Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: Four new species of Cichlidogyrus from Ophthalmotilapia ventralis (Teleostei: Cichlidae), the first record of this parasite family in the basin

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ABSTRACT. Examination of gill parasites from Ophthalmotilapia ventralis (Boulenger, 1898) in Lake Tanganyika (Africa) revealed the presence of four new species of Monogenea, all members of Cichlidogyrus Pellegrin, 1960 (Ancyrocephalidae). In view of the systematic importance of haptoral structure, the fish host shows a remarkable diversity of morphological groups. Cichlidogyrus vandekerkhovei sp. nov. and C. makasai sp. nov. are especially characterized by the unusual length of the dorsal transverse bar auricles, while C. sturmbaueri sp. nov. is distinguished by the unique shape of the accessory piece of its male copulatory organ. Importantly, C. centesimus sp. nov. displays a number of features new to the genus, namely a spirally coiled thickening at the end of the penis, the absence of an accessory piece in the genital apparatus, and a hitherto unknown uncini configuration in the haptor. This is the first record of ancyrocephalid parasites from the Tanganyika basin. Some mechanisms possibly contributing to this yet unknown diversity are discussed, identifying topics deserving further scientific scrutiny.

KEY WORDS. Cichlidogyrus vandekerkhovei sp. nov.; Cichlidogyrus makasai sp. nov.; Cichlidogyrus centesimus sp. nov.; Cichlidogyrus sturmbaueri sp. nov.; Africa.

Of the three major African Great Lakes (Malawi, Victoria and Tanganyika) Lake Tanganyika is the deepest and the oldest (COHEN et al. 1997). This lake is home to the most morphologically and genetically diverse fauna of Cichlidae in the world (GAUD & METZ 1998, SNOEKS 2000). For non-cichlids, the level of endemicity and the extent to which they evolved into species flocks is highest in this lake (SNOEKS 2000, SALZBURGER et al. 2006). The lake harbours species flocks that result from radiations of substrate and food items. Its 34 species, which are classified in 13 genera, are either biparental or maternal mouth-brooders (KONINGS 1998, TAKAHASHI 2003, KOBLMÜLLER et al. 2004, 2008, and references therein). Problems still exist regarding species identification and their respective taxonomical status (HANSSENS et al. 1999). Among these, Ophthalmotilapia Pellegrin, 1904 is paraphyletic and needs taxonomic revision (KOBLMÜLLER et al. 2004). Ophthalmotilapia ventralis (Boulenger, 1898) and O. heterodon (Poll & Matthes, 1962) form a clade with Cichlidogyrus fuscifer (Boulenger, 1898), while O. boops (Boulenger, 1901) and O. nasuta (Poll & Matthes, 1962) are sister taxa to each other.

However, despite the great number of studies on Cichlidae of Lake Tanganyika, studies on their parasite fauna, especially on their monogenean flatworms, are practically inexistent as in many tropical biomes throughout the world (WHITTINGTON 1998, BAKKE et al. 2002, HUSSY et al. 2006, VANHOVE et al. 2011). However, monogeneans are known to depict a considerable biological diversity – the average number of species per host is more than six in West Africa (PARISELLE et al. 2003b) – and they usually depict high host specificity, parasitizing a single or few closely related host species. The high host specificity of monogenean species has recently been evidenced by the discovery of extensive cryptic speciation in the group (POUYAUD et al. 2006). Further evidence for the expected high host specificity is provided by the demonstration that the monogenean fauna may

So far, most species of Monogenea recorded from cichlids belong to Cichlidogyrus Paperna, 1960, with 71 currently described species (Pariselle & Euzet 2009) mostly from West Africa, but also from South and East Africa and West Asia (Levant). Here, we describe the first representatives of the genus from the Tanganyika basin.

MATERIAL AND METHODS

Fish were caught in April 2008 (Zambia and Tanzania) and April 2010 (Democratic Republic of the Congo (DRC)) using gill or hand nets. Species were determined on site by C. Sturmbauer (Karl-Franzens University of Graz, Austria) and by R. Muzumani (Centre de Recherche en Hydrobiologie, Uvira, DRC). The fishes were dissected, as soon as possible, and the right branchial arches were stored in 96% ethanol for further examination. Fish specimens were numbered, fixed, and preserved in ethanol for subsequent species determination.

Additional parasite specimens were collected from O. ventralis hosts captured in Zambia in April 1995 and retrieved from the Royal Museum for Central Africa collections (MRAC 95-96-P-296-301).

In the laboratory, the gills were transferred into clear water in a Petri dish and the monogeneans were detached from these gills using a strong water jet. The worms were individually transferred onto a slide with a mounted needle, directly into a drop of ammonium picrate-glycerine solution (prepared according to Malmberg 1957), covered with a round cover slip, and sealed with Glyceel (Bates 1997). Some worms from the DRC were mounted between slide and coverslip with Malmberg’s solution in the field. Drawings of the sclerotised pieces of the haptor and of the copulatory complex were made using a Leica DM2500 microscope with a camera lucida and a video camera (Leica DFC320). Measurements were obtained with a Leica Application Software v 3.1 and are presented in micrometers with the mean followed by the range and the number of measurements in parentheses. All measurements follow Gussev (1962) (Fig. 1). The method of numbering the haptoral parts is that recommended in ICOPA IV (see Euzet & Prost 1981); the terminology of structures follows Pariselle & Euzet (1995b). PCA analysis was performed using Statistica v. 9 (StatSoft, Inc. 2009).

Figure 1. Measurements used in this study: (DB) dorsal transverse bar: (h) length of dorsal bar auricle; (w) dorsal bar maximum width; (x) dorsal bar total length; (y) distance between auricles; (A) anchor: (a) anchor total length; (b) anchor blade length; (c) anchor shaft length; (d) anchor guard length; (e) anchor point length; (MA) male apparatus: (Ap) accessory piece length; (Pe) penis total length; (He) heel length; (U) uncinuli length; (VB) ventral transverse bar: (w) ventral bar maximum width; (x) length of one ventral bar branch.
TAXONOMY

All four new species of Monogenea found on the gills of specimens of *O. ventralis* belong to *Cichlidogyrus* Paperna, 1960 (according to Paperna 1960 and Parisellet al. 2003a; it should be noted, however, that the absence of an accessory piece in the male copulatory organ (MCO) of *C. centesimus* sp. nov. might indicate the need for revision of the generic diagnosis). These species are described below.

*Cichlidogyrus vandekerkhovei* sp. nov.

Fig. 2

Type host: *Ophthalmotilapia ventralis* (Boulenger, 1898).
Additional hosts: *O. nasuta* (Poll & Matthes, 1962) and *O. boops* (Boulenger, 1901).

Site: gills.

Type locality: Wonzye Point (8°43’30”S, 31°8’0”E) (Lake Tanganyika, Zambia).

Other records: also found on the type host at Kasenga Point (8°43’31”S, 31°8’1”E) (Zambia) and at Kikoti (7°11’27”S, 30°4’0”E) (Democratic Republic of the Congo), and on *O. nasuta* and *O. boops* at Mtosi (7°35’27”S, 30°38’29”E) (Tanzania) (all in Lake Tanganyika).

Material studied: 20 individuals.


Description: Body 254 (163-355, n = 20) long, 83 (69-112, n = 20) wide at mid-body. Pharynx is 23 (17-29, n = 9) wide. Dorsal anchor with very short shaft and more pronounced guard, blade arched, slightly bent in the middle: a = 26 (23-28, n = 28), b = 21 (19-23, n = 25), c = 2 (1-4, n = 25), d = 8 (5-10, n = 25), e = 11 (9-12, n = 28). Dorsal transverse bar straight or slightly arched, small, with extremely long auricles: x = 23 (20-27, n = 20), y = 4 (2-6, n = 20), w = 4 (3-5, n = 20), h = 30 (24-34, n = 36). Ventral anchor, with same shape as dorsal anchor: a = 25 (22-29, n = 27), b = 20 (17-26, n = 27), c = 2 (1-5, n = 27), d = 7 (6-10, n = 27), e = 10 (9-13, n = 27). Ventral transverse bar V-shaped, often twisted: x = 31 (26-35, n = 20), w = 4 (3-5, n = 20). Uncini I small (see Remarks below) = 12 (11-14, n = 10) long; II = 11 (9-
groups, according to the length of their uncinuli (POUYAUD
et al. 1982; Paperna, 1960; Paperna & Euzet, 1964; Pariselle & Euzet, 1994; Birgi & Euzet, 1983; Paperna & Thurston, 1969; Paperna & Euzet, 1990; Pariselle, Bilong Bilong & Euzet, 2003; Pariselle & Euzet, 1995). Indeed, Scutogyrus spp. have a characteristiclly enlarged dorsal bar and a thin oval plate associated to the ventral bar (Pariselle & Euzet 1995a), which are not present in C. vandekerkhovei sp. nov.

Remarks. Despite the presence of very long auricles on the dorsal bar, similar in size to the auricles of species of Scutogyrus Pariselle & Euzet, 1995 (see Fig. 3), C. vandekerkhovei sp. nov. is considered a representative of Cichlidogyrus. Indeed, Scutogyrus spp. have a characteristically large uncinuli.

13, n = 15) long; III to VII short (see Remarks below) = 16 (13-23, n = 67) long. Penis, beginning in a large spherical bulb with well-developed heel, He = 5 (4-6, n = 20), a short and large curved tube of which the diameter narrows abruptly at the distal extremity: Pe = 34 (30-37, n = 20), simple and straight accessory piece with forked end, one extremity shorter than the other, sometimes crossed: Ap = 31 (24-34, n = 20). Vagina not observed.

The specific epithet of the new species, vandekerkhovei, honours the aquatic ecologist Dr. Jochen Vandekerkhove (Belgium), in recognition of his guidance during the early research years of the junior author.

Cichlidogyrus makasai sp. nov.

Fig. 4

Type host: Ophthalmotilapia ventralis (Boulenger, 1898). Additional hosts: O. nasuta (Poll & Matthes, 1962) and O. boops (Boulenger, 1901). Site: gills.

Type locality: Wonzye Point (8°43′30″S, 31°8′0″E) (Lake Tanganyika, Zambia).

Other records: also found on the type host at Kasenga Point (8°43′31″S, 31°8′1″E) (Zambia) and at Kikoti (7°11′27″S, 30°4′0″E) (Democratic Republic of the Congo), and on O. nasuta and O. boops at Mtosi (7°35′27″S, 30°38′29″E) (Tanzania) (all in Lake Tanganyika).

Material studied: 15 individuals.


Description: Body 265 (211-321, n = 15) long, 78 (62-109, n = 15) wide at mid-body. Pharynx is 26 (17-34, n = 9) wide. Dorsal anchor with very short shaft and more pronounced guard, blade arched: a = 21 (19-23, n = 21), b = 19 (17-20, n = 21), c = 2 (1-2, n = 21), d = 6 (4-8, n = 21), e = 9 (8-10, n = 21). Dorsal transverse bar straight or slightly arched, small, with very long auricles: x = 25 (24-28, n = 15), y = 5 (4-7, n = 13), w = 4 (3-5, n = 15), h = 20 (17-23, n = 27). Ventral anchor, same size and shape as dorsal anchor: a = 21 (20-22, n = 21), b = 18 (17-20, n = 22), c = 1 (1-3, n = 22), d = 6 (5-8, n = 22), e = 8 (7-10, n = 22). Ventral transverse bar V-shaped: x = 29 (25-33, n = 27), w = 3 (2-4, n = 14). Uncini I small (see Remarks above) = 11 (9-12, n = 21) long; II = 10 (9-15, n = 18) long; III to VII short (see Remarks below) = 14 (11-17, n = 82) long. Penis beginning in a spherical bulb with pronounced heel, He = 3 (2-4 n = 15), a thin curved tube which tapers distally: Pe = 73 (69-79, n = 15), simple accessory piece slightly bent at distal third, resembles a spanner: Ap = 22 (20-25, n = 15). Vagina not observed.

Remarks. Cichlidogyrus makasai sp. nov. belongs to the same morphological group of C. vandekerkhovei sp. nov. It can be easily distinguished from the latter species by the relatively longer auricles on the dorsal bar (smaller in C. makasai sp. nov. than in C. vandekerkhovei sp. nov.); the dorsal bar auricles are significantly longer than those of all other Cichlidogyrus spp. and shorter than those of Scutogyrus spp. (see Fig. 3).
**Cichlidogyrus makasai** sp. nov. is named after the research assistant Mr. Lawrence Makasa (Zambia), for his participation and contribution to the field expedition in Zambia and Tanzania during which most of the specimens for this study were collected.

**Cichlidogyrus centesimus** sp. nov.

Type host: *Ophthalmotilapia ventralis* (Boulenger, 1898).

Additional hosts: *O. nasuta* (Poll & Matthes, 1962) and *O. boops* (Boulenger, 1901).

Site: gills.

Type locality: Wonzye Point (8°43'30"S, 31°8'0"E) (Lake Tanganyika, Zambia).

Other records: also found on the type host at Kasenga Point (8°43'31"S, 31°8'1"E) (Zambia) and at Kikoti (7°11'27"S, 30°4'0"E) (Democratic Republic of the Congo), and on *O. nasuta* and *O. boops* at Mtosi (7°35'27"S, 30°38'29"E) (Tanzania) (all in Lake Tanganyika).

Material studied: 18 individuals.


Description: Body 379 (263-520, n = 16) long, 90 (59-147, n = 15) wide at mid-body. Pharynx is 30 (17-45, n = 8) wide. Large dorsal anchor with pronounced shaft and very long guard, blade arched: a = 48 (41-55, n = 31), b = 31 (26-38, n = 30), c = 5 (3-10, n = 30), d = 19 (13-24, n = 30), e = 9 (7-12, n = 31). Thick dorsal transverse bar slightly arched: x = 45 (37-52, n = 17), y = 19 (16-22, n = 17), w = 8 (7-10, n = 17), h = 13 (11-16, n = 34). Large ventral anchor, with different shape and smaller size as compared to dorsal anchor, with well-developed guard and shaft,

Figure 4. *Cichlidogyrus makasai* sp. nov.: (Ap) accessory piece; (DB) dorsal transverse bar; (DA) dorsal anchor; (He) heel; (MA) male apparatus; (Pe) penis; (VB) ventral transverse bar; (VA) ventral anchor; (I-VII) uncinuli. Scale bar = 30 µm.
blade regularly arched: \(a = 38 (34-44, n = 29)\), \(b = 36 (32-41, n = 29)\), \(c = 5 (3-10, n = 29)\), \(d = 10 (5-14, n = 29)\), \(e = 11 (9-13, n = 29)\). Thick, long and V-shaped ventral transverse bar: \(x = 41 (22-49, n = 35)\), \(w = 6 (4-7, n = 18)\). Uncinuli I very large = 32 (26-37, \(n = 35\)) long; II with larval size and shape II = 11 (10-12, \(n = 24\)) long; III to VII long (see Remarks below) = 24 (22-30, \(n = 135\)) long. Penis beginning in an ovoid bulb, with a straight and very long heel which is variable in length, \(He = 24 (11-47 n = 17)\), short and straight, the distal external wall marked with spirally coiled thickening forming 4/5 turns: \(Pe = 24 (23-25, n = 17)\), no accessory piece associated with the penis. Vagina not observed.

Remarks. The haptor of *C. centesimus* sp. nov. is unique because it shows long uncinuli I and III to VII (like *C. arthracanthus* Paperna, 1960 and *C. inconsultans* Birgi & Lambert, 1986 (see Pariselle & Euzet 2009). However, compared to these two species the pair I of uncinuli of the new species is larger and thicker, characteristics known only for species with short uncinuli pair III to VII such as *C. albaretii* Pariselle & Euzet, 1998; *C. arfii* Pariselle & Euzet, 1995; *C. berradai* Pariselle & Euzet, 2003; *C. bychowskii* Markevich, 1934; *C. dugeti* Dossou & Birgi, 1984; *C. digitatus* Dossou, 1982; *C. dionchus* Paperna, 1968; *C. euzeti* Dossou & Birgi, 1984; *C. falcifer* Dossou & Birgi, 1984; *C. halimus* Paperna, 1969; *C. longicirrus* Paperna, 1965; *C. mandidiae* Birgi & Lambert, 1986; *C. nuniezii* Pariselle & Euzet, 1998; *C. papernastrema* Price, Peebles & Bamford, 1969; *C. philander* Douëllou, 1993; *C. quaestio* Douëllou, 1993; *C. reversei* Pariselle & Euzet, 2003; *C. sanseoi* Pariselle & Euzet, 2004; *C. yanni* Pariselle & Euzet, 1996.

The male reproductive apparatus of *C. centesimus* sp. nov. is unique within *Cichlidogyrus* because 1) it lacks the accessory piece which is associated with the penis of all other *Cichlidogyrus* spp. known; 2) its penis displays a spirally coiled thickening (a feature observed in other Monogenea species such as *Thaparocleidus chandpuri* Pariselle, Lim & Lambert, 2001).

**Figure 5. Cichlidogyrus centesimus** sp. nov.: (DB) dorsal transverse bar; (DA) dorsal anchor; (He) heel; (MA-a) male apparatus, with long heel; (MA-b) male apparatus, with short heel; (Pe) penis; (VB) ventral transverse bar; (VA) ventral anchor; (I-VII) uncinuli. Scale bar = 30 µm.
The length of the heel of the MCO is highly variable in *C. centesimus* but Principal Component Analysis (PCA) on all morphometric data detected no differences to warrant species delimitation (Fig. 6).

The specific name of *C. centesimus* (Latin for "one hundredth") refers to the fact that the species represents the one-hundredth species described by the senior author (A.P.).

![Figure 6. PCA (first three axes) on all measurements of *C. centesimus* sp. nov. showing no clusters.](image)

**Cichlidogyrus sturmbaueri** sp. nov.  
Fig. 7

**Type host:** *Ophthalmotilapia ventralis* (Boulenger, 1898).  
**Additional host:** *O. nasuta* (Poll & Matthes, 1962).  
**Site:** gills.  
**Type locality:** Wonzye Point (8°43’30"S, 31°8’0"E) (Lake Tanganyika, Zambia).

Other records: also found on the type host at Kasenga Point (8°43’31"S, 31°8’1"E) (Zambia) and on *O. nasuta* at Musamba (7°49’54"S, 30°56’49"E) (Tanzania) (all in Lake Tanganyika).

**Material studied:** 8 individuals.


**Description:** Body 311 (259-366, n = 7) long, 83 (63-115, n = 7) wide at mid-body. Pharynx is 27 (17-42, n = 6) wide. Dorsal anchor small with pronounced shaft and guard: a = 20 (19-21, n = 12), b = 17 (16-20, n = 12), c = 3 (2-3, n = 12), d = 7 (5-7, n = 12), e = 8 (6-9, n = 12). Dorsal transverse bar thin and arched: x = 23 (20-28, n = 8), y = 10 (9-11, n = 8), w = 4 (3-4, n = 8), h = 13 (12-15, n = 16). Ventral anchor similar to dorsal anchor, but slightly larger: a = 24 (23-26, n = 12), b = 20 (18-21, n = 12), c = 3 (2-5, n = 12), d = 9 (7-10, n = 12), e = 8 (7-11, n = 12). V-shaped and thin ventral transverse bar: x = 34 (31-39, n = 16), w = 4 (3-4, n = 8). Uncini i short = 14 (13-16, n = 12) long; II with larval size and shape II = 11 (10-12, n = 7) long; III to VII long = 22 (19-24, n = 23) long. Penis beginning in an ovoid bulb, with short heel, He = 6 (4-7 n = 8), straight: Pe = 36 (34-39, n = 8), h-shaped accessory piece associated with the penis, Ap = 25 (24-28). Vagina not observed.

**Remarks.** The haptoral sclerites of *C. sturmbaueri* sp. nov. belongs to the group with short uncini I and long uncini II to VII such as *C. aegypticus* Ergens, 1981; *C. agnesi* Pariselle & Euzet, 1994; *C. anthemocolpos* Dossou, 1982; *C. bilongi* Pariselle & Euzet, 1996; *C. bonhommei* Pariselle & Euzet, 1998; *C. bouvii* Pariselle & Euzet, 1997; *C. dossoi* Doullou, 1993; *C. dovellouae* Pariselle, Bilong Bilong & Euzet, 2003; *C. ergensi* Dossou, 1982; *C. flexicolpos* Pariselle & Euzet, 1995; *C. gallus* Pariselle & Euzet, 1995; *C. guirali* Pariselle & Euzet, 1997; *C. heni* Pariselle & Euzet, 1998; *C. kouassi* N’Doubou, Thys van den Audenaerde & Pariselle, 1997; *C. legendrei* Pariselle & Euzet, 2003; *C. lemoallei* Pariselle & Euzet, 2003; *C. microscutus* Pariselle & Euzet, 1996; *C. ouedraogoi* Pariselle & Euzet, 1996; *C. paganoi* Pariselle & Euzet, 1997; *C. testificatus* Dossou, 1982; *C. thurstonae* Ergens, 1981; *C. tiberianus* Paperna, 1960 and *C. vexus* Pariselle & Euzet, 1995. *Cichlidogyrus sturmbaueri* sp. nov. is unique since it exhibits a h-shaped accessory piece.

The specific epithet, *sturmbaueri*, is given in honour of Prof. Christian Sturmbauer (Austria), specialist in the evolution of Tanganyika cichlids and team leader of the expedition in Zambia and Tanzania during which most of the host fish used in this study was caught.

**DISCUSSION**

Four new representatives of *Cichlidogyrus*, *C. vandekerkhovei* sp. nov., *C. makasai* sp. nov., *C. centesimus* sp. nov. and *C. sturmbaueri* sp. nov. are described from *Ophthalmotilapia ventralis*. The host species are members of the endemic Tanganyika cichlid tribe Ectodini (Poll, 1986). Although ancyrocephalids are known to be a speciose lineage of parasites of cichlids (Pariselle & Euzet 2003), they have already established diversity hotspots of Cichlidae (see Snooks 2000), these descriptions represent the first records of Ancyrocephalidae and only the second of Monogenea in this basin (Vanhove et al. 2011).

The four species described herein belong to three different morphological groups of *Cichlidogyrus* (Pariselle & Euzet 2003, 2009, Vignon et al. 2011). *Cichlidogyrus centesimus* sp. nov. displays a hitherto unknown combination of characters (and the absence of an accessory piece in the MCO could even indicate the need to revise the generic diagnosis). Hence, based on haptor characteristics, the studied species of *Ophthalmotilapia*.
are host to a remarkable diversity of *Cichlidogyrus* – not in terms of species richness, but in terms of the various morphological groups within *Cichlidogyrus* co-occurring on one individual host species. Pouyaud et al. (2006) suggest that the haptor characteristics are phylogenetically informative to distinguish groups of *Cichlidogyrus* spp., while the genital morphology is more appropriate for species identification.

In view of the current lack of genetic data and comparative material of *Cichlidogyrus* spp. from the Tanganyika basin, it is impossible to estimate to what extent the current diversity in haptor morphology actually represents the presence of distinct lineages of Monogenea. Thus, it is not yet appropriate for species identification.

Furthermore, the unique ecosystem characteristics of Lake Tanganyika also offer opportunities for a high richness of fish parasites. For instance, ecological stability and depth of the lake facilitate its role as an evolutionary reservoir, such as observed for thalassoid gastropods (Wilson et al. 2004) and cichlids (Nishida 1991, Salzburger et al. 2002). Therefore, the monogenean community might, partly, consist of ancient lineages as well. Furthermore, Kohlmüller et al. (2006) suggest that the divergence of hosts belonging to the Tropheini influenced differentiation and led to an accelerated rate of molecular evolution of the brood parasite *Synodontis multipunctata* Boulenger, 1898 (cuckoo catfish) from Lake Tanganyika. Hence, the role of the radiation of Cichlidae in the (co-)evolutionary dynamics of species of *Cichlidogyrus* should also be considered.

Finally, we want to indicate that the species under study, *O. ventralis*, apparently shares several parasite species with its congeners, *O. boops* and *O. nasuta*. These species of *Ophthalmotilapia*, although, do not compose a monophyletic group (Kohlmüller et al. 2004). An apparently broad host spectrum in *Cichlidogyrus* or other representatives of the Monogenea may be the result of cryptic speciation (Zeitara & Lumme 2002, Huyse & Malmberg 2004, Pouyaud et al. 2006, Kuusela et al. 2008). Consequently, studies on the new parasite species, based on molecular
data, are needed, not only to estimate the extent to which the
distinct haptor morphology reflects the existence of various lin-
eages but also to check for possible cryptic speciation leading to
an underestimated host-specificity. Furthermore, parasite data
from other cichlids in the Tanganyika basin are necessary to
formulate hypotheses on the origin and evolutionary history of
this seemingly very diverse fauna of monogenean flatworms.

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