3F8FC11CA6E

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 material. 15 specimens mounted in Hoyer's medium.

Number of hosts examined. 11.

Prevalence. 1/11 = 9%.

Mean intensity. 17/1 = 17.

Abundance. 17/11 = 1.5.

Type-material. Holotype deposited at the Royal Museum for Central Africa, Tervuren, Belgium (RMCA_VERMES_43658), paratypes deposited at the MRAC (number RMCA_VERMES_43659), the MNh, Paris, France (MNHN HEL1825) and the Iziko South African Museum, Cape Town, Republic of South Africa (SAMC) (SAMC-A094636 and SAMC-A094637).

Description. The anatomy is that of Bagrobdella. Total length 621 (543–749; 12), greatest width 182 (113–262; 13); pharynx 64 (42–79; 10). Dorsal anchor a = 71 (67–77; 13), b = 64 (59–70; 13), c = 1 (0–5; 13), d = 10 (7–13; 13), e = 23 (21–26; 13). Dorsal bar x = 88 (77–100; 10), w = 14 (10–18; 10), median projection posteriorly oriented: Y = 55 (51–70; 6). Ventral anchor slightly drilled at the blade proximal extremity: a = 63 (61–67; 13), b = 62 (60–64; 12), c = 6 (4–8; 12), d = 12 (9–17; 12), e = 26 (22–28; 13). Ventral bar: 92 (78–103; 10), w = 14 (11–18; 8), extends in the form of an outgrowth: BL = 7 (5–11; 7), BL = 5 (5–6; 8). This bar has a median projection posteriorly cross-chapped cx = 77 (69–85; 12), cy = 25 (21–31; 12), absence of sclerotized trapeze-shaped. Hook pairs of similar size: I = 26 (23–31; 11), II = 17 (14–19; 10), III and IV = 22 (19–27; 10), and V up to VII = 19 (18–22; 12). MCO 66 (58–71; 11), asymmetrical bulb AL = 16 (11–20; 9), Al = 9 (5–12; 9), is a curved tube with thick wall and constant diameter; a part of the wall extend the penis extremity of about 25%.

Etymology. The species is named after Dr. Maarten Van Steenberge, a researcher at Royal Belgian Institute of Natural Sciences, who is a specialist in African freshwater fish.

Note: The authors of the new taxa are different from the authors of this paper: Article 50.1 and Recommendation 50A of the International Code of Zoological Nomenclature.

Remarks. The new species was placed in Bagrobdella due to the presence of a haptor with dorsal, ventral anchor/bar complexes, 7 pairs of hooks with ancyrocephaline distribution, pairs 1, 3–7 with shanks comprised of 2 subunits, proximal subunit expended, pair 2 with shank of 1 subunit. Ventral bar straight, with long anterior projection associated with lightly sclerotized skirt, dorsal bar straight, with posterior shield-like projection, which are characteristics of the genus (Kritsky and Kulo 1999). Bagrobdella vanhovei sp. nov. is similar to B. auchenoglanii, B. fraudulenta, B. parauchenoglanii and B. anthopenis by (1) having a trapezoidal-shaped (former visible in 15 of the 78 studied specimens) piece associated with the median projection of the ventral bar (Figs 9–14), (2) having seven pairs of hooks of different sizes. 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 sp. nov. is easily distinguished from all species already described by the morphology of its MCO which is unique, being not spirally coiled – when all other species have a coiled MCO, which was a genus character (Kritsky and Kulo 1999), and more, the MCO of B. vanhovei has a sub-terminal opening (at three quarter of the total length), when for all other species the opening is terminal (Figs 15–20).

Bagrobdella vanhovei sp. nov. has two small hard parts that have never been reported before in the description of Bagrobdella species. These are: 1) a structure in the shape of a button between the bar and ventral anchors (Fig. 26), 2) a semi-circular structure toping the extremity of the ventral bar median projection (Fig. 25).
Multivariate analyses

Our dataset contained 68 individuals with 21 quantitative variables (Figs 2–7). Among the 68 individuals, three were considered as illustrative (B. auchenoglanii, B. fraudulenta and B. anthopenis). The first two dimensions of the PCA represent 71.8% of the total dataset inertia. Individuals are clustered into three groups which correspond to the three species included in the analysis (Bagrobdella vanhovei sp. nov., B. vansteenbergei sp. nov., and Bagrobdella parauchenoglanii). Only parameters contributing to more than 95 percent for group separation were displayed on the graph (Fig. 28). The dimension 1 opposes most individuals belonging to the group B. vanhovei sp. nov. (to the right of the graph, characterized by a positive coordinate on the axis) to individuals belonging to the groups B. vansteenbergei and B. parauchenoglanii (from Cameroon), to the left of the graph, characterized by a strongly negative coordinate on the axis).

The individuals belonging to the group B. vanhovei sp. nov. (characterized by a positive coordinate on the axis) are sharing: 1) high values for III_IV, DA_d, DB_w, I, VA_b, DA_a, VB_x, VA_a, VA_d and DA_b (variables are sorted from the strongest); 2) low values for VA_e and Pe (variables are sorted from the weakest).

The dimension 2 opposes individuals belonging to B. parauchenoglanii (characterized by a negative coordinate on the axis) are sharing: 1) high values for the variables Pe, VA_c, DA_c and VB_w (variables are sorted from the strongest); 2) low values for the variables DA_b, DB_w, VB_x, cx, VA_a, DA_a, VA_b, DA_e, III_IV and DA_d (variables are sorted from the weakest).

In the group of B. vansteenbergei sp. nov. (characterized by a negative coordinate on the axis) individuals share: 1) high values for the variables VA_e and DA_e (variables are sorted from the strongest); 2) low values for the variables V_a_VII, DA_c, VA_c, IDB_w, DA_d, DV_d, VB_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 B. vansteenbergei sp. nov.

On the graph, B. auchenoglanii and B. anthopenis are isolated from the other species, while B. fraudulenta grouped with B. parauchenoglanii, even so these two latter species being easily distinguishable (at least by their penis morphology) (Figs 15–20).

Figures 21–26. (21) *Bagrobdella vanhovei* sp. nov. microphotograph in toto; (22) *Bagrobdella vansteenbergei* sp. nov. microphotograph in toto; (23) Microphotograph of the trapezoidal plate in the haptor of *Bagrobdella vanhovei*; (24) Button like structure; (25) Semi-circular structure; (26) Egg (*B. vanhovei* sp. nov). Scale bars: 21 = 100 µm, 22 = 200 µm, 23, 24, 26 = 20 µm, 25 = 50 µm.
DISCUSSION

The non-homogeneous distribution in populations of parasite observed in our fish samples is very common (Dold and Holland 2011), for example it has been observed very recently in the Brachyplatystoma vaillantii Valenciennes, 1840 (Siluriformes, Pimelodidae) in the Amazon (Brito-Junior and Tavares-Dias 2021). As mentioned by Tinsley et al. (2020) the factors responsible for the aggregated distribution of a monogenean species on fish are likely linked to the host factors alone, instead of the heterogeneity in host exposition to parasite infestation stages.

As there is sometimes a correlation between water quality and helminth infections – e.g., in the Cyprinid fish Zargar et al. (2012), overdispersion of B. vanhovei sp. nov. and the absence of B. vansteenbergei sp. nov. 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, B. vansteenbergei sp. nov. could be adapted to the conditions found at the Mouth of Mutambala River, while B. vanhovei sp. nov. could be more resistant to variations of abiotic conditions.

Bagrobdella vanhovei sp. nov. and B. vansteenbergei sp. nov. are both new to science and endemic to Lake Tanganyika. However, the four species of Bagrobdella already known have been described in different localities: B. achenoglanii in Ghana (with a redescription based 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. parauchenoglanii in Cameroon (Akoumba et al. 2017).

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

So, among the five Bagrobdella species described from A. occidentalis, three (B. achenoglanii, B. fraudulenta and B. antho-
penis) are found in the watershed of the Nilo-Sudanian province, when the two others new one (B. vanhovei and B. vansteenbergei) are from the Tanganyika province. As a consequence, either these monogenean species are specific of their ichthyofaunal provinces, or we are in the presence of two different host species (the systematic status of A. occidentalis remains under debate – see for example Geerinckx and Vreven (2013)).

Bagrobdella vanhovei sp. nov. shows a high variability in its morphometrical characters (Fig. 28), with small, medium and large measurements for similar hard parts and this causes individuals of this species to oppose on the two axes of the PCA. These differences, apart from a great variability of specimens from a single species, may likely be due to two 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.

Considering that the two new species have a non-spirally coiled MCO, the Bagrobdella diagnosis should be amended, in fact the more recent given by Kritsky and Kulo (1999) indicate that “Copulatory complex a coiled tube with clockwise rings”.

The research on new species of Monogenea from Siluriformes is ongoing in the Lake Tanganyika.

LITERATURE CITED


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**Author 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.

**Competing Interests**
The authors have declared that no competing interests exist.

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