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A new locality and host record for *Enterogyrus coronatus* (Pariselle Lambert & Euzet (1991) in South Africa and a review of the morphology and distribution of *Enterogyrus* (Ancyrocephalidae) species

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Summary

Examination of 40 specimens of *Pseudocrenilabrus philander philander* (Weber, 1897) collected from Padda Dam (26°10′S; 17°59′E), South Africa revealed the presence of a stomach monogenean of the genus *Enterogyrus* (Paperna, 1963). The monogenean presented a prevalence of 52.5 % and mean intensity of 4.2. The body, surrounded by a thick cuticle which is striated transversally, is dorso-ventrally flattened. The haptor has two pairs of gripi, a lightly sclerotised ventral transverse bar and marginal uncinuli. The dorsal gripus has a bifurcate root and a curved blade which is shorter than the shaft and is larger than that of the ventral gripus. The genetic distance between *E. coronatus* and the present *Enterogyrus* species (0.24 %) confirms the morphological similarities. This study presents a new locality and host record of the genus *Enterogyrus* from South Africa and a review of the morphology and distribution of *Enterogyrus* species is also given.

Keywords: Monogenea; Dactylogyridae*; Enterogyrus*; *Pseudocrenilabrus philander philander*; South Africa

Introduction

African fishes of the family Cichlidae are parasitized by five genera of monogeneans belonging to the family Dactylogyridae. The highly diversified *Cichlidogyrus* Paperna, 1960, *Onchobdella* Paperna, 1968 and *Scutogyrus* Pariselle & Euzet, 1995 represent three genera infecting the gills. The two remaining genera; *Enterogyrus* Paperna, 1963 and *Urogyrus* Bilong Bilong *et al*. 1994 are endoparasitic, infecting the stomach and the urinary bladder, respectively. To date there are eight known species of *Enterogyrus* (Ancyrocephalidae), namely *Enterogyrus cichlidarum* Paperna, 1963; *E. malmbergi* Bilong Bilong, 1988; *E. melenensis* Bilong Bilong, Birgi & Lambert, 1989; *E. barombiensis* Bilong Bilong, Birgi & Euzet, 1991; *E. foratus* Pariselle, Lambert & Euzet, 1991; *E. coronatus* Pariselle, Lambert & Euzet, 1991; *E. amieti* Bilong Bilong, Euzet & Birgi, 1996 and *E. crassus* Bilong Bilong, Birgi & Euzet, 1996. Previous records are mostly from West Africa (Bilong Bilong, 1988; Bilong Bilong *et al*., 1989; Bilong Bilong *et al*., 1991; Pariselle *et al*., 1991; Bilong Bilong *et al*., 1996) while a few records are from Egypt (Eid & Negm, 1987; Khird, 1990) and Israel (Paperna, 1963). Other records are due to introductions of African hosts into other countries (Jiménez-Garcia *et al*., 2001; Jeronimo *et al*., 2010). In South Africa, there has been a single record of *Enterogyrus* species from Middle Letaba Dam, Limpopo Province (Olivier *et al*., 2009).

In all previous descriptions and records of enterogyrids, species determination has been carried out using morphology and size of sclerotised parts of the attachment organs. The reproductive cirrus has been used for resolving species level identification. Nevertheless, molecular data from *Enterogyrus* species is still very limited; with only three sequences of *Enterogyrus* species available on GenBank (Mendlová *et al*., 2010; 2012). The finding of many ancyrocephaline monogenean *Enterogyrus* specimens in the stomach of the southern mouthbrooder, *Pseudocrenilabrus philander philander* (Weber, 1897) prompted the present study. This paper presents the second record of *Enterogyrus* species from southern Africa, and uses both morphological and molecular data to identify the species from Padda Dam.

Materials and methods

Fish were collected in April 2013 on the University of Johannesburg grounds from the Padda Dam (26°10′S; 17°59′E). The dam is situated in the Westdene Tributary System which is one of the origins of the Limpopo River (South Africa). Fish were captured using hand nets and transported to the laboratory where they were kept in a holding tank with aerated dam water. Fish were killed by

Table 1. Taxa used in the phylogenetic analysis of the *Enterogyrus* species in the current study

Parasite species	Host species	Locality	LSU
Enterogyrus coronatus Pariselle, Lambert & Euzet, 1995	*Tilapia dageti* Thys van den Audenaerde, 1967	Senegal, Africa	HQ010030
Enterogyrus sp. 1	*Sarotherodon galilaeus* (Linnaeus)	Senegal, Africa	HQ010032
Enterogyrus sp. 2	*Sarotherodon galilaeus* (Linnaeus)	Senegal, Africa	HQ010031
Onchobdella aframae Paperna, 1968	*Hemichromis fasciatus* Peters, 1857	Senegal, Africa	HQ010033
Onchobdella bopeleti Bilong Bilong & Euzet, 1995	*Hemichromis fasciatus* Peters, 1857	Senegal, Africa	HQ010034

severing the spinal cord immediately posterior to the cranium. The abdominal cavity of each fish was opened via a medio-saggital incision and the digestive system removed. The stomachs were removed, individually placed in Petri dishes containing 0.9 % physiological saline and subsequently examined with the aid of a dissection microscope. The parasites which were anchored to the stomach wall were gently removed with the aid of a needle.

Light microscopy studies

A total of 30 parasites were preserved in 70 % ethanol for light microscopy studies. Ten specimens were mounted on glass slides using Gray and Wess solution (Humason, 1979), covered with a cover slip and sealed with clear nail vanish. Ten additional specimens were stained with Hören's trichrome (Manual of Veterinary Parasitological Techniques, 1986) and five were stained with lignin pink and mounted in lacto phenol. A Zeiss Axioplan 2 imaging light microscope equipped with a camera and operated with Axiovision software (Carl Zeiss, Jena, Switzerland) was used to identify monogeneans through observations, micrographs, drawings and measurements. Measurements (in micrometres) are presented in the following order: (number of measurements) mean ± standard deviation (minimum-maximum). Dimensions of the haptoral structures (Fig. 1) used follow those of Gussev (1962) as amended by Bilong Bilong *et al*. (1989). The haptoral nomenclature follows that of Pariselle & Euzet (1995). A comparison of the standardized measurements of sclerotised parts of known species of *Enterogyrus* (Table 1) was made by hierarchical clustering using IBM SPSS Statistics V. 21 (Statistical Package for Social Sciences, SPSS, Inc.).

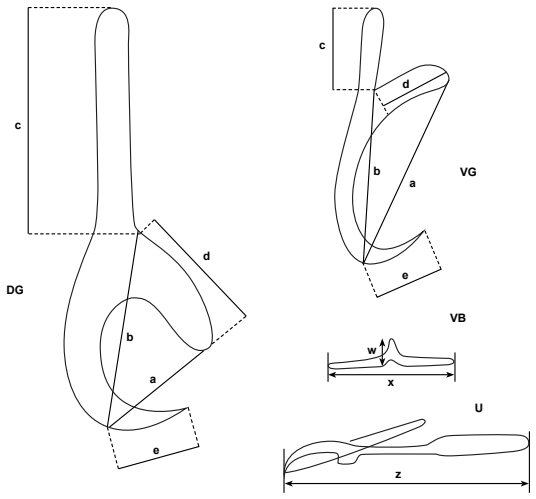

Fig. 1. Measurements of haptoral structures (after Gussev, 1962). a = anchor length b = blade; c = shaft; d = guard; DG = dorsal gripus; e = point; U = uncinulus; VB = ventral transverse bar; VG = ventral gripus; w = width of VB; x = length of VB; z = length of uncinulus

Scanning electron microscopy

A total of 30 specimens were collected in this study, five of which were fixed and preserved in 70 % ethanol (Merck, Germany) for scanning electron microscopy (SEM). Specimens were prepared by dehydrating them in a graded series of ethanol and subsequently in a graded series of hexamethyldisilazane (Merck, Germany) after Dos Santos *et al*. (2013). Samples were then sputter coated with gold and examined with a TESCAN Vega 3 LMH SEM (Brno, Czech Republic) at 5-10kV acceleration voltages.

Genetic Analysis

Five specimens were removed from the stomach wall and digested using a DNeasy ™ Tissue kit (QIAGEN, Netherlands) to extract genomic DNA, as per the manufacturer's instructions. The large subunit region (LSU) of rDNA was amplified using primers C1 and D2 (Hassouna *et al*., 1984). The amplification reaction was performed using a MultiGene Gradient system (Labnet International, Inc., USA) using the parameters set out by Matejusová *et al.* (2001). The PCR products were run on a 1 % agarose gel and sequenced similarly to the method used by Avenant-Oldewage *et al*. (2013).

These sequences were aligned and edited in GENEIOUS Pro™ 5.0 software (Biomatters Ltd, New Zealand). All five sequences collapsed into a single haplotype and this sequence was aligned to the three other sequences for *Enterogyrus* retrieved from GenBank to determine the distinctness of this species. Sequences of two other dactylogyrid monogenea (*Onchobdella*) were recovered from GenBank to be used as outgroups in the phylogenetic reconstructions. The list of all taxa used for the present phylogenetic analyses is shown in Table 1. Sequence alignment was performed using MacClade4 (Maddison & Maddison, 2005) with the resultant alignment analysed using PAUP 4* (Swofford, 2002). Genealogical relationships between taxa were analysed using parsimony, likelihood and distance approaches, with the robustness of their topologies assessed using 1000 bootstrap replicates.

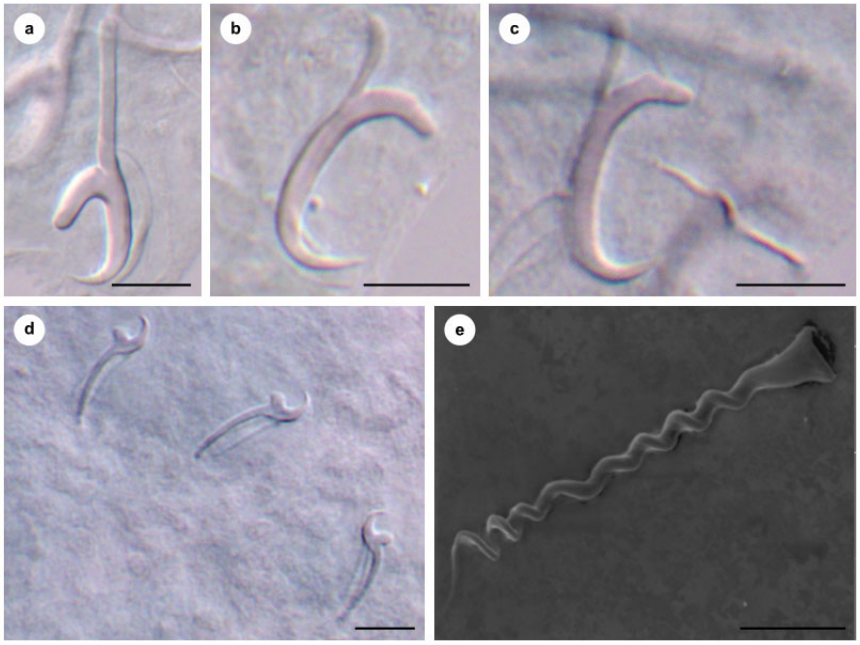

Fig. 2. Micrographs of sclerotised parts of *Enterogyrus coronatus* from the stomach of *Pseudocrenilabrus philander*. A= dorsal gripus; B = ventral gripus; C = uncinuli; D = ventral bar (arrow) E = light micrograph of the cirrus; F = scanning electron micrograph of the cirrus. Scale bars: A = 10 µm; B – D = 5 µm; D = 10 µm

Results

Description (Fig. 2A-E).
Body size is (n = 22) 211 ± 126 (83.4 – 341.1) µm long by 73.2 ± 38 (31.5 – 115.5) µm wide at level of ovary. Small dorso-ventrally flattened, pear shaped. Thick, transversally-striated tegument around body. Anterior to pharynx are four dorsal ocelli: an anterior pair, small and wider spaced; posterior pair, larger than anterior pair, very close or in some specimens, merged on the median plane. Pharynx, medio ventrally positioned, (n = 22) 16.5 ± 7.7 (10.5 – 28.2) µm long and 15.6 ± 7.3 (9.5 – 26.5) µm wide. Haptor is (n = 22) 70.5 ± 22.5 (25.9 – 90.3) µm at widest point and 70.5 ± 22 (26.7 – 92.7) µm long, separated from

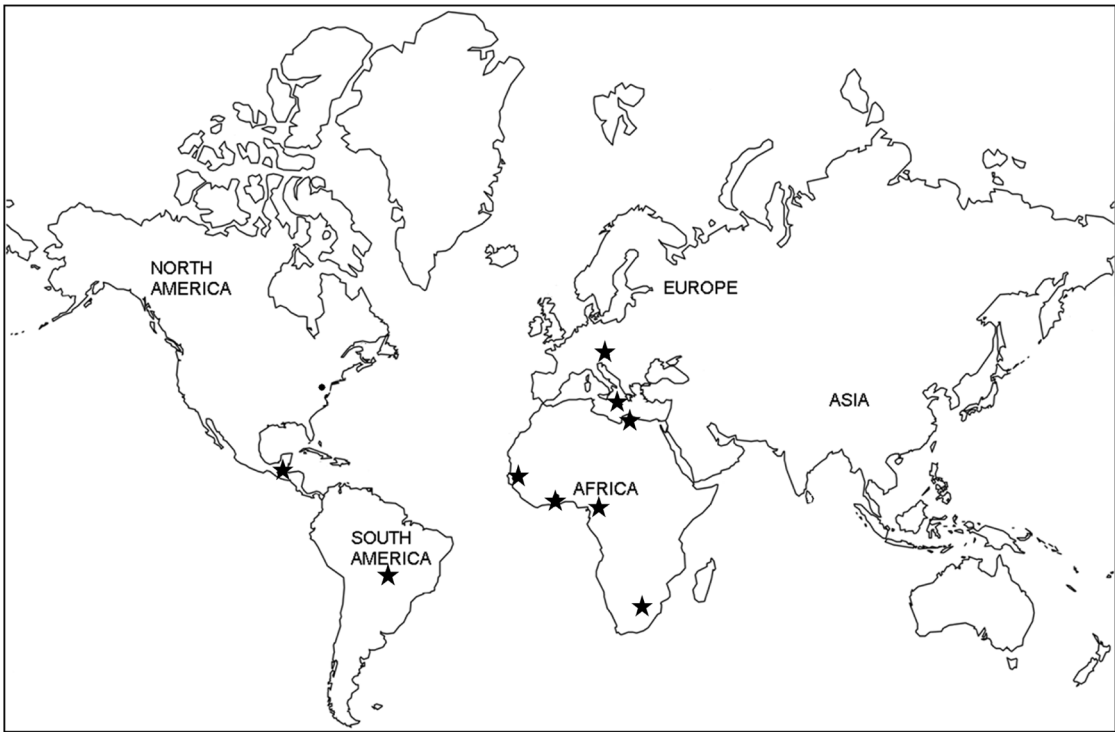

Fig. 3. Geographical distribution of *Enterogyrus* species

Table 2. Previous records of *Enterogyrus* species. (* = species excluded in the morphological analysis in the present study)

***Enterogyrus* spp.**	**Host**	**Locality**	**References**
E.cichlidarum	*Tilapia zilli* (Gervais, 1848)	Rubin River, Israel	Paperna (1963)
		Jordan and coastal systems, Israel.	Paperna (1979)
	Oreochromis niloticus (Linnaeus, 1758)	South of Cameroon	Bilong Bilong *et al*. (1989)
	Sarotherodon galilaeus (Linnaeus 1758)	Nile River	Khird (1990)
	O. niloticus, T. zillii.	Nile River	Khird (1990)
	Oreochromis mossambicus (Peters, 1852)	USA	Noga & Flowers, 1995)
	S. galilaeus sanagaensis (Thys van den Audenaerde, 1966).	Sakbayémé, Sanaga Basin, Cameroon	Bilong Bilong *et al*. (1996)
	T. nyongana Thys van den Audenaerde, 1971.	So'o Nyong Basin, Cameroon	Bilong Bilong *et al*. (1996)
	Pseudocrenilabrus philander philander (Weber, 1857)	Middle Letaba Dam, South Africa	Olivier *et al*. (2009)
	O. mossambicus	Middle Letaba Dam, South Africa	Olivier *et al*. (2009)
	O. niloticus	State of Santa Catarina, Southern Brazil	Jeronimo *et al.* (2010)
	O. niloticus	Kafr El-Sheikh fish farms, Egypt	Eissa *et al.* (2011)
**E.globodiscus*	*Etroplus suratensis* (Block, 1790)	Sri Lanka, India	Kulkarni (1969)
**E.papernai*	*E. suratensis*	Sri Lanka	Gussev & Fernando (1973)
**E.hemihaplochromii*	*Hemihaplochromis multicolor* Schoeller, 1903.	Germany (aquarium)	Bender (1979)
**E.niloticus*	*O. niloticus*	Barher Mouise, Nile River, Egypt	Eid & Negm (1987)
E.malmbergi	*O. niloticus*	Edebda, Sanaga River, Cameroon	Bilong-Bilong (1988)
	O. niloticus	Mérida, México	Jiménez-Garcia *et al*. (2001).
	Cichlasoma callolepis (Regan 1904).	Santa Anita Lagoon, México	Jiménez-Garcia *et al*. (2001).
E.melenensis	*Hemichromis fasciatus* Peters, 1857.	Melen, Yaoundé, Cameroon	Bilong-Bilong, Birgi & Lambert (1989)
		Nyong, Sanaga and Lobé Basins, Cameroon	Bilong-Bilong, Birgi & Lambert (1989)
E. barombiensis	*Pungu maclareni* (Trewavas, 1962)	Barombi Mbo Crater Lake, Cameroon	Bilong-Bilong, Birgi & Euzet (1991)
	Stomatepia pindu Trewavas, 1972	Barombi Mbo Crater Lake, Cameroon	Bilong-Bilong, Birgi & Euzet (1991)
	Konia eisentrauti (Trewavas, 1962)	Barombi Mbo Crater Lake, Cameroon	Bilong-Bilong, Birgi & Euzet (1991)
E. foratus	*S. melanotheronheudelotii* (Duméril, 1859)	Mouth of Casamance River, Senegal.	Pariselle, Lambert & Euzet (1991)
	S. melanotheronmelanotheron Rüppel, 1852	Layo Station, Ebrié Lagoon, Côte d'Ivoire.	Pariselle, Lambert & Euzet (1991)
E. coronatus	*T. guineensis* (Bleeker, 1862)	Layo Station, Ebrié Lagoon, Côte d'Ivoire.	Pariselle, Lambert & Euzet (1991)
	Tilapia dageti Thys van den Audenaerde, 1967	Senegal	Mendlová *et al.* (2010)
E. crassus	*T. nyongana* Thys van den Audenaerde, 1971.	So'o Nyong Basin, Cameroon	Bilong-Bilong , Euzet & Birgi (1996)
E. amieti	*S. galilaeus sanagaensis*	Sakbayémé, Sanaga Basin, Cameroon	Bilong-Bilong , Euzet & Birgi (1996)

Table 3. Comparison of measurements (in µm) of different body parts among known members of the genus *Enterogyrus*

Enterogyrus spp.	*E. cichlidarum*	*E.malmbergi*	*E.melenensis*	*E. barombiensis*	*E. foratus*	*E. coronatus*	*E. crassus*	*E. amieti*	Present specimens
N. specimens	–	20	25	41	64		10	10	30
Body Length	–	721.6	465	525.7	602.68	535.11	799	573	211
Body Width	–	281.5	153	115.7	148.03	115.01	248	139	73.2
Pharynx diameter	–	64.5	38	–	33.37	33.37	77	39	16.5
Haptor Length	–	115.7	65	132.9	–	–	108	103	70.5
Haptor Width	–	234.8	105	108.3	–	–	227	120	70.5
				Dorsal gripus					
a	11.25	23.13	8.49	10.2	12.81	11.38	27.8	12.3	9.3
b	16.7	33.2	13.73	16.7	18.49	16.4	33.8	18.3	13.9
c	18.5	23.3	14.68	12.2	25.9	21.17	23.8	12	17.7
d	8.13	15.4	7.73	8.1	8.37	7.14	17.8	9	8.9
e	5.03	6.45	4.68	4.5	5.89	5.47	7.5	4.8	3.9
				Ventral gripus					
a	13.05	17.6	12.15	11.8	14.17	15	24.8	13.6	12.7
b	11.35	14.1	10.88	10.9	12.61	12.83	21.8	12.3	12.3
c	6.45	9.85	5.98	6	7.63	6.33	12.2	6	5.5
d	5.2	8.93	5.53	5	5.45	5.67	11.2	5.5	5.5
e	4.13	4.83	3.38	4	4.5	4.69	6.6	4.8	3.4
				Transverse bar					
x	16.5	42.9	16.8	16.9	17.86	18.14	53.5	10.7	10.6
w	–	–	–	–	1	1	4	1	1
				Uncinuli					
Pairs I-II	12.5	13.6	12.5	11.9	14.25	14.18	–	–	10.2
Pairs III-VII	12.5	13.6	12.5	11.9	14.25	14.18	19	13.5	10.9
Cirrus length	48.6	44.35	45.7	46.7	50.21	48.6	69	52	47.1
Base width	–	–	–	–	–		10	5.8	7.6
Spiral formula	1-2-3/5-2-3	3-2-3	4-2-3	6-2-4	4-2-3	4-2-1+2	4-2-3	5-2-4	3-2-3

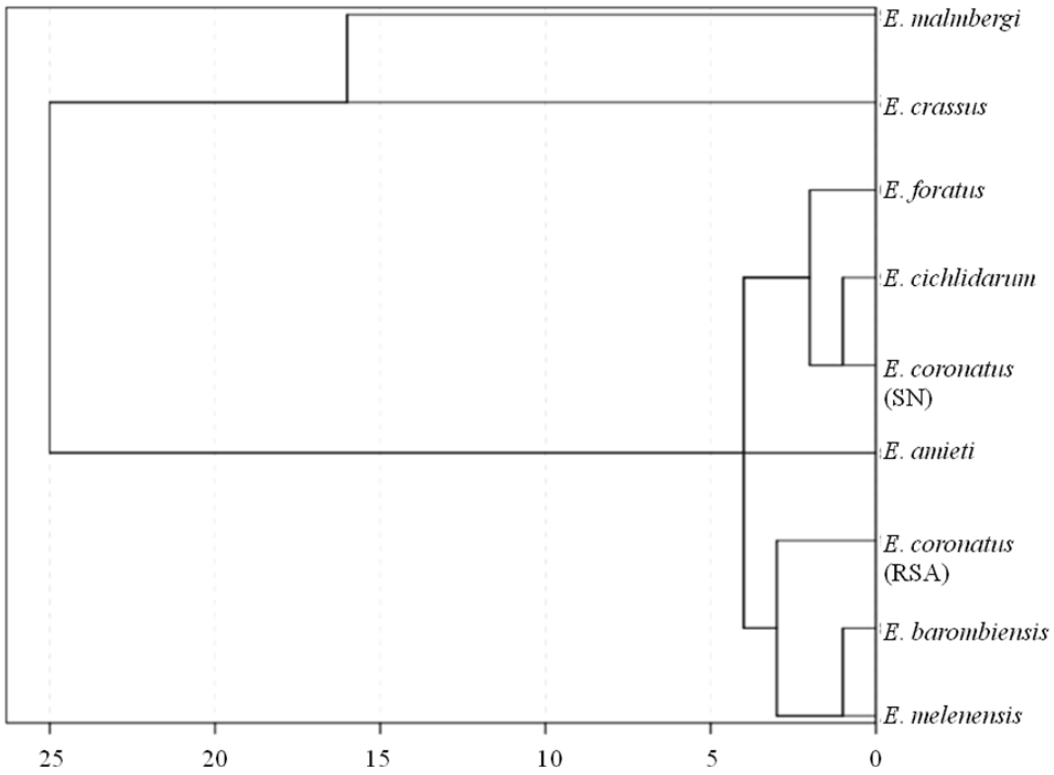

Fig. 4. Dendrogram for Euclidean hierarchical clustering based on standardised morphological data of the taxonomically important metric dimensions of the eight species of *Enterogyrus* described thus far

body by a slight constriction; has a thinner tegument which lacks transverse striations characteristic of main body. Two types of haptors observed in specimens: cup shaped haptor and tongue shaped haptor, each is armed with 14 uncinuli, 2 pairs of gripi (ventral and dorsal) and a lightly sclerotised transverse bar. Tongue shaped haptor comprises two segments: an elongate posterior penduncular segment bearing the dorsal and ventral gripi, the transverse bar and ventral uncinuli I and II; and a bulbous anterior segment with hooklets III – VII positioned in an equatorial sphere with their tips directed anteriorly. Dorsal gripus has shaft (c) which is longer than the blade (b). Cirrus size (n = 10) 7.6 ± 2.7 (5.7 – 13.5) µm wide at its base and (n = 11) 47.1 ± 15.1 (30.7 – 66.6) µm long, tubular, median, situated slightly posterior to pharynx, forming a continuous spiral as in other species of the genus. Between its base and distal extremity are series of 3 spirals, followed by 2 longer spirals, and finally 3 tightly packed distal spirals. The spiral pattern, as proposed by Pariselle *et al*. (1991), can be represented by the formula: 3-2-3. This spirality conforms to that described by Bilong Bilong *et al*. (1991) for *E. malmbergi* (Fig. 2E). This monogenean presented a prevalence of 52.5 %, mean intensity of 4.2 and a mean abundance of 2.2 in Padda Dam.

Although *Enterogyrus globodiscus* (Kulkarni, 1969), *E. papernai* (Gussev & Fernando, 1973), *E. niloticus* (Eid & Negm, 1987) and *E. hemihaplochromii* (Bender, 1979) appear in Table 2, these species have not been included in the morphological comparisons (Table 3). The type species, *E. cichlidarum* was recorded from Israel by Paperna in 1963. Subsequent findings were mostly from West Africa, with some records from Germany (Bender, 1979), America (Noga & Flowers, 1995) and México (Jiménez-Garcia *et al*., 2001) (Table 2, Figure 3).

In order to identify the present specimens which were collected from the stomach of *P. p. philander* in Padda Dam, morphological comparisons with previously recorded *Enterogyrus* species were done (Table 3). There is limited genetic data on *Enterogyrus* species, hence a morphological phylogenetic comparison, based on haptoral metric dimensions was carried out and the results are presented in Figure 4. The metric dimensions of all analysed features of haptoral sclerites of the present species correspond closely with the measurements given for *E. barombiensis* and *E. melenensis* (Table 3, Figure 4). However, *E. barombiensis* is different from *E. melenensis* and the present specimens in that it has a smaller shaft to blade ratio, a characteristic seen in *E. malmbergi*, *E. crassus* and *E. amieti*. On the other hand, *E. cichlidarum*, *E. foratus*, *E. coronatus* have a bigger shaft to blade ratio (Table 3).

In general, there is no conspicuous variability in the dorsal and ventral gripi metric dimensions except in *E. malmbergi* and *E. crassus*, which have larger dimensions than the other six species. The former species also represent species with the longest transverse bars of 42.9 µm and 53.5 µm, respectively. Furthermore, when only the cirrus length is considered, the present species is closely related to all the other species except for *E. crassus* whose cirrus is discernibly longer than that of the other seven species (Table 3). When the spirality of cirri of these three species was

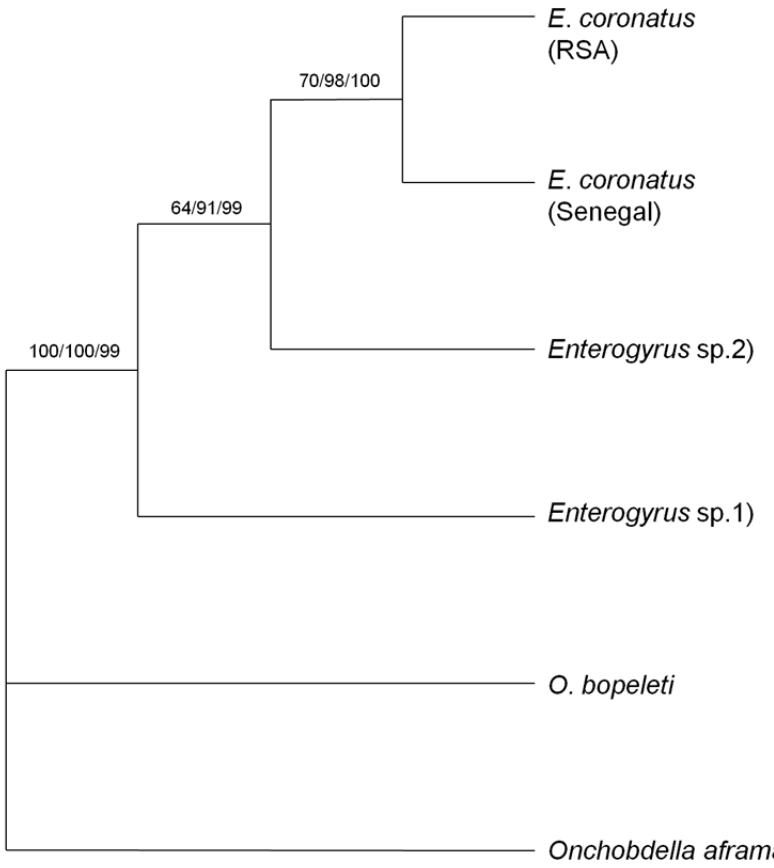

Fig. 5. Neigbour Joining tree based on parsimony methods inferred from the analysis of LSU rDNA sequences of three *Enterogyrus* species, two *Onchobdella* species and the *Enterogyrus* species in the current study. Bootstrap percentages for maximum likelihood, maximum parsimony and distance are shown above branches.

compared with that of the present species, a close resemblance with *E. malmbergi* was discerned. The spiral formulae *E. cichlidarum* is 1-2-3 and 5-2-3 while that of *E. melenensis* (4-2-3), is similar to that of *E. foratus* and *E. crassus*. Based on morphological data alone, the results were not conclusive enough to identify the specimens to species level; thus, molecular work was carried out to resolve these inconsistencies. The obtained genetic sequences of the South African specimens were very closely related to the sequence of *E. coronatus* from Senegal, except for two transitions in the LSU rDNA region. This was confirmed by the phyogenetic reconstruction in Figure 5, which displays similarity between *E. coronatus* from Senegal and that from South Africa. The partial LSU rDNA sequences included four *Enterogyrus* species and two species of *Onchobdella*, *Onchobdella aframae* and *O. bopeleti*, which were included as outgroups (Table 1). Based on the analyses of LSU rDNA sequences, the six species of cichlid parasites formed a clade with *Onchobdella aframae* as the basal taxon (Fig. 5). Irrespective of the method of analysis, similar topologies were obtained and as such only a neighbor-joining tree is presented with the relevant statistical support indicated. There was no nucleotide variability (i.e. p-distance corresponding to 0.0024) between *E. coronatus* found in *Tilapia dageti* from Senegal and *E. coronatus* found in *P. p. philander* from South Africa (Table 4).

Table 4. Pair-wise genetic distances (%) based on the LSU rDNA fragment of *Enterogyrus* from South Africa and other dactylogyrids from African cichlids

	Parasite species	**1**	**2**	**3**	**4**	**5**	**6**
1	*Enterogyrus coronatus* (RSA)	*					
2	*Enterogyrus coronatus*	0.24	*				
3	*Enterogyrus* sp. 1	7.70	7.95	*			
4	*Enterogyrus* sp. 2	3.91	4.15	7.68	*		
5	*Onchobdella bopeleti*	33.90	33.67	34.82	34.22	*	
6	*Onchobdella aframae*	34.53	34.29	35.55	34.71	5.23	*

Discussion

Four species have been omitted in the morphological analyses carried out in this study. The two species, *E. globodiscus* and *E. papernai*, which were both described from *Etroplus suratensis* (Block, 1790) in Asia have two transverse bars compared to one for African and Levantine species. According to Pariselle & Euzet (2009), this difference is sufficient to justify splitting *Enterogyrus* into two genera. Comparison of *Enterogyrus* species specimens from *T. zillii* and O. *niloticus* by Pariselle & Euzet (2009) revealed no differences and thus *E. niloticus* was synonymised with *E. cichlidarum*. *Enterogyrus hemihaplochromii* was described in an unpublished thesis; hence this name is a *nomen nudum* (Pariselle & Euzet, 2009). Thus, to date there are eight *Enterogyrus* species. The type species (*E. cichlidarum*) was recorded from Israel by Paperna in 1963. Subsequent findings were mostly from West Africa. The genus is confined to African cichlids but some *Enterogyrus* species have been introduced into Germany (Bender, 1979), America (Noga & Flowers, 1995) and in México (Jiménez-Garcia *et al*. (2001) together with their African introduced hosts.

The posterior uncinuli (pairs I and II) of the present specimens are discernibly smaller and thinner than anterior uncinuli (pairs III to VII). This observation was consistent for all specimens in the present study and supplements the observations made by Bilong Bilong *et al*. (1996) for *E. amieti*. In spite of the fact that posterior and anterior uncinuli of the other known species are indicated as of equal lengths, careful analyses of illustrations reflect larger, thicker anterior uncinuli and smaller, thinner posterior uncinuli. This seems to be a constant feature for all species of *Enterogyrus*.

The organization of the haptor and morphology of the sclerotised structures of the present specimens is characteristic of the genus *Enterogyrus*. Although the specimens observed in this study possessed either a cup shaped or a tongue shaped haptor (Pariselle *et al*., 1991), the number and shape of the sclerotised parts of the haptor remained unaltered. The presence of two types of haptors was also observed in specimens of *E. amieti* described by Bilong Bilong *et al*. (1996). It is highly possible that the difference in haptor shape was brought about by specimen orientation during the preparation, fixing and mounting procedures. Some specimens may have retracted their haptors resulting in a cup shaped structure, while others may have extended their haptors have resulting in the tongue shaped structure.

Enterogyrus coronatus was originally described from *Tilapia guineensis* (Bleeker, 1862) in Côte d'Ivoire and subsequently recorded from *Tilapia dageti* Thys van den Audenaerde, 1967 in Senegal by Mendlová *et al*. (2010; 2012). The occurrence of this enterogyrid in *P. p. philander* from South Africa provides a new locality and host record. This is also the first time the parasite has been collected from a mouthbrooder. The majority of enterogyrid species recorded thus far show strict specificity towards their host. For example, *E. crassus* was found only in *Tilapia nyongana* Thys van den Audenaerde, 1971; *E. amieti* only in *Sarotherodon galilaeus sanagaensis* Thys van den Audenaerde, 1966; *E. foratus* only in *Sarotherodon melanotheron heudelotii* (Duméril, 1859) and *E. melenensis* only in *Hemichromis fasciatus* Peters, 1857. *Enterogyrus malmbergi* has been recorded in *Oreochromis niloticus* (Linnaeus, 1758). It is worth mentioning that under natural conditions, a single specimen of *E. malmbergi* was recovered from *Cichlasoma callolepis*, a cichlid native to México (Jiménez-Garcia *et al*. (2001). Four cichlid species (*Tilapia zilli*, *O. niloticus*, *S. galilaeus sanagaensis*, *and T. nyongana*) have been noted to host *E. cichlidarum* under natural conditions (Eid & Negm, 1987; Bilong Bilong, *et al*., 1989; Khird, 1990; Paperna, 1963; Paperna, 1979; Bilong Bilong *et al*., 1996; Olivier *et al*., 2009; Jeronimo *et al*., 2010; Eissa *et al*., 2011). In addition, *E. barombiensis* has been recorded from *Stomatepia pindu* Trewavas, 1972 and from *Konia eisentrauti* (Trewavas, 1962) under natural conditions. Similar to *E. cichlidarum* and *E.barombiensis*; *E. coronatus* is thus a broad spectrum parasite; which infects both mouthbrooders (genus *Pseudocrenilabrus*) and substrate brooders (genus *Tilapia*).

Simultaneous occurrence of two congeneric *Enterogyrus* species; one with a broad spectrum and another with an oïoxenous specificity, has previously been reported by Bilong Bilong (1988) and Bilong Bilong *et al*. (1996). In the present study, only one enterogyrid species was recorded from *P. p. philander*. Similarly, only one oïoxenous *Cichlidogyrus philander* was recorded from the gills of the same host within the same locality (le Roux *et al*., 2011), even though a greater richness for other cichlidogyrid species has been reported in other localitites. For example, in *Cichlidogyrus* species, richness of 17 has been reported from *T. guineensis* (Pouyaudi *et al*., 2006).

Monogenean species determination is generally carried out using morphology and size of sclerotised parts of the haptoral and reproductive organs. Morphological characters have been used to infer phylogenetic relationships between monogenean species (Pouyaudi *et al*., 2006). Given the limited genetic data on *Enterogyrus* species, a morphological comparison approach, based on haptoral sclerites was followed in this study. This approach is open to extensive reconsideration once genetic data for most species are collected, as such data will add additional informative characters to morphological data. Although based on morphological data, the dendrogram obtained in this study provides the first interpretation on phylogenetic relationships among the enterogyrids. This should not be regarded as a definitive hypothesis on enterogyrid evolutionary history; the tree provides a phylogenetic hypothesis that may be tested based on molecular data.

Pouyaudi *et al*. (2006) suggests that the morphology of *Ciclidogyrus* and *Scutogyrus* haptoral sclerites is more useful for inferring phylogenetic relationships than the morphology of their reproductive organs. On the other hand, reproductive organs are more suitable for resolving species-level identification, presumably because of its faster rate of change (Pouyaudi *et al*., 2006). The use of the cirrus in *Enterogyrus* species determination was problematic in this study in view of the fact that different spe-

cies presented inconspicuous differences in the length and spirality of the cirrus. For example, *E. melenensis*, *E. foratus* and *E. crassus* have a similar spirality of 4-2-3. This spiral formula is almost similar to that of *E. coronatus* which is 4-2-1+2. It is noteworthy that *E. cichlidarum* has a spiral formula of 1-2-3 and 5-2-3 while *E. barombiensis* and *E. amieti* have unique spirality formulae. These inconsistencies make it difficult to identify enterogyrids to species level. In addition, based on measurements of both haptoral and reproductive morphology structures, the present specimens were found to be closely related to *E. barombiensis* and *E. melenensis*. However, *E. barombiensis* is distinct in having a dorsal gripus with a shaft that is shorter than the blade. The only morphological characters that *E. coronatus* shares with the present specimens are: bigger shaft to blade ratio and cirrus length, characteristics which are also shared with *E. melenensis*.

Furthermore, the present specimens are much smaller than *E. coronatus* type specimens collected from Côte d'Ivoire, thus, plasticity could be a possible explanation for this, demonstrating that geography, host related and environmental factors may influence the morphology of the hard parts. However, comprehensive sampling needs to be done across a wide range of geographical locations in order to make a more meaningful conclusion.

Although metrical dimensions of all analysed features of the haptoral sclerites and cirrus length and spirality of the specimens in this study did not correspond to those of *E. coronatus*, molecular work proved to be more useful in determining this species. The number of differences observed in the LSU rDNA region and the genetic distance of only 0.24 % between *E. coronatus* and the present species confirms that this species is *E. coronatus*. Studies on all enterogyrid species, based on molecular work data are needed to accurately determine enterogyrids to species level. Furthermore, enterogyrid data from other cichlids are necessary to formulate hypotheses on the origin and evolutionary history of these seemingly closely related fauna of monogenean flatworms.

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References

AVENANT-OLDEWAGE, A., LE ROUX, L. E., MASHEGO, S. N., JANSEN VAN VUUREN, B. (2013): *Paradiplozoon ichthyoxanthon* n. sp. (Monogenea: Diplozoidae) from *Labeobarbus aeneus* (Cyprinidae) in the Vaal River, South Africa. *J. Helminthol.* Available on CJ02013. DOI: 10.101 7/S0022149X12000879

BENDER, H. G. (1979): Zur Morphologie und Biologie von *Enterogyrus hemihaplochromii* n. sp. (Trematodes, Monogenea, Dactylogyridae, Ancyrocephalinae), einem Endoparasiten bei *Hemihaplochromis multicolor* (Teleostei, Cichlidae). PhD Thesis, *Zulassungsarbeit*, *West Germany: Zoologisches Institut der Universität Hohenheim*

BILONG BILONG, C. F. (1988): *Enterogyrus malmbergi* n. sp, (Monogenea - Ancyrocephalidae) parasite de l'estomac du Cichlidae *Tilapia nilotica* Linné, 1757 au Sud-Cameroun. *Ann. Fac. Sc. Bio-Biochim*., 5: 51 – 58

BILONG BILONG, C. F., BIRGI, E., LAMBERT, A. (1989): *Enterogyrus melenensis* n.sp. (Monogenea, Ancyrocephalidae), parasite stomacal de *Hemichromis fasciatus* Peters, 1857 (Téléostéen, Cichlidae) du Sud-Cameroun. *Revue Zool. Afr.*, 1031: 99 – 105

BILONG BILONG, C. F., BIRGI, E., EUZET, L. (1991): *Enterogyrus barombiensis* n. sp. (Monogenea, Ancyrocephalidae) parasite stomacal de trios Cichlidae endemiques lac de cratère Barombi Mbo (Cameroun). *Ann. Parasitol. Hum. Comp.*, 66: 105 – 108

BILONG BILONG, C. F., EUZET, L., BIRGI, E. (1996): Monogenean stomach parasites of cichlid fishes from Cameroon: Two new species of the genus *Enterogyrus* Paperna, 1963 (Ancyrocephalidae). *Syst. Parasitol.*, 34(1): 37 – 42. DOI: 10.10007/BF01531208

DOS SANTOS, Q. M., JANSEN VAN VUUREN, B., AVENANT-OLDEWAGE, A. (2013): *Paradiplozoon vaalense* n. sp. (Monogenea: Diplozoidae) from the gills of moggel, *Labeo umbratus* (Smith, 1841), in the Vaal River System. South Africa. *J. Helminthol*. Available on CJ02013. DOI: 10.1017/S0022149X1300059X

EID, N., NEGM, M. (1987): Some morphological study on a new species of endoparasitic monogenetic Trematode "*Enterogyrus niloticus*" in the intestine of *Tilapia nilotica*. *J. Egypt. Vet. Med. Assoc.,* 47(1 – 2): 79 – 86

EISSA, I. A. M., GADO, M. S., IAILA, A. M., ZAKI, V. H., NOOR EL-DEEN, A. E. (2011): Field studies on prevailing internal parasitic diseases in male hybrid *Tilapia* in relation top monosex *Tilapia* at Kafr El-Sheikh Governorate fish farms. *J. Am. Sci.*, 7 (3): 722 – 728

GUSSEV, A. V. (1962): Monogenoidea. In: I. E BYCHOVSKAYA-PAVLOVSKAYA *et al*. (Ed) *Key to parasites of freshwater fish of the USSR. Moscow-Leningrad*. Moscow-Lenigrad: Academiya Nauk SSSR, 919 pp. (Translated from Russian by Israel Program for Scientific Translations, Ser. No. 1136, Jerusalem, 1964)

GUSSEV, A.V., FERNANDO, C. H. (1973): Dactylogyridae (Monogenoidea) from stomach of fishes. *Folia Parasitol.*, 20(3): 207 – 212

HASSOUNA, N., MICHOT, B., BACHELLERIE, J. P. (1984): The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Res.*, 12(8): 3563 – 3583

HUMASON, G. L. (1979): *Animal Tissue Techniques*. 4th Edition, San Francisco, USA, Freeman and Co., 661pp.

JERONIMO, G. T., SPECK, G. M., MARTINS, M. L. (2010): First report of *Enterogyrus cichlidarum* Paperna 1963 (Monogenoidea: Ancyrocephalidae) on Nile Tilapia *Oreochromis niloticus* cultured in Brazil. *Neotrop. Helminthol.*, (4 (1): 75 – 80

JIMÉNEZ-GARCIA, M. I., VIDAL-MARTINEZ, V. M., LÓPEZ-JIMÉNEZ, S. (2001): Monogeneans in introduced and native cichlids in México: evidence for transfer. *J. Parasitol.*, 87(4): 907 – 909. DOI: 10.2307/3285151

KHIDR, A. A. (1990): Population dynamics of *Enterogyrus cichlidarum* (Monogenea: Ancyrocephalinae) from the stomach of *Tilapia* species in Egypt. *Int. J. Parasitol.*, 20(6): 741 – 745

KULKARNI, T. (1969): Studies on the monogenetic trematodes of fishes found in Hyderabad, Andhra Pradesh (India) - Part II. *Rivista di Parassitologia*, 30(2): 263 – 282

MADDISON, D. R., MADDISON, W. P. (2005): *MacClade* Version 3 [computer software]. Sunderlan, MA: Sinauer Associates

MATEJUSOVÁ, I., KOUBKOVA, B., D'AMELIO, S., CUNNINGHAM, C. (2001): Genetic characterization of six species of diplozoids (Monogenea; Diplozoidae). *Parasitology*, 123(5): 465 – 474

MENDLOVÁ, M., PARISELLE, A., VYSKOČILOVÁ, M., ŠIMKOVÁ, A. (2010): Molecular phylogeny of monogeneans parasitizing African freshwater Cichlidae inferred from LSU rDNA sequences. *Parasitol. Res.* 107(6): 1405 – 1413. DOI: 10.1007/s00436-010-2008-6

MENDLOVÁ, M., DESDEVISES, Y., CIVÁŇOVÁ, K., PARISELLE, A., ŠIMKOVÁ, A. (2012): Monogeneans of West African Cichlid Fish: Evolution and Cophylogenetic Interactions. PLoS ONE 7(5): e37268. Retrieved August 23, 2013 from PLoS ONE database DOI: 10.1371/journal.pone.0037268

NATION, J. L. (1983). A New Method Using Hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. *Stain Technol.*, 58(6): 347 – 351. DOI: 10.3109/10520298309066811

NOGA, E. D., FLOWERS J. R. (1995): Invasion of *Tilapia mossambica* (Cichlidae) viscera by the monogenean *Enterogyrus cichlidarum*. *J. Parasitol.*, 81: 815 – 817. DOI: 10.2307/3283988

OLIVIER, P. A. S., LUUS-POWELL, W. J., SAAYMAN, J. E. (2009): Report on some monogenean and clinostomid infestations of freshwater fish and waterbird hosts in Middle Letaba Dam, Limpopo Province, South Africa. *Onderstepoort J. Vet. Res.*, 76: 187 – 199. DOI: 10.4102/ojvr.v76i2.44

PAPERNA, I. (1963): *Enterogyrus cichlidarum* n. gen. n. sp., a monogenetic trematode parasitic in the intestine of fish. *Bull. Res. Counc. Isr.*, 11B: 183 – 187

PAPERNA, I. (1979): Monogenea of inland water fish in Africa. *Ann. Musee Roy. Afr. Centr.*, 226: 1 – 131

PARISELLE, A., LAMBERT, A., EUZET, L. (1991): A new type of haptor in mesoparasitic monogeneans of the genus *Enterogyrus* Paperna, 1963, with a description of *Enterogyrus foratus* n. sp. and *E. coronatus* n. sp, stomach parasites of cichlids in West Africa. *Syst. Parasitol.*, 20: 211 – 220. DOI: 10.1007/BF00009785

PARISELLE, A., EUZET, L. (1995). Gill parasites of the genus *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae) from *Tilapia guineensis* (Bleeker, 1862), with descriptions of six new species. *Syst. Parasitol.*, 30 (3): 187 – 198. DOI: 10.1007/BF00010469

PARISELLE, A., EUZET, L. (2009): Systematic revision of dactylogyridean parasites (Monogenea) from cichlid fishes in Africa, the Levant and Madagascar. *Zoosystema* 31: 849 – 898. DOI: 10.5252/z2009n4a6

PARISELLE, A., BOEGER, W., SNOEKS, J., BILONG BILONG C., MORAND, S., VANHOVE, M. (2011): The monogenean parasite fauna of cichlids: a potential tool for host biogeography. *Int. J. Evol. Biol.*, 2011, 15 pp. DOI: 10.4061/2011/471480

POUYAUD, L., DESMARAIS, E., DEVENEY, M., PARISELLE, A. (2006): Phylogenetic relationships among monogenean gill parasites (Dactylogyridea, Ancyrocephalidae) infesting tilapiine hosts (Cichlidae): systematic and evolutionary implications. *Mol. Phylogenet. Evol.*, 38: 241 – 249. DOI: 10.1016/j.ympev.2005.08.013

SWOFFORD, D. L. (2002): *PAUP**- Phylogenetic Analysis Using Parsimony. v.4.0b10 [computer software]. Sunderlan, MA: Sinauer Associates

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