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Species of *Gyrodactylus* von Nordmann, 1832 (Platyhelminthes: Monogenea) from cichlids from Zambezi and Limpopo river basins in Zimbabwe and South Africa: evidence for unexplored species richness

Petra Zahradníčková · Maxwell Barson · Wilmien J. Luus-Powell · Iva Přikrylová

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Abstract New findings on *Gyrodactylus* spp. parasitising African cichlids in southern Africa are presented, comprising data from Zimbabwe and South Africa. Morphometry of opisthaptoral hard parts in combination with nuclear ribosomal DNA sequences confirmed the presence of six species of *Gyrodactylus* von Nordmann, 1832. Three new species are described from fishes in Zimbabwe: *Gyrodactylus chitandiri* n. sp. from the gill arches of *Coptodon rendalli* (Boulenger) and *Pseudocrenilabrus philander* (Weber); *Gyrodactylus occupatus* n. sp. from the fins of *Oreochromis niloticus* (L.), *Pharyngochromis acuticeps* (Steindachner) and *P. philander*; and *Gyrodactylus parisellei* n. sp. from the fins of *O. niloticus*, *P. philander* and *Tilapia* sp. *Gyrodactylus nyanzae* Paperna, 1973 was also identified from the gills of *O. niloticus* and *C. rendalli* collected from two localities in Zimbabwe; these findings represent new host and locality records for this parasite. *Gyrodactylus sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011 was identified from *P. philander* collected in South Africa and Zimbabwe thereby providing new host and locality records for this parasite. Finally, *Gyrodactylus yacatli* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011 was collected from the fins of *O. niloticus* and *P. philander* studied in Zimbabwe; this represents the first record of this species from the continent of Africa. Notably, this study improves upon the knowledge of *Gyrodactylus* spp. parasitising cichlids from these southern African regions. All species studied were recorded from at least two different cichlid host species indicating trend for a wide range of *Gyrodactylus* hosts in Africa. Accordingly, this supports the idea of intensive host switching in the course of their evolution.

P. Zahradníčková · I. Přikrylová (✉)
Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
e-mail: ivaprik@sci.muni.cz

M. Barson
Department of Biological Sciences, University of Zimbabwe, PO Box MP167, Mt. Pleasant Harare, Harare, Zimbabwe

W. J. Luus-Powell · I. Přikrylová
Department of Biodiversity, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa

I. Přikrylová
Water Research Group, School of for Environmental Sciences and Development, North West University, Private Bag X6001, Potchefstroom 2531, South Africa

Introduction

Cichlidae is one of the families with the highest species diversity among teleost fishes, with more than 1,660 known species, including 1,130 African representatives (Froese & Pauly, 2015). From the African continent, the highest cichlid diversity (more than 700

species considered valid) is recognised from the east African Great Lakes, such as Lake Malawi, Lake Tanganyika and Lake Victoria. These lakes display a high degree of endemicity which can vary between 50–85% of total known fish species recognised in the lakes (Snoeks, 2000; Turner et al., 2001; Joyce et al., 2011; Froese & Pauly, 2015). Despite the isolation of these lakes, very similar ecologically structured populations of fish with specific morphological adaptations have developed during a period of about 10 million years (Duponchelle et al., 2008; Mittermeier et al., 2011). African cichlids exhibit high ecological diversification, such that there are species in various trophic levels, and their reproductive strategies (i.e. brood-care patterns) are numerous (Keenleyside, 1991). The Limpopo and Zambezi river basins currently provide habitats for 33 cichlid species from the subfamily Pseudocrenilabrinae Fowler and tribes Coptodonini, Haplochromini, Hemichromini, Oreochromini and Tilapiini (*sensu* Salzburger et al., 2005; Koblmüller et al., 2008; Schwarzer et al., 2009; Dunz & Schliewen, 2013; Froese & Pauly, 2015). All species, except for introduced *Oreochromis andersonii* (Castelnau) and *Oreochromis macrochiri* (Boulenger), are referred to as endemic to Limpopo River basins (Froese & Pauly, 2015). In Africa, the majority of cichlids are native. However, fish farming had led to increased introduction of invasive alien species through breeding stock, such as *Oreochromis niloticus* (L.). The local fish populations are now endangered due to competition, hybridisation (Cambray & Swartz, 2007; Kazembe et al., 2010) and the possible introduction of non-native parasites into the system (Barson et al., 2008).

Viviparous monogeneans of the genus *Gyrodactylus* von Nordmann, 1832 are relatively small parasites predominantly found on the skin, fins and gills of fish, but they have also been recorded on body surface of amphibians (Paetow et al., 2009). They have a very simplified body, and an extremely short and unique life-cycle. They combine parthenogenesis and hyperviviparity thereby enabling rapid multiplication on their hosts (Bakke et al., 2007). Worldwide, more than 495 species of *Gyrodactylus* have been described, with 466 considered valid (Harris et al., 2004; Shinn et al., 2011). To date 33 *Gyrodactylus* spp. have been described from the African continent, the majority of which have been described during the late 1960's and 1970's (Paperna, 1968; Price & Gery, 1968; Ergens, 1973; Prudhoe & Hussey, 1977). Recently, these parasites have been studied extensively, resulting in a rapid increase in the number of new species reported from Africa (Christison et al., 2005; Nack et al., 2005; Přikrylová et al., 2009, 2012a, b; García-Vásquez et al., 2011; Vanhove et al., 2011). In Africa, species of *Gyrodactylus* are known from fishes belonging to nine families, but predominantly from the Cichlidae and Claridae (see El-Naggar & El-Abbassy, 2003; Christison et al., 2005; El-Abbassy, 2005; El-Naggar, 2007; García-Vásquez et al., 2007, 2010).

From the Limpopo River basin, there is only a single species recorded, *Gyrodactylus transvaalensis* Prudhoe & Hussey 1977, described from *Clarias gariepinus* (Burchell) (see Prudhoe & Hussey, 1977). No species of *Gyrodactylus* is known either from cichlids, or any other fish host from the Zambezi River basin.

Molecular data for African *Gyrodactylus* spp. are still very limited, as data for only 14 species are available, of which eight species have been obtained from cichlids (García-Vásquez et al., 2007, 2011; Přikrylová et al., 2009, 2012a, b; Vanhove et al., 2011). These data represent just a fraction of the 166 molecularly characterised *Gyrodactylus* spp., a number representing worldwide knowledge on the genetic diversity of *Gyrodactylus*, for which 2,279 entries are currently available in the GenBank database. The internal transcribed spacer (ITS) region of the nuclear rRNA gene is, for the majority of species of *Gyrodactylus*, the most frequently sequenced molecular marker supplementing the morphological characterisation of the species. In addition to the ITS region, the positions of several African gyrodactylid genera have been shown in a phylogenetic study based on the data for the small subunit (SSU) of the rRNA gene (Přikrylová et al., 2013). The same study confirmed a previously revealed polyphyletic origin of the African *Gyrodactylus* spp., inferred from ITS rDNA data (Vanhove et al., 2011). The combination of both nuclear markers (ITS and SSU) was shown to be suitable for studying phylogenetic relationships within the Gyrodactylidae (see Gilmore et al., 2012; Přikrylová et al., 2013).

The present study contributes to the knowledge of the distribution and interspecific relationships among *Gyrodactylus* spp. of African cichlid hosts by providing detailed morphological and molecular characterisation of three new species.

Materials and methods

Collection of host and parasite material

A total of 136 specimens of ten cichlid species were examined for the presence of gyrodactylid parasites. The hosts were collected in August 2011, March 2012 and August 2012 from the following three localities in Zimbabwe [Chirundu, Zambezi River (16°32′6.61″S, 28°52′4.98″E; August 2011); Lake Kariba (16°4′51.63″S, 28°52′4.98″E; August 2011 and 2012); Lake Chivero (17°52′16.11″S, 30°48′3.81″E, August 2012)] and one in South Africa [Nwanedi River (22°39′40.99″S, 30°22′32.15″E, March 2012)]. Fishes were sampled by seine netting or electrofishing, and identified according to Skelton (2001). Details on collected cichlid species including the basin of their occurrence, their total body length and number of *Gyrodactylus* spp. collected are provided in Table 1. Individuals of *Gyrodactylus* were collected from the fins and gills of host fishes using dissection needles. Specimens were fixed in ammonium picrate glycerine (GAP) (Malmberg, 1970) and mounted on slides for subsequent morphological analyses. Selected specimens were cut transversally; the anterior part of the parasite's body was fixed in 96% ethanol for molecular analyses and the posterior part fixed in GAP for morphological analyses.

Morphological analyses

Morphometric analyses were performed at the Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic. Specimens of *Gyrodactylus* were studied using a phase-contrast microscope (Olympus BX50). Hard parts were drawn with the aid of a drawing attachment and the drawings digitised and arranged using Adobe Photoshop CS6 and Adobe Illustrator CS6 version 13.0. Measurements of the hamuli and bars were taken according to Přikrylová et al. (2008), and those of the body and marginal hooks according to Christison et al. (2005). The following measurements of the hard parts of the monogeneans were taken: hamulus total length (HTL); hamulus point length (HPL); hamulus shaft length (HSL); hamulus root length (HRL); ventral bar

Table 1 An overview of the cichlid fishes examined in the present study from Zambezi and Limpopo drainage basins and *Gyrodactylus* spp. recovered. Total length of fish (TL), number of host fish examined/infected (N/n), species and number of individuals of *Gyrodactylus* (n) recovered per species are provided

Host species	Tribe	Basin	TL	N/n	Gyrodactylus spp. (n)
Coptodon rendalli (Smith)	C	ZR	108 (68–160)	9/6	*G. chitandiri* n. sp. (71); *G. nyanzae* (1)
Oreochromis niloticus (L.)	O	ZR	87 (50–205)	21/9	*G. nyanzae* (19); *G. occupatus* n.sp. (5); *G. parisellei* n. sp. (6); *G. yacatli* (3)
Oreochromis mortimeri Trawas	O	ZR	96 (62–130)	7/0	
Oreochromis mossambicus (Peters)	O	LR	162 (87–250)	4/0	
Pharyngochromis acuticeps (Steindachner)	H	ZR	62 (38–126)	13/7	*G. occupatus* n. sp. (1)
Pseudocrenilabrus philander (Weber)	H	LR	55 (48–82)	9/3	*G. sturmbaueri* (5)
	H	ZR	56 (31–75)	45/17	*G. chitandiri* n. sp. (2); *G. occupatus* n. sp. (3); *G. parisellei* n. sp. (4); *G. sturmbaueri* (1); *G. yacatli* (1)
Sargochromis codringtonii (Boulenger)	H	ZR	265 (215–314)	6/0	
Serranochromis macrocephalus (Boulenger)	H	ZR	277 (214–280)	4/0	
Tilapia sparrmanii Smith	T	LR	43 (36–50)	2/0	
	T	ZR	80 (37–138)	15/1	*Gyrodactylus* sp. (1)
Tilapia sp.	T	ZR	115	1/1	*G. occupatus* n. sp. (5); *G. parisellei* n. sp. (1)

Abbreviations: Cichlid tribes: C, Coptodini; H, Haplochromini; HE, Hemichromini; O, Oreochromini; T, Tilapiini. Drainage basins: LR, River Limpopo Basin; ZR, River Zambezi Basin

median length (VBL); ventral bar membrane length (VBML); ventral bar width (VBW); dorsal bar length (DBL); dorsal bar width (DBW); marginal hook total length (MHTL); marginal hook sickle length (MHSL); marginal hook handle length (MHHL); marginal hook sickle distal width (MHSDW); marginal hook sickle proximal width (MHSPW); marginal hook sickle aperture distance (MHSAD). Metrical characteristics were obtained using Micro Image (MicroImage 4.0 Olympus). All measurements are given in micrometres and are presented as the range with the mean and the number of measurements in parentheses. For a comparative study of the African *Gyrodactylus* spp., type-material was obtained from the Royal Museum for Central Africa, Tervuren, Belgium as follows: *Gyrodactylus cichlidarum* Paperna, 1968 (holotype M.T.35.584), *Gyrodactylus nyanzae* Paperna, 1973 (holotype M.T.35.513) and *Gyrodactylus sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011 (paratype M.T.37.670). Additional material was obtained from the Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic: *Gyrodactylus ulinganisus* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011 (paratype M-479/1).

For statistical analyses, the original measurements of *Gyrodactylus ergensi* Přikrylová, Matějusová, Musilová & Gelnar, 2009 and *Gyrodactylus malalai* Přikrylová, Blažek & Gelnar, 2012 were also included. A Principal Components Analysis (PCA) was carried out on the covariance matrix of the measurements of opisthaptoral hard parts with implementation of Euclidean similarity index using PAST, version 2.14 (Hammer et al., 2001). Body size and ventral bar membrane length were excluded from the PCA, as the soft body was expansible, and the membrane edge was difficult to visualize such that it produced a large range of measure values. Thus measurements for 14 morphometric features were included in the analyses.

DNA extraction and amplification

Total genomic DNA was extracted using the Qiagen Blood and Tissue Isolation kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol; DNA was eluted in 50 µl. The ITS region of the rDNA was amplified using the primers ITS-1F (5′-GTT TCC GTA GGT GAA CCT-3′) (Rokicka et al., 2007) and ITS-2R (5′-TCC TCC GCT TAG TGA TA-3′) (Matějusová et al., 2001), in a Mastercycler eP gradient thermocycler (Eppendorf, Hamburg, Germany). Each amplification reaction contained 1 µl of template DNA, 1× PCR buffer, 1.25 mM $MgCl_2$, 100µM dNTPs, 0.1 mg/ml BSA (Bovine Serum Albunin), 0.5 µM of each primer (Generi Biotech, Hradec Králové, Czech Republic) and 1.5U Taq polymerase in a total volume of 20 µl. PCR amplicons were visualised on Gold View stained agarose gel (1%) and purified using the High Pure PCR Product Purification Kit (Roche, Basel, Switzerland). Sequencing was carried out using the PCR primers with the Big Dye Chemistry Cycle Sequencing Kit v.3.1 and an ABI 3130 Genetic Analyser automated sequencer (Applied Biosystems, Foster City, California, USA).

Sequence alignment and phylogenetic analyses

Nine sequences for *Gyrodactylus* spp. from cichlids were retrieved from the GenBank database, and aligned with the newly-obtained sequences using Clustal W multiple alignment program (Thompson et al., 1994) in MEGA v.6.0 (Tamura et al., 2013) (see Table 2 for details). *Gyrodactylus alekosi* Přikrylová, Blažek & Vanhove, 2012 (FR850682), a parasite of *C. gariepinus*, was chosen as the outgroup. For trimming the resulting alignment, trimAl v.1.2 (Capella-Gutiérrez et al., 2009) was used. After trimming the aligned sequences, a 666 nt long alignment was retained. The optimal model of molecular evolution was estimated using jModelTest v.0.1.1 (Posada, 2003; Guindon & Gascuel, 2003). Based on the corrected Akaike Information Criterion (AICc) (Hurvich & Tsai, 1989), the transversion + gamma-shape parameter (TVM + Γ) model was selected. To allow subsequent implementation in the phylogenetic software, the model with the second best corrected AIC score was chosen, namely the General Time Reversible + gamma-shape (GTR + Γ) model, with a gamma-shape parameter of 0.58. Maximum likelihood (ML) searches were performed in PhyML v.3.0 (Guindon & Gascuel, 2003) under the optimised model. Nodal support was assessed through 1,000 bootstrap pseudoreplicates using the nearest-neighbour interchange branch swapping algorithm. Bayesian inference (BI) analysis, also using the GTR + Γ model, was carried out with MrBayes v.3 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Posterior probabilities were calculated over 1.10^6 generations, sampling the Markov chain every 100 generations.

Table 2 List of *Gyrodactylus* spp. reported from cichlid hosts. Country of record and accession numbers for the sequences available in the GenBank database are provided

Species	Cichlid host	Tribe	Country	Acc. no.	Reference
G. aegypticus El-Naggar & El-Tantawy, 2003[a]	*Coptodon zilii* (Gervais)	T	Egypt		El-Naggar & El-Tantawy (2003)
G. cichlidarum Paperna, 1968[b]	*Hemichromis fasciatus* Peters	HE	Ghana		Papena (1968)
	Hemichromis bimaculatus Gill	HE	Ghana		Paperna (1968)
	Oreochromis aureus (Steindachner)	O	Ghana		Paperna (1979)
	Oreochromis niloticus (L.)	O	UK	DQ124228	García-Vásquez et al. (2010)
	Sarothedoron galilaues (L.)	O	Ghana		Paperna (1979)
		O	Ghana		Paperna (1979)
		O	Senegal		Paperna (1979)
	Sarotherodon melanotheron (Duméril)	O	Ghana		Paperna (1979)
	Tilapia guineensis (Günther)	T	Ghana		Paperna (1979)
	Coptodon zilii (Gervais)	C	Ghana		Paperna (1968), Paperna (1979)
G. ergensi Přikrylová, Matějusová, Musilová & Gelnar, 2009	*Sarothedoron galilaues* (L.)	O	Senegal	FN394985	Přikrylová et al. (2009)
	Oreochromis niloticus (L.)	O	Senegal		Přikrylová et al. (2009)
G. haplochromi Paperna, 1973	*Haplochromis angustifrons* Boulenger	H	Uganda		Paperna (1973)
G. hildae García-Vásquez, Hansen, Christison, Bron & Shinn, 2011	*Oreochromis niloticus* (L.)	O	Etiopia	FJ231869	García-Vásquez et al. (2011)
G. malalai Přikrylová, Blažek & Gelnar, 2012	*Coptodon zilii* (Gervais)	T	Kenya		Přikrylová et al. (2012a)
	Oreochromis niloticus (L.)	O	Kenya		Přikrylová et al. (2012a)
		O	Sudan	FR695484-5	Přikrylová et al. (2012a)
G. nyanzae Paperna, 1973	*Oreochromis variabilis* (Boulenger)	O	Uganda		Paperna (1973)
G. shariffi Cone, Arthur & Bondad-Reantaso, 1995	*Oreochromis niloticus* (L.)	O	Philippines		Cone et al. (1995)
G. sturmbaueri Vanhove, Snoeks, Volckaert & Huyse, 2011	*Simochromis diagramma* (Günther)	TR	Zambia	HQ21477-80	Vanhove et al. (2011)
G. thlapi Christison, Shinn & Van As, 2005	*Pseudocrenilabrus philander* (Weber)	H	Botswana		Christison et al. (2005)
G. thysi Vanhove, Snoeks, Volckaert & Huyse, 2011	*Simochromis diagramma* (Günther)	TR	Zambia	HQ214481	Vanhove et al. (2011)

Table 2 continued

Species	Cichlid host	Tribe	Country	Acc. no.	Reference
G. ulinganisus García-Vásquez, Hansen, Christison, Bron & Shinn, 2011	*Oreochromis mossambicus* (Peters)	O	South Africa	FJ231870	García-Vásquez et al. (2011)
G. yacatli García-Vásquez, Hansen, Christison, Bron & Shinn, 2011	*Oreochromis niloticus* (L.)	O	Mexico		García-Vásquez et al. (2011)
G. zimbae Vanhove, Snoeks, Volckaert & Huyse, 2011	*Simochromis diagramma* (Günther)	TR	Zambia	HQ214482	Vanhove et al. (2011)
	Ctenochromis horei (Günther)	H	Zambia		Vanhove et al. (2011)
Gyrodactylus sp.	*Hemichromis bimaculatus* Gill	HE	Sudan	HF548666.1	Přikrylová et al. (2013)

Abbreviations: C, Coptodini; H, Haplochromini; HE, Hemichromini; O, Oreochromini; T, Tilapiini; TR, Tropheini

[a] Species regarded as *nomen nudum* (Christison et al., 2005); [b] Species synonym - *G. niloticus* Cone, Arthur & Bondad-Reantaso, 1995 (García-Vásquez et al., 2007)

One-fourth of the samples were discarded as "burn-in". Neighbour Joining (NJ) analysis was carried out with MEGA 5.2 based on uncorrected p-distances using a bootstrap resampling procedure with 1,000 replicates. Conversion of alignment files was carried out using ALTER v.1.2 (Glez-Peña et al., 2010) and the trees were drawn with FigTree v.1.3 (Rambaut, 2008).

Results

A total of 190 specimens of the genus *Gyrodactylus* were collected from six cichlid species *Coptodon rendalli* (Smith), *O. niloticus*, *Pharyngochromis acuticeps* (Steindachner), *Pseudocrenilabrus philander* (Weber), *Tilapia sparrmanii* Smith and *Tilapia* sp. Six species of *Gyrodactylus* were identified among the studied specimens. The summarised results of the present study are given in Table 1. The records of *Gyrodactylus yacatli* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011 on *O. niloticus* and *P. philander* in Zimbabwe represent the first records of this parasite in Africa. The morphology of the opisthaptoral hard parts, supported by the molecular data revealed the presence of three new species, described here as *Gyrodactylus chitandiri* n. sp., *Gyrodactylus occupatus* n. sp. and *Gyrodactylus parisellei* n. sp. Sixty-five specimens of *Gyrodactylus* spp. were not included in the present study as they represent parasites with a discrepant morphological type of hamuli and their identification is still being considered. In most of the cases, there was a single individual of *Gyrodactylus* spp. per host specimen. Infections with both, closely related and non-related species of *Gyrodactylus*, were present on the same host species, e.g. *O. niloticus* infected with *G. occupatus* n. sp. and *G. parisellei* n. sp. and *C. rendali* parasitised by *G. nyanzae* and *G. chitandiri* n. sp. Morphological descriptions and (where applicable) molecular characterisation of all six species are provided below.

Family Gyrodactylidae Cobbold, 1864
Genus *Gyrodactylus* von Nordmann, 1832

Gyrodactylus chitandiri n. sp.

Type-host: *Coptodon rendalli* (Smith) (Perciformes: Cichlidae).
Other host: *Pseudocrenilabrus philander* (Weber) (Perciformes: Cichlidae).
Type-locality: Chirundu, River Zambezi (16°32′6.61″S, 28°52′4.98″E), Zimbabwe.
Other locality: Lake Kariba (16°4′51.63″S, 28°52′4.98″E), Zimbabwe.
Site on host: Gills (*C. rendalli*) and fins (*P. philander*).
Type-material: Holotype and two paratypes (IPCAS Coll. No. M-587) are deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice,

Czech Republic. A further two paratypes are deposited in the Natural History Museum, London, UK (NHMUK 2015.3.20.1-2) and one paratype is deposited in the invertebrate collection of the Royal Museum for Central Africa, Tervuren, Belgium (RMCA 37.786).
Representative DNA sequence: A sequence (666 nt) covering partial ITS1 (274 nt), the 5.8S rDNA (157 nt) and partial ITS2 (235 nt) is deposited in the European Nucleotide Archive (ENA) under accession number LN849942.
Etymology: The specific name, *chitandiri*, is the Shona word for parasite.

Description (Figs. 1.1, 1.2, 2.1, 3.4, 3.7)

[Based on 71 specimens, measured under coverslip pressure; see Table 3 for measurements of opisthaptoral hard parts.] Body 409–677 (653; n = 59) long, 63–168 (131; n = 59) wide at level of uterus. Prohaptor with 2 finger-like spike sensilla. Pharyngeal bulb 29–71 × 32–57 (49 × 46; n = 60). Excretory bladders present. Intestinal caeca not extending beyond level of uterus. Male copulatory organ 10–21 (15; n = 22) long, 11–20 (17; n = 22) wide, located close to pharyngeal bulb, armed with 2 principle, 2 medium and 3–4 small spines. Opisthaptor clearly differentiated from rest of body, armed with a complex of hard structures. Hamuli robust, small, each with short root that inclines laterally. Ventral bar rectangular, with small anterolateral processes and tongue-shaped membrane. Dorsal bar simple. Marginal hook sickle proper rises forwards from base, curves in the second third at right angle; sickle point reaches toe edge; sickle base rhomboid, with rounded corners.

Molecular characterisation

Partial ITS1-5.8S-ITS2 rDNA sequence was generated from one specimen only. A BlastN search in the GenBank database (March 2015) using the entire sequence revealed no identical hits. *Gyrodactylus chitandiri* n. sp. appeared most closely related to *G. sturmbaueri* from *P. philander* collected in South Africa (present study) and *G. sturmbaueri* from *Simochromis diagramma* (Günther) from Zambia (Vanhove et al., 2011) based on the uncorrected p-distances (Table 4), 3.9 and 4.4%, respectively, and its position in the phylogram (Fig. 6).

Remarks

The hamuli and marginal hook dimensions in *G. chitandiri* n. sp. are similar to those in *G. sturmbaueri* as given by Vanhove et al. (2011). The two species, however, differ substantially in the shape of the marginal hook sickles. The sickle proper in *G. chitandiri* n. sp. is thicker and inclines forward (*vs* more robust proper sickles with a short point in *G. sturmbaueri*) and the base is less prolonged downward and rounded (*vs* markedly prolonged and downward declining toe with a short upper line of base in *G. sturmbaueri*) (see Fig. 2.1 and 2.2).

Gyrodactylus nyanzae Paperna, 1973

Type-host: *Oreochromis variabilis* (Boulenger) (Perciformes: Cichlidae).
Other hosts: *Oreochromis niloticus* (L.) and *Coptodon rendalli* (Smit) (both Perciformes: Cichlidae).
Type-locality: Lake Victoria, Uganda
Other localities: Chirundu, River Zambezi (16°32′6.61″S, 28°52′4.98″E); Lake Kariba (16°4′51.63″S, 28°52′4.98″E), Zimbabwe (both present study).
Site of host: Gills.
Voucher material: Four voucher specimens (IPCAS Coll. No.M-590) are deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic. A further two voucher specimens are deposited in the Natural History Museum, London, UK (NHMUK 2015.3.20.6-7).
Representative DNA sequences: A sequence (778 nt) covering partial ITS1 (323 nt), the 5.8S rDNA (157 nt) and partial ITS2 (298 nt) is deposited in the European Nucleotide Archive (ENA) under accession number LN849939.

Redescription (Figs. 1.3, 1.4, 2.11, 2.12, 3.1)

[Based on 20 specimens, measured under coverslip pressure; see Table 3 for measurements of opisthaptoral hard parts.] Body 479–848 (652; n = 16) long, 97–239 (131; n = 16) wide at level of uterus. Prohaptor bears 2 finger-like spike sensilla. Pharyngeal bulb 30–56 × 30–45 (40 × 36; n = 15); secretory glands present. Intestinal caeca not extending beyond level of

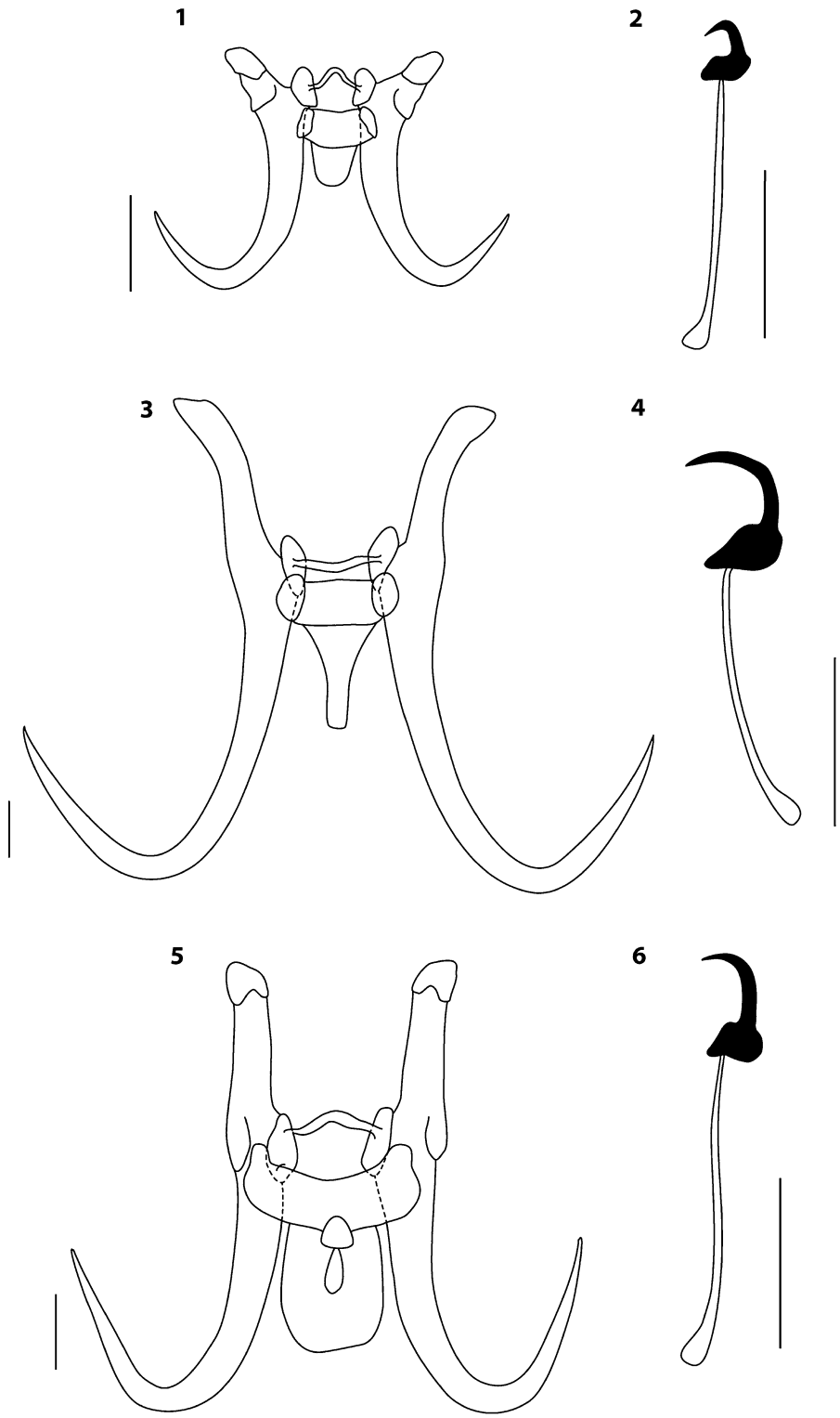

Fig. 1 Opisthaptoral hard parts of *Gyrodactylus* spp. Hamuli, ventral and dorsal bar (2, 4, 6) and marginal hook (1, 3, 5). 1, 2, *Gyrodactylus chitandiri* n. sp.; 3, 4, *G. nyanzae*; 5, 6, *G. occupatus* n. sp. (5-6). *Scale-bars*: 10 μm

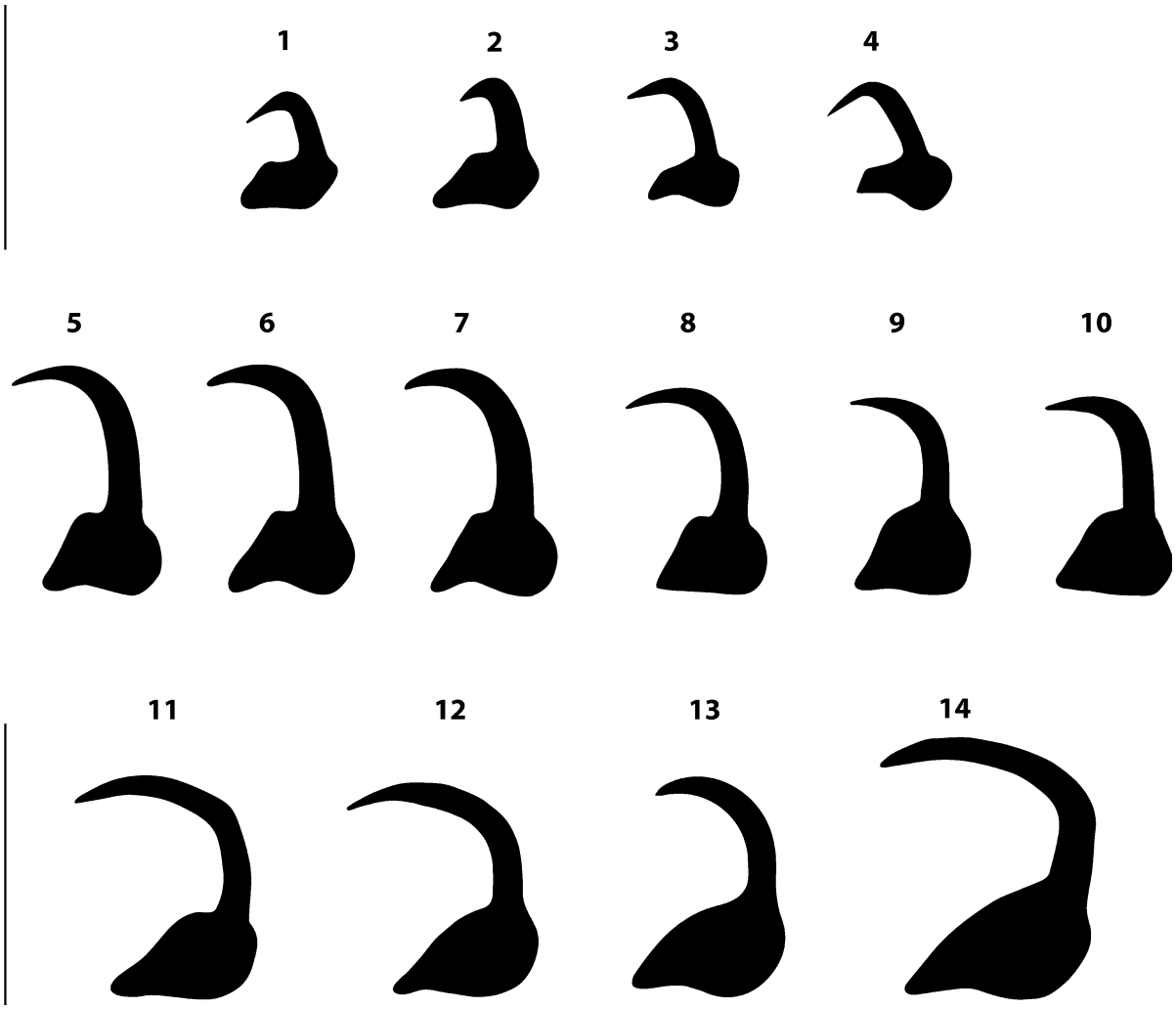

Fig. 2 Drawings of marginal hook sickles of *Gyrodactylus* spp. from African cichlid hosts. 1, *G. chitandiri* n. sp.; 2, *G. sturmbaueri*; 3, 4, *G. yacatli* [3, present study; 4, drawing after the original species description by García-Vásquez et al. (2011)]; 5, *G. occupatus* n. sp.; 6, 7, *G. ulinganisus* (6, present study; 7, paratype); 8, *G. parisellei* n. sp.; 9, 10, *G. cichlidarum* (holotype); 11, 12, *G. nyanzae* (11, present study; 12, holotype); 13, *G. ergensi*, drawing after the original species description by Přikrylová et al. (2009); 14, *G. malalai*, drawing after the original species description by Přikrylová et al. (2012a, b). *Scale-bars*: 10 μm

uterus. Male copulatory organ posterolateral to pharyngeal bulb, 9–16 (11; n = 10) long, 9–14 (11; n = 10) wide, armed with 1 large and 5 small spines. Hamuli slender, long, with laterally directed roots. Ventral bar without anterolateral processes, membrane gradually narrowed in lower part slightly extended and blunt-ending. Dorsal bar simple rod-shaped. Sickle proper of marginal hook rises from teardrop-shaped base slightly inclined backwards, curves at an obtuse angle forward in second third of its length, sickle point oriented downward, slightly extending beyond edge of toe.

Molecular characterisation

Partial ITS1-5.8S-ITS2 rDNA sequence was generated from three specimens. ITS region displayed intraspecific variation. Two haplotypes of *G. nyanzae* were isolated from specimens parasitising *O. niloticus* and *C. rendalli* which differed by two transitions.

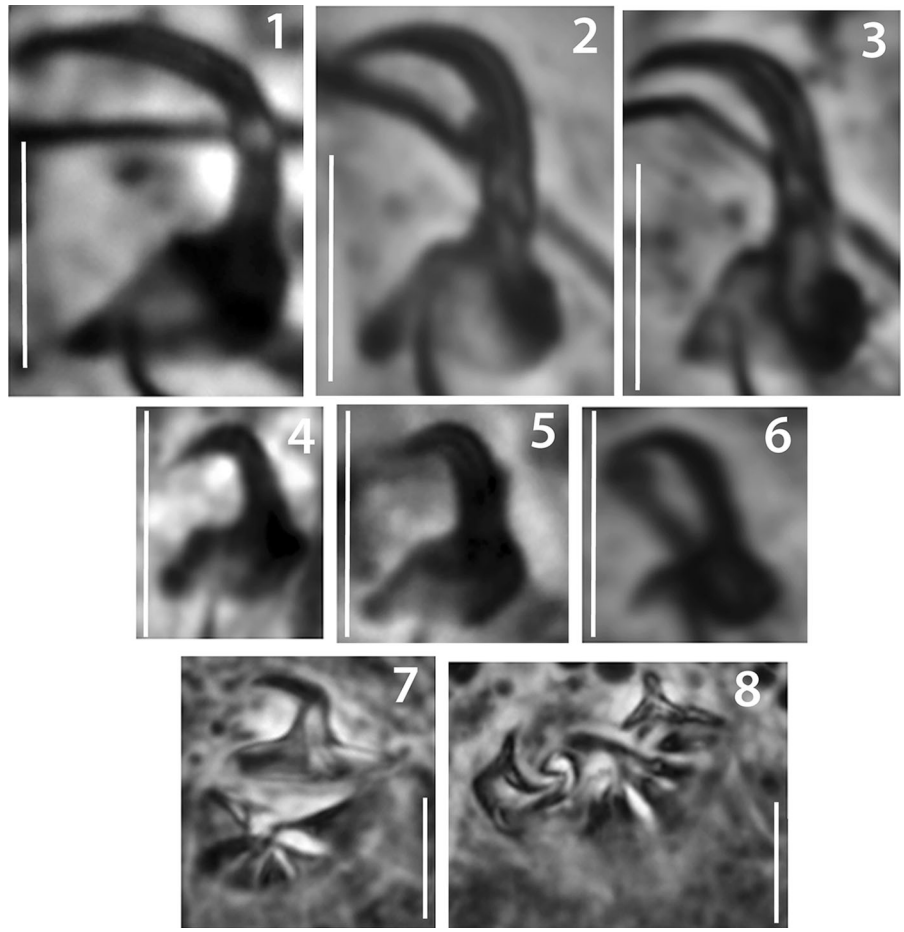

Fig. 3 Photomicrografs of marginal hooks sickle (1–6) and male copulatory organ (7, 8) of *Gyrodactylus* spp. from African cichlid hosts. 1, *G. nyanzae*; 2, *G. occupatus* n. sp.; 3, *G. parisellei* n. sp.; 4, 7, *G. chitandiri* n. sp. (4, 7); 5, 8, *G. sturmbaueri*; 6, *G. yacatli*. *Scale-bars*: 5 μm

A BlastN search in GenBank database (March 2015) using the entire sequence revealed no identical hits. *Gyrodactylus nyanzae* resulted as most closely related to *G. ergensi* from *Sarotherodon galilaeus* L. collected in Senegal (Přikrylová et al., 2009) and *G. malalai* from *O. niloticus* collected in Kenya (Přikrylová et al., 2012), based on the uncorrected p-distances (Table 4), 3.9% for both species, and its position in the phylogram (Fig. 6).

Remarks

Among the known *Gyrodactylus* spp. from African cichlid fishes *G. nyanzae* possesses one of the largest opisthaptoral hard parts. The shape of the marginal hook sickles, hamuli and bars of *G. nyanzae* specimens collected from two hosts, *O. niloticus* and *C. rendalli*, and from two localities in Zimbabwe were identical to that of the examined holotype (Fig. 2.11, 2.12). The measurements of the hamuli (point length, shaft length, and root length) of specimens of the present study correspond with the measurements reported for the holotype re-examined here (see Table 3).

***Gyrodactylus occupatus* n. sp.**

Type-host: *Oreochromis niloticus* L. (Perciformes: Cichlidae).

Table 3 Comparative metrical data for opishaptoral hard parts of selected species of *Gyrodactylus*

	G. cichlidarum (n = 1) Holotype*	*G. chitandiri* n. sp. (n = 71) Present study	*G. nyanzae* (n = 20) Present study	*G. nyanzae* (n = 1) Holotype*	*G. occupatus* n. sp. (n = 14) Present study	*G. parisellei* n. sp. (n = 11) Present study
HTL	55.6	25.7–28.9 (26.8)	79.7–88.3 (84.9)	91.0	66.1–73.8 (69.4)	48.5–53.5 (52.1)
HPL	23.1	8.7–11.9 (10.3)	31.8–37.0 (34.1)	34.5	26.8–33.4 (29.8)	19.8–23.5 (22.1)
HSL	39.3	22.8–26.2 (24.0)	60.0–64.7 (63.2)	62.5	45.0–49.2 (46.9)	35.5–38.8 (37.6)
HRL	20.3	7.9–10.9 (9.3)	30.9–37.0 (34.0)	37.0	24.7–29.8 (27.0)	16.2–19.9 (18.6)
VBW	18.9	7.7–10.3 (9.1)	18.4–23.7 (21.4)	24.5	21.5–24.1 (22.6)	17.0–21.2 (18.5)
VBL	5.3	2.8–5.1 (4.4)	7.2–9.6 (7.1)	7.5	7.7–13.3 (8.7)	8.9–10.6 (9.5)
VBML	13.9	2.6–7.7 (3.6)	15.3–22.1 (18.0)	11.5	14.7–21.2 (17.2)	11.7–13.8 (12.3)
DBW	13.0	5.9–9.7 (7.7)	12.1–19.4 (16.3)	–	15.7–19.3 (16.8)	13.4–15.8 (14.2)
DBL	1.6	0.4–1.0 (0.8)	1.2–2.2 (1.7)	–	1.2–2.3 (1.7)	1.2–1.8 (1.5)
MHTL	–	15.9–22.0 (20.6)	25.2–30.3 (28.0)	35.0	31.6–37.9 (34.0)	27.8–32.5 (30.0)
MHHL	20.3	12.2–18.6 (16.8)	18.0–22.4 (20.6)	25.5	23.8–28 (26.3)	20.5–24.8 (22.7)
MHSL	7.2	3.5–4.9 (4.1)	7.0–8.0 (7.6)	9.5	7.6–8.1 (7.8)	7.3–7.8 (7.6)
MHSPW	4.2	2.6–4.2 (3.5)	5.2–6.6 (5.9)	5.0	4.2–4.9 (4.6)	3.6–4.7 (4.1)
MHSDW	–	2.0–3.2 (2.4)	4.5–5.7 (5.1)	–	3.8–4.9 (4.6)	3.4–4.7 (3.9)
MHSAD	6.9	2.4–3.7 (3.1)	6.5–7.6 (7.0)	9.5	6.4–7.6 (7.4)	6.4–7.4 (7.6)

Abbreviations: 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

* Re-examination of the holotype

Table 4 Uncorrected pairwise genetic distances (p-distance in %) between species of *Gyrodactylus* included in the phylogenetic analysis (666 nt)

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	***G. chitandiri* n. sp.**														
2	***G. occupatus* n. sp.**	7.9													
3	***G. parisellei* n. sp.**	7.9	5.0												
4	*G. cichlidarum*	6.6	3.6	2.7											
5	*G. ergensi*	6.5	6.2	6.6	6.3										
6	*G. hildae*	30.0	28.6	29.4	29.4	29.1									
7	*G. malalai*	6.6	6.9	6.6	6.2	2.1	29.3								
8	***G. nyanzae***	5.9	6.8	7.4	7.1	3.9	28.9	3.9							
9	***G. sturmbaueri***	3.9	7.3	7.6	7.1	5.4	29.9	6.2	5.1						
10	*G. sturmbaueri*	4.4	7.7	8.0	7.6	5.9	29.9	6.6	5.6	0.8					
11	*G. thysi*	32.4	32.9	33.9	32.6	32.9	33.2	32.4	32.1	33.1	32.9				
12	*G. ulinganinsus*	7.9	3.9	4.5	4.1	6.6	29.4	6.9	7.1	7.6	8.0	33.1			
13	*G. zimbae*	34.4	33.5	34.4	33.9	33.6	36.3	34.2	33.3	34.1	34.1	39.5	34.2		
14	*Gyrodactylus* sp.	32.5	31.5	32.4	31.9	31.7	31.8	31.7	31.5	32.7	32.5	15.5	31.0	36.7	
15	*G. alekosi*	25.2	24.6	24.0	23.7	24.5	29.8	23.7	24.0	24.9	25.2	33.4	24.5	33.9	32.8

Other hosts: *Pharyngochromis acuticeps* (Steindachner, 1866); *Pseudocrenilabrus philander* (Weber); *Tilapia* sp. (all Perciformes: Cichlidae).
Type-locality: Lake Chivero (17°52′16.11″S, 30°48′3.81″E), Zimbabwe.
Site on host: Fins.
Type-material: Holotype and two paratypes (IPCAS Coll. No.M-588) are deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic. One paratype is deposited in the Natural History Museum, London, UK (NHMUK 2015.3.20.3) and one paratype is deposited in the invertebrate collection of the Royal Museum for Central Africa, Tervuren, Belgium (RMCA 37.787).
Representative DNA sequence: A sequence (783 nt) covering partial ITS1 (332 nt), the 5.8S rDNA (157 bp) and partial ITS2 (294 nt) is deposited in the European Nucleotide Archive (ENA) under accession number LN849940.
Etymology: The specific name, *occupatus*, refers to its ability to infect a wide spectrum of fish hosts.

Description (Figs. 1.5, 1.6, 2.5, 3.2)

[Based on 14 specimens, measured under coverslip pressure; see Table 3 for measurements of opisthaptoral hard parts.] Body elongate, 639–728 (684; n = 2) long, 128–139 (134; n = 2) wide at level of uterus. Prohaptor with single pair of cephalic lobes, each bearing a spike sensillum. Pharyngeal bulb 37–64 × 34–49 (58 × 47; n = 5). Male copulatory organ subspherical, 17 × 13 (n = 1); composition of spines not observed. Intestinal caeca and secretory glands not visible. Hamuli slender, with conspicuously prolonged roots. Ventral bar with small rounded posterior processes and tongue-shaped ventral membrane with medial teardrop-shaped ridge; dorsal bar simple rod-shaped. Marginal hook sickle proper, clearly separated from base, rises from rounded foot upwards and gradually curves; sickle point slightly extended beyond edge of finger-shaped toe.

Molecular characterisation

Partial ITS1-5.8S-ITS2 rDNA sequences were generated from six specimens, their length varied from 656 to 783 nt. The sequences were identical for the six specimens. A BlastN search in GenBank database (March 2015) using the entire sequences revealed no identical hits. *Gyrodactylus occupatus* n. sp. appeared most closely related to *G. cichlidarum* from *O. niloticus* collected in an aquarium at University of Stirling, United Kingdom (García-Vásquez et al., 2007) and *G. ulinganisus* from *Oreochromis mossambicus* (Peters) (García-Vásquez et al., 2011) based on the uncorrected p-distances (Table 4), 3.6% and 3.9%, respectively. *Gyrodactylus occupatus* n. sp. formed a well-supported cluster with *G. cichlidarum*, *G. ulinganinsus* and *G. pariesellei* n. sp. but the exact position of *G. occupatus* n. sp. was not well resolved (see Fig. 6).

Remarks

The shape of the hamuli and ventral bar of *Gyrodactylus occupatus* n. sp. is similar to that of *G. parisellei* n. sp., *G. cichlidarum* as given by García-Vásquez et al. (2007) and *G. ulinganisus* as provided by García-Vásquez et al. (2011). *Gyrodactylus occupatus* n. sp. differs from these species in the dimensions of the hamuli and the shape of the marginal hooks. The size of all four hamuli features in *G. occupatus* n. sp. is greater than that of *G. parisellei* n. sp. (Table 3). The marginal hook sickle proper of *G. occupatus* n. sp. is slightly longer, more robust and curves at a more acute angle (*vs* sickle proper slightly thinner, arising from the base firstly upward and then turning in *G. parisellei* n. sp.; see Fig. 2.5, 2.8). Marginal hook sickle of *G. cichlidarum* arises from the base upward and turns in a more open angle with tip going directly forward (*vs* sickle proper with a more rounded turn and tip heading more downward in *G. occupatus* n. sp.; see Figs. 2.5, 2.9, 2.10).

Gyrodactylus parisellei **n. sp.**

Type-host: *Pseudocrenilabrus philander* (Weber) (Perciformes: Cichlidae).
Other hosts: *Oreochromis niloticus* L.; *Tilapia* sp. (both Perciformes: Cichlidae).
Type-locality: Lake Kariba (16°4′51.63″S, 28°52′4.98″E), Zimbabwe.
Other locality: Lake Chivero (17°52′16.11″S, 30°48′3.81″E), Zimbabwe.

Site on host: Fins.
Type-material: Holotype and two paratypes (IPCAS Coll. No.M-589) are deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic. An additional two paratypes are deposited in the Natural History Museum, London, UK (NHMUK 2015.3.20.4-5) and one paratype is deposited in the invertebrate collection of the Royal Museum for Central Africa, Tervuren, Belgium (RMCA 37.788).
Representative DNA sequence: A sequence (781 nt) covering partial ITS1 (353 nt), the 5.8S rDNA (157 nt) and partial ITS2 (291 nt) is deposited in the European Nucleotide Archive (ENA) under accession number LN849941.
Etymology: The species is named in honour of Dr. Antoine Pariselle, French Research Institute for Development, Marseille, France, for his significant contribution to the knowledge of monogenean parasites of African cichlids.

Description (Figs. 2.8, 3.3, 4.1, 4.2)

[Based on 11 specimens, measured under coverslip pressure; see Table 3 for measurements of opisthaptoral hard parts.] Body elongate, with well-separated opisthaptor; total body length 385–747 (476; n = 5), width 68–125 (93; n = 5) at level of uterus. Cephalic region bi-lobed, each lobe bearing a spike sensillum. Pharyngeal bulb 38–51 × 35–42 (41 × 36) (n = 7), surrounded by secretory glands, extends into intestinal caeca. Male copulatory organ bulbous, 10–13 (12; n = 2) long, 9–12 (10; 9–12; n = 2) wide, armed with single large principal spine and 5 small spines in single row. Hamuli of slender appearance, with well-developed roots. Ventral bar trapezoid, with small lateral processes and tongue-shaped membrane with medial teardrop-shaped ridge. Dorsal bar simple rod-shaped. Marginal hook base relatively long, rounded, with pronounced finger-like toe. Sickle proper clearly separated from foot, rising from base upwards, curving nearly at a right angle at mid-length; sickle point partially extends to toe edge.

Molecular characterisation

Partial sequence of ITS1-5.8S-ITS2 rDNA was amplified and sequenced from one specimen only. A BlastN search in GenBank database (March 2015) using entire sequence revealed no identical hits. *Gyrodactylus parisellei* n. sp. appeared to be closely related to *G. cichlidarum* from *O. niloticus* collected in aquarium at University of Stirling, UK (García-Vásquez et al., 2007) and *G. ulinganisus* from *O. mossambicus* (García-Vásquez et al., 2011) based on the uncorrected p-distances, 2.7% and 4.5%, respectively (Table 4). *Gyrodactylus cichlidarum* resulted as the closest sister taxon to *G. parisellei* n. sp. in a well-supported cluster with *G. ulinganinsus* and *G. occupatus* n. sp. (see Fig. 6).

Remarks

The overall shape of the hamuli of *G. parisellei* n. sp., with upward heading long root and ventral bar with tongue-shaped membrane, is similar to that of *G. cichlidarum* as shown by García-Vásquez et al. (2007) and *G. ulinganisus* as provided by García-Vásquez et al. (2011), but the hamuli of the *G. parisellei* n. sp. are smaller than those of *G. ulinganisus* (hamulus total length 48.5–53.3 *vs* 59.0–65.0 μm; hamulus point length 19.8–23.5 *vs* 27.0–30.0 μm). The new species differs from *G. cichlidarum* in the shape of the marginal hooks. The shaft of the marginal hook sickle in *G. parisellei* n. sp. start off slightly backwards, the heel is rounded and extends slightly downward (*vs* proper sickle rising upward, heel ending rounded, but not sloping in *G. cichlidarum*). The shaft and point of the marginal hook sickle in *G. parisellei* n. sp. are not as broad as those of *G. cichlidarum* (see Fig. 2.8, 2.9, 2.10). The hamuli point of *G. parisellei* n. sp. is shorter than that of *G. cichlidarum* (19.8–23.5 *vs* 23.3–27.9 μm).

Gyrodactylus sturmbaueri Vanhove, Snoeks, Volckaert & Huyse, 2011

Type-host: Simochromis diagramma (Günther) (Perciformes: Cichlidae).
Other host: *Pseudocrenilabrus philander* (Weber) (Perciformes: Cichlidae).
Type-locality: Lake Tanganyika, Zambia
Other localities: Lake Kariba (16°4′51.63″S, 28°52′4.98″E), Zimbabwe; River Nwanedi (22°39′40.99″S, 30°22′32.15″E), South Africa.
Site on host: Fins.

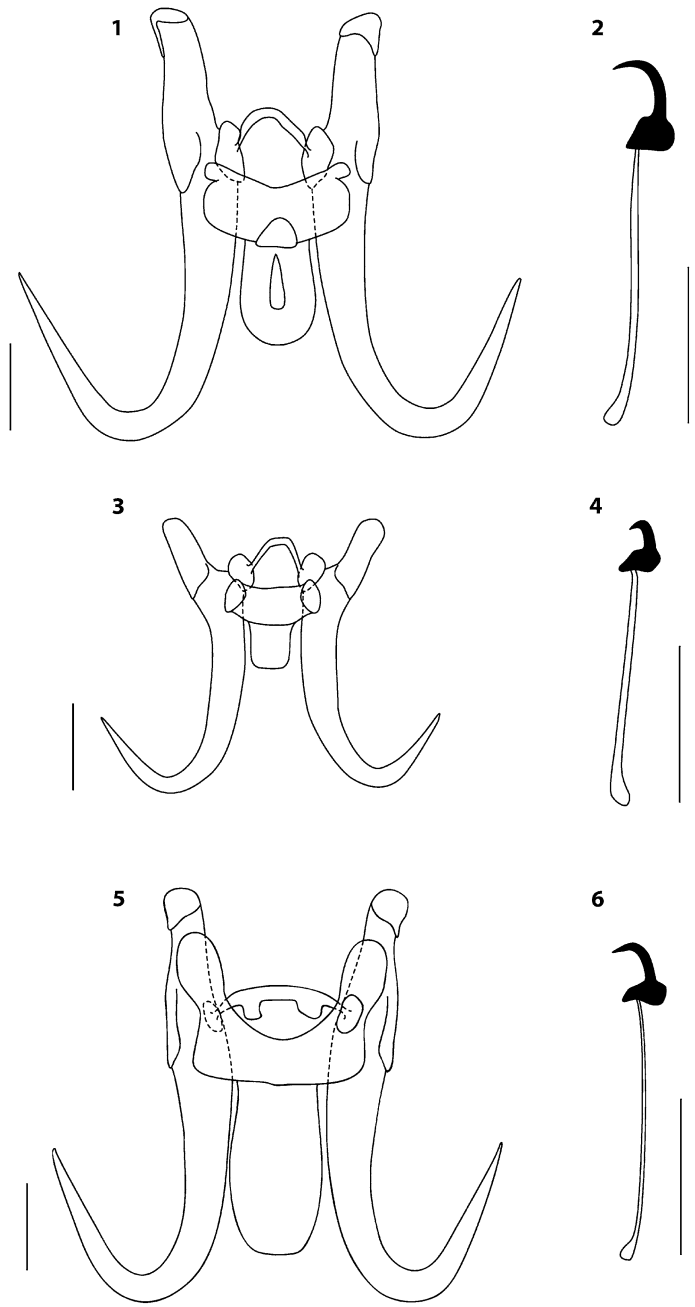

Fig. 4 Opisthaptoral hard parts of *Gyrodactylus* spp. Hamuli, ventral and dorsal bar (1, 3, 5) and marginal hook (2, 4, 6). 1, 2, *Gyrodactylus parisellei* n. sp.; 3, 4, *G. sturmbaueri*; 5, 6, *G. yacatli*. *Scale-bars*: 10 μm

Voucher material: Four voucher specimens (IPCAS Coll. No.M-591) are deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic and an additional two voucher specimens are deposited in the Natural History Museum, London, UK (NHMUK 2015.3.20.8-9).

Representative DNA sequence: A sequence (758 nt) covering partial ITS1 (321 nt), the 5.8S rDNA (157 nt) and partial ITS-2 (280 nt) is deposited in the European

Nucleotide Archive (ENA) under accession number LN849938.

Amended description (Figs. 2.2, 3.5, 3.8, 4.3, 4.4)

[Based on six specimens, measured under coverslip pressure; see Table 5 for measurements of opisthaptoral hard parts.] Body 397–608 (586; n = 5) long, 52–115 (106; n = 5) wide at level of uterus. Prohaptor with 2 spike sensilla. Male copulatory organ 10–15 (12; n = 3) long, 10–15 (13; n = 3) wide, armed with 1 principle, 2 medium-sized and 3–4 small spines. Pharyngeal bulb 43–49 × 34–46 (46 × 40) (n = 2) wide, surrounded by secretory glands. Intestinal caeca not observed. Opisthaptor clearly differentiated from rest of body. Hamuli robust, with well-pronounced roots. Ventral bar simple, without anterolateral processes and with short tongue-shaped membrane. Dorsal bar narrow, rod-shaped. Marginal hook sickle proper rises from rhomboid-shaped base upwards, second third rounded at right angle; sickle point short, not extending to toe edge; foot rounded, separated from sickle proper, base narrowed in part where handle reaches base; toe rounded, gradually extends to level of joining sickle proper.

Molecular characterisation

Partial ITS1-5.8S-ITS2 rDNA sequence was generated from one specimen only. The sequence is the closest to that for *G. sturmbaueri* from *Simochromis diagramma* (Günther) collected in Zambia (Vanhove et al., 2011) downloaded from GenBank from which it differs by six substitutions [two in ITS1 (one transition and one transversion); three in ITS2 (one transition and two transversions) and one transversion in 5.8S], resulting in 0.8% uncorrected p-distance between these haplotypes with different geographical and host origin.

Remarks

The shape of the marginal hook sickle of the present specimens of *G. sturmbaueri* is identical to that of the re-examined paratype. The lengths of hamulus shaft and root of *G. sturmbaueri* in the present material

Table 5 Comparative metrical data for opishaptoral hard parts of selected species of *Gyrodactylus*

Measurements	***G. sturmbaueri*** (n = 6) Present study	***G. sturmbaueri*** (n = 1) Paratype*	***G. ulinganisus*** (n = 1) Paratype*	***G. yacatli*** (n = 4) Present study	***G. yacatli*** (n = 4) García-Vásquez et al. (2011)
HTL	29.1–34.5 (31.3)	28.8	64.9	47.8–50.6 (49.4)	47–49 (48.4)
HPL	11.2–13.6 (12.4)	9.8	29.5	20.1–22.0 (21.0)	22–23 (22.7)
HSL	25.2–27.6 (24.8)	25.3	46.2	34.0–36.6 (35.3)	31–33 (32.2)
HRL	8.8–11.3 (10.2)	8.8	23.3	16.0–17.3 (16.5)	16–18 (16.9)
VBW	8.6–12.9 (10.8)	10.2	26.0	18.6–26 (22.5)	20–21 (20.4)
VBL	5.2–6.1 (4.9)	3.8	11.1	16.3–16.8 (16.6)	23–26 (24.7)
VBML	4.6–6.6 (5.1)	–	13.2	15.3–18.7 (17.0)	11–12 (11.6)
DBW	7.3–9.6 (8.7)	–	16.7	14.5–17.3 (16.0)	–
DBL	0.7–0.9 (0.8)	–	2.0	2.9–3.8 (3.5)	–
MHTL	17.7–22.5 (20.1)	–	33.1	20.9–23.4 (22.2)	22–24 (22.3)
MHHL	13.9–16.3 (15.3)	–	24.9	16.9–19.2 (18.3)	17–20 (18.0)
MHSL	3.9–4.8 (4.5)	4.4	7.8	4.3–5.0 (4.6)	4–5 (4.5)
MHSPW	2.1–3.0 (2.5)	2.0	4.5	3.1–3.3 (3.2)	3–4 (3.3)
MHSDW	3.3–4.0 (3.7)	3.1	4.0	2.0–2.5 (2.2)	3–4 (3.2)
MHSAD	2.7–4.7 (3.8)	3.9	7.4	3.6–4.0 (3.7)	4–5 (4.2)

Abbreviations: 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

* Re-examination of types

correspond with measurements of the paratype specimen (see Table 5). The dimensions of the hard parts of *G. sturmbaueri* and *G. chitandiri* n. sp. partially overlap, but the two species differ notably in the shape of the marginal hooks. The sickle proper in *G. sturmbaueri* is slightly longer and thicker, the foot is more elongated downward and rounded, the base is narrowed close to where the handle reaches the base and the toe is more gradually extended (*vs* thinner sickle proper with a longer tip and upper part of the toe, and rhomboid shape of the base in *G. chitandiri* n. sp.) (see Fig. 2.1, 2.2).

Gyrodactylus yacatli García-Vásquez, Hansen, Christison, Bron & Shinn, 2011

Type-host: Oreochromis niloticus (L.) (Perciformes: Cichlidae).
Other host: *Pseudocrenilabrus philander* (Weber) (Perciformes: Cichlidae).
Type-locality: Mexico (aquaculture).
Other localities: Chirundu, River Zambezi (16°32′6.61″S, 28°52′4.98″E), Lake Kariba (16°4′51.63″S, 28°52′4.98″E), Zimbabwe.
Site on host: Fins.
Voucher material: Two voucher specimens (IPCAS Coll. No. M-480) are deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic and an additional two voucher specimens are deposited in the Natural History Museum, London, UK (NHMUK 2015.3.20.10-11).

Amended description (Figs. 2.3, 2.4, 3.6, 4.5, 4.6)

[Based on 4 specimens, measured under coverslip pressure; see Table 5 for measurements of opisthaptoral hard parts.] Body elongated, with clearly differentiated opisthaptor. Total body length 394–459 (430; n = 3), width at level of uterus 75–83 (77; n = 4). Cephalic lobes with secretory glands, surrounding pharyngeal bulb. Pharyngeal bulb 23–32 × 19–29 (27 × 24) (n = 2). Male copulatory organ not observed. Ventral bar large, with 2 pronounced anterolateral processes with circular structure in centre of upper part; membrane distinct, tongue-shaped. Dorsal bar narrowest in central part, extended downward laterally. Marginal hooks of slender appearance, sickle proper clearly separated from foot, points forward, curves slightly; sickle point not extending beyond edge of toe, foot rounded, widest in upper third, elongate in downward direction and gradually continuing into medially bevelled toe.

Remarks

DNA fragment of interest failed to amplify. *Gyrodactylus yacatli* was first described from the gills and fins of *O. niloticus* cultured in Mexico but the origin of the fish stock was not clarified (García-Vásquez et al., 2011). The hamuli total length, point length and root length of *G. yacatli* in the present specimens correspond with the measurements given in the original species description (García-Vásquez et al., 2011). Other similar measurements of the hard parts include marginal hook total length, handle length and sickle length. The shape of the marginal hooks of the present specimens is identical to that in the original description (see Fig. 2.3, 2.4).

Morphometric analyses

The PCA ordination plot (Fig. 5) of the specimens of *Gyrodactylus* spp. provides a multidimentional illustration of the intraspecific and interspecific variability of the metric parameters of opisthaptoral hard parts used. Data for 14 morphomertic features for a total of 113 individuals of *Gyrodactylus* spp. were included in the analysis. The first principal component explained 95% of the data variability to which the second component contributed little (just over 2%). The first component was associated with the size of the hamuli, predominantly the total length of hamuli and the shaft) and the second component was associated with the dimensions of the ventral bar dimensions). Symbols in the PCA diagram represent the relative position of each individual and reflect the resemblance of conspecific specimens and those with similar morphological type of opisthaptoral hard parts.

Phylogenetic analyses

In total, 15 haplotypes were included in the final alignment. All nine sequences of *Gyrodactylus* spp. from African cichlids available in the GenBank

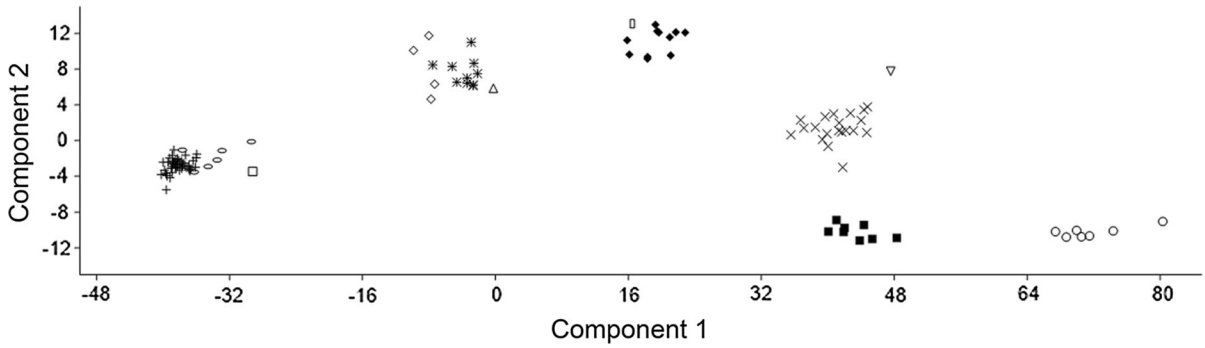

Fig. 5 Plot of the specimens in the two dimensional plane of the principal component analysis based on the measurements of opisthaptoral hard parts in selected *Gyrodactylus* spp. Key to species: *G. chitandiri* n. sp. (+); *G. cichlidarum* (Δ); *G. ergensi* (■); *G. malalai* (○); *G. nyanzae* (present study: ×, holotype: ▽); *G. occupatus* n. sp. (◆); *G. parisellei* n. sp. (*); *G. sturmbaueri* (present study:, paratype: □); *G. ulinganisus* (); *G. yacatli* (◇)

database were included in the analyses. The 666 nt long alignment contained 391 variable sites of which 243 were parsimony informative. There were no differences in the topology between NJ, ML and BI trees (Fig. 6). The three new species described above were part of one well-supported cluster, among which two main clades are apparent: one grouping *G. sturmbaueri* and *G. chitandiri* n. sp. together with three species parasitic on members of the Coptodini, *G. nyanzae*, *G. ergensi* and *G. malalai*, and the other comprising four species, a highly supported sister-species pair (*G. parisellei* n. sp. and *G. cichlidarum*)

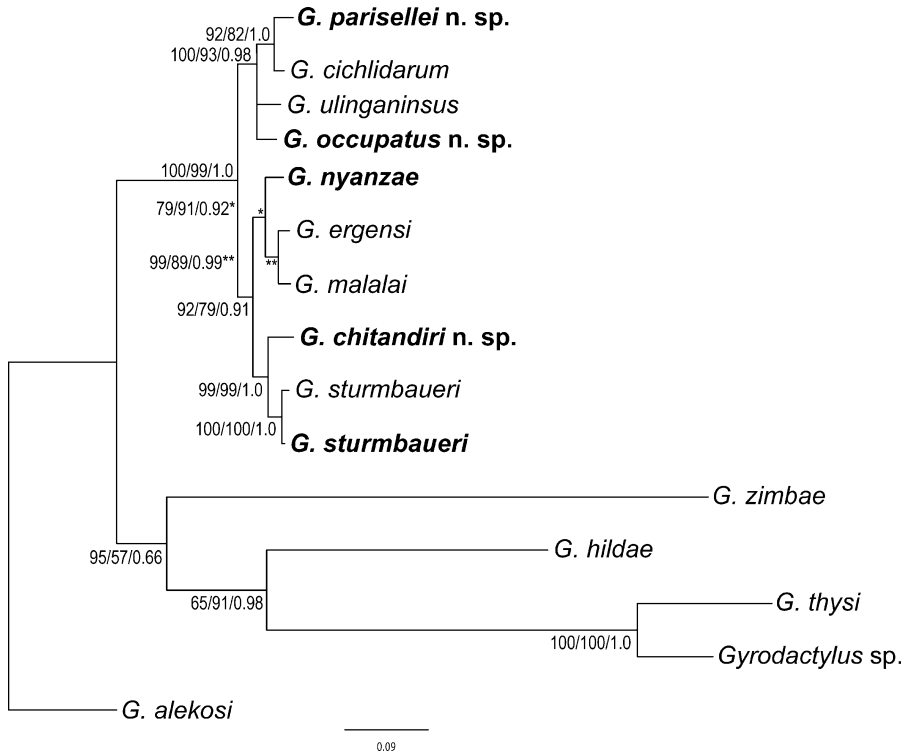

Fig. 6 Phylogram for *Gyrodactylus* spp. based on Bayesian Inference (BI) analysis of ITS rDNA gene sequences. Tree topologies from the Neighbor Joining (NJ) and Maximum Likelihood (ML) analyses were identical; nodal support values are presented as NJ/ML/BI. Sequences generated in the present study are highlighted in bold

plus *G. occupatus* n. sp. and *G. ulinganisus*, the latter with not well-resolved positions within the clade. Another well-supported clade was also evident, comprising *Gyrodactylus hildae* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011, *G. zimbae* Vanhove, Snoeks, Volckaert & Huyse, 2011, and two closely-related sister taxa, *G. thysi* Vanhove, Snoeks, Volckaert & Huyse, 2011 and *Gyrodactylus* sp. from Sudanese *Hemichromis bimaculatus* Gill. All distances between the species included in the phylogenetic analyses are given in Table 4. The smallest differences were noted between sequences for *G. cichlidarum* and *G. parisellei* n. sp. (p-distance of 2.1%) and between sequences for *G. malalai* and *G. ergensi* (p-distance of 2.7%). The close relationships of these species are evident based on their clustering with high values of nodal support (Fig. 6). The observed genetic distances between *G. zimbae* and *G. hildae*, *G. thysi* + *Gyrodactylus* sp. and the group of nine species, clustering with high nodal support, were 36.3, 36.7–39.5 and 33.3–34.4%, respectively, indicating a very distantly related species.

Discussion

African cichlids and *Gyrodactylus* spp.

Cichlids are attractive model organisms for the study of evolutionary events, selective processes and adaptive radiation because of their independent rapid speciation, leading to the origin of numerous endemic species, enhanced by isolation of the flock populations (Sturmbauer, 1998; Murray, 2001; Genner et al., 2007; Duponchelle et al., 2008; Sturmbauer et al., 2011). Nevertheless, little attention has been devoted to studying the parasite fauna of cichlids. According to Hecht & Endemann (1998), the most important pathogens of non-salmonid fishes are most likely ectoparasites causing lesions and bacteria and fungi which are associated with causing infection. Susceptibiliy to infection may subsequently increase mortality rates, especially under high density of fish in artificial farming conditions (Van As & Basson, 1984). Clinical infections of *Gyrodactylus* sp. affecting cichlids have been reported from Kenya (Paperna, 1996). The potential pathogenicity of parasites from the genus *Gyrodactylus* differs considerably among species (Bakke et al., 2007), and accordingly the precise species identification is crucial for recognition of the potential risks and subsequent treatment. The first species of *Gyrodactylus* described from African cichlids was *G. cichlidarum* found on *Sarothedoron galilaues* (L.) (type-host), and recorded from three other host species in Ghana by Paperna (1968). To date, 12 *Gyrodactylus* spp. were discovered from various cichlids across the entire African continent and only two from non-African cichlids (Cone et al., 1995; García-Vásquez et al., 2011). The results of the present study, with new species descriptions and several new host and locality records, notably expand upon the current knowledge on the parasites of the genus *Gyrodactylus* from Africa. However, our current knowledge still represents a small fraction of what is known about species richness of another dactylogyrid genus, *Cichlidogyrus* Paperna, 1960, with more than 90 species. This indicates that different evolutionary strategies of the two groups of monogenean parasites of cichlids may have affected their contemporary species richness.

The finding of *G. sturmbaueri* on a host far from Lake Tangynyika illustrates that the cichlid-monogenean host-parasite system should be studied in more detail. The endemicity in Lake Tanganyika in general is very high throughout many taxa (Snoeks, 2000), and also for monogenean parasites (Bukinga et al., 2012; Pariselle et al., 2015). Vanhove et al. (2011) proposed that the migration and parasite exchange between fishes from riverine and lake systems could be one of the scenarios which resulted in the occurrence of highly distinct *Gyrodactylus* spp. lineages on a single host. This might also explain how *G. sturmbaueri* might have spread from the lake into the riverine system or *vice versa*, and then be found from a distant locality such as South Africa.

It is known that the tribe Tropheini to which the type-host of *G. sturmbaueri* belongs, are derived from a generalist riverine ancestor, and *Pseudocrenilabrus* is ancestral to the Tropheini and the modern Haplochromini (see Salzburger et al., 2005). The role of *P. philander* in the current distribution of various *Gyrodactylus* spp. in the studied area seems to be unquestionable, considering that the present study recorded five out of six species from this host. This finding provides a baseline for a new line of inquiry related to if *P. philander* served as a "device", either for spreading of the parasites or as an original host on which the speciation of parasites occurred. In South Africa, the

effects of humans act on the current distribution of *P. philander* and its parasites should be taken into consideration as well, as the evidence of extralimital origin, where individuals were transported beyond the limits of their native range and directly released into a novel environment, is well documented (Ellender & Weyl, 2014). Another host example, which most probably notably contributed to the current richness and distribution of *Gyrodactylus* spp. on the African continent, is the Nile tilapia, *O. niloticus.* Nile tilapia is a non-native fish in many African regions where it has been introduced and intensively cultured and seems to be highly susceptible to infections with *Gyrodactylus* spp. The known records of *Gyrodactylus* spp. from this host, together with the results of the present study, confirm that *O. niloticus* serves as host for seven African *Gyrodactylus* spp., and for which it served as a carrier for the distribution into novel environment and/or as an island for speciation.

Host switching, the ability of viviparous gyrodactylids to "jump" from one host to another as adults (Cable et al., 2002), is an important mechanism of diversification (Boeger et al., 2003) and a very common mode of speciation (Ziętara & Lumme, 2002). The case of nine closely related *Gyrodactylus* spp. grouping in a well-supported clade, might be a possible explanation of how these species can occur on hosts from five different tribes (*sensu* Dunz & Schliewen, 2013).

The present study confirms that careful attention should be given during the morphological identification of *Gyrodactylus* spp. parasitising African cichlids, which are primarily distinguished based on the shape of marginal hook sickles and metric parameters, with emphasis on the marginal hook sickles, hamuli and connecting bars. Two of the new species, *G. occupatus* n. sp. and *G. parisellei* n. sp., were recovered from the same host species, site on host, and in the same localities. Although some morphological similarities between these two species were observed, their differentiation based on morphometric parameters is feasible. Therefore careful attention should be given to distinguish between morphologically similar species, such as *G. occupatus* n. sp. and *G. ulinganisus* as well as *G. parisellei* n. sp. and *G. cichlidarum*, to avoid misidentification.

Molecular and phylogenetic analyses

Recent studies discussing phylogenetic relationships among the species of the Gyrodactylidae proposed the polyphyly of *Gyrodactylus* from African cichlids (Vanhove et al., 2011; Přikrylová et al., 2013), but both studies were based mainly on ITS rDNA sequences. The latter study also used an analysis based on the 18S rDNA sequences but with a limited number of representatives of the "cichlid" *Gyrodactylus* spp. included. The present study focused on the gyrodactylid parasites of cichlids and thus we did not address the findings of the studies cited above. The grouping into clades is supported by the morphological similarities of the opisthaptoral hard parts of the species, similar to the patterns observed and discussed by Přikrylová et al. (2013). The sister species *G. parisellei* n. sp., *G. occupatus* n. sp., *G. cichlidarum* and *G. ulinganisus* are similar in the overall shape of the hamuli and the morphology of the ventral bar. The group formed by *G. nyanzae*, *G. malalai* and *G. ergensi* represents parasites bearing hamuli of a very slender appearance. The newly-described species, *G. chitandiri* n. sp., clustered together with *G. sturmbaueri*, and both species also possess a similar type of hamuli, i.e. small, with short roots. The remaining four species, *G. zimbae*, *G. hildae*, *G. thysi* + *Gyrodactylus* sp., represented genetically distant species and, from morphological point of view, are very variable and different from the species studied here.

Currently four haplotypes differing by up to 3 nt sites in ITS2 rDNA are available in the nucleotide database for *G. sturmbaueri* (see Vanhove et al., 2011). Over the region comprising ITS1-5.8S-ITS2 cluster, the uncorrected pairwise genetic distance was 0.8% between *G. sturmbaueri* sampled in South Africa and Zambia (HQ214480). This does not exceed the limit value of 1% difference between two sequences considered as an impetus for species delineation, as proposed by Ziętara & Lumme (2002). Even a higher value of 1.2%, observed between specimens of *G. rysavyi* Ergens, 1973 from Senegal and Mozambique by Přikrylová et al. (2012b) did not mean the presence of two species. The genetic, as well as morphological differences observed, were considered as consequence of their distant geographic origin and as a result of incipient speciation because of isolation-by-distance (Přikrylová et al., 2012b). This seems to be the case for the genetic differences observed between specimens of *G. sturmbaueri* from the present study and the specimen sequenced by Vanhove et al. (2011).

The description of three new species, i.e. *G. chitandiri* n. sp., *G. occupatus* n. sp. and *G. parisellei* n.

sp., increases the number of *Gyrodactylus* spp. described from freshwater fishes in Africa to 37 and those from African cichlids to 15 species. Taking into account the relatively small samples of fish examined in the present study, and considering the enormous diversity of African cichlids, we suggest that the number of *Gyrodactylus* spp. parasitising these hosts may be much higher than currently reported.

The importance of the host itself cannot be neglected, as hosts can play important roles in the speciation and distribution of the parasites. Use of genetic methods has become a necessary approach in the taxonomic studies of gyrodactylid parasites. This approach is especially helpful for the detection of cryptic species often showing very small differences in the shape and dimensions of the hard parts, and also might help to reveal existing phenotypic plasticity within species. Considering each *Gyrodactylus* species was found on at least two cichlid hosts in our study, this may indicate wider host specificity than currently reported and also demonstrate the importance of host switching in species diversification within *Gyrodactylus*.

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

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