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VERSITA

Gyrodactylus malalai sp. nov. (Monogenea, Gyrodactylidae) from Nile tilapia, *Oreochromis niloticus* (L.) and Redbelly tilapia, *Tilapia zillii* (Gervais) (Teleostei, Cichlidae) in the Lake Turkana, Kenya

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Abstract

Gyrodactylus malalai sp. nov. is described from the fin surface of cichlid fishes *Oreochromis niloticus* (L.) and *Tilapia zillii* (Gervais) caught in Lake Turkana (Kenya). The new species morphologically resembles *Gyrodactylus nyanzae* Paperna, 1973, but can be readily distinguished by the shape of the marginal hook sickles and the size of its hamuli. The sequence data of rDNA spanning partial 18S, internal transcribe spacer 1 and 2 and the 5.8S gene is unique within GenBank. Genetically, as most similar *Gyrodactylus ergensi* Přikrylová, Matějusová, Musilová et Gelnar, 2009 was found (97.5%). Moreover, a specimen of *G. cichlidarum* from *O. niloticus*, and a specimen *G. ergensi* from *Sarotherodon galilaeus* (L.) were collected during sampling in Kenya. Likewise, additional sampling of *O. niloticus* from the Blue Nile in Sudan revealed the presence of the newly described species. These findings represent the first records of gyrodactylids in both African countries.

Keywords

New species, *Gyrodactylus*, rDNA, East Africa, cichlids, Kenya, Sudan

Introduction

Lake Turkana is the largest lake in the East African Rift Valley situated in arid northeastern Kenya. Turkana is a closed-basin lake with only one permanent inlet, the River Omo, delivering water from the Ethiopian highlands. This brackish lake is inhabited by 48 species of fish (including seven cichlids), of which 10 are endemic to the lake (Hopson and Hopson 1982).

Recently, cichlid fish have become an important aliment source, which is demonstrated by the increase in tilapia production during the past 10 years from 1.19 million tonnes in 2000 up to 3.1 million tonnes in 2009 (FAO). Intensive cultured conditions provide an ideal environment for spread of a wide range of pathogenic organisms, including ectoparasitic gyrodactylids (Fryers and Iles 1972, Roberts and Sommerville 1980). Generally, gyrodactylosis is a serious disease in cichlid aquacultures worldwide (García-Vásquez *et al.* 2010). Monogenean parasites of cichlid fish have been intensively studied for the last few decades (Pariselle and Euzet 1995, 2003, 2009), with the main focus on the numerous viviparous species (García-Vásquez *et al*. 2007, 2010). More recently, a number of *Gyrodactylus* spp. have been discovered not only on cichlids resulting in the description of 12 new species (Přikrylová *et al*. 2009, Vanhove *et al.* 2011, García-Vásquez *et al*. 2011, Přikrylová *et al*. 2012). The total number of *Gyrodactylus* species described from African fish is generally low (total 33), and only 10 of them parasitize cichlid fish (Table I). These species represent only a fragment of the total number of valid *Gyrodactylus* species descriptions (Harris *et al*. 2004), when the expected global diversity of the genus *Gyrodactylus* was suggested to be 20 000 gyrodactylid species (Bakke *et al*. 2002, 2007).

Molecular data from African gyrodactylids is still very limited. Twelve sequences only of African *Gyrodactylus* species parasitising freshwater fish are so far available in the GenBank.

The first parasitological investigation in Lake Turkana provided a unique opportunity to collect a variety of monogenean

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Table I. Species of *Gyrodactylus* von Nordmann, 1832 recorded to date from African cichlids with type host fish species and country of observance are given

Species	Type-host	Country
G. cichlidarum Paperna, 1968	*Sarotherodon galilaeus galilaeus* (L.)	Ghana
G. ergensi Přikrylová, Matějusová, Musilová et Gelnar, 2009	*Sarotherodon galilaeus* (L.)	Senegal
G. haplochromi Paperna, 1973	*Haplochromis angustifrons* Boulenger	Uganda
G. hildae García-Vásquez, Hansen, Christison, Bron et Shinn, 2011	*Oreochromis niloticus niloticus* (L.)	Ethiopia
G. nyanzae Paperna, 1973	*Oreochromis variabilis* (Boulenger)	Uganda
G. sturmbaueri Vanhove, Snoeks, Volckaert et Huyse, 2011	*Simochromis diagramma* (Günther)	Zambia
G. thlapi Christison, Shinn et As, 2005	*Pseudocrenilabrus philander philander* (Weber)	Botswana
G. thysi Vanhove, Snoeks, Volckaert et Huyse, 2011	*Simochromis diagramma* (Günther)	Zambia
G. ulinganisus García-Vásquez, Hansen, Christison, Bron et Shinn, 2011	*Oreochromis mossambicus* (Peters)	South Africa
G. zimbae Vanhove, Snoeks, Volckaert et Huyse, 2011	*Simochromis diagramma* (Günther)	Zambia

parasites, including viviparous gyrodactylids, and, consequently, to discover as yet undescribed species. This paper presents a molecular and morphological description of a new species of the genus *Gyrodactylus* Nordmann, 1832 and the first identification of viviparous monogeneans on the inland fish in Kenya and Sudan.

Materials and methods

During September 2008 and 2009, live Mango tilapia, *Sarotherodon galilaeus*, Nile tilapia, *Oreochromis niloticus* and Redbelly tilapia, *Tilapia zillii*, were obtained from local fishermen on three sites of Lake Turkana, Kenya (Table II). Fishes were kept in tanks with aerated lake water and dissected as soon as possible after capture (within two days). Parasitological dissection of the fish was carried out in a mobile laboratory established near to the collection sites. During another sampling trip in January 2010, specimens of *O. niloticus* were collected from two localities in Sudan (see Table II). Monogenean parasites were removed from the surface of the fins, body and the gills of host fishes. The haptoral parts of the worms were excised and fixed with Malmberg´s medium (Malmberg 1970) onto microscope slides for subsequent morphological analysis. The remaining parts of the parasite´s bodies were stored in absolute ethanol SPECTRANAL (Allied-Signal, Riedel-de Haën). The specimens collected in Sudan were placed directly in absolute ethanol and the slides for morphological analysis were subsequently prepared in the home laboratory according to the methods previously used by Rokicka *et al*. (2007).

The morphological analysis of the collected parasites was carried out in the Laboratory of Parasitology at the Department of Botany and Zoology, Masaryk University, Brno, Czech Republic, focused on the morphology of the hard parts using a phase-contrast microscope (Olympus BX51). The hard parts of the worms were drawn with the aid of a drawing attachment. The measurements of hamuli and bars were taken according to Přikrylová *et al*. (2008), and the measurements of the parasite´s whole body and marginal hooks were taken according to Christison *et al*. (2005). All measurements are in micrometers unless stated otherwise and presented as mean with a standard deviation and range plus number of measurements in parentheses. For comparative studies of the African species of *Gyrodactylus*, type material of 14 species were obtained and studied, as mentioned in Přikrylová *et al*. (2009).

For the molecular analysis, ethanol from microtubes containing parasites´ body parts was evaporated in a vacuum centrifuge and DNA was extracted using Qiagen Blood and a TissueIsolation kit according to the manufacturer´s protocol. The DNA was eluted in 50 µl. The Internal Transcribed Spacer (ITS) region of the ribosomal DNA was amplified with ITS1F (5'-GTTTCCGTAGGTGAACCT-3') (Rokicka *et al*. 2007) and ITS2R (5'-TCCTCCGCTTAGTGATA-3') (Matějusová *et al*. 2001), and a partial fragment of 18S was amplified using primers S1 (5'-ATTCCGATAACGAACGAGACT-3'), which anneal in the internal region of the 18S gene (Sinnappah *et al.* 2001) and ITSR3A (5'-GAGCCGAGTGATCCACC-3') (Matějusová *et al*. 2001), in a Mastercycler eP gradient (Eppendorf).

Each amplification reaction contained 1 µl of template DNA, 1× PCR buffer, 1.25 mM $MgCl_2$, 100 µM dNTPs, 0.1 mg/ml BSA (Bovine Serum Albumin), 0.5 µM of each primer (Generi Biotech) and 1.5 U of Taq polymerase in a total volume of 20 µl.

The 5 µl of PCR product was vizualised on GoldView stained agarose gel (1%); the remaining 15 µl PCR product was purified using High Pure PCR Product Purification Kit (Roche). For sequencing, an identical primer for reverse and forward sequences, as in amplification reactions, was used together with a Big Dye Chemistry Cycle Sequencing Kit (version 3.1) and an ABI 3130 (Genetic Analyzer, Applied Biosystems). The obtained nucleic acid sequences were subjected to Basic Local Alignment Search Tool searches (BLASTN searches) (Altschul *et al*. 1990) to identify any matches or closely related *Gyrodactylus* species. Seven sequences of the African *Gyrodactylus* species were downloaded and aligned togethter with our sequences in Clustel W implement-

ed in MEGA 5 (Tamura *et al*. 2011). The included species were as follows: *Gyrodactylus cichlidarum* Paperna, 1968 (DQ124228), *Gyrodactylus ergensi* Přikrylová, Matějusová, Musilová et Gelnar, 2009 (FN394985), *Gyrodactylus hildae* García-Vásquez, Hansen, Christison, Bron et Shinn, 2011 (FJ231869), *Gyrodactylus sturmbaueri* Vanhove, Snoeks, Volckaert et Huyse, 2011 (HQ214477), *Gyrodactylus thysi* Vanhove, Snoeks, Volckaert et Huyse, 2011 (HQ214481), *Gyrodactylus ulinganisus* García-Vásquez, Hansen, Christison, Bron et Shinn, 2011 (FJ231870), *Gyrodactylus zimbae* Vanhove, Snoeks, Volckaert et Huyse, 2011 (HQ214482). Pair-wise genetic distances were computes in MEGA 5 according to the evolutionary model that was selected by jModelTest 0.1.1. (Posada 2008).

Results

Three *Gyrodactylus* spp. were indentified among the specimens collected from the studied cichlid fish. From the Kenyan *O. niloticus* and *T. zillii* a new species *Gyrodactylus malalai* sp. nov. was described. A detailed morphological and molecular description is provided below. On the Kenyan *S. galilaeus*, *G. ergensi* was recorded. Among the parasites collected from the Kenyan *O. niloticus*, one specimen was identified as *G. cichlidarum*, because the morphological and metrical characters of the haptoral sclerites of this individual were comparable to those among specimens of García-Vásquez *et al*. (2007). Examination of *O. niloticus* in Sudan revealed in record of specimens bearing haptoral sclerites of the same morphological type and metrical dimensions as those described as a new species in Kenya. The measurements of all studied gyrodactylids are presented in Table III. The presented findings represent the first record of the genus *Gyrodactylus* on fish in Kenya and in Sudan.

***Gyrodactylus malalai* sp. nov.**

Type host: *Oreochromis niloticus* L. (Perciformes, Cichlidae). Other host: *Tilapia zillii* Gervais.

Type locality: Turkana Lake (Kalokol, Kenya) N 3°33.58′, E 35°55.24′.

Other locality: Blue Nile (Sennar, Sudan) N 13°32.81′, E 33°38.17′.

Site on the host: fins.

Collection date: September 2008 and 2009, January 2010.

Deposition of type material: Canada balsam fixed specimens, holotype and paratype slides are deposited at the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic (accession number M-512). The nucleic acid sequence is deposited in the GenBank under accession number FR695484.

Etymology: Named after Dr. John Malala, research officer in the Lake Turkana Station, Kalokol (Kenya Marine and Freshwater Research Institute), recognising his contribution during the field study on Lake Turkana.

Morphological description (Fig. 1, Table III)

A new parasite species is described based on 18 individuals. Flattened specimens 792 ± 74.8 (666–876, n = 7) long, 118 ± 13.7 (98–136, n = 7) wide at the level of anterior beginning of uterus. Pharyngeal bulb 42.5 ± 6.2 (34–49.5, n = 7) long and 37.2 ± 6.4 (26.5–44, n = 7) wide. Excretory bladders observed in head part of the body. Intestinal crura not extending beyond anterior edge of testes. Male copulatory organ (MCO) positioned posteriorly to pharyngeal bulb, 15.6 ± 2 (13.5–18, n = 4) long and 13.2 ± 2.2 (11–15, n = 4) wide. When observed, armed with one larger spine and four or five smaller spines (Fig. 1B). Measurements of the haptoral sclerites are given in Table III (presented in comparison to other *Gyro-*

Table II. Host fish species, number of collected/infected specimen, mean total length ± standard deviation in cm of the cichlid fishes examined and collection dates and localities are given

Host	N/n	TL (cm)	Date	Loc
Oreochromis niloticus	40/6	23.2 ± 3.29	September 2008	Turkana Lake, Kenya
				Kalokol (N 3°33.58′, E 35°55.24′)
				Loiyangalani (N 2°51.25′, E 36°41.86′)
				Todonyang (N 4°26.15′, E 35°56.38′)
	21/3	23.2 ± 7.78	September 2009	Turkana Lake (Loiyangalani, Kalokol, Todonyang)
	11/2	17.5 ± 2.78	January 2010	Sennar, Sudan (Blue Nile N 13°32.81′, E 33°38.17′)
				Kosti, Sudan (White Nile N 13°09.77′, E 32°39.80′)
Sarotherodon galilaeus	17/2	21.5 ± 3.84	September 2008	Turkana Lake (Kalokol, Loiyangalani)
	13/1	22.5 ± 4.27	September 2009	Turkana Lake (Kalokol, Loiyangalani)
Tilapia zillii	12/0	20.1 ± 1.48	September 2008	Turkana Lake (Kalokol, Loiyangalani)
	11/2	20.5 ± 4.07	September 2009	Turkana Lake (Kalokol, Loiyangalani, Todonyang)

N/n – number of collected/infected specimens, TL – mean total length, Loc – localities.

Table III. Comparison of the measurements (mean ± standard deviation with range in parentheses, in μm) of the haptoral hard parts of *Gyrodactylus* spp. collected from *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zillii* with measurements made on the holotype of *G. nyanzae* and with measurements of *G. ergensi* reported by Přikrylová *et al.* (2009) and of *G. cichlidarum* reported by García-Vásquez *et al.* (2007)

Measurement	*G. malalai* sp. nov.(n = 18) present study	*G. nyanzae* (n = 1) holotype	*G. ergensi* (n = 14) present study	*G. ergensi* (n = 18) Přikrylová *et al.* (2009)	*G. cichlidarum* (n = 1) present study	*G. cichlidarum* (n = 20) García-Vásquez *et al.* (2007)
HTL	109 ± 3.9 (102–116.5)	91	83 ± 3.5 (77–89)	88.4 ± 2.7 (85–93.5)	60	54.3 ± 3.4 (46.6–59.9)
HPL	40.2 ± 2.9 (36–49)	34.5	31 ± 1 (29–32.5)	35.5 ± 1.3 (33.5–37.5)	24.5	25.9 ± 1.3 (23.3–27.9)
HSL	74.5 ± 2.8 (68.5–78)	62.5	59.5 ± 1.7 (57–62)	(60.5–70.5) 64.5 ± 2.4	39.5	32.7± 2.2 (26.9–35.1)
HRL	45 ± 5.5 (32.5–54)	37	33.9 ± 3.2 (28–38.5)	35.2 ± 2.2 (31–40)	21	19.8 ± 1.8 (15.6–22.2)
VBL	8.3 ± 0.6 (7.5–9.5)[c]	7.5	7.4 ± 0.7 (6–8.5)	7.1 ± 0.6 (6–8)	6.5	7.6 ± 0.9 (5.9–9.5)
VBML	14.5 ± 1.4 (12.5–16.5)[d]	11.5	11.2 ± 0.7 (10–13)[d]	11.8 ± 0.9 (10.5–14)	13.5	12.9 ± 1.1 (11–14.3)
VBW	16 ± 1.5 (23.5–28.5)[c]	24.5	18 ± 1.2 (16.5–20)	19.3 ± 1 (18–21.5)	21.5	22.9 ± 1.8 (20.1–26.9)
DBL	2.3 ± 0.2 (2–2.5)[f]		1.6 ± 0.2 (1.5–2)[e]	1.8 ± 0.2 (1.5–2)	1.7	1.5 ± 0.2 (1.2–1.8)
DBW	23.5 ± 1.1 (22.5–25)[g]		15.7 ± 1.8 (13.5–19)[e]	16.8 ± 0.9 (15.5–18)	18	21.5 ± 3 (18.2–25.3)
MHTL	32 ± 0.6 (31.5–33)[e]	35	27 ± 1.5 (25–29.5)	28 ± 1 (26.5–29.5)	28	28.2 ± 1.4 (24.3–30.3)
MHSL	8.5 ± 0.3 (8–9)[b]	9.5	6.7 ± 0.3 (6 –7)	7.1 ± 0.3 (6.5–7.5)	8.2	7.5 ± 0.4 (6.9–8.4)
MHHL	23.5 ± 0.7 (23–24.5)[c]	25.5	20.5 ± 1.3 (19–23)	20.9 ± 1.1 (18.5–22.5)	20.5	21.3 ± 1 (17.8–22.8)
MHSDW	7.3 ± 0.5 (6.5–8)[b]		4.4 ± 0.2 (4–5)	4.4 ± 0.2 (4–5)	4.8	4.9 ± 0.5 (4.1–6.3)
MHSPW	6 ± 0.3 (5.5–6.5)[c]	5	4.8 ± 0.8 (4–6)	4.6 ± 0.2 (4–5)	4.3	4.4 ± 0.3 (3.9–5.2)
MHSAD	8 ± 0.5 (7.5–9)[b]	9.5	6.2 ± 0.3 (5.5–6.5)[a]	6.6 ± 0.3 (6–7)	7.5	7 ± 0.3 (6.4–7.9)

[a]n = 13; [b]n = 12; [c]n = 11; [d]n = 10; [e]n = 9; [f]n = 7; [g]n = 6. HTL – hamulus total length; HPL – hamulus point length; HSL – hamulus shaft length; HRL – hamulus root length; VBL – ventral bar median length; VBML – ventral bar membrane length; VBW – ventral bar width; DBL – dorsal bar length; BDW – dorsal bar width; MHTL – marginal hook total length; MHSL – marginal hook sickle length; MHHL – marginal hook handle length; MHSDW – marginal hook sickle distal width; MHSPW – marginal hook sickle proximal width; MHSAD – marginal hook sickle aperture distance.

dactylus species of cichlid fish). Hamuli slender, long, well developed root with rounded edges. Root, close to the joining with shaft descend in slightly flattened area. Dorsal bar simple. Ventral bar without processes, ventral bar membrane of tongue form. Broad sickle shaft of the marginal hook sickle rises at slightly backwards angle from sickle foot, curves in a wide angle in the sickle shaft. Sickle point directed forwards, terminating beyond toe. Sickle foot of raindrop shape; upper line gradually continues into the toe. Heel moderately sloped, foot bottom in wavy shape ends in the toe.

Molecular characterization

The total fragment (1217 bp) consists of the 3' end of the 18S subunit (472 bp), the ITS1 (344), the 5.8S (157 bp), the ITS2 (244 bp). The obtained sequences of *G. malalai* sp. nov. from Kenya and Sudan were identical, except for one transition at 184 bp of the ITS1 region, when Sudanese specimens have A instead of G in Kenyan specimens. A BLASTN search (September 2010) revealed other similar sequences of different *Gyrodactylus* species. *Gyrodactylus malalai* sp. nov. based on the pair-wise distances (Tamura 3-parameter p-inv = 0.33 correct-

ed distances; Table IV) is most closely related to *G. ergensi* (2.5%).

Comments

Morphological differentiation: The shape of hamuli of *G. malalai* sp. nov. is similar to those of *G. ergensi* and *G. nyanzae*, of a slender appearance with a long root, but the hamuli of *G. malalai* sp. nov. are larger (mean total length 109 μm) than in *G. ergensi* and *G. nyanzae* (88.4 μm and 91 μm, respectively). The mentioned species differ in the shape of the marginal hook sickle. *G. ergensi* has a rounded curved sickle proper, while *G. malalai* sp. nov. sickle proper curves in a wide angle and has the sickle proper longer. Although the size of the marginal hook sickle, *G. malalai* sp. nov. and *G. nyanzae* are similar, but they differ in the shape of their marginal hook sickle

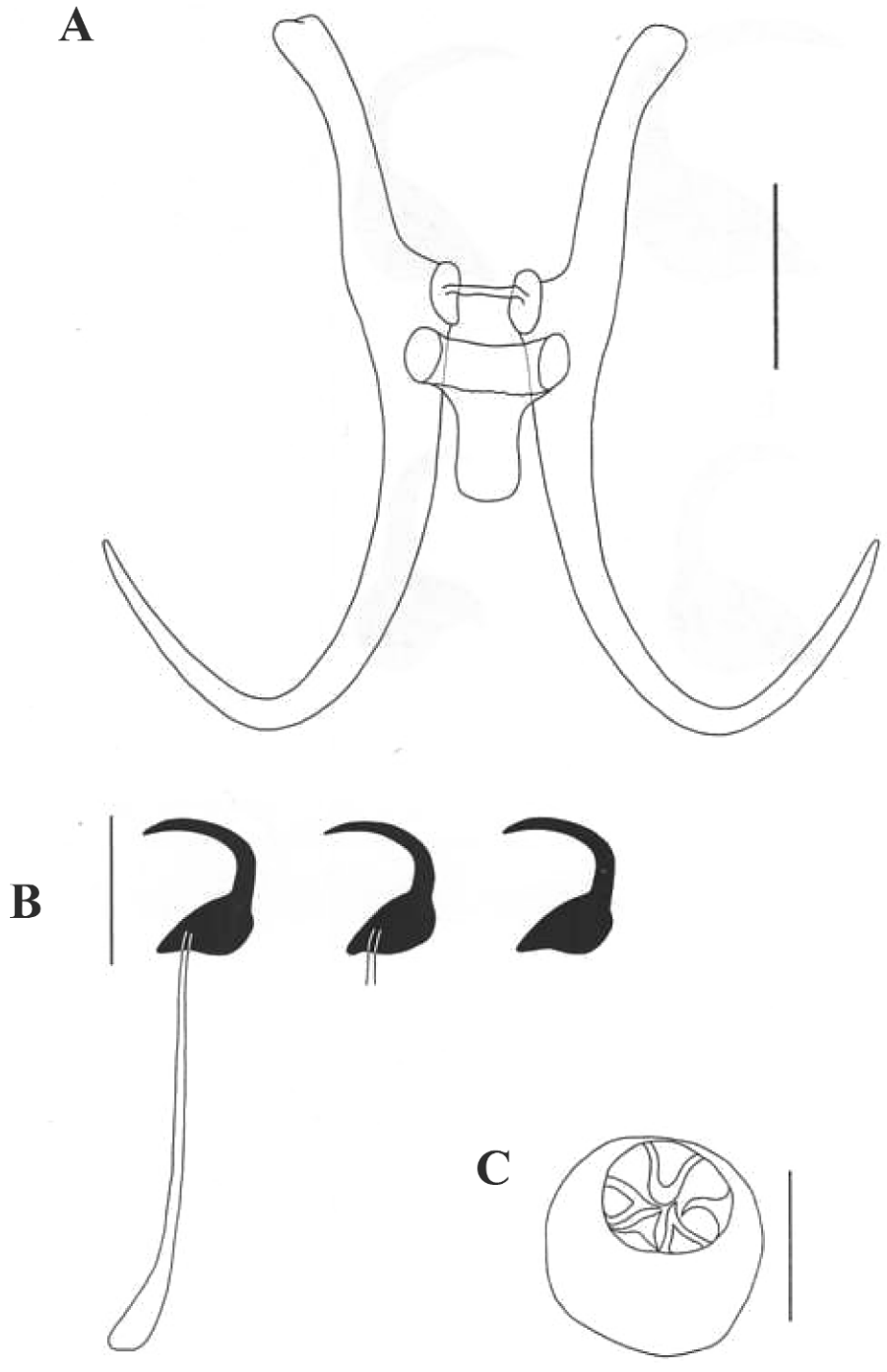

Fig. 1. *Gyrodactylus malalai* sp. nov. described from the *Oreochromis niloticus* L. **A.** An attachment complex: hamuli, ventral and dorsal bars. **B.** Marginal hooks. **C.** Detail of the cirrus. Scale bars = 30 μm (in A) and 10 μm (in B and C)

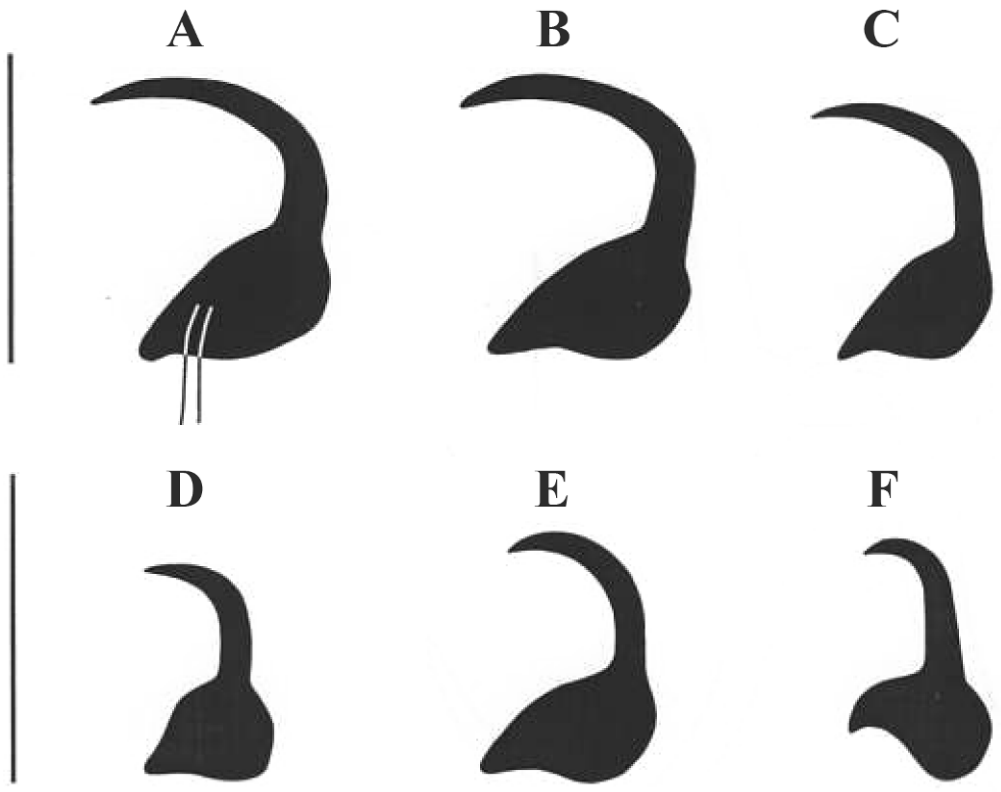

Fig. 2. Drawings of the marginal hook sickles of five *Gyrodactylus* spp. of cichlid fishes: **A** – *G. malalai* sp. nov. (Kenyan specimen). **B** – *G. malalai* sp. nov. (Sudanese specimen). **C** – *G. nyanzae* (holotype). **D** – *G. ergensi*. **E** – *G. cichlidarum* (holotype). **F** – *G. thlapi* (paratype). Scale bars = 10 μm

(Fig. 2). Compared with *G. nyanzae*, *G. malalai* sp. nov. has a more robust marginal hook sickles and the orientation of the sickle proper differs.

A clear differentitation of *G. malalai* sp. nov. from *G. ergensi* was confirmed by a molecular study. Altogether, there are 17 differences between the ITS1 and ITS2 of *G. malalai* sp. nov. and *G. ergensi*. These substitutions can be attributed to 7 in ITS1 (2 transitions and 5 transversions) and 10 in ITS2 (5 transitions and 5 transversions). The number of differences observed in the ITS regions suggest that these two species are different, and the observed 2.5% difference in ITS overreach values of 1% suggested by Ziętara and Lumme (2003) as a cut-off for delineation of *Gyrodactylus* species. Based on the differences in the pair-wise corrected distances between *G. malalai* sp. nov. and the other African *Gyrodactylus* species of cichlids can be divided into two groups (Table IV). Apart the closely related species *G. ergensi*, one group is defined by a closely related species where the genetic distances are 7.3–7.9% between the new and other species (*G. cichlidarum*, *G. sturmbaueri* and

Table IV. Pair-wise genetic distances based on the ITS1, 5.8S and ITS2 rDNA fragment of *Gyrodactylus malalai* sp. nov. and another *Gyrodactylus* species from African cichlids (Tamura 3 parameter + I model)

Gyrodactylus species	1	2	3	4	5	6	7
1. *G. malalai* sp. nov.							
2. *G. ergensi*	0.025						
3. *G. cichlidarum*	0.073	0.073					
4. *G. sturmbaueri*	0.077	0.067	0.083				
5. *G. ulinganisus*	0.079	0.078	0.049	0.095			
6. *G. hildae*	0.429	0.421	0.435	0.433	0.416		
7. *G. thysi*	0.462	0.460	0.480	0.491	0.488	0.442	
8. *G. zimbae*	0.481	0.468	0.481	0.500	0.478	0.463	0.380

G. ulinganisus) and a second group, of less related species (*G. hildae*, *G. thysi* and *G. zimbae*) with genetic distances 43–48%.

Discussion

Little is known about the species diversity of African species, because researchers, to date, have focused on the Northern hemisphere *Gyrodactylus* spp. Recent studies (Vanhove *et al*. 2011, Přikrylová *et al.* 2012, present study) indicate Africa to be a hotspot of fish parasite biodiversity with numerous species as yet undescribed. As our knowledge of monogenean biodiversity is a clear reflection of previous sampling effort, the predictions of parasite biodiversity in Asia and South America might also be high, following the conservative estimates of Bakke *et al*. (2002).

Based on the observations in Přikrylová *et al*. (2009), this research found the hamuli of *G. malalai* sp. nov., having a slender appearance, morphologically very similar to those of *G.nyanzae* and *G. ergensi.* The new species differs from these two in the total hamuli length, having slightly larger than *G. nyanzae* and *G. ergensi*. The new species also differ in all other measurements taken from the hamuli (see Table III). From Figure 2, it is clear that the shape of the marginal hook sickle of *G. malalai* sp. nov. is morphologically different from those of other *Gyrodactylus* species parasitizing cichlids. The marginal hook sickles of *G. malalai* sp. nov. are similar in size to those of *G. nyanzae*, but not in shape. The new species has a marginal hook with broad sickle shaft which rises at a slightly backwards angle from the sickle foot and curves in a wide angle in the sickle shaft. While the sickle shaft of *G. nyanzae* rises uprightly and bends in a wider angle than in *G. malalai* sp. nov. In both species the sickle point direct forward and terminate beyond the toe. Generally in comparison to *G. nyanzae*, the new species has a more robust marginal hook.

Gyrodactylus cichlidarum was originally described from *Sarotherodon galilaeus* L., but several cichlid species of different genera (*Haplochromis*, *Oreochromis*, *Tilapia*, *Tristramella*) have been noted to host this parasite under natural conditions (Shinn *et al*. 2010). Similarly, *O. niloticus* is a well known host to *G. cichlidarum*, since an infection of this species on the yolk sac of *O. niloticus niloticus* resulted in high mortality in an isolated aquarium facility at the University of Stirling (García-Vásquez *et al*. 2007). The metrical dimensions of nearly all analysed features of the haptoral sclerites of *G. cichlidarum* from the present study correspond with the measurements given by García-Vásquez *et al*. (2007). The measurements of 12 of the 15 analysed features of haptoral sclerites of *G. cichlidarum* in the present study fit into the range presented in the study of García-Vásquez *et al*. (2007), see Table III. Only one discrepancy was observed, when the length of the hamuli shaft in our study was observed to be larger (39.5 μm) than in García-Vásquez *et al*. (2007; 26.9–35.1 μm). The shape of the marginal hook sickles of *G. cichlidarum* collected in Kenya has been confirmed to be the same as in the holotype specimen of this species and those individuals presented by García-Vásquez *et al*. (2007). The third species identified in our study was *G. ergensi*. From the measurements presented in Table III, it is clearly visible that nearly all haptoral sclerite measurements (except hamulus point length) overlap with the measurements presented in the original species description (Přikrylová *et al*. 2009). The shape of the haptoral sclerites, especially the shape of the marginal hooks, was found to be identical to those of individuals recently collected and described from *S. galilaeus* in Senegal (Přikrylová *et al*. 2009).

The first molecular characteristics of *Gyrodactylus* parasitising African freshwater fish was published by García-Vásquez *et al*. (2007). Recent investigations of African gyrodactylid parasites have produced more molecular data (Přikrylová *et al*. 2009, 2012; García-Vásquez *et al*. 2011; Vanhove *et al*. 2011). In addition, Vanhove *et al*. (2011) constructed phylogram based on 432 bp of 5.8S DNA and partial ITS2 region only, showing the position of 3 *Gyrodactylus* species found on the cichlid *Simochromis diagramma* (Günther, 1894). Unfortunately, the phylogenetic position of the *G. sturmbauerei*, *G. ergensi* and *G. cichlidarum* clade was not definitively resolved in the Vanhove *et al*. (2011). In the present study, genetic distances determine *G. ergensi* as the closely related species to *G. malalai* sp. nov. and thus phylogenetic position of the new species could be easily deduced from results of Vanhove *et al*. (2011). Undoubtedly, deeper phylogenetical analysis could produce more information concerning African *Gyrodactylus* species relationships. Nevertheless, sufficient data for this kind of analysis is still not available. Phylogenetic analysis were based mainly on the ITS and 5.8S DNA region´s sequences (Cable *et al*. 1999, Huyse *et al*. 2003, Matějusová *et al*. 2003). The results of Vanhove *et al*. (2011) confirm that ITS regions are not ideal for wider comparison among the family Gyrodactylidae, because of their apparent rapid evolution (Hillis *et al*. 1996). As a better solution the sequencing of 18S region could be suggested, unfortunately very limited number of gyrodactylids has been sequenced for this region to date (Matějusová *et al*. 2003).

Molecular comparison of *G. ergensi* and *G. malalai* sp. nov. sufficiently showed that these gyrodactylid parasites represent two valid species. This was additionally supported by the differences observed in morphometry of the haptoral sclerites as mentioned above.

Present study shows that considerable effort is needed to bring more comprehensive view of African fish parasite diversity and much more descriptions of the new species are expected. In this moment, the total number of *Gyrodactylus* species described from the African continent, including new species in the present study, comprise of 34 species. The molecular characterisation of newly described *Gyrodactylus* species is now an indispensable part of new species descriptions, in very short period we may have sufficient molecular data for further analysis.

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