First description of monogenean parasites in Lake Tanganyika: the cichlid *Simochromis diagramma* (Teleostei, Cichlidae) harbours a high diversity of *Gyrodactylus* species (Platyhelminthes, Monogenea)

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SUMMARY

Lake Tanganyika harbours the most diverse endemic cichlid fish assemblage of Africa, but its monogenean fish parasites have not been investigated. Here we report, for the first time, on the *Gyrodactylus* parasites in this hotspot of fish biodiversity. Haptor morphometrics and nuclear ribosomal DNA sequences revealed 3 new species on Zambian *Simochromis diagramma*: *Gyrodactylus sturmbaueri* n. sp., *G. thysi* n. sp. and *G. zimbae* n. sp. Their distinct morphology and strong genetic differentiation suggest that they belong to distant lineages within the genus *Gyrodactylus*, and phylogenetic reconstructions suggest affinities with other genera of gyrodactylids. Additional U-shaped haptoral plates in *G. thysi* n. sp. and a second large spine-like structure in the male copulatory organ of *G. zimbae* seem to represent new features for the genus. Such large diversity on a single host species can probably be explained by host-switching events during the course of evolution, in agreement with the generally accepted concept that ecological transfer is an important aspect of gyrodactylid speciation. Additional parasitological surveys on other host species, covering a broader phylogenetic and geographical range, should clarify the evolutionary history of Gyrodactylidae on cichlids in the African Great Lake and other parts of Africa.

Key words: Africa, cichlids, Gyrodactylidae, Lake Tanganyika, Monogenea, *Simochromis diagramma*, speciation, species description, species flock, Tropheini.

INTRODUCTION

Lake Tanganyika is the largest and oldest of the East African lakes (Cohen *et al.* 1997). With about 250 cichlid and 75 other fish species, it is not the most speciose lake (Snoeks, 2000). However, its cichlid fauna displays the highest ecological and morphological diversity. The level of endemicity and the extent to which non-cichlid fishes developed into species flocks surpass the other major lakes in the region, Malawi and Victoria (Coulter, 1991*b*; Snoeks, 2000; Salzburger *et al.* 2002). Considering the substantial number of potential host species in tropical hotspots of biodiversity, such as the East African Great Lakes (Galis and Metz, 1998; Snoeks, 2000), it is anticipated that parasitological surveys will lead to the discovery of many new parasite species (e.g., Whittington, 1998).

Monogenea are very common flatworm parasites of bony fish (though other aquatic cold-blooded vertebrates, some invertebrate taxa, and *Hippopotamus* are possible hosts as well), of which they usually infect the gills and fins; they have a one-host life-cycle (Pugachev *et al.* 2009). Within this class, several genera, of which *Gyrodactylus* von Nordmann, 1832 and *Cichlidogyrus* Paperna, 1960 are well-known examples, are pathogens of fish in aquaculture. The genus *Gyrodactylus* is expected to comprise tens of thousands of species when using the rather conservative estimate of, on average, 1 species per species of fish host (Bakke *et al.* 2002, 2007; Harris *et al.* 2004). Hence, we can assume that only a small fraction of its global diversity has been described thus far. Although *Gyrodactylus* is expected to be so speciose, almost no species have been described from cichlids, in contrast to *Cichlidogyrus*. Pariselle and Euzet (2009) recorded 71 species of *Cichlidogyrus* in Africa and the Middle East, mainly from cichlids. The number of *Gyrodactylus* species from African freshwater fish amounts to only 18, according to Christison *et al.* (2005), of which only 4 were collected from cichlids. *Gyrodactylus thlapi* Christison, Shinn and van As, 2005 was described from the cichlid *Pseudocrenilabrus philander philander* (Weber, 1897) in Botswana. *G. cichlidarum* Paperna,

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1968 was collected from various tilapiine hosts, *G. haplochromii* Paperna, 1973 was described from *Haplochromis angustifrons* Boulenger, 1914 in Lake George, and *G. nyanzae* Paperna, 1973 from *Oreochromis variabilis* (Boulenger, 1906) in Lake Victoria. The only record of *Gyrodactylus* from the Great Lake is by Blais *et al.* (2007), yet without species assignment. According to Paperna (1979), *G. cichlidarum* has the widest host range of the 3 species known at that time. Recently, *G. ergensi* was described by Přikrylová *et al.* (2009*a*) from *Sarotherodon galilaeus* (Linnaeus, 1758) and *O. niloticus* in Senegal. Two additional species parasitizing African cichlids were described by Cone *et al.* (1995) from cultured Nile tilapia in the Philippines, namely *G. niloticus and G. shariffi*. The former was reported by García-Vásquez *et al.* (2007) to be synonymous to *G. cichlidarum*. Despite the paucity of knowledge about African gyrodactylids, they may be economically important, as they have the potential to be notoriously pathogenic (Paperna, 1996), for example, in captive tilapia (García-Vásquez *et al.* 2007).

Within the Gyrodactylidae, several morphological criteria, such as the distribution of marginal hooks, the presence of additional haptoral elements, and the arrangement of male copulatory organ (MCO) spines, are used to support new gyrodactylid genera and to hypothesize about the relationships among them (e.g., Luus-Powell *et al.* 2003; Vianna *et al.* 2007). However, Kritsky and Boeger (2003) found several gyrodactylid genera to cluster among *Gyrodactylus* spp. in phylogenetic analyses, indicating that the latter is paraphyletic. Hence, the status of genera within the family, and their interrelationships, are far from understood. Africa has recently seen the discovery of several morphologically deviant gyrodactylid genera, such as *Mormyrogyrodactylus* Luus-Powell, Mashego and Khalil, 2003 (MCO structure and armament, accessory bars) and *Diplogyrodactylus* Přikrylová, Matějusová, Musilová, Gelnar and Harris, 2009 (no dorsal bar, two types of marginal hooks, tubular MCO, a pair of muscular adhesive discs). The (re)descriptions mention some similarities with, among others, the African clawed toad parasite *Gyrdicotylus* Vercammen-Grandjean, 1960 and with *Macrogyrodactylus* Malmberg, 1957, a common parasite on African fish (see also Vianna *et al.* 2007). Hence, further research into diversity and phylogeny of African Gyrodactylidae seems important.

Lake Tanganyika's cichlids are classified into tribes, based on Poll (1986). One of these tribes, the endemic Tropheini, comprises a highly diverse assemblage, mostly of rock-dwelling algae scrapers. They are maternal mouth-brooders with a range of trophical morphological adaptations (Poll, 1986; Sturmbauer *et al.* 2003). Most of the representatives studied are host to *Cichlidogyrus* spp. attached to their gills and to few or no *Gyrodactylus* (nobis). An interesting exception is the genus *Simochromis* Boulenger, 1898. Its members, comprising 5 species, are grazers inhabiting shallow rocky habitats with a wide distribution throughout Lake Tanganyika (Meyer *et al.* 1996; Koblmüller *et al.* 2010). A considerable number of *Gyrodactylus* individuals were found on the largest species in the genus, *S. diagramma* (Günther, 1983), often living in co-existence on one host or in one gill chamber with *Cichlidogyrus* spp. The present study, including the description of 3 new species, represents the first record of Monogenea in Lake Tanganyika, and is the second paper to include molecular data for African *Gyrodactylus* sampled in their natural habitat.

MATERIALS AND METHODS

Sampling

Host cichlid fish were collected using hand and gill nets in the littoral rocky habitat at Kalambo Lodge (8°37′S, 31°12′E) (Zambia). They were identified to species level by C. Sturmbauer (Karl-Franzens University, Graz, Austria) and stored in pure ethanol by species. In the laboratory, the body, fins and gills, as well as the recipient's ethanol, were inspected for parasites under an Olympus SZX12 stereomicroscope. Monogenea were removed with a dissection needle. They were either stored in 5 μl of H_20 for molecular work, or mounted on a slide for morphological analysis. On some specimens morphological and genetic data were collected simultaneously by severing the haptor from the body. In this case the anterior end was stored awaiting genetic processing, whereas the haptor was used for morphological research. In some cases the complete individual was used for genetic analysis, after photographing the animal, allowing *a posteriori* comparison between morphology and DNA sequencing results. Taxon and author names of fish in this study follow Eschmeyer and Fricke (2009).

Morphometric analysis

Specimens were mounted on a slide in milli-Q water and fixed under a cover-slip using ammonium picrate-glycerine (Malmberg, 1970). On some individuals, partial digestion through proteinase K treatment was carried out following Harris and Cable (2000) to render the hard parts more visible. Pictures and measurements were taken based on Shinn *et al.* (2004) using an Olympus BX50 microscope at a magnification of 100× (oil immersion, 10× ocular) and Olympus DP-Soft 3.2 software. Principal Component Analysis on the correlation matrix of the haptor morphometrics in Statistica 8.0 (StatSoft, Inc, 2008) allowed detection of clusters representing morphospecies. Because of intra-individual variation of the marginal hook shaft and the flexibility of the

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dorsal bar, the marginal hook total length and shaft length and the dorsal bar length were omitted from the PCA. Body size parameters were also omitted because of the ethanol fixation process (causing, for example, distortion), and because of the varying degree of flattening of the body. Unfortunately, the ethanol fixation we used in this study did not allow a reliable study of the soft body parts, although these are considered systematically informative (Malmberg, 1970; Pugachev *et al*. 2009). Hence, our morphological results are limited to the hard body parts.

Molecular and phylogenetic analysis

DNA was extracted after addition of 5μl of a double-concentrated lysis solution containing 1 × PCR buffer (Eurogentec), 0·45% Tween 20 (Merck), 0·45% NP40 (Calbiochem) and $60\,\mu g.L^{-1}$ proteinase K (Sigma). Enzymatic digestion was carried out at 65 °C for 25 min, followed by inactivation of the enzyme at 95 °C for 10 min. Polymerase Chain Reaction (PCR) was performed using a GeneAmp PCR system 2700 thermocycler (Applied Biosystems). The reaction volume of 25 μl contained 2·5 μl of 10× PCR buffer (Eurogentec), 2·5 μl of dNTPs (2 mM) (Amersham Pharmacia Biotech), 1 μl of $MgCl_2$ (50 mM) (Eurogentec), 0·2 μl of *Taq* Polymerase (Eurogentec), 1 μl of each primer (20 μM) (Eurogentec), 1 μl of template DNA, topped up with milli-Q water. Primers used were ITS1A (5′-GTAACAAGGTTTCCGTAGGTG-3′) and ITS2 (5′-TCCTCCGCTTAGTGATA-3′) (Matějusová *et al*. 2001), spanning the first and second internal transcribed spacers (ITS-1 and ITS-2) and intervening 5·8S rDNA. This region has been proven to be useful in classifying and distinguishing *Gyrodactylus* spp. in agreement with species identification and delineation based on morphology (Matějusová *et al*. 2001; Meinilä *et al*. 2002; Ziętara *et al*. 2002; Huyse *et al*. 2003, 2004; Ziętara and Lumme, 2004). After an initial denaturation for 3 min at 96 °C, samples were subjected to 35 cycles of 50 s at 95 °C, 50 s at 52 °C and 50 s at 72 °C. After a final elongation of 7 min at 72 °C, samples were cooled to 4 °C. PCR products were purified with NucleoFast (Macherey-Nagel), according to the manufacturer's guidelines, and sequenced applying a 1/8 dilution of the Big Dye Terminator 3.1 sequencing protocol (Applied Biosystems). Finally products were run on an ABI PRISM 3130 Avant Genetic Analyser automated sequencer (Applied Biosystems). In addition to the initial PCR primers, the internal primers ITSR3A (5′-GAGCCGAGTGATCCACC-3′) and ITS2F (5′-TGGTGGATCACTCGGCTCA-3′) (Matějusová *et al*. 2001) were used.

For the phylogenetic analyses, we retrieved the 3 currently known sequences from African *Gyrodactylus* species: *G. cichlidarum* isolated from captive United Kingdom *Oreochromis niloticus niloticus* (Linnaeus, 1758), *G. ergensi* from *Sarotherodon galilaeus* collected in Niokolo Koba National Park, Senegal and *G. eyipayipi* Vaughan, Christison, Hansen and Shinn, 2010 from captive greater pipefish *Syngnathus acus* Linnaeus, 1758. Furthermore, in order to situate the new species within the known diversity of *Gyrodactylus* spp. and the Gyrodactylidae at large, representatives were included of each of the subgenera described by Malmberg (1970), with some extra taxa to include all clades retrieved by Ziętara and Lumme (2004), including the 3 sequenced South American species. The other Gyrodactylidae genera of which sequences are available were also added (Table 1).

Sequences were aligned by MUSCLE (Edgar, 2004) using default distance measures and sequence weighting schemes. The resulting alignment was trimmed with trimAl v.1.2 (Capella-Gutiérrez *et al*. 2009), making use of an automated trimming heuristic optimized for subsequent Maximum Likelihood (ML) phylogenetic tree reconstruction. The ITS region is challenging to align as a whole, especially due to ITS-1, which is known to display substantial length variation that limits meaningful alignments to subgenera of *Gyrodactylus* (Huyse and Volckaert, 2002; Ziętara and Lumme 2002, 2004). Due to the lack of signal in the ITS-1 region (and to a lesser extent ITS-2) when comparing distant *Gyrodactylus* species, some authors restrict the comparison to the coding and thus more conserved 5·8S rDNA. Hence, alignment and trimming (omitting all but a small portion of the spacer region) was carried out separately for the 5·8S rDNA gene and for ITS-2, retaining a dataset with a length of 157 and 275 bp, respectively. In subsequent analyses, 5·8S rDNA was combined with ITS-2, whereas ITS-1 was left out (following e.g. Ziętara *et al*. 2002; Kritsky and Boeger, 2003). Using an ML optimized base tree, jModelTest 0.1.1 (Posada, 2008; see also Guindon and Gascuel, 2003; Felsenstein, 2005) was used to estimate the optimal model of molecular evolution. Based on the corrected Akaike Information Criterion (AICc) (Hurvich and Tsai, 1989), the TVM (Posada, 2003) + Γ model was selected for the 5·8S rDNA + ITS-2 dataset. In view of the subsequent implementation in phylogenetic software, we opted for the model with the second best corrected Akaike score, namely the GTR (Tavaré, 1986; Rodriguez *et al*. 1990) + Γ model, with a gamma-shape parameter of 0·42. Under this model, a maximum likelihood (ML) search was carried out in PhyML v.3.0 (Guindon and Gascuel, 2003), assessing nodal support through 1000 bootstrap samples using the nearest-neighbour interchange branch swapping algorithm. PAUP* v.4.01b (Swofford, 2001) with the PaupUp interface (Calendini and Martin, 2005) was used for the maximum parsimony method (MP), in which gaps

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Table 1. List of published sequences used in this study

Affiliation	Species	GenBank Accession number	Reference
Gyrodactylus (*Gyrodactylus*)	*G. carassii* Malmberg, 1957	AY278033	Ziętara and Lumme (2004)
	G. elegans von Nordmann, 1832	AY278034	
Gyrodactylus (*Mesonephrotus*)	*G. arcuatus* Bychowsky, 1933[a]	AF328865	Ziętara *et al*. (2002)
	G. ostendicus Huyse and Malmberg, 2004	AY338439	Huyse *et al*. (2003)
	G. nipponensis Ogawa & Egusa, 1978[a,b]	AB063295	Hayward *et al*. (2001)
Gyrodactylus (*Metanephrotus*)	*G. branchicus* Malmberg, 1964[a]	AY061977	Ziętara and Lumme (2003)
	G. rarus Wegener, 1910[a]	AY061976	
Gyrodactylus (*Limnonephrotus*)	*G. pungitii* Malmberg, 1964[c]	AF484543	Ziętara and Lumme (2002)
	G. sprostonae Ling, 1962	AY278044	Ziętara and Lumme (2004)
Gyrodactylus (*Paranephrotus*)	*G. flesi* Malmberg, 1957[a]	AY278039	
	G. micropsi Gläser, 1974	AF328868	Ziętara *et al*. (2002)
	G. rugiensis Gläser, 1974	AF328870	
Gyrodactylus (*Neonephrotus*)	*G. anguillae* Ergens, 1960[d]	AB063294	Hayward *et al*. (2001)
African species	*G. cichlidarum* Paperna, 1968	DQ124228	García-Vásquez *et al*. (2007)
	G. ergensi Přikrylová, Matějusová, Musilová and Gelnar, 2009	FN394985	Přikrylová *et al*. (2009*a*)
	G. eyipayipi Vaughan, Christison, Hansen and Shinn, 2010	FJ040183	Vaughan *et al*. (2010)
South American species[e]	*G. bullatarudis* Turnbull, 1956	AJ011410	Cable *et al*. (1999)
	G. poeciliae Harris and Cable, 2000	AJ001844	Harris and Cable (2000)
	G. turnbulli Harris, 1986	AJ001846	Cable *et al*. (1999)
Other Gyrodactylidae	*Acanthoplacatus* sp.	AF465784	Kritsky and Boeger (2003)
	Diplogyrodactylus martini Přikrylová, Matějusová, Musilová, Gelnar and Harris, 2009	AM943008	Přikrylová *et al*. (2009*b*)
	Fundulotrema foxi (Rawson, 1973)	GQ918278	S. D. King, direct submission
	Gyrdicotylus gallieni Vercammen-Grandjean, 1960	AJ001843	Cable *et al*. (1999)
	Gyrodactyloides bychowskii Albova, 1948	AJ249348	Bruno *et al*. (2001)
	Macrogyrodactylus clarii Gussev, 1961	GU252711	Barson *et al*. (2010)
	M. clarii × *M. heterobranchii* from Zimbabwe	GU252712	
	M. clarii × *M. heterobranchii* from Kenya	GU252713	
	M. congolensis Prudhoe, 1957 from Kenya	GU252716	
	M. congolensis from Senegal	GU252717	
	M. heterobranchii N'douba and Lambert, 1999	GU252714	
	M. karibae Douëllou and Chishawa, 1995	GU252715	
	M. polypteri Malmberg, 1957	AJ567672	Matějusová *et al*. (2003)

[a] Shown to be a member of a clade including members of the *G*. (*Mesonephrotus*), *G*. (*Paranephrotus*) and *G*. (*Metanephrotus*) subgenera, as well as previously unassigned species, by Ziętara and Lumme (2004).
[b] Suggested to belong to this subgenus by Ziętara and Lumme (2004) based on phylogenetic results.
[c] Member of the *wageneri*-group of *G*. (*Limnonephrotus*).
[d] Reported to cluster with the *rugiensis*-group of *G*. (*Paranephrotus*) in Ziętara and Lumme (2004).
[e] Clustering together in Kritsky and Boeger (2003) and Ziętara and Lumme (2004).

were treated as a fifth character, and for calculating the genetic distances according to the model selected. The model was also used in Bayesian analysis, implemented in MrBayes v.3 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Posterior probabilities were calculated over 2.10^6 generations (after which stationarity of the Markov chain had been reached, indicated by a standard deviation of split frequencies of $5{\cdot}10^{-3}$, absence of a trend in the probabilities plotted against the generations, and by a Potential Scale Reduction Factor (Gelman and Rubin, 1992) converging towards 1), while sampling the Markov chain at a frequency of 100 generations. We discarded 25% of the samples as 'burn-in'. Files containing alignments were converted using ALTER (Glez-Peña *et al*. 2010).

RESULTS

Species descriptions

***Gyrodactylus sturmbaueri* n. sp.** (Fig. 1 left; Fig. 2 (A, B, H); Table 2)
Type-host: Simochromis diagramma (Günther, 1894).
Site of infection: gills, possibly fins or skin. Most specimens were attached to the gill filaments, but as

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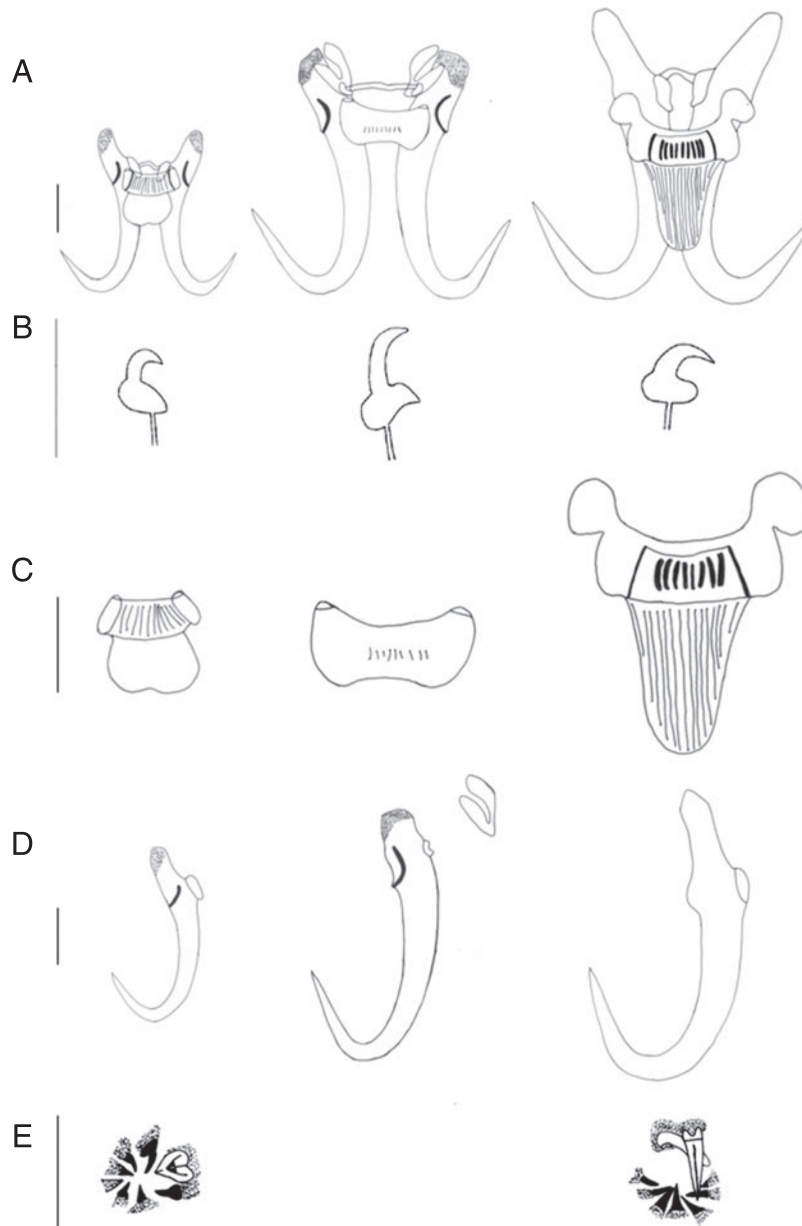

Fig. 1. Hard parts of *Gyrodactylus sturmbaueri* n. sp. (left), *G. thysi* n. sp. (middle) and *G. zimbae* (right). (A) Overview; (B) marginal hook sickle; (C) ventral bar; (D) hamulus; (E) MCO spines (not observed in *G. thysi* n. sp.). All scale bars = 20 μm.

some individuals were found detached in the preservation ethanol, we cannot exclude other infection sites.

Type-locality: Kalambo Lodge, Lake Tanganyika, Zambia (S 8°37′, E 31°12′).

Studied material: 32 mounted individuals were measured; of 5 of those, the anterior end of the body was used for molecular analysis. This, and partial proteolytic digestion of some of the mounted individuals, prevented measurements on the entire body in some specimens. Five additional specimens were sequenced.

Type-material: the holotype is deposited at the Natural History Museum, London, U.K. (2010.9.15.2). Paratypes are deposited at the Royal Museum for Central Africa, Tervuren, Belgium (37671) and at the South African Museum, Cape Town, Republic of South Africa (SAMCTA-29493).

Sequence data: a fragment of 900 bp was amplified and sequenced, containing the last 42 bp of the 18S rDNA gene, the ITS-1 (339 bp), the 5·8S rDNA gene (157 bp), the ITS-2 (303 bp) and the first 59 bp of the 28S rDNA gene. Three sites in ITS-2 were polymorphic. Sequences were deposited in GenBank (Accession numbers HQ214477-HQ214480).

Etymology: named after Professor Dr Christian Sturmbauer (Austria), specialist in the evolution of Tanganyika cichlids, and team leader of the expedition during which the host fish were caught.

Diagnosis (average (range), in micrometer): Cover-slip-flattened specimens with 2 anterior lobes, 455·9 (380·4–570·6) long and 216·8 (150·6–302·0)

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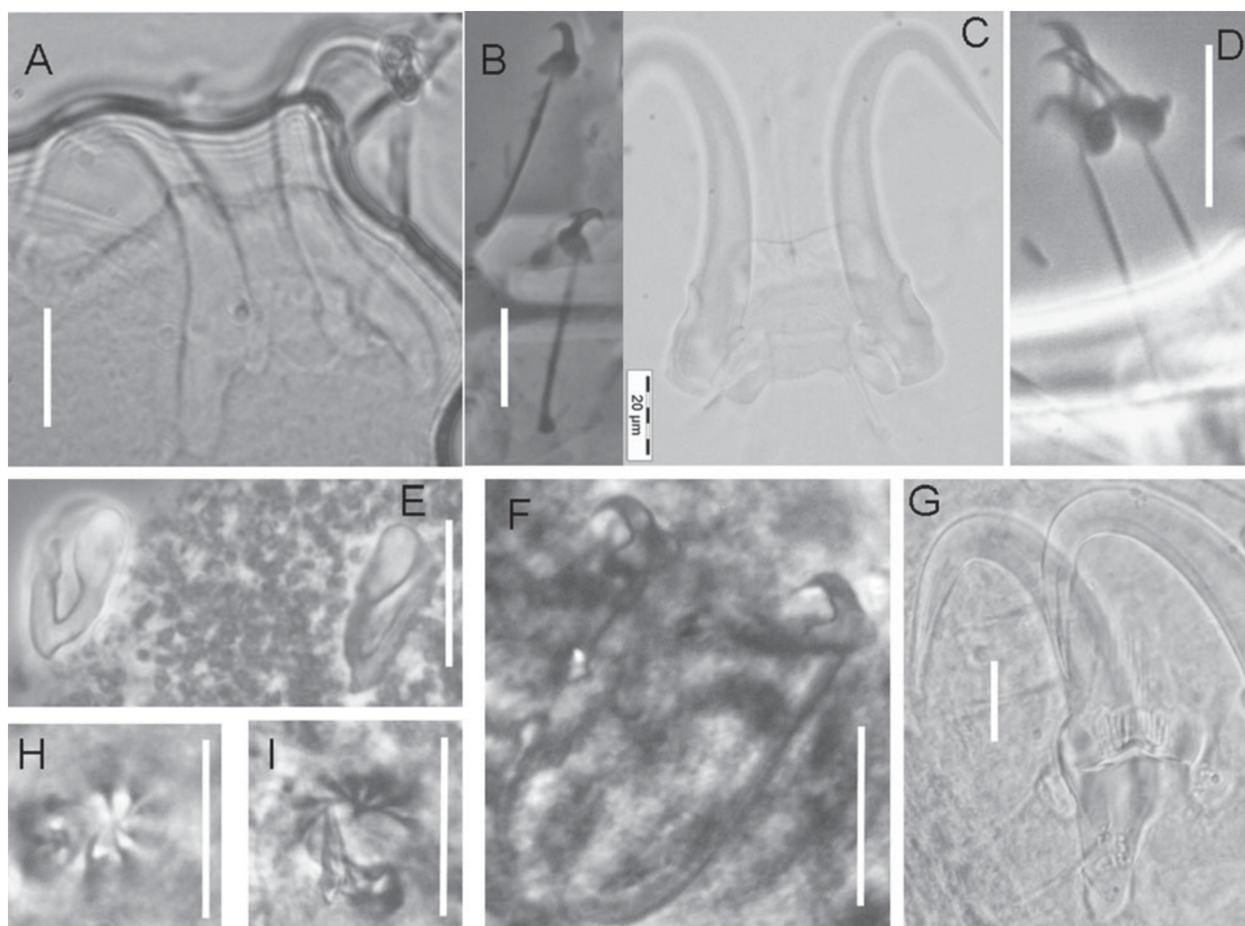

Fig. 2. Hard parts pictures of *Gyrodactylus sturmbaueri* n. sp. (A) Hamuli and bars; (B) marginal hooks; (H) MCO *G. thysi* n. sp.; (C) hamuli, bars and additional structures; (D) marginal hooks; (E) detail of detached additional structures and *G. zimbae* n. sp.; (F) marginal hooks; (G) hamuli and ventral bar; (I) MCO. All scale bars = 20 μm.

wide. MCO armed with a large apical spine of length 7·4 (6·1–9·2) and 7 smaller spines arranged in one row. One 'medial' spine of length 5·7 (4·3–6·7) and 2 'subterminal' spines 6·1 (5·2–7·3) long, are more slender than the two 6·5 (5·2–8·3) long 'terminal' ones (terminology of García-Vásquez *et al*. 2007) and than 2 spines positioned closer to the apical spine, with a length of 6·3 (4·8–8·2). Hamulus slender, 58·8 (55·8–63·9) long, with aperture 31·0 (29·0–33·3) and point of length 23·5 (20·9–29·3). Hamulus inner curve length is 1·6 (0·7–2·8). Hamulus shaft 43·0 (39·1–45·5) long, with a width of 9·6 (7·1–11·0) at the proximal and 5·5 (4·5–7·0) at the distal end. Root of hamulus 17·0 (14·5–19·9) long. Ventral bar 20·0 (16·3–23·6) long and 21·3 (18·0–23·9) wide in total. Median portion clearly striated, with a length of 8·4 (6·9–10·3). Ventral bar membrane 10·2 (7·1–13·6) long, two-lobed. Ventral bar processes small and stubby, 1·4 (1·0–2·0) long, process to mid-length 1·5 (0·9–2·3). Dorsal bar simple, 22·6 (16·4–32·5) long and 1·5 (1·2–1·9) wide. Marginal hooks with a total length of 39·0 (33·2–51·0) and a shaft length of 30·3 (24·8–42·7). Sickle of marginal hook 8·5 (7·0–10·1) long, with a proximal width of 6·7 (4·7–8·0) and a distal width of 4·6 (3·1–5·7). The foot of the marginal hook sickle comprises a considerable part of the total sickle length; toe 2·8 (2·3–3·6) long, with tip extending beyond sickle point. Marginal hook aperture 7·6 (6·2–8·8).

***Gyrodactylus thysi* n. sp.** (Fig. 1 middle; Fig. 2 (C, D, E); Table 2)

Type-host: Simochromis diagramma (Günther, 1894).

Additional host: Simochromis cfr. *diagramma* (Günther, 1894).

Site of infection: probably fins or skin. All specimens were retrieved from the preservation ethanol, and none was found attached to the gills, making a location at the outer surface of the fish most likely.

Type-locality: Kalambo Lodge, Lake Tanganyika, Zambia (S 8°37′, E 31°12′).

Studied material: 5 mounted individuals were measured; the anterior end of the body of 2 of these was used for molecular analysis. This, and partial proteolytic digestion of 4 of the mounted individuals, allowed measurements of body size in only a single specimen.

Type-material: the holotype is deposited at the Natural History Museum, London, U.K. (2010.9.15.3). Paratypes (partially digested) are

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Table 2. Comparison of morphological body and haptor measurements (in μm, average ± standard deviation with range in parentheses) of *Gyrodactylus sturmbaueri* n. sp., *G. thysi* n. sp. and *G. zimbae* n. sp.

	G. sturmbaueri n. sp. ($n = 32$)	*G. thysi* n. sp. ($n = 5$)	*G. zimbae* n. sp. ($n = 12$)
Total body length	455·9 ± 64·6[a] (380·4–570·6)	472·6[g]	1208·8 ± 575·1[h] (610·2–2342·1)
Total body width	216·8 ± 35·4[a] (150·6–302·0)	107·7[g]	440·5 ± 178·0[h] (201·8–771·3)
Hamulus aperture	31·0 ± 1·1 (29·0–33·3)	43·3 ± 5·4 (39·3–52·7)	43·9 ± 2·2 (40·4–46·4)
Hamulus proximal shaft width	9·6 ± 0·8 (7·1–11·0)	17·1 ± 1·0 (16·3–18·7)	19·5 ± 1·4 (17·1–21·7)
Hamulus point length	23·5 ± 1·6 (20·9–29·3)	43·4 ± 1·1 (41·7–44·5)	46·9 ± 1·6 (43·6–49·3)
Hamulus distal shaft width	5·5 ± 0·6 (4·5–7·0)	8·1 ± 1·1 (7·2–9·9)	11·0 ± 1·2 (9·2–13·7)
Hamulus shaft length	43·0 ± 1·4 (39·1–45·5)	71·1 ± 2·2 (67·3–73·3)	71·9 ± 1·3 (70·3–74·8)
Hamulus inner curve length	1·6 ± 0·5 (0·7 ± 2·8)	5·7 ± 1·5 (4·0–7·4)	2·7 ± 1·1 (1·2–4·7)
Hamulus root length	17·0 ± 1·4 (14·5–19·9)	18·5 ± 1·0 (17·6–20·2)	35·3 ± 2·1 (32·9–39·7)
Hamulus total length	58·8 ± 1·9 (55·8–63·9)	92·9 ± 1·7 (90·5–94·6)	105·1 ± 3·4 (99·8–111·7)
Ventral bar total length	20·0 ± 2·0[a] (16·3–23·6)	17·9 ± 2·5 (15·5–21·7)	49·9 ± 4·9[i] (38·8–55·7)
Ventral bar total width	21·3 ± 1·6[b] (18·0–23·9)	38·0 ± 6·7 (27·9–46·3)	58·8 ± 3·4 (53·0–64·6)
Ventral bar process to mid-length	1·5 ± 0·3[b] (0·9–2·3)	4·7 ± 1·3 (3·4–6·7)	13·3 ± 2·0 (9·0–15·1)
Ventral bar median length	8·4 ± 0·9[b] (6·9–10·3)	12·3 ± 1·6 (10·7–14·9)	12·5 ± 1·5 (10·7–15·0)
Ventral bar process length	1·4 ± 0·3[c] (1·0–2·0)	2·6 ± 0·7 (1·9–3·5)	10·4 ± 1·0 (9·0–12·0)
Ventral bar membrane length	10·2 ± 1·6[a] (7·1–13·6)	/	33·7 ± 2·1[i] (29·0–35·9)
Dorsal bar total length	22·6 ± 3·3[d] (16·4–32·5)	35·7 ± 3·8 (32·3–40·9)	23·3 ± 3·8[j] (19·7–29·1)
Dorsal bar width	1·5 ± 0·2[b] (1·2–1·9)	2·7 ± 0·1 (2·5–2·8)	1·7 ± 0·2[h] (1·4–2·0)
Marginal hook total length	39·0 ± 3·2[e] (33·2–51·0)	46·6 ± 3·6 (40·6–49·6)	61·0 ± 2·7[i] (58·2–67·0)
Marginal hook shaft length	30·3 ± 3·2[e] (24·8–42·7)	32·8 ± 3·9 (26·5–36·6)	51·9 ± 2·8[i] (48·5–58·6)
Marginal hook sickle length	8·5 ± 0·8[f] (7·0–10·1)	11·6 ± 1·0 (10·7–13·0)	9·4 ± 0·7[i] (8·6–10·7)
Marginal hook sickle proximal width	6·7 ± 0·8[f] (4·7–8·0)	8·3 ± 0·6 (7·5–9·0)	8·9 ± 0·8[i] (7·8–9·8)
Marginal hook toe length	2·8 ± 0·3[f] (2·3–3·6)	5·4 ± 0·9 (4·2–6·6)	4·8 ± 0·3[i] (4·3–5·1)
Marginal hook sickle distal width	4·6 ± 0·6[f] (3·1–5·7)	6·5 ± 1·0 (5·8–7·9)	7·9 ± 0·6[i] (7·3–9·2)
Marginal hook aperture	7·6 ± 0·7[f] (6·2–8·8)	13·4 ± 0·9 (11·9–14·3)	9·0 ± 0·4[i] (8·2–9·4)
MCO apical spine length	7·4 ± 0·9[k] (6·1–9·2)	/	14·6 ± 1·3[l] (13·6–16·8)
Length of 'associated' blunt MCO spine	/	/	13·1 ± 0·3[m] (12·9–13·3)
Length of small spines closest to apical spine	6·3 ± 1·3[j] (4·8–8·2)	/	/
MCO 'terminal' spine length	6·5 ± 1·1[k] (5·2–8·3)	/	6·2 ± 0·9[h] (5·2–7·5)
MCO 'subterminal' spine length	6·1 ± 0·7[k] (5·2–7·3)	/	6·3 ± 0·8[j] (5·2–7·2)
MCO 'medial' spine length	5·7 ± 0·7[k] (4·3–6·7)	/	6·0 ± 0·6[n] (5·5–6·8)

[a] $n = 23$; [b] $n = 28$; [c] $n = 27$; [d] $n = 29$; [e] $n = 30$; [f] $n = 31$; [g] $n = 1$; [h] $n = 7$; [i] $n = 11$; [j] $n = 6$; [k] $n = 15$; [l] $n = 5$; [m] $n = 2$; [n] $n = 4$.

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deposited at the Royal Museum for Central Africa, Tervuren, Belgium (37670) and at the South African Museum, Cape Town, Republic of South Africa (SAMCTA-29494).

Sequence data: a fragment of 898 bp was amplified and sequenced, containing the last 42 bp of the 18S rDNA gene, the ITS-1 (291 bp), the 5·8S rDNA gene (158 bp), the ITS-2 (348 bp) and the first 59 bp of the 28S rDNA. Both sequences were identical; the sequence was deposited in GenBank (Accession number HQ214481).

Etymology: named after Professor Emeritus Dirk F. E. Thys van den Audenaerde (Belgium), eminent researcher of African fish, and honorary director of the Royal Museum for Central Africa, for his promotion of ichthyological research in Africa.

Diagnosis (average (range), in micrometer): Cover-slip-flattened specimen 472·6 long and 107·7 wide. MCO not observed. Hamulus elongate, 92·9 (90·5–94·6) long, with aperture 43·3 (39·3–52·7) and point of length 43·4 (41·7–44·5). Hamulus inner curve length 5·7 (4·0–7·4). Hamulus shaft 71·1 (67·3–73·3) long, with a width of 17·1 (16·3–18·7) at the proximal and 8·1 (7·2–9·9) at the distal end. Root of hamulus reduced, 18·5 (17·6–20·2) long. A somewhat U-shaped structure covers the dorsal bar attachment points and part of the hamulus root. Ventral bar 17·9 (15·5–21·7) long and 38·0 (27·9–46·3) wide, with a somewhat halter-shaped median portion of 12·3 (10·7–14·9) long. Ventral bar membrane not observed. Ventral bar processes relatively small and blunt, 2·6 (1·9–3·5) long, process to mid-length 4·7 (3·4–6·7). Dorsal bar 35·7 (32·3–40·9) long. The central part of the dorsal bar 2·7 (2·5–2·8) wide, relatively broad as compared to the attachment zones to the hamulus. Marginal hooks elongate, 46·6 (40·6–49·6) long with a shaft length of 32·8 (26·5–36·6). Outwards pointing sickle of marginal hook 11·6 (10·7–13·0) long, with a proximal width of 8·3 (7·5–9·0) and a distal width of 6·5 (5·8–7·9). Curved toe of marginal hook sickle 5·4 (4·2–6·6) long. Marginal hook aperture 13·4 (11·9–14·3).

Remarks: Some metrics from the 2 sequenced individuals are quite different. Since their sequences are identical, we treat them as the same species. The fact that we did not observe a ventral membrane is very likely an artifact of proteolytic digestion; we cannot confirm its absence based on the limited number of mounts in this study. The pair of U-shaped structures at the base of the hamuli has never been recorded before in any other *Gyrodactylus* species.

***Gyrodactylus zimbae* n. sp.** (Fig. 1 right; Fig. 2 (F, G, I); Table 2)

Type-host: Simochromis diagramma (Günther, 1894).

Additional hosts: Simochromis cfr. *diagramma* (Günther, 1894), *Ctenochromis horei* (Günther, 1894).

Site of infection: mainly fins. Most studied specimens were either attached to the fins (pectoral, caudal and anal) or retrieved from the preservation ethanol. The single specimen on *Ctenochromis horei* was found on the filaments of the second gill arch.

Type-locality: Kalambo Lodge, Lake Tanganyika, Zambia (S 8°37′, E 31°12′).

Studied material: 12 mounted individuals were measured; of 2 of those, the anterior end of the body was used for molecular analysis. This, and partial proteolytic digestion of some of the mounted individuals prevented measurements of body size on some specimens. Five additional specimens were sequenced. Their pictures were taken before DNA extraction, allowing *post-factum* measurements to ensure their position within the morphometric range of *Gyrodactylus zimbae*. Those measurements were not included in the descriptive statistics.

Type-material: the holotype is deposited at the Natural History Museum, London, U.K. (2010.9.15.4) Paratypes are deposited at the Royal Museum for Central Africa, Tervuren, Belgium (37672), and at the South African Museum, Cape Town, Republic of South Africa (SAMCTA-29495).

Sequence data: a fragment of 1000 bp was amplified and sequenced, containing the last 42 bp of the 18S rDNA, the ITS-1 (372 bp), the 5·8S rDNA gene (158 bp), the ITS-2 (369 bp) and the first 59 bp of the 28S rDNA. All sequences were identical; the sequence was deposited in GenBank (Accession number HQ214482).

Etymology: named after Justina Kasabila Zimba (Zambia), research officer in charge of the Lake Tanganyika Research Station in Mpulungu, for her contributions at the field laboratory during the sampling of the fish and their parasites.

Diagnosis (average (range), in micrometer): Two anterior lobes. Considerable body size with a length of 1208·8 (610·2–2342·1) and width of 440·5 (201·8–771·3). MCO with a large apical spine of length 14·6 (13·6–16·8) with broad base, and seemingly associated with a blunt spine of comparable size range 13·1 (12·9–13·3). One row of 7 smaller MCO spines, each similarly thick, of length 6·2 (5·2–7·5) (two 'terminal' pairs), 6·3 (5·2–7·2) ('subterminal' pair) and 6·0 (5·5–6·8) ('medial' spine) (terminology based on García-Vásquez *et al*. 2007). Hamulus firm, 105·1 (99·8–111·7) long, with aperture 43·9 (40·4–46·4) and point of 46·9 (43·6–49·3) long. Hamulus inner curve length 2·7 (1·2–4·7). Hamulus shaft 71·9 (70·3–74·8) long, with a width of 19·5 (17·1–21·7) at the proximal and 11·0 (9·2–13·7) at the distal end. Root of hamulus 35·3 (32·9–39·7) long. Ventral bar 49·9 (38·8–55·7) long and 58·8 (53·0–64·6) wide in total, with a median length of 12·5 (10·7–15·0) and an elongate membrane of length 33·7 (29·0–35·9). Both ventral bar and membrane with clear striae. Ear-shaped ventral bar processes 10·4 (9·0–12·0) long, processes to mid-length

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13·3 (9·0–15·1). Dorsal bar 23·3 (19·7–29·1) long and 1·7 (1·4–2·0) wide, attachment zones to hamulus of irregular shape. Marginal hooks with a total length of 61·0 (58·2–67·0) and a shaft of 51·9 (48·5–58·6) long. Sickle of marginal hook 9·4 (8·6–10·7) long, with a proximal width of 8·9 (7·8–9·8) and a distal width of 7·9 (7·3–9·2). Sickle point extends beyond toe tip. Toe of marginal hook sickle blunt, 4·8 (4·3–5·1) long. Marginal hook aperture 9·0 (8·2–9·4).

Morphometric analysis

In addition to the *Gyrodactylus* spp. found on *Simochromis diagramma and S.* cfr. *diagramma*, we recorded 4 other congeners in Lake Tanganyika (Table 4). The specimen on *Ctenochromis horei* was identified as *Gyrodactylus zimbae* n. sp. from genetic data, confirming its position in a PCA plot (Fig. 3, visualizing the resemblance of the specimens found on other host species to the species described in this study). The specimens on *Cyathopharynx furcifer* (Boulenger, 1898) and *Lobochilotes labiatus* (Boulenger, 1898) strongly resemble *Gyrodactylus zimbae* n. sp. (Fig. 3). None of those specimens was included in the species diagnosis above, since we cannot assess with certainty whether they fall within the normal size range of the species under study, display phenotypic plasticity as adaptation to a different host, or represent a new species. Unfortunately, no material of these specimens was available for molecular analysis. The parasite infecting the gills of *Tropheus moorii* Boulenger, 1898 clusters close to *Gyrodactylus sturmbaueri*, but clear qualitative differences (e.g. relatively longer hamulus root) were observed, leading us to conclude that it belongs to a different species.

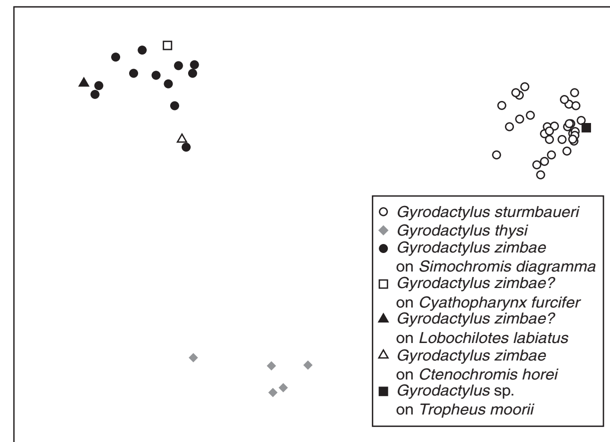

Fig. 3. Plot of principal component analysis (horizontal axis: PC 1, vertical axis: PC 2) on measurements of 32 *Gyrodactylus sturmbaueri* n. sp., 5 *G. thysi* n. sp. and 12 *G. zimbae* n. sp. on the type-hosts, including 4 *Gyrodactylus* spp. on other host species. Body size, marginal hook total length, shaft length, and dorsal bar length were not included in the PCA.

Molecular and phylogenetic analysis

BLAST searches came up with 2 close matches spanning the complete ITS-1, 5·8S and ITS-2 region, for *G. sturmbaueri* n. sp., displaying 92% overall identity to *G. ergensi* and 91% to *G. cichlidarum*. These are the only sequenced *Gyrodactylus* species from cichlid hosts and from African freshwater fish. Genetic distances (based on 432 bp of 5·8S rDNA and ITS-2) between all species in the analysis are shown in Table 3. The distance between *G. sturmbaueri* n. sp. (and its 2 close relatives) and *G. thysi* n. sp. is similar to the distance to African gyrodactylid genera *Macrogyrodactylus* and *Gyrdicotylus*, to most of the other *Gyrodactylus* spp., and to *Fundulotrema* (namely 30–41%). The genetic distance to *G. zimbae* n. sp. is 41–45%. The difference between the 3 new species and the other genera ranges from 41 to 49% for *Diplogyrodactylus martini* and is above 45% for *Gyrodactyloides and Acanthoplacatus*.

A phylogenetic reconstruction is shown in Fig. 4. The 2 known tilapiine parasites firmly group together with *G. sturmbaueri* n. sp. in all analyses. The sequence of *G. zimbae* n. sp. seems to be more distinct, as it shows a higher similarity to that of various non-African representatives than the other African species, especially in ITS-2. It seems to cluster with species of *G.* (*Paranephrotus*), *G.* (*Mesonephrotus*) and *G.* (*Metanephrotus*), as well as *Fundulotrema foxi* and the South American *Gyrodactylus* spp. The other African species, *G. eyipayipi*, appears to be most closely related to members of the *rugiensis* group of *G.* (*Paranephrotus*) and *G.* (*Neonephrotus*), which in turn clusters together with *G.* (*Limnonephrotus*), confirming the findings of Huyse *et al.* (2003) and Ziętara and Lumme (2004). *Acanthoplacatus* sp. is basal to this clade. The representatives of *G.* (*Gyrodactylus*) cluster together, with strong support, but the position of this subgenus and that of *Gyrodactyloides bychowskii* vary according to the method of phylogenic reconstruction, and remained unresolved.

DISCUSSION

Although the African Great Lakes are acknowledged hotspots of biodiversity, especially because of the well-studied cichlid species flocks (Galis and Metz, 1998; Snoeks, 2000), they have been almost entirely neglected in parasite research. Among all African cichlids, 69 *Cichlidogyrus* species have been described, but only 5 *Gyrodactylus* spp. (Christison *et al.* 2005; Přikrylová *et al.* 2009*a*). Apart from studies on a few Digenea, Cestoda and Acanthocephala (Prudhoe, 1951), the helminths of Lake Tanganyika have been greatly overlooked or ignored. The 3 species descriptions represent the first monogenean records from Lake Tanganyika.

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Table 3. Gamma-corrected pairwise genetic distances (in %) between the taxa included in the phylogenetic analysis, for a fragment of 432 bp (5·8S rDNA and partial ITS-2)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	*Gyrodactylus sturmbaueri* n. sp.																																		
2	*G. thysi* n. sp.	39																																	
3	*G. zimbae* n. sp.	43	52																																
4	*G. cichlidarum*	8	34	41																															
5	*G. ergensi*	7	37	45	8																														
6	*G. eyipayipi*	37	44	41	39	39																													
7	*G. bullatarudis*	41	43	35	33	33	48																												
8	*G. poeciliae*	46	45	36	44	40	53	14																											
9	*G. turnbulli*	41	39	36	38	39	48	26	27																										
10	*G. carassii*	34	42	52	34	36	40	56	52	48																									
11	*G. elegans*	31	46	54	33	34	44	49	48	41	8																								
12	*G. arcuatus*	40	36	28	36	39	39	27	27	27	42	44																							
13	*G. ostendicus*	36	33	24	31	36	32	24	25	28	37	39	5																						
14	*G. nipponensis*	36	33	25	33	36	32	25	23	27	37	38	5	2																					
15	*G. branchicus*	33	34	16	29	31	32	22	22	22	34	38	9	8	9																				
16	*G. rarus*	33	34	16	29	31	32	22	22	22	33	37	9	8	9	0																			
17	*G. pungitii*	38	42	37	38	38	20	37	36	32	38	38	34	27	27	25	25																		
18	*G. sprostonae*	31	37	37	32	33	16	40	38	34	38	40	35	26	26	24	23	6																	
19	*G. flesi*	38	39	26	37	35	35	26	25	30	39	43	18	17	17	12	12	26	24																
20	*G. micropsi*	39	45	41	38	37	11	44	49	42	38	42	37	29	28	30	31	18	13	33															
21	*G. rugiensis*	37	46	41	38	38	13	46	45	41	38	43	38	31	29	29	29	19	13	31	6														
22	*G. anguillae*	39	43	44	40	39	11	46	54	46	37	43	41	34	33	33	33	19	13	31	4	6													
23	*Acanthoplacatus* sp.	59	61	50	56	55	36	48	49	47	57	55	49	43	43	39	40	29	32	43	29	31	32												
24	*Diplogyrodactylus martini*	49	45	41	49	46	35	39	39	37	43	43	36	37	35	32	33	43	38	32	33	30	31	51											
25	*Fundulotrema foxi*	36	34	24	34	34	35	22	26	24	42	37	14	14	15	10	11	24	26	18	33	33	35	35	39										
26	*Gyrdicotylus gallieni*	36	38	40	33	33	36	34	34	39	29	29	36	33	33	30	30	32	30	34	41	43	42	52	40	37									
27	*Gyrodactyloides bychowskii*	45	46	49	50	50	42	46	51	42	39	38	39	40	39	32	31	37	33	36	42	39	42	64	49	36	49								
28	*Macrogyrodactylus clarii*	33	22	36	32	33	28	29	31	30	23	22	24	21	20	21	20	29	23	23	26	27	28	39	31	26	24	31							
29	*M. clarii* × *M. heterobranchii* (Kenya)	38	27	40	38	39	32	36	33	36	27	27	27	23	22	25	25	35	29	26	30	32	32	48	33	32	27	39	0						
30	*M. clarii* × *M. heterobranchii* (Zimbabwe)	33	22	36	32	33	28	29	31	30	23	22	24	21	20	21	20	29	23	23	26	27	28	39	31	26	24	31	0	0					
31	*M. congolensis* (Kenya)	38	29	40	38	41	35	35	35	31	30	28	28	26	26	24	23	34	29	27	34	34	35	47	35	28	28	31	6	6	6				
32	*M. congolensis* (Senegal)	38	29	40	38	41	35	35	35	31	30	28	28	26	26	24	23	34	29	27	34	34	35	47	35	28	28	31	6	6	6	0			
33	*M. heterobranchii*	37	26	39	37	38	32	35	34	35	27	26	27	24	23	25	24	36	29	27	31	33	33	47	34	31	27	38	0	0	0	6	6		
34	*M. karibae*	32	25	32	32	35	28	28	30	26	26	23	22	22	22	19	19	28	23	23	30	29	30	40	31	22	23	25	5	5	5	1	1	5	
35	*M. polypteri*	30	27	36	31	34	35	31	36	37	27	27	31	30	30	25	25	38	32	29	35	34	36	49	35	31	26	32	9	10	9	6	6	9	6

Table 4. Records of *Gyrodactylus* spp. in Lake Tanganyika on hosts other than *Simochromis diagramma* or *S.* cfr. *diagramma*

Location	Host species	Infection site	Material available	Species
Kalambo Lodge	*Ctenochromis horei* (Günther, 1984)	Gills (second gill arch)	Picture, sequence (ITS-1, 5·8S rDNA, ITS-2)	*G. zimbae*
Kalambo Lodge	*Cyathopharynx furcifer* (Boulenger, 1898)	Gills (first gill arch)	Mounted specimen	*G. zimbae*?
Mbita Island	*Lobochilotes labiatus* (Boulenger, 1898)	Pelvic fin	Mounted specimen	*G. zimbae*?
Chaitika Point	*Tropheus moorii* (Boulenger, 1898)	Gills (fourth gill arch)	mounted specimen	*G.* sp.

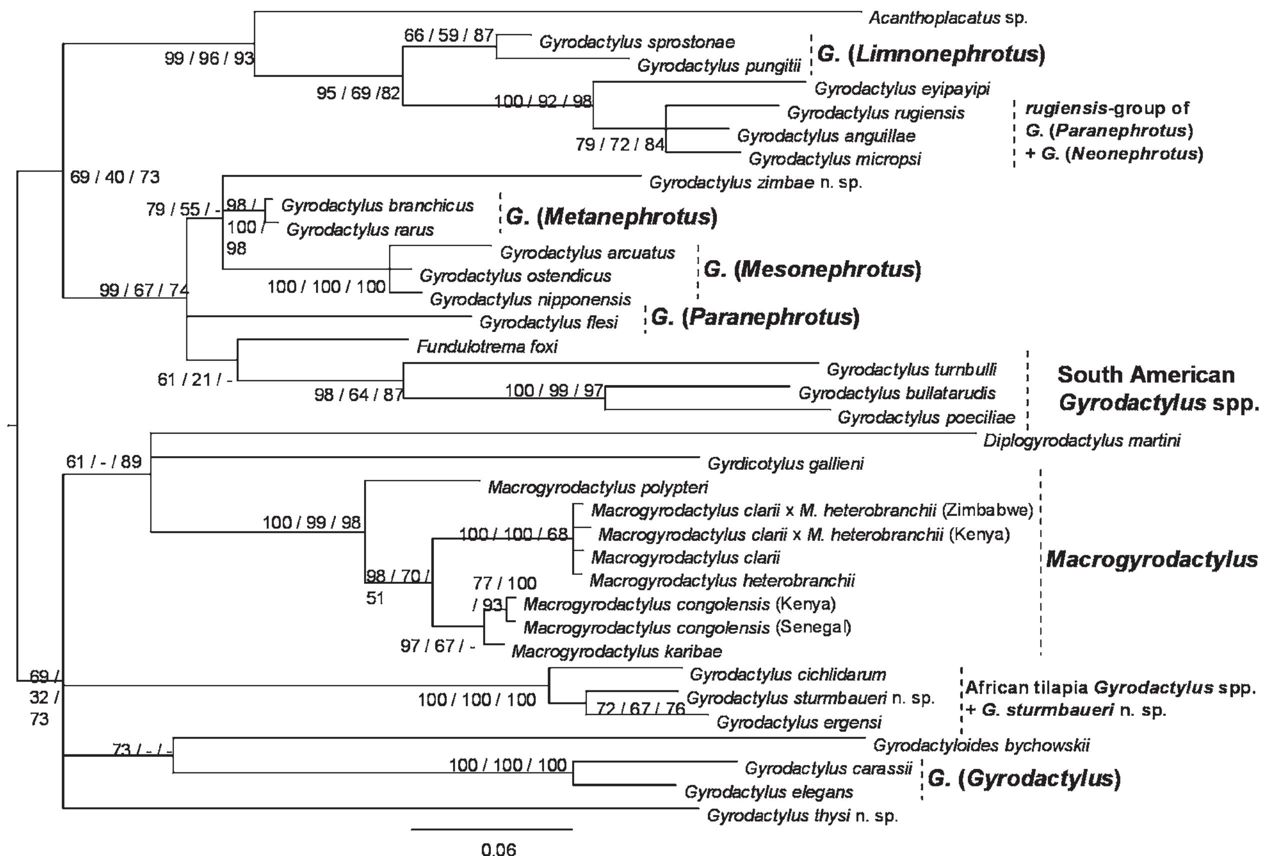

Fig. 4. Phylogram constructed from 5·8S rDNA (157 bp) and partial ITS-2 sequences (275 bp) for gyrodactylid genera, and subgenera and selected species of *Gyrodactylus*. Statistical node support is consequently shown as follows: Bayesian posterior probability/maximum likelihood bootstrap/maximum parsimony bootstrap. Branch lengths correspond to the expected number of substitutions per site under Bayesian inference.

Comparisons with other Gyrodactylus *spp.*

Based on molecular data, *G. sturmbaueri* n. sp. appears relatively closely related to *G. cichlidarum* from *Oreochromis niloticus* and to *G. ergensi* from *Sarotherodon galilaeus and O. niloticus*. Even though these are the only available sequences of African species so far, an overall identity of over 90% in the ITS rDNA region, and an identical 5·8S rDNA gene, do suggest a very close evolutionary relationship between the 3 species. Morphologically, however, they are quite distinct: *G. cichlidarum* displays slightly smaller haptoral elements, a more slender hamulus, a more curved ventral bar, and a more pointed marginal hook sickle, with the point extending beyond the toe. *G. sturmbaueri* n. sp. is also distinct from *G. ergensi*, for instance, in the longer and more slender hamulus and the shorter marginal hook shafts of the latter species. The number of small MCO spines also differs between the 3 species (7 in *G. sturmbaueri* n. sp. *versus* 6 in *G. cichlidarum* and 5 in *G. ergensi*). *G. zimbae* n. sp. seems relatively large in comparison to the known African *Gyrodactylus* fauna, as it is twice as large as most species described so far (Christison *et al.* 2005). The presence of a blunt, spine-like structure whose basis is associated

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with the large apical spine (it was observed both next to and partly under the other large spine, possibly as an artifact of flattening) is not known from other gyrodactylids.

The haptor morphology of *G. thysi* n. sp. is quite similar to *G. thlapi* described on *Pseudocrenilabrus philander philander*. This is especially true for the elongated marginal hook with a broad, poorly angling point, a curved foot with pointed toe, a long, rounded heel and the position of the origin of the marginal hook shaft. The simple dorsal bar with relatively small attachment zones and the shape of the ventral bar remind of *G. thlapi*. The dimensions of *G. thlapi* are, however, smaller for all haptoral elements. An interesting feature is the reduced hamulus root. Christison *et al*. (2005) stated that, although the influence of this reduction in root length on attachment to the host surface is unknown, it may be compensated for by the longer attachment planes of the ventral bar, allowing sufficient rotation of the hamuli over the ventral bar (see Shinn *et al*. (2003) for the mechanism of attachment and the role of the different haptoral elements). Although still a matter of speculation, the U-shaped structures we observed near the hamulus roots of *G. thysi* might also have a role in compensating for the short root, perhaps in association with the muscular retraction of the hamuli towards the body of the worm. In one of our mounts, the haptor became detached from the body, while the U-shaped structures remained attached to the body. This might suggest a function in the interplay between haptoral parts and body (musculature). In any case, additional haptoral elements of such shape have not been recorded before, though other *Gyrodactylus* spp. seem to have structures associated with the hamulus root (Pugachev *et al*. 2009). More species descriptions and molecular data are needed to clarify the interrelationships with other species, and to establish whether the observed similarities represent true relationships or parallel evolution.

Phylogenetic positioning within the gyrodactylid family

Genetic distances suggest that species currently assigned to *Gyrodactylus* differ as much from each other as from other gyrodactylid genera. Together with the phylogenetic reconstructions, this supports the view of Kritsky and Boeger (2003) that *Gyrodactylus* is not a natural grouping. Representatives of *Acanthoplacatus, Gyrdicotylus, Gyrodactyloides* and *Fundulotrema* cluster within *Gyrodactylus* spp. both in our analyses and in the study of Kritsky and Boeger (2003). However, the ITS region is not ideal for phylogenetic analysis at this level, because of its apparent rapid evolution (Hillis *et al*. 1996). Therefore, artificial groupings due to long-branch attraction cannot be excluded.

The position of *G. thysi* n. sp. is not well supported in the 5·8S rDNA + ITS-2 analysis, but, based on the pairwise genetic distances, it is most closely related to *Macrogyrodactylus*. The limited amount of data needs to be considered, but it is noteworthy that the aforementioned genus is characterized by additional haptoral parts, as is *G. thysi* n. sp. Other genera considered to be related to *Macrogyrodactylus* on the basis of the haptoral arrangement have recently been described both in Africa (*Mormyrogyrodactylus*) and America (*Scutalatus* Vianna, Boeger and Dove, 2007) (Luus-Powell *et al*. 2003; Vianna *et al*. 2007). It would be interesting to investigate their relationship with less rapidly evolving markers and to test the monophyly of *Gyrodactylus*. Indeed, to cite Kritsky and Boeger (2003), this genus as used to date could be a '*catch-all' taxon for species lacking distinctive morphological traits*.

The position of the *G. sturmbaueri* n. sp., *G. ergensi* and *G. cichlidarum* clade is not well resolved, but they cluster firmly, and share an identical 5·8S sequence. They could belong to 1 subgenus, as this gene is believed to be useful to distinguish among the 6 subgenera of *Gyrodactylus* that were described by Malmberg (1970) based on the excretory system (Ziętara *et al*. 2002; Huyse *et al*. 2003), and is also used by García-Vásquez *et al*. (2007) for the subgenus affiliation of *G. cichlidarum*. Morphological analysis of soft parts is paramount to formally investigate whether raising this clade to subgenus level would be justified. The most divergent new species is *G. zimbae*. It clusters, mainly supported by BI, together with members of a diverse clade identified by Ziętara and Lumme (2004). In contrast to the study of Ziętara and Lumme (2004), but corroborating Kritsky and Boeger (2003), the South American *Gyrodactylus* spp. are also related to this group, as is *Fundulotrema*. Hence, the geographical and ecological diversity of this clade could be larger than already put forward by Ziętara and Lumme (2004). No affinities where shown between the 3 Tanganyika species and *G. eyipayipi*, the only African marine species known so far (Vaughan *et al*. 2010). This species clustered closely with members of the *rugiensis*-group which infects North Atlantic gobies.

The origin of the Gyrodactylus *species on* Simochromis *cichlids*

The large genetic distances between the 3 new *Gyrodactylus* species (in comparison to species sharing 1 host in the studies of Matějusová *et al*. 2001; Ziętara and Lumme, 2002; Huyse *et al*. 2003), as well as the phylogenetic reconstructions, suggest that they belong to distinct lineages within the genus *Gyrodactylus*, and that they cannot have diverged within their present type-host. Moreover, applying a molecular clock for *Gyrodactylus* of 5·5%.my^{-1} (Ziętara and Lumme, 2002), the origin of the 3 *Gyrodactylus* spp. has been dated at more than

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4 MYA (based on the highest distance in the 5·8S rDNA + ITS-2 fragment, but this could be an underestimation due to our inability to align the entire ITS region). Consequently, it predates the split between the Tropheini and the other 'modern haplochromines', which was dated at 2.8 million years ago (Koblmüller *et al*. 2010).

How can the occurrence of such highly distinct *Gyrodactylus* lineages on a single host be explained? One of the following scenarios or a combination of them can be put forward.

(1) Lake Tanganyika as an evolutionary reservoir for ancient lineages

Due to the lake's depth and stability, it harbours several lineages of thalassoid gastropods that became extinct elsewhere in Africa, serving as a reservoir for subsequent colonizations (Wilson *et al*. 2004). This function also holds for the major cichlid lineages (Nishida, 1991; Salzburger *et al*. 2002) and therefore for Monogenea possibly too.

(2) Host migration and parasite exchange within Lake Tanganyika

The possibility of viviparous Gyrodactylidae to change hosts as adults (Cable *et al*. 2002) is an important trigger for diversification (Boeger *et al*. 2003) and a common, if not their most important, mode of speciation (Harris, 1993; Ziętara and Lumme, 2002). Due to an increased chance of parasite encounters, host geographical range and vagility has been suggested to be positively correlated with parasite species richness (Gregory, 1990; Mwita and Nkwengulila, 2008). Because of the high mobility of *Simochromis* and given their limited level of stenotopy, such a phenomenon seems possible for *Simochromis* hosts as well. This could also explain the near-absence of geographical races in *Simochromis* (Brichard, 1978; Meyer *et al*. 1996; Konings, 1998). However, due to a complete lack of other *Gyrodactylus* material from the Tanganyika basin, it is at present impossible to hypothesize whether such host-switches took place in the present-day *Simochromis* species, or in ancestral lineages.

(3) Host migration and parasite exchange with riverine species

The *Gyrodactylus* diversity on *Simochromis* might also originate to some extent from colonizations from outside the lake, as is the case in Tanganyika crustacean fish parasites, for which multiple invasions into the lake are probable (Coulter, 1991*a*). It is interesting to remember the morphological resemblances between *G. thysi* n. sp. and the *Pseudocrenilabrus philander* parasite *G. thlapi* that occurs in the Okavango Delta, Botswana. Fish dispersal pathways must have existed at some point in time between African waterways in general and the Great Lakes in particular (Greenwood, 1983; Salzburger *et al*. 2005). Moreover, Tropheini are derived from a generalist riverine ancestor, and *Pseudocrenilabrus* is ancestral to the Tropheini and the modern Haplochromini (Salzburger *et al*. 2005). It would be interesting to investigate, with molecular data, the influence of phylogenetic or ecological affinities of the hosts on the evolution of their parasites (as Barson *et al*. (2010) did for *Macrogyrodactylus*).

Host switching might also explain the close relation between *G. sturmbaueri* n. sp. and *G. ergensi and G. cichlidarum*, parasites of various Tilapiini occurring in the Levant, North, West and West-Central Africa (Trewavas, 1983). The typical *Simochromis* habitat is the rocky littoral, but it is also abundant in the shallow muddy habitats, often associated with nearby river estuaries. It shares these habitats, and its herbivorous substrate grazing lifestyle, with *Oreochromis tanganicae* (Günther, 1894). This is the only *Oreochromis* in the lake proper, though congeners like *O. karomo* (Poll, 1948) and *O. niloticus* also occur in the Tanganyika basin (Brichard, 1978; Konings, 1998). Based on the limited data available, it is impossible, however, to infer or reconstruct host-switching pathways.

Host and site specificity

Our sampling strategy did not allow a clear distinction between fin and gill parasites, but based on haptor morphology we suspect a difference in infection sites: fins for *Gyrodactylus zimbae* n. sp. and *G. thysi* n. sp., and gills for *G. sturmbaueri* n. sp. A smaller haptor is characteristic for gill species (Malmberg, 1970). It is interesting that *G. zimbae* n. sp. was found on the gills of a non-*Simochromis* host, namely *Ctenochromis horei*. We might be observing an accidental host and site-switch, and as a wide range of host specificity exists within *Gyrodactylus* (Bakke *et al*. 2002, 2007), niche change may happen relatively quickly between closely related *Gyrodactylus* species (Huyse *et al*. 2003) and even within a species (e.g. *G. arcuatus* (Raeymaekers *et al*. 2008)). Although the identification of *G. zimbae* n. sp. on *C. horei* was confirmed molecularly and morphologically, the specimens on *C. furcifer and Lobochilotes labiatus*, resembling *G. zimbae* n. sp., were not available for molecular analysis. Although Paperna (1979) stated that some African *Gyrodactylus* exhibit low host-specificity, we might be dealing with cryptic species, as similar-looking specimens from different hosts have already been shown to be different species (Geets *et al*. 1999; Ziętara and Lumme, 2002; Huyse and Malmberg, 2004; Kuusela *et al*. 2008). Therefore we need molecular confirmation, before inferring a host-switch to the quite distantly related cichlid *Cyathopharynx furcifer* that belongs to the tribe Ectodini (Poll, 1986).

CONCLUSIONS

These are the first records of Monogenea in Lake Tanganyika. The Tropheini cichlid *Simochromis*

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diagramma was found to be infected with 3 new *Gyrodactylus* species, highly distinct in morphology and DNA sequence. Except for genetic similarity between *G. sturmbaueri* n. sp., *G. ergensi* and *G. cichlidarum*, and some morphological resemblance between *G. thysi* n. sp. and *G. thlapi*, no close affiliations were found with other *Gyrodactylus* species. The divergence between the 3 species, of which some display morphological features unknown from the genus, and their position in a molecular phylogeny of Gyrodactylidae, suggests affinities with other genera and the non-monophyly of the genus *Gyrodactylus*. The reservoir function of Lake Tanganyika for ancient lineages, in combination with ecological transfers from unrelated hosts (within the lake proper, or from or towards riverine systems) could be responsible for this unusual combination of *Gyrodactylus* species on a single host species. A more specific scenario, however, cannot be inferred based on the present data. Other fish species from Lake Tanganyika and surrounding river systems should be investigated for the presence of *Gyrodactylus*, in order to compile sufficient data for meaningful comparisons and to infer the phylogenetic relationships of gyrodactylids in Africa.

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