

Redescription of *Cichlidogyrus tiberianus* Paperna, 1960 and *C. dossoui* Douëllou, 1993 (Monogenea: Ancyrocephalidae), with special reference to the male copulatory organ

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Abstract The flatworms of the genus *Cichlidogyrus* Paperna, 1960 (Monogenea: Ancyrocephalidae) are gill parasites of freshwater fish, affecting predominantly the family Cichlidae. *Cichlidogyrus tiberianus* Paperna, 1960 and *Cichlidogyrus dossoui* Douëllou, 1993 are among the most widely distributed species of the genus, occurring in several African river basins and infecting many different host species, including the economically important Nile tilapia *Oreochromis niloticus* (Linnaeus) and redbreast tilapia *Coptodon rendalli* (Boulenger). Despite their wide distribution, *C. tiberianus* and *C. dossoui* have so far been studied only by light microscopy. In this paper they are

redescribed on the basis of scanning electron microscopy of newly-collected material. The new material was obtained from redbreast tilapia caught in the Luapula River (D. R. Congo). The haptor sclerites and genitalia are redescribed and illustrated in detail. Special attention is given to the complex morphology of the male copulatory organ.

Introduction

Cichlidogyrus Paperna, 1960 is a diverse genus of monogenean gill parasites, comprising more than 100 nominal species. They parasitize a wide range of cichlid fishes, including the economically important Nile tilapia *Oreochromis niloticus* (Linnaeus) and redbreast tilapia *Coptodon rendalli* (Boulenger) (Pariselle & Euzet, 2009). Originally restricted to the rivers and lakes of Africa and the Levant, *Cichlidogyrus* now occurs in tilapia farms worldwide (e.g. Lopez, 1991; Lizama et al., 2007; Aguirre-Fey et al., 2015) and has also become established in the wild in several Asian and American countries (e.g. Jiménez-García et al., 2001; Maneepitaksanti & Nagasawa, 2012). Fish infected with *Cichlidogyrus* often show hyperplasia of the gill lamellae (Roberts & Somerville, 1982) and heavy infections may result in death, especially in young fish (Kabata, 1985).

The wide distribution of these parasites has prompted research into their control and management

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(e.g. Shaharom-Harrison, 1987; Dotta et al., 2015) as well as more basic research into their biology and evolution. For example, in recent years, molecular studies have shed some light on the phylogenetic relationships within the genus (e.g. Pouyaud et al., 2006; Řehulková et al., 2013). Progress has also been made in other areas, such as the study of the co-evolutionary relationships between *Cichlidogyrus* species and their hosts (Mendlová et al., 2012; Vanhove et al., 2015) and the morphological evolution of the haptor, the attachment organ that anchors the parasite to the gills (Vignon et al., 2011; Messu Mandeng et al., 2015).

Yet, in spite of these important advances, many aspects of the taxonomy and evolution of *Cichlidogyrus* remain only superficially known. A case in point is our limited understanding of the remarkable genital morphology of the genus. In *Cichlidogyrus* the male copulatory organ (MCO) shows great variation in shape, ranging from the relatively simple, needle-like MCO of *C. centesimus* Vanhove, Volckaert & Pariselle, 2011 to the very complex, spiralling MCO of *C. sanseoi* Pariselle & Euzet, 2004 (see Pariselle & Euzet, 2004; Vanhove et al., 2011). The female genitalia also display a high degree of variation (Pariselle & Euzet, 2009). Unfortunately, the vast majority of *Cichlidogyrus* spp. has been studied only as whole-mounts under a compound microscope, impeding a detailed analysis of the genitalia. Obviously, the routine use of higher-resolution techniques such as scanning electron microscopy (SEM) or histology would shed more light on the genital morphology and reproductive biology of *Cichlidogyrus*.

Here we focus on *C. tiberianus* Paperna, 1960 and *C. dossoui* Douëllou, 1993. These species are among the most widely distributed of the genus. *Cichlidogyrus tiberianus* has been reported from 15 different host species in 13 countries, including Israel, Egypt, several countries in western, central, and southern Africa, and the Philippines (Paperna, 1960; Pariselle & Euzet, 2009; and references given below). *Cichlidogyrus dossoui* also has a broad geographical and host range, occurring on nine different cichlid species in Zimbabwe, Zambia, South Africa, Cameroon, Mexico and Panama (Douëllou, 1993; Pariselle & Euzet, 2009; and references given below). The two species often co-occur on the same host individual (pers. obs.; Vanhove et al., 2013) and as such are good candidates to study competition and niche

differentiation in *Cichlidogyrus*. Unfortunately, such studies would be hampered by the current scarcity of morphological data. *Cichlidogyrus tiberianus* and *C. dossoui* have so far been studied only by light microscopy (Paperna, 1960; Ergens, 1981; Dossou, 1982; Lopez, 1991; Douëllou, 1993), and their genitalia and haptors have been illustrated only by line drawings, with the exception of two low-magnification photographs of *C. tiberianus* published by Lopez (1991). Furthermore, the type-material of *C. tiberianus* is missing and presumably lost (S. Rothman, pers. commun.). The lack of detailed morphological data could easily lead to misidentifications, because the two species differ in only a few characters. Thus, there is a clear need for additional taxonomical and morphological studies.

In the present paper *C. tiberianus* and *C. dossoui* are redescribed based on recent material from the D. R. Congo. For each species we present an overview of previous records, a new diagnosis and more than 30 SEM photographs illustrating the sclerites and genitalia. The complex morphology of the MCO is described and discussed in detail, with special attention being given to the asymmetry and chirality (handedness) of this organ.

Materials and methods

Two specimens of *Coptodon rendalli* were caught in the Luapula River off Kashobwe (9°40'16"S, 28°37'20"E, former province of Katanga, Democratic Republic of the Congo, September 2014). Both fish measured 166 mm in total length; their standard length was 125 mm and 132 mm, respectively. They were sacrificed using an overdose of tricaine methanesulfonate (MS222) and their right gills were dissected, fixed and stored in pure ethanol. The fish were deposited in the ichthyological collection of the Royal Museum for Central Africa (RMCA-MRAC). Their gills were screened under a Leica ES2 stereomicroscope and monogeneans were removed using fine dissecting needles. The worms were then subjected to proteinase K digestion and processed for SEM (Fannes et al., 2015). Measurements were taken from SEM-photographs using the software tpsDig version 2.17 (Rohlf, 2013); the metrics taken are shown in Fig. 1. The measurement of the accessory piece length follows Douëllou (1993). The measurements are

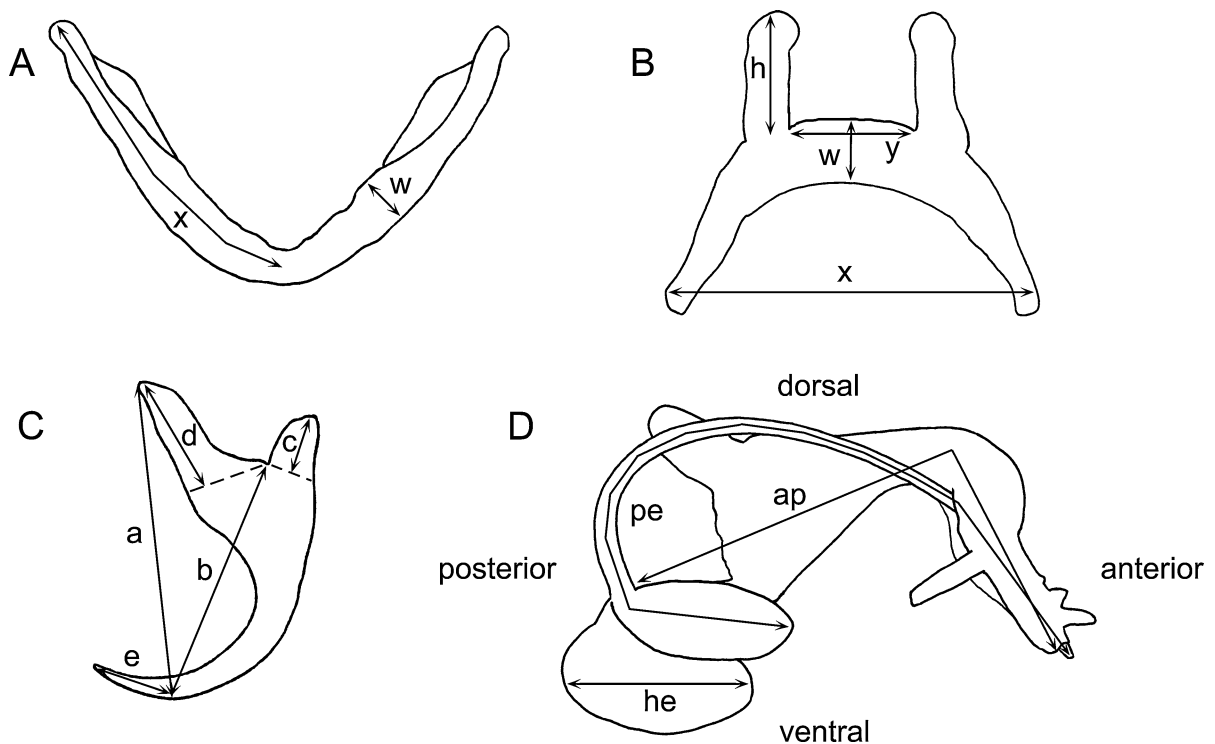


Fig. 1 Measurements used in this study. A, Ventral transverse bar (x, length of one ventral bar branch; w, ventral bar maximum width); B, Dorsal transverse bar (x, dorsal bar total length; w, dorsal bar maximum width; h, length of dorsal bar auricle; y, distance between auricles); C, Anchor (a, anchor total length; b, anchor blade length; c, anchor shaft length; d, anchor guard length; e, anchor point length); D, Male copulatory organ (ap, accessory piece length; pe, penis length; he, heel length)

expressed in micrometres and given as the range followed by the mean and number of measurements in parentheses. The SEM stubs have been deposited in the RMCA invertebrate SEM collection (codes S-11, S-12).

Host nomenclature follows Eschmeyer & Fricke (2015). In order to facilitate the description of the MCO we introduce a directional terminology in which the tip, tube and bulb of the penis are considered to mark the anterior, dorsal and posterior parts of the MCO, respectively (Fig. 1D). The definition of anteroposterior and dorsoventral axes also allows us to define a left and right side for the MCO, necessary to describe its asymmetry. Evidently, this terminology is introduced purely for convenience and does not necessarily reflect the true anatomical positions.

High-resolution versions of the published images, as well as numerous additional images, have been uploaded to MorphBank (www.morphbank.net). These images, which can each be enlarged several times before pixelating, are made available in order to

illustrate the intraspecific variation, and to allow other researchers to re-examine and re-interpret our data. The images of *C. tiberianus* can be found in Morphbank collection 859781; those of *C. dossoui* in collection 859782.

Family Ancyrocephalidae Bychowsky, 1937
Genus *Cichlidogyrus* Paperna, 1960

***Cichlidogyrus tiberianus* Paperna, 1960**

Type-host: *Coptodon zillii* (Gervais) (Cichlidae).

Type-locality: Sea of Galilee, Israel.

Records: All from Cichlidae. Israel: *C. zillii*, *Astatotilapia flavijosephi* (Lortet), *Tristramella simonis* (Günther), *Tristramella sacra* (Günther) (in latter two species experimental infection only) (see Paperna, 1960, 1964); Egypt: *C. zillii*, *Oreochromis niloticus* (Linnaeus) (see Ergens, 1981; Hagraas et al., 2000; Ibrahim & Soliman, 2011; Soliman & Ibrahim, 2011; Ibrahim, 2012); Senegal, Guinea, Ivory Coast and D.

R. Congo: *C. zillii*, *Coptodon rendalli* (Boulenger), *Coptodon guineensis* (Günther), *Coptodon coffea* (Thys van den Audenaerde), *Coptodon dageti* (Thys van den Audenaerde), *Coptodon walteri* (Thys van den Audenaerde) (see Pariselle & Euzet, 1995, 1996, 2009; N'Douba et al., 1997; Pouyaud et al., 2006; Mendlová et al., 2012; present study); Ghana: *C. zillii* (see Paperna, 1965, 1969); Benin: *C. zillii* (see Dossou, 1982); Cameroon: *C. guineensis*, *Coptodon kottae* (Lönnberg), *Coptodon gutturosa* (Stiassny, Schlieuwen & Dominey), *Coptodon bakossiorum* (Stiassny, Schlieuwen & Dominey), *Pelmatolapia mariae* (Boulenger) (see Pariselle et al., 2013); Uganda: *C. zillii*, *C. rendalli* (see Paperna & Thurston, 1969; Thurston, 1970; Paperna, 1979); Zambia: *C. rendalli*, *Tilapia sparrmanii* Smith (see Vanhove et al., 2013); Zimbabwe: *C. rendalli* (see Douëllou, 1993); Philippines: *C. zillii*, *O. niloticus* (see Natividad et al., 1986; Bondad-Reantaso & Arthur, 1990; Lopez, 1991). The records from *O. niloticus* may be misidentifications and may in fact refer to *C. thurstonae* Ergens, 1981 (see Pariselle & Euzet, 2009 and references therein).

Material studied: 8 individuals (RMCA invertebrate SEM collection, S-11) taken from the gills of two specimens of *C. rendalli* (MRAC Vert-2015.014.P.00001, 00002) caught in the Luapula River off Kashobwe, D. R. Congo (9°40'16"S, 28°37'20"E, 7.ix.2014, water temperature 22°C).

Redescription (Figs. 2, 3)

Haptoral sclerites (Fig. 2A–H). Dorsal anchors with well-developed shaft and guard (Fig. 2C–E); total length 23–25 (24, n = 3); blade length 18–20 (19, n = 3); shaft length 5–7 (6, n = 3); guard length 9–11 (10, n = 3); point length 7–10 (8, n = 3). Dorsal bar with concave and convex side; concave side showing narrow groove between auricle bases (Fig. 2A); very small, round structures present near base of each auricle in some specimens (Fig. 2A); dorsal bar total length 24–25 (25, n = 2); maximum width 4–4 (4, n = 2); length of auricle 7–8 (8, n = 2); distance between auricles 8–9 (8, n = 2). Ventral anchors with relatively short shaft and guard (Fig. 2F–H), clearly distinct in shape from dorsal anchors; total length 34–35 (34, n = 4); blade length 33–34 (33, n = 4); shaft length 5–6 (6, n = 4); guard length 7–9 (8, n = 4); point length 12–12 (12, n = 4). Ventral bar: length of one ventral bar branch 23–23 (23, n = 2); maximum width 3–3 (3, n = 2).

Genitalia (Fig. 3A–F). Penis consisting of ovoid bulb and curved, gradually tapering tube; length 57–60 (59, n = 3). Accessory piece relatively large, with length 36–39 (37, n = 6). Proximal part of accessory piece situated on left side of MCO, relatively broad in shape, with prominent extension (here called the proximal extension; Fig. 3A, C). Near middle of accessory piece a ridge-like structure that continues in a conspicuous, approximately 5 µm long extension (here called the middle extension; Fig. 3A, B, D). Distal part of accessory piece shaped like inverted groove (Fig. 3B, D). Terminal part of penis tube in this groove and thus not visible (but in some specimens tip of penis protruding out of groove; Fig. 3B). Terminal margin of groove showing numerous denticles; most denticles small and triangular but some (in particular dorsalmost ones) larger and more elongated, exhibiting a finger- or spine-like shape (Fig. 3D). Heel rounded, more or less oval in shape, with length 10–14 (12, n = 6). Left surface of heel appearing slightly convex (Fig. 3A, E), right surface appearing concave (Fig. 3C). Heel connecting with accessory piece via narrow, strap-like structures (Fig. 3E). Vagina tube-shaped, usually forming a coil. Narrow, bar-like structure associated with tube (Fig. 3F; the bar may be the sclerotised edge of the vaginal pore).

Remarks

The combined presence of the following features distinguishes *C. tiberianus* from all other members of the genus: uncinuli I short (*sensu* Pariselle & Euzet, 2003, 2009); III to VII long (*sensu* Pariselle & Euzet, 2003, 2009); ventral anchors with short shaft and guard (Fig. 2F–H), clearly distinct in shape from dorsal anchors; vagina a narrow tube, usually with a coil (Fig. 3F); penis with ovoid bulb and long, curved tube (Fig. 3A, C); accessory piece as in Fig. 3A–D, with well-developed proximal extension and long middle extension. It can be readily distinguished from *C. dossoui* by the longer middle extension, the slightly narrower, coiled vagina and the ventral anchors with short shaft and guard.

Cichlidogyrus dossoui Douëllou, 1993

Type-host: *Coptodon rendalli* (Boulenger) (Cichlidae).

Type-locality: Lake Kariba, Zimbabwe.

Records: All from Cichlidae. Zimbabwe: *C. rendalli*, *Oreochromis mortimeri* (Trewavas), *Serranochromis macrocephalus* (Boulenger) (see Douëllou, 1993);

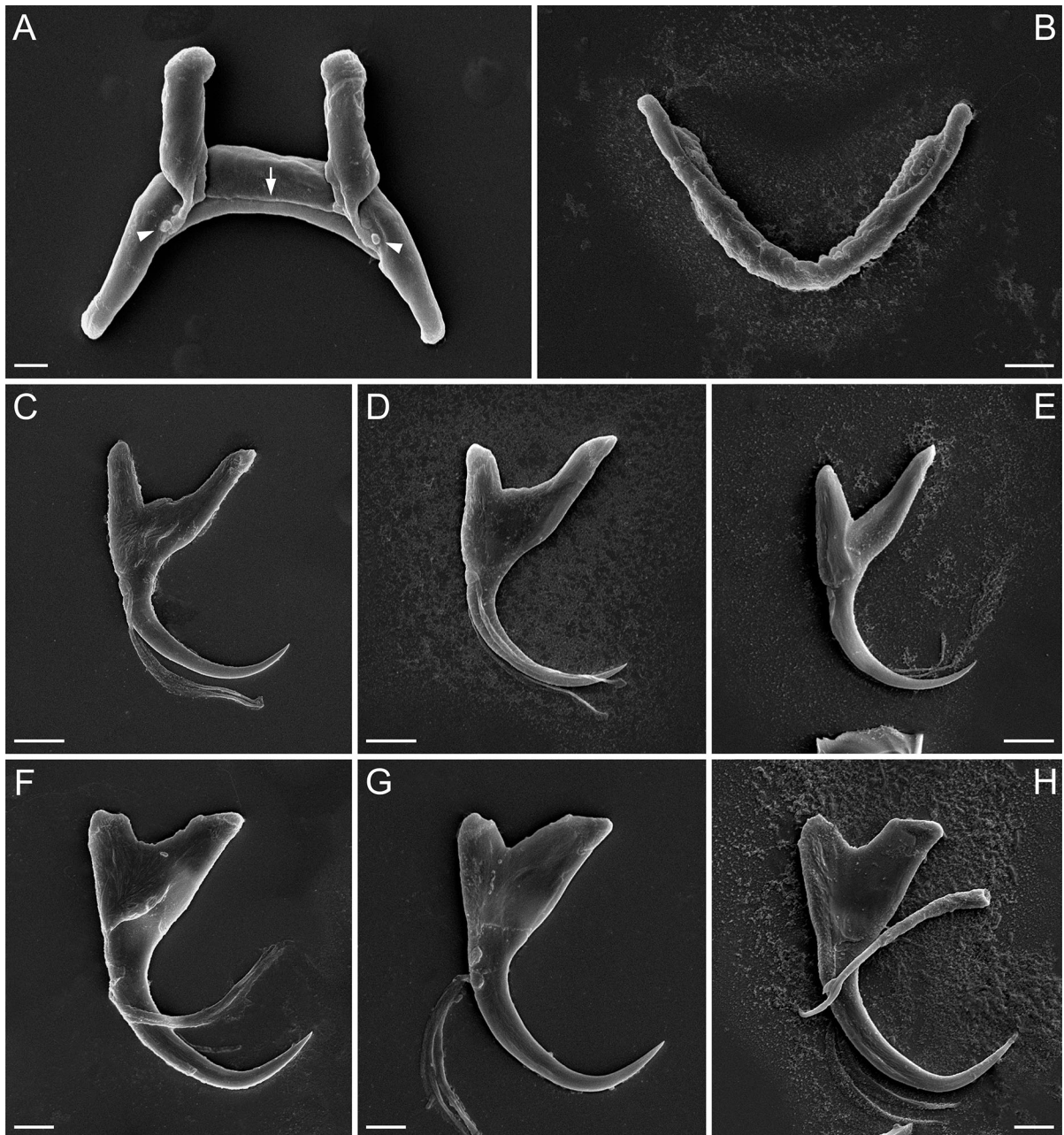


Fig. 2 Haptoral sclerites of *Cichlidogyrus tiberianus* Paperna, 1960. A, Dorsal bar, concave surface (arrow: groove; arrowheads: round structures); B, Ventral bar; C–E, Dorsal anchors of different specimens; F–H, Ventral anchors of different specimens (note uncinulus in H). D–F have been flipped horizontally for consistency with other figures. Scale-bars: A, 2 μm ; B–H, 5 μm

South Africa: *Oreochromis mossambicus* (Peters) (see Madanire-Moyo et al., 2011, 2012); Zambia: *Tilapia sparrmanii* Smith, *C. rendalli* (see Vanhove et al., 2013); D. R. Congo: *C. rendalli* (present study); Cameroon: *Coptodon guineensis* (Günther), *Coptodon camerunensis* (Lönnberg) (see Pariselle et al., 2013);

Mexico: *Oreochromis niloticus* (Linnaeus), *Oreochromis aureus* (Steindachner), *O. mossambicus* (see López-Jiménez, 2001; Salgado-Maldonado & Rubio-Godoy, 2014 and references therein; Aguirre-Fey et al., 2015; Paredes-Trujillo et al., 2016); Panama: *O. niloticus* (see Roche et al., 2010).

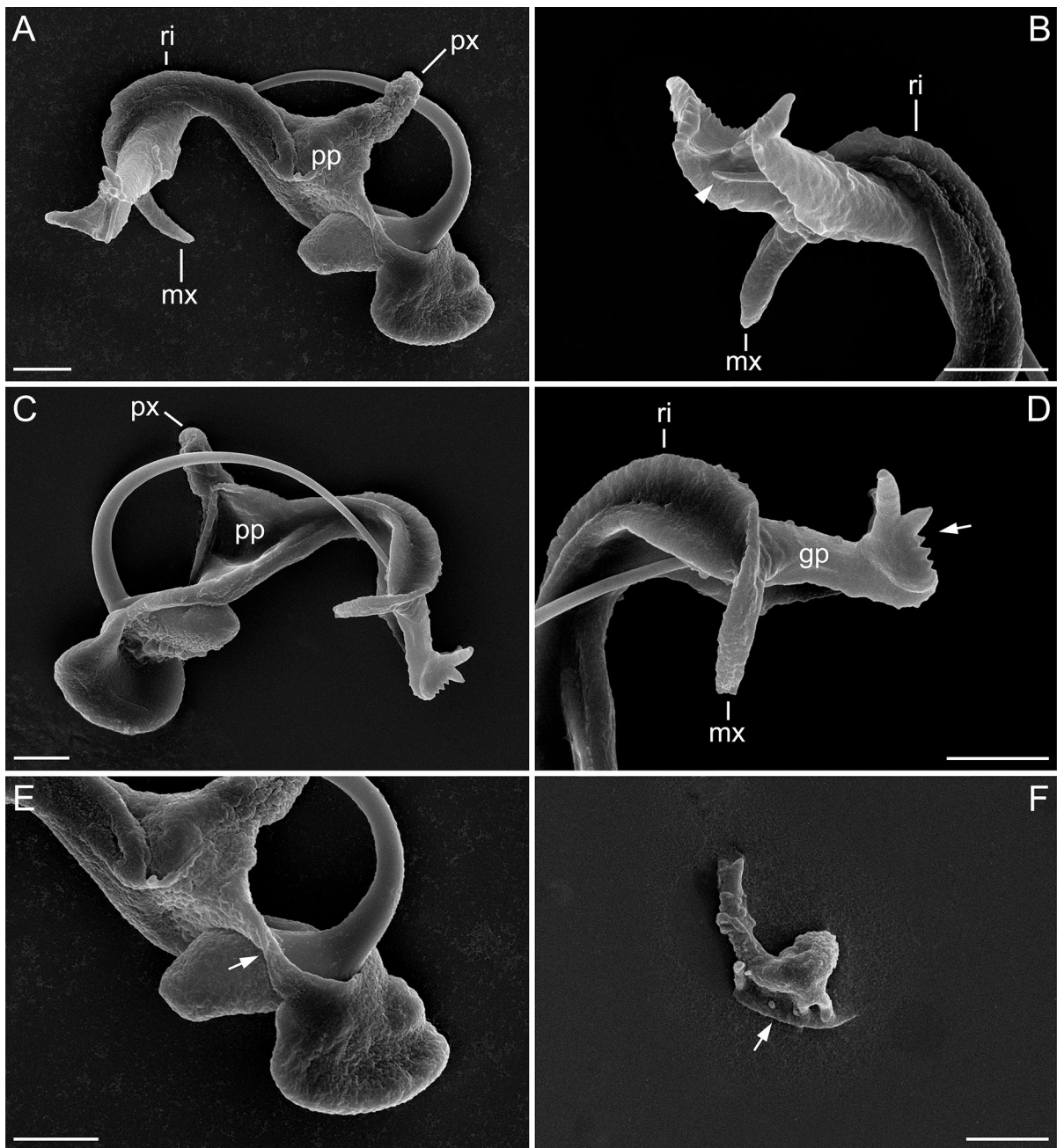


Fig. 3 Genitalia of *Cichlidogyrus tiberianus* Paperna, 1960. A, MCO, left view; B, Anterior part of MCO, left view (*arrowhead*: tip of penis tube); C, MCO, right view; D, Anterior part of MCO, right view (*arrow*: denticles); E, Penis bulb and heel, left view (*arrow*: strap-like structure); F, Vagina (*arrow*: bar-like structure). *Abbreviations*: gp, groove-like part of accessory piece; mx, middle extension; pp, proximal part of accessory piece; px, proximal extension; ri, ridge. *Scale-bars*: 5 μ m

Material studied: 11 individuals (RMCA invertebrate SEM collection, S-12) taken from the gills of two specimens of *C. rendalli* (MRAC Vert-

2015.014.P.00001, 00002) caught in the Luapula River off Kashobwe, D. R. Congo (9°40'16"S, 28°37'20"E, 7.ix.2014, water temperature 22°C).

Redescription (Figs. 4, 5)

Haptoral sclerites (Fig. 4A–I). Dorsal anchors with well-developed shaft and guard (Fig. 4D, E); total length 27–29 (28, n = 4); blade length 22–25 (23, n = 4); shaft length 6–9 (8, n = 4); guard length 10–11 (10, n = 4); point length 9–10 (9, n = 4). Dorsal bar with

concave and convex side; concave side showing narrow groove between auricle bases (Fig. 4A, B); total length 35–35 (35, n = 2); maximum width 5–5 (5, n = 2); length of auricle 11–12 (12, n = 2); distance between auricles 8–9 (9, n = 2). Ventral anchors larger than dorsal anchors, with well-developed shaft and guard (Fig. 4F–H); total length 35–36 (35, n = 4);

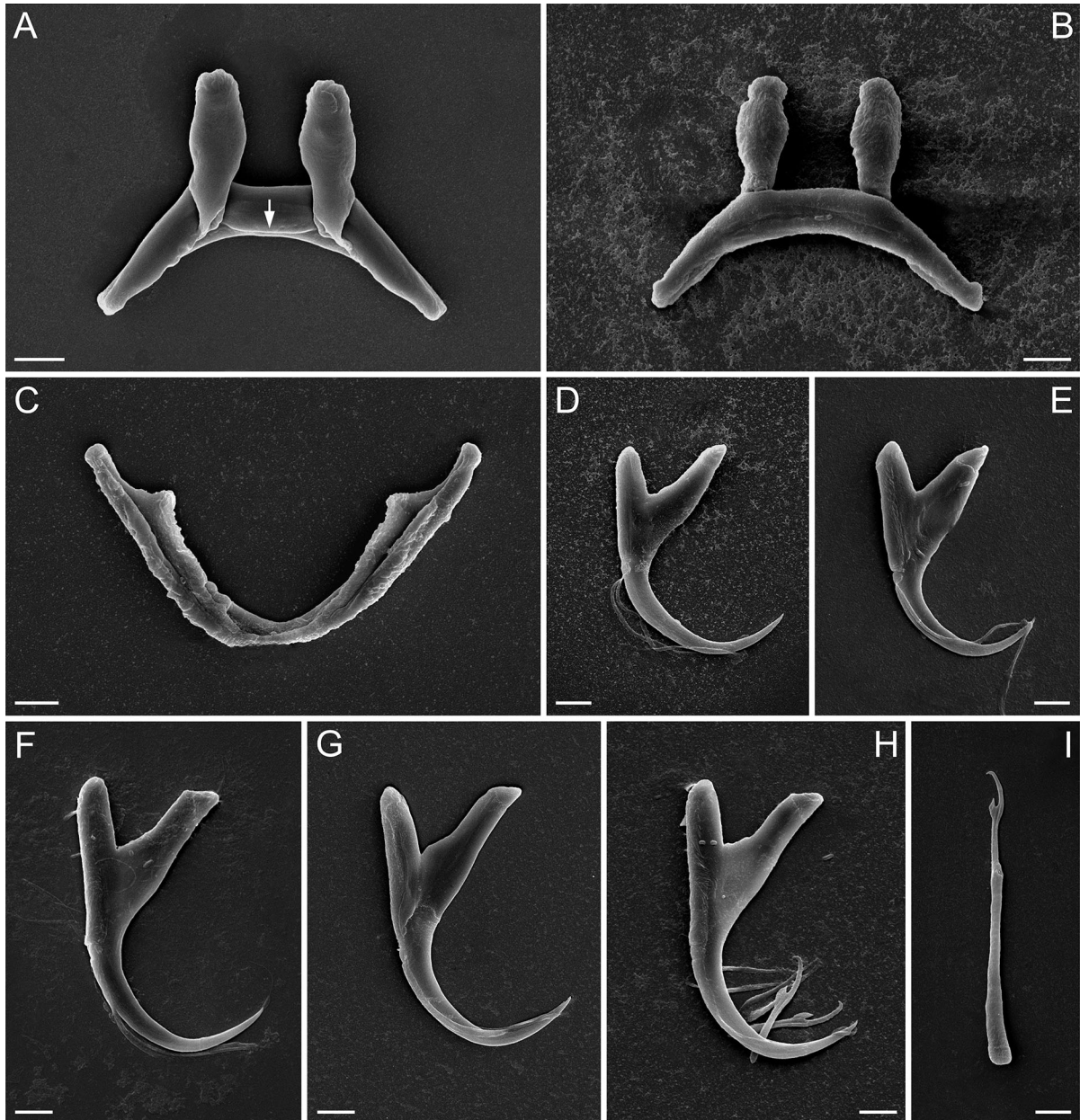


Fig. 4 Haptoral sclerites of *Cichlidogyrus dossoui* Douëllou, 1993. A, Dorsal bar, concave surface (arrow: groove); B, Dorsal bar, convex surface; C, Ventral bar; D, E, Dorsal anchors of different specimens; F–H, Ventral anchors of different specimens (note uncinuli II in H); I, Uncinulus. E, G and H have been flipped horizontally for consistency with other figures. Scale-bars: 5 μ m

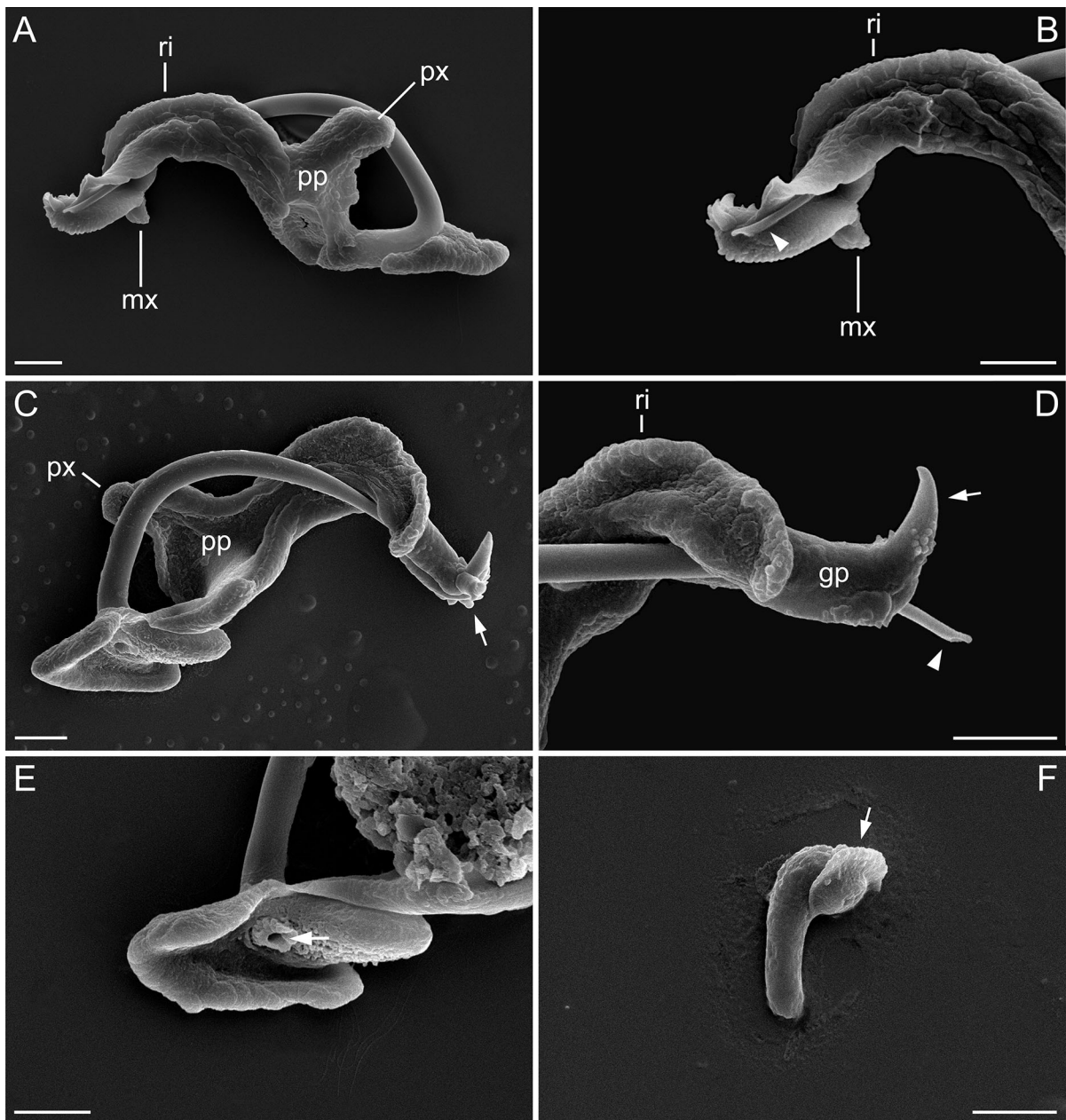


Fig. 5 Genitalia of *Cichlidogyrus dossoui* Douëllou, 1993. A, MCO, left view; B, Anterior part of MCO, left view (*arrowhead*: tip of penis tube); C, MCO, right view (*arrow*: denticles); D, Anterior part of MCO, right view (*arrowhead*: tip of penis tube; *arrow*: dorsally pointing tip of accessory piece); E, Penis bulb and heel, right view (*arrow*: opening); F, Vagina (*arrow*: ridge-like structure). *Abbreviations*: gp, groove-like part of accessory piece; mx, middle extension; pp, proximal part of accessory piece; px, proximal extension; ri, ridge. *Scale-bars*: 5 μ m

blade length 28–30 (29, $n = 4$); shaft length 7–10 (9, $n = 4$); guard length 11–13 (12, $n = 4$); point length 12–13 (13, $n = 4$). Ventral bar: length of one ventral bar branch 30–32 (31, $n = 2$); maximum width 4–5 (5, $n = 2$).

Genitalia (Fig. 5A–F). MCO similar to that of *C. tiberianus* but middle extension of accessory piece much shorter (Fig. 5A, B) and heel narrower (Fig. 5A, C, E). Proximal part of accessory piece on left side of MCO (Fig. 5A, C). Terminal tip of accessory piece

pointed dorsally in most specimens (Fig. 5D). Some specimens showing a small opening on right surface of penis bulb (Fig. 5E; this opening may be the entry point of ducts coming from the *vesicula seminalis* and prostatic reservoirs). Penis length 58–62 (59, $n = 5$), accessory piece length 38–44 (41, $n = 7$), heel length 8–15 (11, $n = 7$). Vagina tube-like, relatively short; at mid-length showing a bend of about 90° (Fig. 5F). One specimen with a ridge-like structure (arrow in Fig. 5F; this ridge may be a remnant of the edge of the vaginal pore).

Remarks

The combined presence of the following features distinguishes *C. dossoui* from all other members of the genus: uncinuli I short (*sensu* Pariselle & Euzet, 2003, 2009); III to VII long (*sensu* Pariselle & Euzet, 2003, 2009; Fig. 4I); ventral anchors with well-developed shaft and guard (Fig. 4F–H); vagina tube-like, showing a bend of about 90° near its middle (Fig. 5F); penis with ovoid bulb and long, curved tube (Fig. 5A, C); accessory piece as in Fig. 5A–C, with well-developed proximal extension and very short middle extension. It can be readily distinguished from *C. tiberianus* by the shorter middle extension, the slightly broader vagina without coils and the ventral anchors with well-developed shaft and guard.

Discussion

The MCOs of *C. tiberianus* and *C. dossoui* are relatively small structures with a complex shape. Hitherto, these organs have only been studied by light microscopy in whole-mounted specimens (Paperna, 1960; Ergens, 1981; Dossou, 1982; Lopez, 1991; Douëllou, 1993). Furthermore, although some of these authors have provided detailed descriptions and excellent drawings, they usually did not label their figures, making it sometimes difficult to interpret their descriptions. The present descriptions are based on SEM examination of a relatively large number of specimens, allowing a detailed analysis of the variation in MCO shape. We found that the MCOs of *C. tiberianus* and *C. dossoui* differ mainly in the length of the middle extension of the accessory piece, which is considerably longer in the former species (Figs. 3A, B, D, 5A, B). Douëllou's (1993) descriptions and

drawings suggest that *C. tiberianus* differs from *C. dossoui* in having an accessory piece with a distinctly forked tip. However, the current results do not fully support such a view. Although some of our images appear to show a forked tip (e.g. Fig. 3B), others do not (see e.g. Fig. 3A and some of the images available online). We suspect that the appearance of the tip of the accessory piece depends to a large extent on the angle of view and hence may be of limited value as a diagnostic feature.

Judging from the published illustrations, at least ten *Cichlidogyrus* species have a MCO similar to that of *C. tiberianus* and *C. dossoui*. These species include *C. anthemocolpos* Dossou, 1982; *C. bonhommei* Pariselle & Euzet, 1998; *C. bouvii* Pariselle & Euzet, 1997; *C. douellouae* Pariselle, Bilong Bilong & Euzet, 2003; *C. ergensi* Dossou, 1982; *C. gillesi* Pariselle, Bitja Nyom & Bilong Bilong, 2013; *C. hemi* Pariselle & Euzet, 1998; *C. kouassii* N'Douba, Thys van den Audenaerde & Pariselle, 1997; *C. legendrei* Pariselle & Euzet, 2003; and *C. vexus* Pariselle & Euzet, 1994. Like the two species examined here, they have an ovoid bulb, a curved tube and a large accessory piece with a prominent proximal extension. Moreover, in addition to having similar MCOs, these species also share other characteristics with *C. tiberianus* and *C. dossoui*, such as short uncinuli I, long uncinuli III–VII and a similarly shaped dorsal bar (Dossou, 1982; Pariselle & Euzet, 1994, 1997, 1998, 2003; N'Douba et al., 1997; Pariselle et al., 2003, 2013). Whether all these species are closely related remains to be determined, but molecular phylogenetic studies have provided tentative support for a close relationship between *C. tiberianus*, *C. douellouae* and *C. ergensi* (see Mendlová et al., 2012; Messu Mandeng et al., 2015). Unfortunately, *C. dossoui* has not yet been included in any phylogenetic analysis.

The MCOs of *C. tiberianus* and *C. dossoui* are highly asymmetrical structures, with the proximal part of the accessory piece being situated on one side of the penis tube. In the present study all examined specimens carried the proximal part on the left side of the tube ('left-handed' MCOs). This suggests that the male genitalia of *C. tiberianus* and *C. dossoui* are examples of directional asymmetry, i.e. asymmetry in which only one of the two mirror images is present, with the exception of very rare mutants (Palmer, 1996; Schilthuizen, 2013). The fact that both *C. tiberianus* and *C. dossoui* were found to be left-handed does not

necessarily mean that all species with similar male genitalia are also left-handed. In genera with asymmetric genitalia, chiral reversal (where a species has genitalia that are the mirror image of those of congeners) is relatively common (Schilthuisen, 2013). Hence, when describing or redescribing species of *Cichlidogyrus*, it remains important to indicate whether the MCO is left- or right-handed.

As noted in the Introduction, *C. tiberianus* and *C. dossoui* often co-occur on the same host individual. Their co-occurrence and similar male genitalia render them potentially vulnerable to hybridization, but no evidence for hybrids has ever been reported. This raises the question as to how these species remain reproductively isolated. One possibility is that *C. tiberianus* and *C. dossoui* are adapted to different microhabitats within the gills. In the gill-parasitic monogenean *Dactylogyrus* Diesing, 1850, species occupying different microhabitats tend to have dissimilar attachment organs (Šimková et al., 2002). *Cichlidogyrus tiberianus* and *C. dossoui* show clear differences in the shape of the ventral anchors (Figs. 2F–H, 4F–H), suggesting that they may indeed be adapted to different microhabitats. However, direct evidence for this hypothesis (such as a difference in spatial distribution between *C. tiberianus* and *C. dossoui*) is currently lacking. Furthermore, it is possible that the relatively small genital differences are sufficient to reproductively isolate the two species. This last hypothesis raises questions about the mechanics of copulation in *Cichlidogyrus*, which are poorly understood and in need of further study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed. Sampling was carried out under attestation de recherche no. 863/2014 from the Faculté des Sciences Agronomiques of the Université de Lubumbashi.

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