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ORIGINAL PAPER

An overview of the *Gyrodactylus* (Monogenea: Gyrodactylidae) species parasitizing African catfishes, and their morphological and molecular diversity

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Abstract An overview of *Gyrodactylus* infecting catfishes from the African continent is provided, including new data from Sudan, Senegal, Kenya and Mozambique. Haptoral sclerite morphometry and nuclear ribosomal DNA sequences revealed the presence of eight *Gyrodactylus* species. On Senegalese *Synodontis nigrita*, *Gyrodactylus synodonti* n. sp. and *Gyrodactylus nigritae* n. sp. are described. These are the first reports of gyrodactylid parasites from mochokid hosts. From the fins of North African catfish *Clarias gariepinus* collected in Mozambique, *Gyrodactylus alekosi* n. sp. and *Gyrodactylus rysavyi* were identified. *G. rysavyi* was also reported from Kenyan *C. gariepinus* and Senegalese *Clarias anguillaris*. From the fins of *C. anguillaris* studied in Senegal, two more species, *Gyrodactylus transvaalensis* and *Gyrodactylus gelnari* n. sp. were recognised. In addition, *Gyrodactylus turkanaensis* n. sp. from the gills of Kenyan *C. gariepinus* was described and an undescribed *Gyrodactylus* sp. was recorded from Sudanese representatives of the same host. Detailed morphometrical and molecular comparisons of the species are presented and discussed. The study highlights the hitherto understudied diversity of viviparous monogenean parasites throughout Africa.

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Introduction

With over 3,100 valid species, the catfishes (order Siluriformes) are among the most successful teleosts (Sabaj et al. 2003). The numerous recent discoveries demonstrate that their biodiversity is yet far from understood (Lundberg et al. 2000). Several representatives of the order are of economic value in view of their importance in aquaculture, with *Clarias gariepinus* (Burchell, 1822) as prime African example (Haylor 1993; Chocha Manda 2010). Their biological and phylogenetic diversity, as well as their predominantly freshwater habitat and global distribution, render siluriforms a key target group in ecological, evolutionary and biogeographic research (Lundberg et al. 2007). Another fruitful scientific approach can be catfish parasitology, certainly when applied to monogenean flatworm parasites, in view of their close relationship to their host species because of their one-host lifecycle and high host specificity. Indeed, research on Monogenea from catfish has proven useful both in disentangling monogenean speciation mechanisms (Pariselle et al. 2003) and in reconstructing catfish biogeographical history (Barson et al. 2010). Moreover,

several monogenean genera have proven to be pathogenic to economically important cultured catfish. Examples include *Quadriacanthus* Paperna, 1961 and *Gyrodactylus* von Nordmann, 1832 (Kabata 1985; Obiekezie and Taege 1991; Paperna 1996).

Monogeneans belonging to *Gyrodactylus* are viviparous skin and gill parasites of many freshwater and marine fishes. Only 28 *Gyrodactylus* species have been described from African fishes, of which only seven are known from catfishes (Christison et al. 2005; Nack et al. 2005; Přikrylová et al. 2009a; Vaughan et al. 2010; Vanhove et al. 2011a; García-Vásquez et al. 2011). This is a limited number compared to the more than 400 valid *Gyrodactylus* spp. worldwide (Harris et al. 2004), or to the 78 species of *Cichlidogyrus* Paperna, 1960 in Africa, Madagascar and the Levant (Pariselle and Euzet 2009; Vanhove et al. 2011b; Gillardin et al. 2011). *C. gariepinus* is known to host five African *Gyrodactylus* species. Two of them, *Gyrodactylus alberti* Paperna, 1973 and *Gyrodactylus clarii* Paperna, 1973 where described from Uganda (Paperna 1973); another two, *Gyrodactylus rysavyi* Ergens, 1973 and *Gyrodactylus groschafti* Ergens, 1973 were contemporaneously described from Egypt (Ergens 1973); and finally, South Africa saw the description of *Gyrodactylus transvaalensis* Prudhoe and Hussey, 1977. Another clariid host, *Gyrodactylus camerunensis* Lönnberg, 1895 yielded two new gyrodactylid species in Cameroun: *G. camerunensis* Nack, Bilong Bilong and Euzet, 2005 and *Gyrodactylus nyongensis* Nack, Bilong Bilong and Euzet, 2005. *Clarias jaensis* Boulenger, 1909 and *Clarias pachynema* Boulenger, 1903 were mentioned as additional host fishes for these two *Gyrodactylus* species. So far, no other records exist of *Gyrodactylus* parasitizing African catfishes.

During a survey of fish parasites on several localities in four different African countries, eight *Gyrodactylus* spp. were collected from catfishes of the families Clariidae and Mochokidae. Two flatworm species, parasitizing clariid hosts, could be assigned to *G. transvaalensis* and *G. rysavyi* on the basis of haptoral hard part morphology. The remaining six represent new *Gyrodactylus* spp., the descriptions of which are presented here. The internal transcribed spacer (ITS) region of the nuclear rDNA is the molecular marker complementing morphological characterisation of the species. Since its first application in *Gyrodactylus* taxonomy (Cunningham et al. 1995), the ITS region has widely been used in combination with species descriptions, because of the variability in its subregions ITS-1 and ITS-2 (Cable et al. 2005; Rokicka et al. 2009; Mullen et al. 2010; Vanhove et al. 2011a). The present study provides new data about monogenean parasite diversity in Africa, and complements what little information, if any, is available from the studied areas (Khalil and Polling 1997).

Materials and methods

Specimen collection and preparation

Monogenean parasites belonging to *Gyrodactylus* were collected from the catfishes *Synodontis nigrita* Valenciennes, 1840 (Teleostei: Siluriformes: Mochokidae), *C. gariepinus* and *Clarias anguillaris* (Linnaeus, 1758) (Teleostei: Siluriformes: Clariidae) during parasitological investigations of freshwater fishes in four African countries carried out between November 2004 and January 2010. Details on the localities and collection dates are provided in Table 1. Parasites were removed from the hosts' fins, body surface and gills. Their haptors were excised, fixed with ammonium picrate–glycerine (Malmberg 1970) and mounted on a slide for subsequent

Table 1 Localities, collection period and number of *Gyrodactylus* spp. analysed in the present study (N = number of specimens used for morphometric study, n = number of specimens analysed by molecular methods)

Country	Locality	Host species	Date of collection	*N/n*
Senegal	Niokolo Koba National Park Niokolo Koba River (13°03.92′ N, 13°10.14′ W)	*S. nigrita*	March 2007	58/7
	Niokolo Koba National Park Mare Simenti (13°01.79′ N, 13°17.6′ W)	*C. anguillaris*	March 2006, 2007 and 2008	29/3
Kenya	Lake Turkana, Kalokol (03°33.58′ N, 35°55.24′ E)	*C. gariepinus*	September 2008	21/0
	Lake Turkana, Todonyang village (4°26.15′ N, 35°56.38′ E)	*C. gariepinus*	September 2008 and 2009	11/0
Mozambique	temporal stream close to the village Bala Bala (24°19.34′ S, 33°02.36′ E)	*C. gariepinus*	February 2009	11/11
Sudan	Sennar, Blue Nile (13°32.81′ N, 33°38.17′ E)	*C. gariepinus*	January 2010	2/2

morphological analysis. The anterior ends of the parasite bodies were stored in SPECTRANAL absolute ethanol (Allied-Signal, Riedel-de Haën). Specimens collected in Sudan were directly fixed in absolute ethanol and slides were prepared in the laboratory following Rokicka et al. (2007).

Morphometric analysis

Morphological analysis of the collected parasite specimens was performed in the Laboratory of Parasitology, Department of Botany and Zoology, Masaryk University, Brno, Czech Republic, based on morphology of the hard parts using a phase-contrast microscope (Olympus BX51). Hard parts were drawn with the aid of a drawing attachment. Measurements of hamuli and bars are those suggested by Přikrylová et al. (2008), and body size parameters and marginal hook measurements were taken based on Christison et al. (2005). All measurements are in micrometer, unless otherwise stated, and are presented as the mean with the range in parentheses. For comparative investigation of African *Gyrodactylus* species, type material was obtained as reported in Přikrylová et al. (2009b) and studied by the principal author. Principal Component Analysis (PCA) was carried out on the correlation matrix of haptor morphometrics using PAST (Hammer et al. 2001).

DNA extraction and amplification

Preservation ethanol was evaporated in a vacuum centrifuge, after which DNA was extracted using the Qiagen Blood and Tissue Isolation kit, according to the manufacturer's protocol. DNA was eluted in 50 μl. The ITS region of the rDNA was amplified with the primers ITS-1F (5′-GTTTCCGTAGGTGAACCT-3′; Rokicka et al. 2007) and ITS-2R (5′-TCCTCCGCTTAGTGATA-3′; Matějusová et al. 2001), in a Mastercycler eP gradient thermocycler (Eppendorf). Each amplification reaction contained 1 μl of template DNA, 1× PCR buffer, 1.25 mM $MgCl_2$, 100 μM dNTPs, 0.1 mg/ml μl bovine serum albumin, 0.5 μM of each primer (Generi Biotech) and 1.5 U of Taq polymerase in a total volume of 20 μl.

Five microliters of PCR product was visualised on Gold View stained agarose gel (1%) and the remaining 15 μl was purified using the High Pure PCR Product Purification Kit (Roche). Sequencing, using identical primers as in initial amplification, was carried out with the Big Dye Chemistry Cycle Sequencing Kit v.3.1 and an ABI 3130 Genetic Analyser automated sequencer (Applied Biosystems).

Sequence alignment and phylogenetic analyses

Sequences were aligned using MUSCLE v.3.8 (Edgar 2004) under default distance measures and sequence weighting schemes. For trimming the resulting alignment, trimAl v.1.2 (Capella-Gutiérrez et al. 2009) was used. The optimal model of molecular evolution was estimated by jModelTest v.0.1.1 (Posada 2008; see also Guindon and Gascuel 2003, Felsenstein 2005). Based on the corrected Akaike Information Criterion (Hurvich and Tsai 1989), the TVM (Posada 2003) + Γ model was selected. To allow subsequent implementation in phylogenetic software, the model with the second best corrected Akaike score was chosen, namely the GTR (Tavaré 1986; Rodriguez et al. 1990) + Γ model, with a gamma-shape parameter of 0.2. Reconstruction of a model-averaged phylogeny showed that tree topology did not display phylogenetic uncertainty because of model selection. Hence, model choice should not influence the results. In PhyML v.3.0 (Guindon and Gascuel 2003), a maximum likelihood (ML) search was performed under the optimised model. Nodal support was assessed through 1,000 bootstrap samples using the nearest-neighbour interchange branch swapping algorithm. For the maximum parsimony (MP) method, PAUP* v.4.01b (Swofford 2001) was used with the PaupUp interface (Calendini and Martin 2005). A heuristic search was carried out using 1,000 replicates of nearest-neighbour interchange branch swapping; gaps were treated as fifth state. Pairwise genetic distances, according to the selected model, were calculated by the same software. Bayesian inference (BI), also using the GTR + Γ model, was implemented in MrBayes v.3 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Posterior probabilities were calculated over 1.10^6 generations, sampling the Markov chain every 100 generations. One-fourth of the samples was discarded as "burn-in". Conversion of alignment files was carried out using ALTER v.1.2 (Glez-Peña et al. 2010) and a tree was drawn in FigTree v.1.3 (http://tree.bio.ed.ac.uk/software/figtree).

Results

Gyrodactylus parasites were found on three catfish species, *Synodontis nigrita*, *C. gariepinus* and *C. anguillaris*. Eight *Gyrodactylus* spp. were identified. From Senegelase *S. nigritae*, two new species, *G. nigritae* n. sp. and *G. synodonti* n. sp., were described. These two species infect different sites of the host's body. While specimens of *G. nigritae* n. sp. were only collected on barbels, *G. synodonti* n. sp. specimens were consistently found on fins. These species are the first *Gyrodactylus* representatives recorded on mochokid catfish. On Senegalese *C. anguillaris*, two known gyrodactylid species were observed, namely *G. rysavyi* and *G. transvaalensis*. A third one appeared to be a new species based on the morphology of its haptoral parts, and is described as *Gyrodactylus gelnari* n. sp. The only Senegalese catfish *Gyrodactylus* representative from which

molecular data were successfully obtained was *G. rysavyi*. A different gyrodactylid parasite altogether was found on Kenyan *C. gariepinus*. Morphological characters and metrics suggest that it represents a new species, hereafter described as *Gyrodactylus turkanaensis* n. sp. On Mozambican *C. gariepinus*, *G. rysavyi* and another unknown *Gyrodactylus* were identified. Haptoral morphology, supported by the molecular data, demonstrate the latter represents a new species, described here as *Gyrodactylus alekosi* n. sp. Sampling in Sudan revealed the presence of gyrodactylid parasites on *C. gariepinus* fins, of which only two specimens could be retrieved and sequenced. While molecular and morphological characterisations of these specimens strongly support their assignment to another new species, a formal species description could not be prepared due to lack of material. Morphological descriptions and (when applicable) molecular characterisation of all species analysed are provided, in alphabetical order, below.

Descriptions

Gyrodactylus alekosi n. sp.

Description based on seven excised haptors. Hence, body dimensions nor internal morphology could be investigated. The external morphology was partially observed. Body elongate with clearly separate haptor, prohaptor with a single pair of cephalic lobes. Dimensions of the haptoral sclerites are given in Table 2. Hamuli sturdy, with flattened area on inner part of root (Fig. 1a). Simple dorsal bar. Ventral bar ribbed at bar proper and bar membrane. Robust shaft of marginal hook sickle rises straight from the base and curves regularly (Figs. 1b, 2c). Sickle base with short blunt toe and pronounced heel slanted with rounded terminal edge.

Molecular characterisation. A 930-bp fragment covering ITS-1 (388 bp), 5.8S rDNA (156 bp), ITS-2 (383 bp) and 28S (3 bp) was successfully sequenced from four specimens and submitted to GenBank under accession number FR850682. The entire sequence was identical for the four specimens. A BlastN (Zhang et al. 2000) search in GenBank in September 2010 using the entire sequence revealed no identical or close hits.

Type host: C. gariepinus (Burchell, 1822) (Siluriformes: Clariidae)

Type locality: temporal stream close to Bala Bala village (24°19.34′ S, 33°02.36′ E), southern Mozambique.

Infection site: Fins.

Type material: Collected February 2009. Holotype and two paratypes deposited in the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic (Accession number M-518).

Etymology: The specific epithet honours Alekos, a special friend of the first author.

Table 2 Comparison of the measurements (in μm, average with range in parentheses) of the haptoral hard parts of *Gyrodactylus* spp. collected from *S. nigrita* and *C. gariepinus* from the current study with the measurements taken on paratype material of *G. rysavyi*

Measurement	*G. alekosi* n. sp (*n*=7) present study	*G. gelnari* n. sp. (*n*=22) present study	*G. nigritae* n. sp. (*n*=35) present study	*G. rysavyi* (*n*=25) present study	*G. rysavyi* (*n*=3) paratypes re-examination
HTL	51.1 (50.5–53)^{m}	71.4 (69–75)	45.5 (43–51.5)	98.6 (89–106.5)	91.8 (90–93)
HPL	26.4 (25–27.5)	35.7(33.5–38)	26.3 (25–28.3)	48.9 (45.5–53)	46.4 (45–47.5)
HSL	29 (28.5–30)	44.4 (42.5–47)	28.5 (27–31)	59.7 (54.5–64)	57.8 (57.5–58.5)
HRL	26.3 (25–29)^{m}	34.4 (31.5–37.5)	23.4 (19–30)	49 (38–57)	42.5 (40–44)
VBL	5.3 (4.5–5.5)^{n}	6.6 (5.5–8)^{g}	4.3 (3.5–5.5)^{a}	8.3 (6–10.5)^{e}	6 (5.5–6.5)
VBML	8.9 (8–10)^{n}	17 (14.5–19)^{g}	8.6 (7–12)^{a}	21.8 (16–27)^{f}	19.7 (19–21.5)
VBW	15.5 (13.5–17.5)^{n}	19.5 (17.5–22)^{h}	14.2 (13–15)^{a}	33.4 (28.5–38.5)^{e}	29.9 (28–31.5)
DBL	1.9	1.9 (1.5–2.5)^{h}	1.3 (1–1.5)^{c}	2.8 (1.8–3.5)^{h}	2.3 (2–2.5)
DBW	14.6	19 (17.5–20.5)^{g}	12.9 (10–16)^{d}	23.7 (19–28)^{h}	21.5 (19.5–22.5)
MHTL		33.3 (32–34.5)^{l}	25.7 (24–28)^{b}	32.6 (30.5–36.5)^{g}	30.7
MHSL	4.7 (4.5–5)^{m}	4.3 (3.5 -5)^{j}	5.5 (5–6.5)^{d}	4.2 (4–5)^{f}	4.3 (4–4.5)
MHHL	18.5 (18–19)^{o}	29 (27.5–31)^{i}	20.3 (18.5–22)^{c}	28.4 (26.2–32)^{f}	26.8 (26.5–27)
MHSDW	3.6(3.5–4)^{m}	4.3 (4–5)^{j}	4 (3.5–4.5)^{d}	3.5 (3–4)^{k}	
MHSPW	4 (4–4.5)^{m}	3.5 (3–4)^{j}	3.8 (3.5–4.5)^{d}	3.6 (3–4)^{g}	3.4 (3–4)
MHSAD	5.1 (5–5.5)^{m}	5.7 (5.5–6)^{k}	5.3 (5–6)^{d}	4.5 (4–5)^{f}	4.3 (4–5)

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

Number of specimens measured: a=33, b=32, c=30, d=28, e=23, f=22, g=21, h=20, i=17, j=13, k=12, l=11, m=5, n=3, o=2

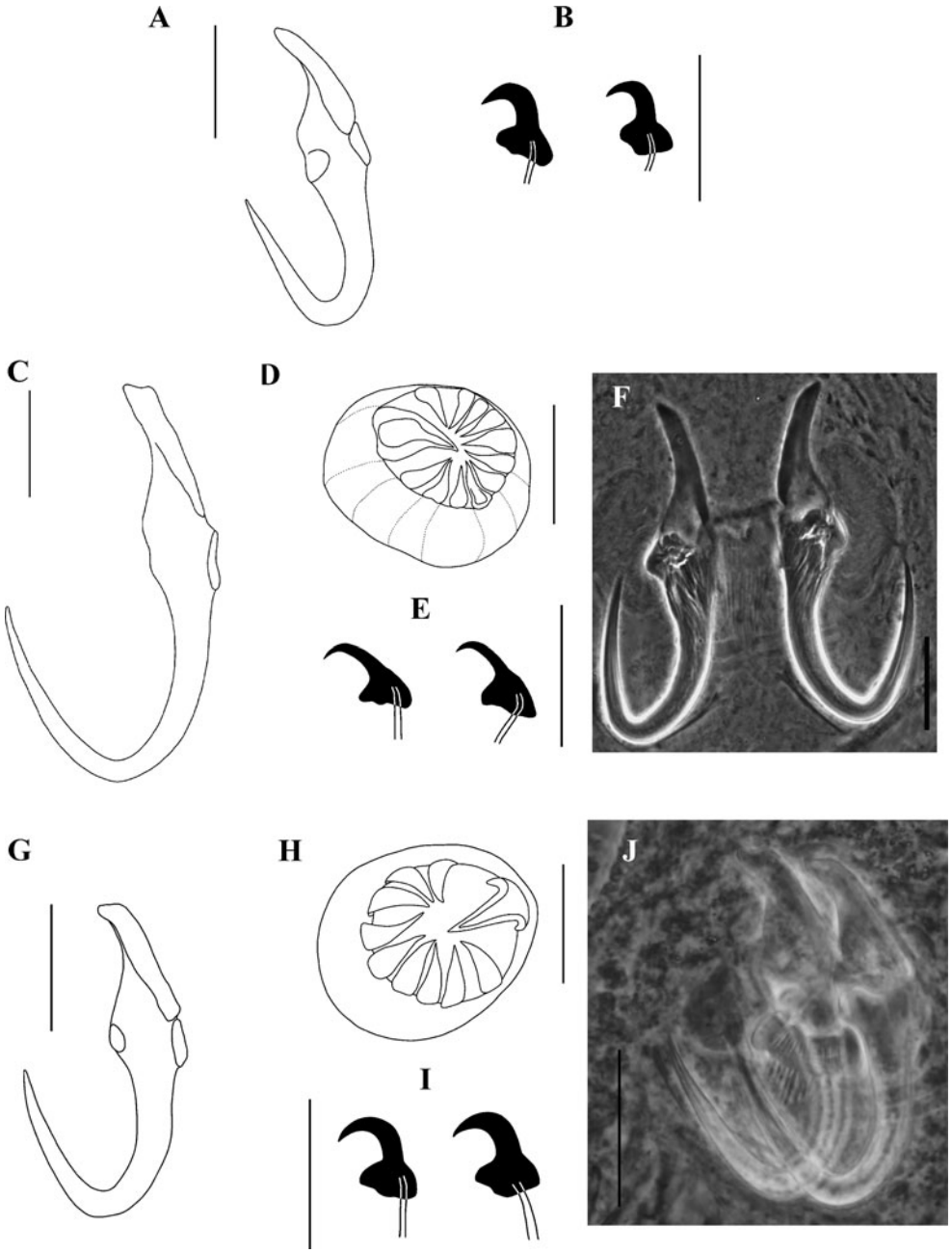

Fig. 1 Line drawings and phase-contrast photomicrographs of *Gyrodactylus alekosi* n. sp. (**a**–**b**), *Gyrodactylus gelnari* n. sp. (**c**–**f**) and *Gyrodactylus nigritae* n. sp. (**g**–**j**). **a** Hamulus. **b** Marginal hook sickles. **c** Hamulus. **d** Detail of the cirrus. **e** Marginal hook sickles. **f** Hamuli complex with detail of the ventral bar. **g** Hamulus. **h** Detail of the cirrus. **i** Marginal hook sickles. **j** Hamuli complex with detail of the ventral bar. *Scale bars*: **a**, **c**, **f**, **g**, **j** 20 μm; **b**, **d**, **e**, **h**, **i** 10 μm

Remarks. Hamuli dimensions of *G. alekosi* n. sp. are similar to those of *G. camerunensis* (total hamuli length: 50.5–53 vs. 45–64; hamulus point length: 25–27.5 vs. 20–28), but both species differ substantially in the shape of their marginal hook sickles. The shaft of the marginal hook sickle of *G. alekosi* n. sp. starts perpendicular to the foot, is regularly curved, and more sturdy than that of *G. camerunensis* which has a thinner sickle proper rising in a slanted manner.

G. gelnari n. sp.

Description based on 22 coverslip-flattened specimens. Elongated body of length 802 (688–925, n=6), width at level of uterus 128 (88–179, n=6). Excretory bladders present. Gut not extending beyond level of testes. Pharyngeal bulb 58 (47–69, n=10) long, 54 (43–54, n=10) wide across the posterior bulb. Male copulatory organ (MCO, Fig. 1d) spherical, posterior to the pharyngeal bulb, 16 (13–18, n=9) in diameter. MCO armed with one principal spine and a single row of 10–11 smaller spines. Measurements of the haptoral sclerites are given in Table 2. Hamuli slender with flattened area on the inner part of the root (Fig. 1c, f). Dorsal bar simple. Surface of the ventral bar proper and its membrane ribbed (Fig. 1f). Narrow shaft of the marginal hook sickle distinctively points forward from the foot and curves slightly at a wide angle towards the point, the latter extending beyond the toe (Fig. 1e). Heel slanted down-

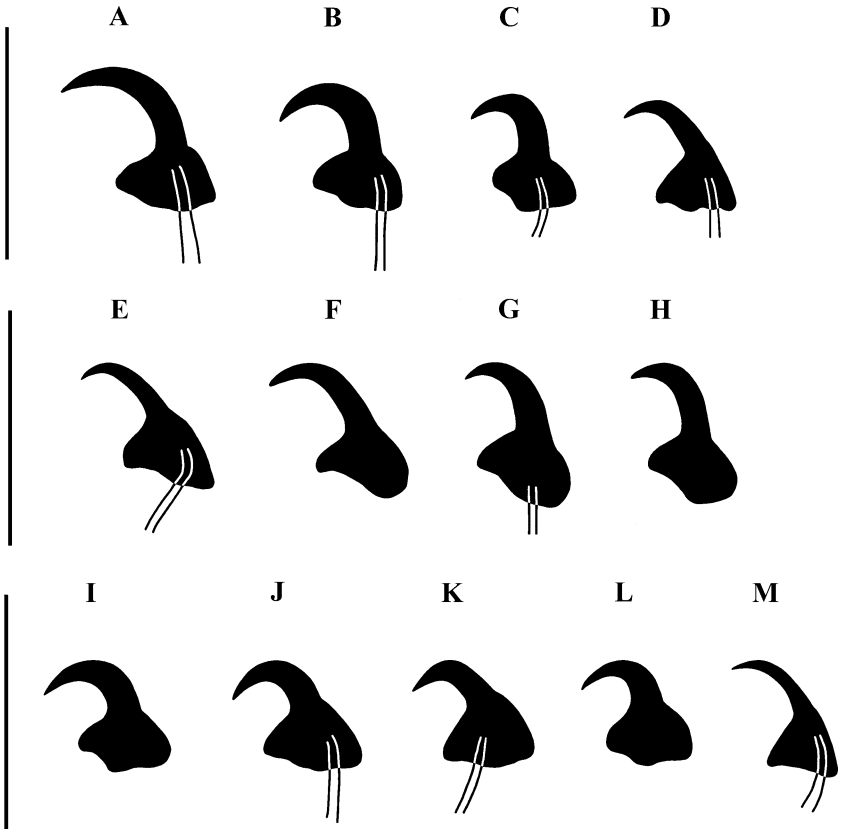

Fig. 2 Drawings of the marginal hook sickles of *Gyrodactylus* spp. of African catfish hosts. **a** *G. synodonti* n. sp. **b** *G. nigritae* n. sp. **c** *G. alekosi* n. sp. **d** *G. turkanaensis* n. sp. **e** *G. gelnari* n. sp. **f** *G. camerunensis* (paratype specimen 263HG Ti148). **g** *G. transvaalensis* (present study). **h** *G. transvaalensis* (paratype specimen 1978.11.3.1–12). **i** *G. rysavyi* (Senegalese specimen). **j** *G. rysavyi* (Kenyan specimen). **k** *G. rysavyi* (Mozambican specimen). **l** *G. rysavyi* (paratype specimen m-134/1). **m** *Gyrodactylus* sp. *Scale bars* 10 μm

wards with a slightly rounded edge. Muscular disc lateral to the hamuli.

Type host: C. anguillaris (Linnaeus, 1758) (Siluriformes: Clariidae)

Type locality: Mare Simenti, Niokolo Koba National Park (13°01.79′ N, 13°17.6′ W), Senegal

Infection site: Fins.

Type material: Collected March 2007. Holotype and two paratypes deposited in the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic (Accession number M-520).

Etymology: the specific epithet honours Prof. Milan Gelnar, the principal investigator supervising the first author, in recognition of his support of her research ideas.

Remarks. The shape of the marginal hook sickle of *G. gelnari* n. sp. is similar to that of *G. camerunensis*, its shaft similarly rising shaft from the base, but the sickle shaft of *G. gelnari* n. sp. is thinner than in *G. camerunensis*. The heel of the marginal hook sickle of *G. camerunensis* is more elongate and distinctively rounded than in *G. gelnari* n. sp. (Fig. 2e and f). Moreover, the hamuli of *G. gelnari* n. sp. (mean total length 71.4 μm) are larger than those of *G. camerunensis* (mean 54 μm, Nack et al. 2005). Based on the dimensions of the haptoral sclerites, *G. gelnari* n. sp. resembles *G. turkanaensis* n. sp. (Tables 2 and 3), but differences in marginal hook sickle morphology can be observed (Fig. 2e and d). Although the sickles proper of both species point forward, the profile of the marginal hook sickle foot of *G. turkanaensis* n. sp. is regularly triangular, smoothly joining into the foot of the sickle. In contrast, in *G. gelnari* n. sp., the foot is more elongate and clearly separated from the sickle proper. Regrettably, samples were not adequately preserved for molecular analysis, thus genetic data on *G. gelnari* n. sp. cannot be presented.

G. nigritae n. sp.

Description based on 32 coverslip-flattened specimens and three excised haptors of sequenced individuals. Body elongate, 819 (589–1079, n=32) long, width at level of uterus 135 (98–209, n=32). Prohaptor with single pair of cephalic lobes. Pharyngeal bulb 51 (37–67, n=32) long, 47 (31–53, n=32) wide across posterior bulb. Excretory bladders present. Gut extending beyond level of testes. MCO (Fig. 1h) posterior to pharyngeal bulb, 18 (14–22, n=13) in diameter. MCO armed with one principal spine and a single row of seven to nine smaller spines. Ribbed ventral bar proper and ventral bar membrane (Fig. 1j). Measurements of the haptoral sclerites are given in Table 2. Hamuli sturdy because of widened joint of shaft and root; flattened area on inner part of root (Fig. 1g). Simple dorsal bar. Ventral bar without lateral processes, surface of ventral bar proper ribbed, tongue-shaped ventral bar membrane. Marginal hook sickle shaft broad, slightly

Table 3 Comparison of the measurements (in μm, average with range in parentheses) of the haptoral hard parts of *Gyrodactylus* spp. collected from *C. gariepinus* and *C. anguillaris* from the present study with the measurements made on paratype specimens of *G. transvaalensis*

Measurement	*G. synodonti* n. sp. (*n*=23) present study	*G. turkanaensis* n. sp. (*n*=21) present study	*G. transvaalensis* (*n*=8) present study	*G. transvaalensis* (*n*=2) re-examination	*Gyrodactylus* sp. (*n*=2) present study
HTL	80.3 (75–87)	82.3 (77–87.5)	43.5 (41.5–44.5)	(41.5–45)	78
HPL	37.2 (34–40.5)	36.4 (34–38.5)	22.3 (20–23)	(20.5–23)	(36.5–39)
HSL	47.2 (43–54.5)	49.3 (47.5–51)	27.8 (25.5–30)	(27.5 -28.5)	54
HRL	41.9 (37–46)	42.2 (34–44.5)	20 (20–22)	(14.5–17)	39.5
VBL	8 (7–10)	7.4 (5.5–8.5)[c]	3.9 (3.5–4.5)[l]	4.5	
VBML	17.7 (15.5–21.5)	19.3 (17–21)[c]	8.1 (7.5–9)[m]		
VBW	25 (23–28)[a]	20.1 (18–22.5)[c]	11.4 (9.5–13.5)[l]	10.5	
DBL	2.3 (2–3)[a]	2.2 (2–2.5)[d]	1.1 (1–1.5)[o]		
DBW	19.9 (17–24.5)[d]	18.5 (16.5–21)[f]	13.2 (12–14.5)[o]		
MHTL	27.9 (26.5–29.5)[f]	30.8 (29–32)[n]	21.5 (20.5–22)[l]	23	
MHSL	6.3 (5.5–7)[e]	4 (3.5–5)[i]	5 (4.5–5.5)[l]	(5–5.5)	(4–4.5)
MHHL	21.7 (19.5–22.5)[h]	27.4 (26–28.5)[f]	16 (15.5–17)[k]		
MHSDW	4.4 (3.5–5.5)[e]	3.9 (3–4.5)[j]	4 (4–4.5)[l]		(3.1–3.3)
MHSPW	3.8 (3.5–4)[e]	3.4 (3–3.5)[i]	3.7 (3.5–4)[l]	4	(3.8–4.3)
MHSAD	6.6 (6–7)[e]	5.4 (4.5–6)[j]	5.7 (5.5–6)[l]	(5.5–5.7)	(5.3–5.7)

Number of specimens measured: a=21, b=20, c=19, d=17, e=16, f=15, g=13, h =12, i=11, j=10, k=7, l=6, m=5, n=4, o=3

pointing forwards, with regularly curved sickle and narrowing point extending beyond the toe (Fig. 1i). Sickle foot ovate in profile, rounded at the top and waved at the bottom.

Molecular characterisation. A 870 bp fragment covering ITS-1 (338 bp), 5.8S rDNA (156 bp) and ITS-2 (376 bp) was sequenced from three specimens and submitted to GenBank under accession number FR850686. The whole sequence was identical for the three specimens. A BlastN search in GenBank in September 2010 using the entire sequence revealed no identical or close hits.

Type host: Synodontis nigrita Valenciennes, 1840 (Siluriformes: Mochokidae)

Type locality: Niokolo Koba River, Passage Koba (13°03.92′ N, 13°10.14′ W), Niokolo Koba National Park, Senegal.

Infection site: Barbels.

Type material: Collected March 2007. Holotype and two paratypes deposited in the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic (Accession number M-517).

Etymology: The specific epithet is derived from the specific epithet of the host fish.

Remarks. G. nigritae n. sp. is similar to *G. transvaalensis* in the overall size of the hamuli. However, *G. nigritae* n. sp. differs from the latter species in hamulus point length (mean 26.3 vs. 22.3 μm) and marginal hook total length (mean 25.7 vs. 21.5 μm). In addition, these species' marginal hook sickles have a different shape. The sickle proper of *G. nigritae* n. sp. is rather robust and curves regularly, into a pronounced point with closed aperture. Conversely, the sickle proper of *G. transvaalensis* is slender and its point terminates immediately after curving slightly. The heel of *G. transvaalensis* is rounded, and elongate in downward direction, while that of *G. nigritae* n. sp., despite also being rounded at the top of the heel, is not that elongate and more symmetrical to the toe.

G. rysavyi Ergens, 1973

Description based on 25 coverslip-flattened (11 Kenyan, 4 Mozambican, 10 Senegalese) and six sequenced (four Mozambican, two Senegalese) specimens. Body elongate with clearly separate haptor, total body length 1,315 (1052–1674, *n*=7), width at level of uterus 171 (134–204, *n*=7). Cephalic region bilobed, each lobe containing a spike sensillum. Excretory bladders present. Pharyngeal bulb 81 (61–113, *n*=10) long, 74 (56–105, *n*=10) wide across the posterior part. MCO posterior to pharyngeal bulb, 23.5 (19–29, *n*=8) in diameter, armed with one large principal spine and 11 thin small spines in a single row. Intestinal crura not extending beyond anterior edge of testes. Hamuli slender because root substantially narrows after its widened joint with the shaft (Fig. 3a). Flattened area on inner part of root. Simple dorsal bar. Ventral bar without lateral processes, surface of ventral bar proper and ventral bar membrane ribbed (Fig. 3b). Measurements of the haptoral sclerites are given in Table 2. Broad sickle shaft of marginal hook sickle points forwards from the sickle foot, and immediately curves regularly. Sickle point downwardly directed, extending beyond the toe. Toe and heel of the sickle foot slantedly rounded.

Molecular characterisation. A 943-bp PCR product covering ITS-1 (383 bp), 5.8S (157 bp), ITS-2 (383 bp) and 28S

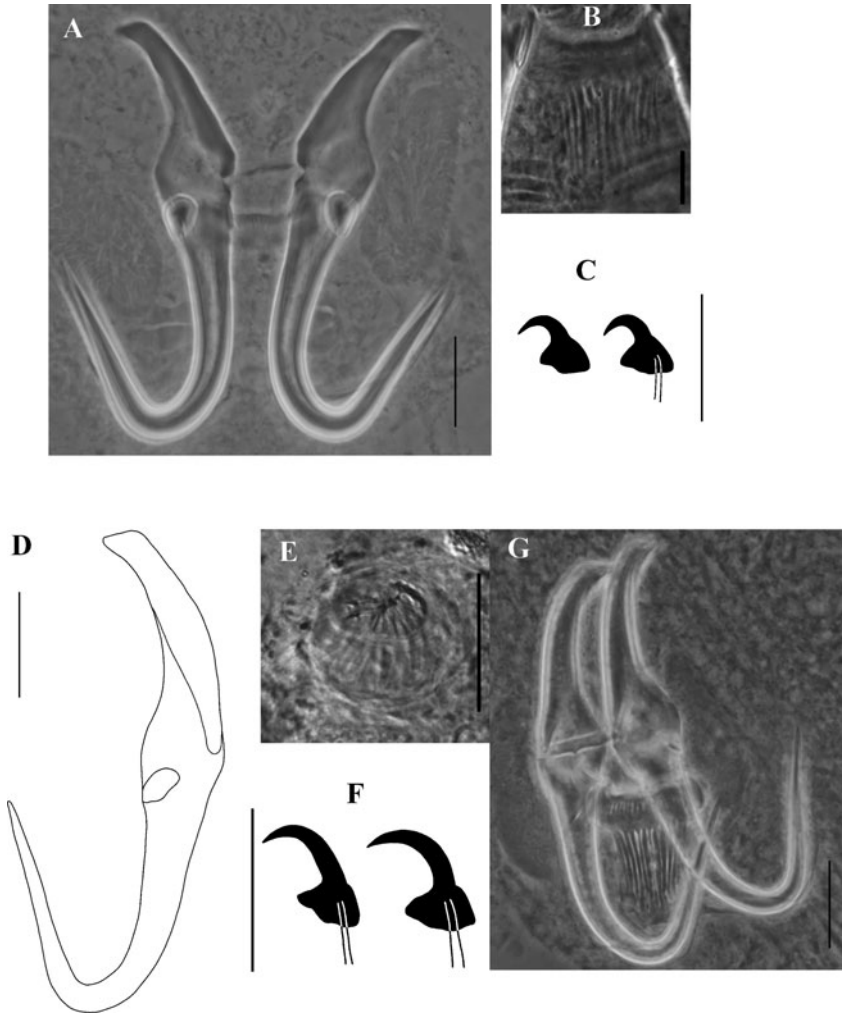

Fig. 3 Line drawings and phase-contrast photomicrographs of *Gyrodactylus rysavyi* Ergens, 1973 (**a**–**c**), *Gyrodactylus synodonti* n. sp. (**d**–**g**). **a** Hamuli. **b** Detail of the ventral bar. **c** Marginal hook sickles. **d** Hamulus. **e** Detail of the cirrus. **f** Marginal hook sickles. **g** Hamuli complex with detail of the ventral bar. *Scale bars*: **a**, **d**, **e**, **g** 20 μm; **b**, **c**, **f** 10 μm

(20 bp) was sequenced from four Senegalese specimens and submitted to GenBank under accession number FR850679. The entire sequence was identical in these four specimens. An additional sequence of a 872 bp PCR product covering ITS-1 (342 bp), 5.8S (157 bp) and ITS-2 (373 bp) was obtained from three specimens collected in Mozambique and submitted to GenBank under accession number FR850681. Pairwise genetic distances are summarised in Table 4. The differences in ITS sequences of *G. rysavyi* of both countries are as follows: the partial ITS-1 differs in seven individual positions (four substitutions and three deletions), and the ITS-2 sequences differ in six bp. A BlastN search in GenBank in September 2010 using the entire sequence revealed no identical or close hits.

Type host: C. gariepinus (Burchell, 1822) (Siluriformes: Clariidae)

Other host: C. anguillaris (Linnaeus, 1758) (Siluriformes: Clariidae)

Type locality: Nile River, Cairo, Egypt

Other localities: Mare Simenti, Niokolo Koba National Park (13°01.79′ N, 13°17.6′ W), Senegal; Kalokol (13°01.79′ N, 13°17.6′ W), Todonyang village (4°26.15′ N, 35°56.38′ E),

Table 4 Gamma-corrected pairwise GTR distances (in %) between the species included in the phylogenetic analysis, for a 881 bp dataset consisting of partial first and second internal transcribed spacers (ITS-1 and ITS-2) and intervening 5.8S rDNA

		1	2	3	4	5
1	*Gyrodactylus synodonti* n. sp.					
2	*G. nigritae* n. sp.	1.9				
3	*G. alekosi* n. sp.	19.6	18.3			
4	*Gyrodactylus* sp. (Sudan)	20.6	19.3	13.8		
5	*Gyrodactylus rysavyi* (Senegal)	20.9	19.6	13.8	1.7	
6	*Gyrodactylus rysavyi* (Mozambique)	20.6	19.6	13.3	2.5	1.2

Turkana Lake, Kenya; temporal stream close to Bala Bala village (24°19.34′ S, 33°02.36′ E), southern Mozambique.
Infection site: Fins.
Remarks. The shape of the marginal hook sickles of *G. rysavyi* specimens collected from three distant localities is identical to that of the re-examined paratypes (Fig. 2). The length of hamulus shaft and point of *G. rysavyi* from the present study correspond with measurements on the paratype material. Other agreements in the measurements include all analysed features of the marginal hooks. There were some small discrepancies in the total hamuli length, this characteristic being larger (89–106.5 μm) in the present study than in the paratype material (90–93 μm). The species can be discriminated from all other *Gyrodactylus* spp. parasitizing clariids, by the size of its sclerites, the latter being larger than all studied species. PCA (Fig. 4) does show clustering according to region of origin. However, the types, originating from Egypt, cluster together with the geographically distant Senegalese specimens. Moreover, the Mozambican specimens are positioned amidst the other locations, all three of which (Senegal, Lake Turkana and Nile) are part of the Nilo-Sudanese ichthyofaunal province (Lévêque 1997). Hence, morphometric groups from various ichthyogeographical regions seem to overlap. On top of the arguments mentioned, it is also the current sample size that does not allow to raise our *G. rysavyi* populations to species level.

Gyrodactylus synodonti n. sp.
Description based on 19 coverslip-flattened specimens and four excised haptors of sequenced specimens. Body

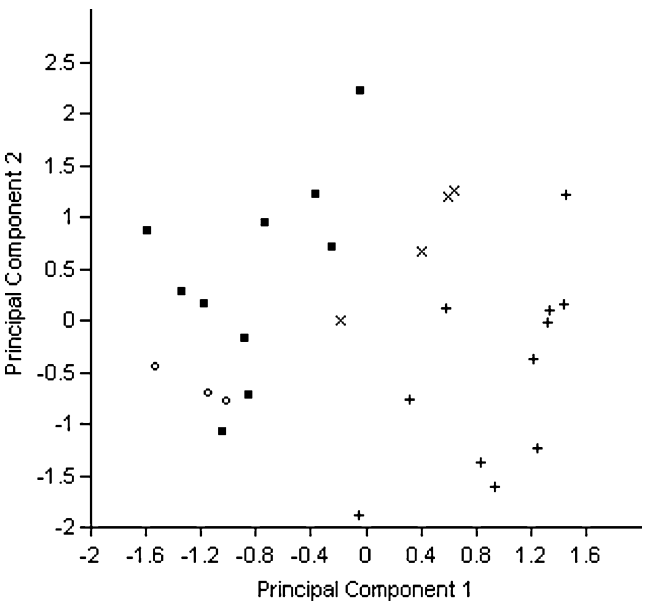

Fig. 4 Plot of principal component analysis on measurements of the haptoral hard parts of *Gyrodactylus rysavyi*, for 11 Kenyan (*plus sign*), four Mozambican (*ex symbol*), ten Senegalese (*filled square*) and three type specimens (*open circle*)

elongate, 842 (602–1080, *n*=13) long and 113 (67–164, *n*=13) wide at level of uterus. Prohaptor with single pair of cephalic lobes. Excretory bladders present. Pharyngeal bulb 45 (35–61, *n*=11) long, 41 (25–58, *n*=11) wide. MCO (Fig. 3e) posterior to pharyngeal bulb, 19 (16–23, *n*=8) in diameter. MCO armed with one principal spine and a single row of seven to nine smaller spines. Intestinal crura not extending beyond anterior edge of testes. Measurements of the haptoral sclerites are given in Table 3. Slender hamuli with well-defined long root. Hamuli root slightly curved outwards, inner part of root flattened. Dorsal bar simple. Ventral bar with small processes; surface of ventral bar proper and its membrane ribbed (Fig. 3g). Sickle proper of marginal hook points slightly forward and is relatively slender, turns slightly into a quite open arch (Fig. 3f). Sickle point directed forwards, terminating beyond toe. Toe elongate and blunt. Muscular disc lateral to hamuli.
Molecular characterisation. An 868-bp fragment covering ITS-1 (338 bp), 5.8S rDNA (156 bp) and ITS-2 (374 bp) was sequenced from four specimens and submitted to GenBank under accession number FR850684. The entire sequence was identical for all four specimens. A BlastN search in GenBank in September 2010 using the entire sequence revealed no identical or close hits.
Type host: Synodontis nigrita Valenciennes, 1840 (Siluriformes: Mochokidae)
Type locality: Niokolo Koba River, Passage Koba (13°03.92′ N, 13°10.14′ W), Niokolo Koba National Park, Senegal.
Infection site: Fins.
Type material: Collected March 2007. Holotype and two paratypes deposited in the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic (Accession number M-516).
Etymology: The specific epithet is derived from the generic name of the host fish.
Remarks. G. synodonti n. sp. is similar to *G. turkanaensis* n. sp. (see Table 3) in hamuli dimensions. The two species can be discriminated by the marginal hook total length (mean: 25.7 vs. 30.8 μm, respectively). The difference in shapes of their marginal hook sickles are distinctive: *G. synodonti* n. sp. has a broader, regularly curved sickle proper, while in *G. turkanaensis* n. sp. it is thinner, rising from the sickle foot at a forward angle. The sickle foot of *G. turkanaensis* n. sp. has a triangular profile and joins smoothly with the sickle proper, whereas in *G. synodonti* n. sp. the sickle toe is more elongate, and the foot is clearly separated from the sickle proper.

G. transvaalensis Prudhoe and Hussey, 1977
Description based on eight coverslip-flattened specimens. Elongate body of total length 687 (547–856, *n*=3), width at level of uterus 100 (84–124, *n*=3). Cephalic region bilobed,

each lobe containing a spike sensillum. Presence of excretory bladders observed. Pharyngeal bulb 46 (39–51, n=3) long, 42 (38–45, n=3) wide. MCO posterior to pharyngeal bulb, 14.5 (n=1) in diameter. Arrangement of MCO spines not discernible on whole mounts. Intestinal crura not extending beyond anterior edge of testes. Measurements of the haptoral sclerites are presented in Table 3. Hamuli rather sturdy as root widens after its joint with shaft (Fig. 5a, d). Root gently narrowing towards the proximal end. Inner part of root flattened. Dorsal bar simple. Ventral bar with without lateral processes; ventral bar proper ribbed. Ventral bar membrane tongue-shaped (Fig. 5b). Marginal hook sickle foot ovate in profile, with slightly triangular toe and blunt heel pointing downwards. Sickle proper rises at slightly forward angle; sickle point narrow and angled almost perpendicularly, just extending beyond toe (Figs. 5c, 2g).

Type host: C. gariepinus (Burchell, 1822) (Siluriformes: Clariidae)

Other host: C. anguillaris (Linnaeus, 1758) (Siluriformes: Clariidae)

Type locality: Confluence of Olifants and Elands Rivers, South Africa.

Other localities: Mare Simenti, Niokolo Koba National Park (13°01.79′ N, 13°17.6′ W), Senegal.

Infection site: Fins.

Remarks. While the Senegalese *G. transvaalensis* specimens from *C. anguillaris* were collected at a large geographic distance from the species' type locality, their general morphology and hamuli dimensions correspond well to the re-examined *G. transvaalensis* type material from South African *C. gariepinus*. This also goes for the marginal hook sickle shape (Fig. 2g and h).

G. turkanaensis n. sp.

Description based on 21 coverslip-flattened specimens. Elongate body of total length 900 (739–1088, n=6), width at level of uterus 140 (98–225, n=6). Prohaptor with single pair of cephalic lobes. Excretory bladders present. Pharyngeal bulb 64 (54–76, n=12) long, 56 (41–71, n=12) wide. MCO posterior to pharyngeal bulb, 19 (15–25, n=7) in diameter. Detailed arrangement of MCO spines not discernible on whole mounts. Intestinal crura extending beyond anterior edge of testes. Measurements of the haptoral sclerites are given in Table 3. Hamuli slender, with root narrowing substantially after joining shaft (Fig. 5e,h). Hamuli with flattened area on inner part of root. Dorsal bar simple. Ventral bar without lateral processes, surface of ventral bar proper and ventral bar membrane ribbed (Fig. 5b). Marginal hook sickle foot of triangular profile; heel continuous with sickle shaft, the latter positioned at a forward angle. Sickle point short and thin, extending beyond the toe. Toe slant, slightly rounded. Muscular disc lateral to hamuli.

Type host: C. gariepinus (Burchell, 1822) (Siluriformes: Clariidae)

Type locality: Kalokol, Lake Turkana (13°01.79′ N, 13°17.6′ W), Kenya.

Infection site: Gills.

Type material: Collected September 2008. Holotype and two paratypes deposited in the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic (Accession number M-519).

Etymology: The specific epithet is derived from the name of the type locality.

Remarks. Although *G. turkanaensis* n. sp. resembles *G. synodonti* n. sp. in hamuli size, clear differences between these two species are observed in the marginal hook sickle shape: *G. turkanaensis* n. sp. has a thinner sickle proper, rising at a forward angle, while *G. synodonti* n. sp. has a broad, regularly curved sickle proper. The sickle foot of *G. turkanaensis* n. sp. is of triangular profile with the heel continuing into the sickle proper, while in *G. synodonti* n. sp. the sickle foot has a more elongate toe and the sickle proper is clearly separated from the foot. The marginal hook sickle shape of *G. turkanaensis* n. sp. resembles that of *G. groschafti* the most, but the two species differ significantly by the size of their hamuli complex, with the hamuli total length of *G. turkanaensis* n. sp. exceeding that of *G. groschafti* (82.3 vs. 34.2 μm, respectively).

Gyrodactylus sp.

Only two excised haptors of sequenced specimens were available. Therefore, body dimensions nor internal morphology can be presented. Measurements of the haptoral sclerites are given in Table 3. Hamuli slender because of long, well-defined root with flattened area on inner part (Fig. 5j). Dorsal bar simple. Ventral bar without lateral processes. Marginal hook sickle foot of triangular profile; toe and heel blunt (Fig. 5k). Very narrow sickle shaft sharply angles forwards, turns into a very open, narrow, short point extending beyond the toe. Heel continuous with shaft.

Molecular characterisation. A 910 bp PCR product covering ITS-1 (380 bp), 5.8S (157 bp), ITS-2 (373 bp) was sequenced from two specimens and submitted to GenBank under accession number FR850688. The entire sequence was identical for both specimens. A BlastN search in GenBank in September 2010 using the entire sequence revealed no identical or close hits.

Host: C. gariepinus (Burchell, 1822) (Siluriformes: Clariidae)

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

Infection site: Fins.

Remarks. Due to the limited number of studied specimens, the formal description of this unknown species is not

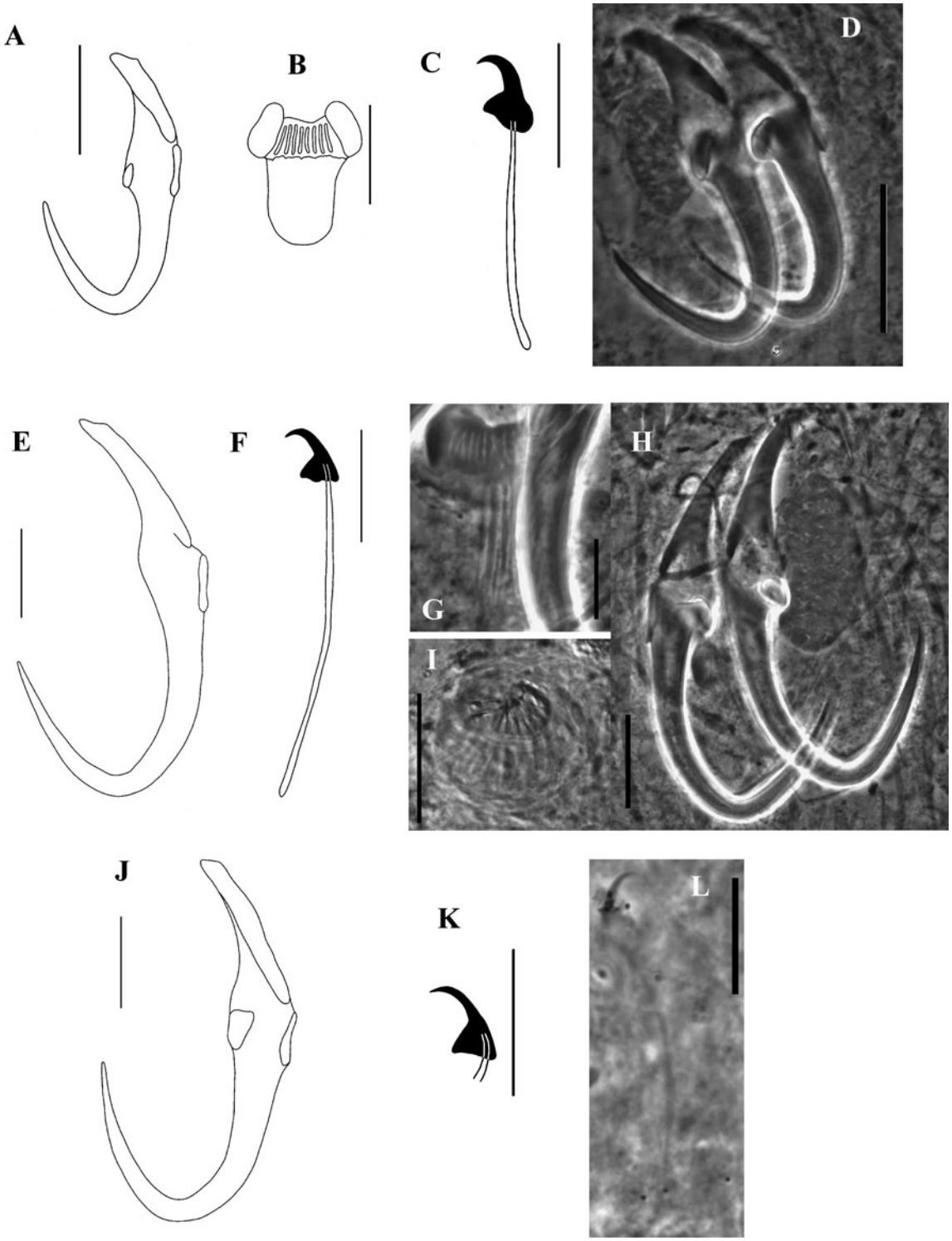

Fig. 5 Line drawings and phase-contrast photomicrographs of *Gyrodactylus transvaalensis* Prudhoe and Hussey 1977 (Senegal) (**a**–**d**), *Gyrodactylus turkanaeansis* n. sp. (**e**–**h**) and *Gyrodactylus* sp. (Sudan) (**j**–**l**). **a** Hamulus. **b** Detail of the ventral bar. **c** Marginal hook sickle. **d** Hamuli complex. **e** Hamulus. **f** Marginal hook. **g** Detail of the ventral bar. **h** Hamuli complex. **i** Detail of the cirrus. **j** Hamulus. **k** Marginal hook sickle. **l** Marginal hook. *Scale bars*: **a**, **d**, **e**, **h**, **j** 20 μm; **b**, **c**, **f**, **g**, **i**, **k**, **l** 10 μm

presented here. The size of the haptoral sclerites is similar to that of *G. turkanaensis* n. sp., but the species differ in their marginal hook sickle shape: *Gyrodactylus* sp. has a more narrow sickle shaft than *G. turkanaensis* n. sp. Furthermore, the sickle shaft of *Gyrodactylus* sp. is slightly curved resulting in a more open sickle aperture, and its

sickle point is very short, whereas *G. turkanaensis* n. sp. has a more distinctively curved shaft and a longer point.

Sequences and phylogenetic analyses

After trimming of the aligned sequences, 881 bp were retained in the combined dataset of ITS-1, 5.8S rDNA and ITS-2. The matrix contained 170 variable sites of which 121 were parsimony-informative. There were no differences in topology between the BI, ML and MP trees (Fig. 6). Three lineages are apparent. Apart from *G. alekosi* n. sp., there are two highly supported clades: one grouping both *Synodontis* parasites, namely *G. synodonti* n. sp. and *G. nigritae* n. sp., and another one clustering *G. rysavyi* with the yet undescribed species parasitizing Sudanese *C. gariepinus*. Gamma-corrected pairwise genetic distances (Table 4) are 1.9% between both *Gyrodactylus* spp. from *Synodontis*, and 1.2–2.5% in the clade containing *G. rysavyi*.

The 5.8S rDNA gene was identical in all species under study except for *G. alekosi* n. sp. In terms of overall genetic distances, this species is more similar to the other *Clarias* parasites (*Gyrodactylus* sp. and *G. rysavyi*) than to *Synodontis* parasite clade (*G. nigritae* n. sp. and *G. synodonti* n. sp.).

Discussion

The comparison of gyrodactylids of catfishes from four distant African countries provides an opportunity to observe morphological and molecular diversity of these parasites on a continental scale. Furthermore, the present study delivers original data on *Gyrodactylus* species distribution on catfishes from Africa. The description of five new species brings the total number of *Gyrodactylus* species described from African fishes to 33 (Christison et al. 2005; Nack et al. 2005; Přikrylová et al. 2009b; Vaughan et al. 2010; Vanhove et al. 2011a; García-Vásquez et al. 2011).

Gyrodactylus spp. on mochokid catfishes

The finding of *Gyrodactylus* species on Senegalese *S. nigrita* represents the first record of these parasites on a mochokid host. Two new species identified from this host, *G. nigritae* n. sp. and *G. synodonti* n. sp., are clearly distinguished by the size of their haptoral sclerites and also by the shape of their marginal hook sickle (Tables 1 and 2 and Fig. 2). Moreover, different infection sites were observed for the two species. *G. synodonti* n. sp., bearing larger hamuli, was only collected from the hosts' fins, while *G. nigritae* n. sp., with smaller hamuli, was found on barbels only. A difference in hamulus size according to infection site is often observed in *Gyrodactylus* (Malmberg 1970; Huyse and Malmberg 2004).

Comparisons between *Gyrodactylus* spp. on clariid catfishes

Our finding of two *Gyrodactylus* spp. on *C. gariepinus* from Mozambique represents the first record of gyrodactylid parasites from this country. One of those Mozambican species is *G. rysavyi*, originally described from the Nile River in Egypt (Ergens 1973) and also retrieved in Senegal and Kenya (current study). Its type host is *C. gariepinus*, which has an almost pan-African distribution (Teugels 1986). *C. gariepinus* largely overlaps in its West-African distribution with the mudfish *C. anguillaris*, another possible host to *G. rysavyi*, hence the wide geographical

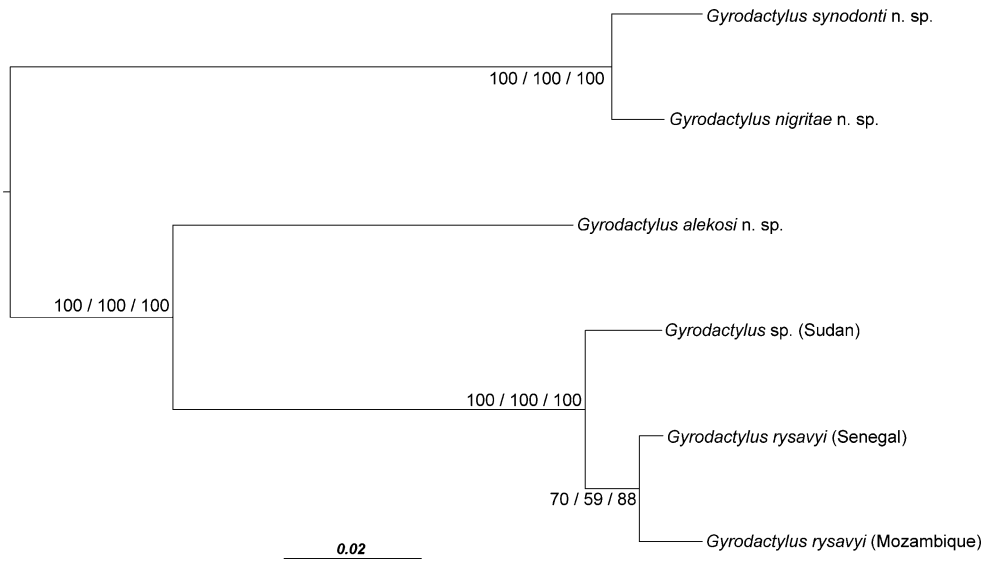

Fig. 6 Midpoint rooted phylogram for the *Gyrodactylus* spp. under study, constructed from an 881-bp dataset consisting of partial first and second internal transcribed spacers (ITS-1 and ITS-2) and intervening 5.8S rDNA. Statistical node support is consistently shown as follows: Bayesian posterior probability/maximum likelihood bootstrap/maximum parsimony bootstrap. Branch lengths correspond to the expected number of substitutions per site under Bayesian inference

range of *G. rysavyi* or its occurrence on other clariid hosts is not surprising.

For *G. gelnari* n. sp. and *G. turkanaensis* n. sp., only morphometrical data were obtained. Differences in systematically important characters between them and with known African congeners are deemed sufficient for their designation as new species. While the haptoral sclerites of *G. gelnari* n. sp. and *G. turkanaensis* n. sp. are morphometrically similar, the shape of their marginal hook sickles differ, enabling to clearly distinguish between them.

New data on distribution and morphometrics are presented for *G. transvaalensis*. In the original description (Prudhoe and Hussey 1977), the number of studied specimens is unclear. Moreover, the associated drawings do not allow morphological comparison, especially as regards details of the marginal hook sickle. Our reanalysis of paratype specimens provides more precise information, facilitating recognition of *G. transvaalensis*. Hamuli total length, shaft length and point length of the new *G. transvaalensis* specimens correspond with the paratype material and, as far as shaft and point length are concerned, with the original measurements by Prudhoe and Hussey (1977). However, there were some discrepancies in root length, explaining the difference in the reported hamulus total length. The values reported here for the root (20–22 μm) exceed those in both Prudhoe and Hussey (1977; 15–20 μm) and in the paratype specimens (14.5–17 μm). The agreement between marginal hook metrics of our specimens and re-examined paratype specimens is shown in Table 3.

Only two *Gyrodactylus* sp. specimens from Sudan were studied. Nevertheless, the morphological differences in marginal hook sickle shape between them and African congeners demonstrate that they represent another hitherto unknown species. The shape of the marginal hook sickle of *Gyrodactylus* sp. is most reminiscent to *G. turkanaensis* sp. n., but its sickle proper is thinner and its point less curved in *Gyrodactylus* sp. Due to the limited number of studied specimens, the species can at present not be formally described, and more material should be morphometrically studied. However, molecular data on *Gyrodactylus* sp. are already presented.

Some of the new species, namely *G. synodonti* n. sp., *G. gelnari* n. sp. and *G. turkanaensis* n. sp., share an additional haptoral feature: a pair of muscular discs situated on the side of the hamuli. These three species have quite large hamuli (of mean total length 80.3, 71.4 and 82.3 μm, respectively), and this feature is likely to support the parasite's attachment to its host. However, such discs have not been observed in *G. rysavyi*, another species of considerable hamuli size (mean total length 101.2 μm). Quite similar haptoral position and structures were observed in *Diplogyrodactylus martini* Přikrylová, Matějusová, Musilová, Gelnar and Harris, 2009 (Přikrylová et al. 2009a). As Africa has seen the discovery of more unusual haptoral structures e.g. accessory bars in *Mormyrogyrodactylus gemini* Luus-Powell, Mashego and Khalil, 2003 (Luus-Powell et al. 2003); additional haptoral plates in *Gyrodactylus thysi* Vanhove, Snoeks, Volckaert and Huyse, 2011 (Vanhove et al. 2011a), it is expected that this continent hosts a remarkable diversity in haptoral organisation among gyrodactylids.

Most *Gyrodactylus* spp. parasitising catfishes can apparently be divided into two morphotypes based on their hamulus shape. Furthermore, most of them have the same type of ventral bar, with ribbed ventral bar proper and ventral bar membrane (Figs. 3 and 5). Another character shared by the same species is the flattened area on the inner part of the hamulus root. On this basis, the following species can be regarded as belonging to a first morphotype: *G. clarii*, *G. groschafti*, *G. rysavyi*, *G. transvaalensis*, *G. camerunensis* and all newly described species. *Gyrodactylus fusci* Ky, 1968, a parasite of Hong Kong catfish, *Clarias fuscus* Lacepède, 1803, should be included, too. From the original description (Ky 1968, drawing 1B), it is evident that this species' hamuli and ventral bar are of the same morphological type as the above-mentioned African *Gyrodactylus* spp. Asian *G. fusci* displays a similar hamulus total length (70–81 μm: Ky 1968) to *G. synodonti* n. sp. and *G. turkanaensis* n. sp. (75–87 μm and 77–87.5 μm, respectively), but these species differ substantially in the size of their marginal hooks. Marginal hook total length in *G. fusci* (42–47 μm) considerably exceeds the 26.5–29.5 μm and 29–32 μm of, respectively, *G. synodonti* n. sp. and *G. turkanaensis* n. sp. The second morphotype can be characterised by large slender hamuli, their point considerably narrowing after emerging from the shaft. As representatives of this group, *G. alberti* and *G. nyongensis* can be mentioned. These two species have a proportionally short marginal hook handle (MHHL) in comparison with the length of their marginal hook sickle (MHSL): *G. alberti* 14.8 μm and 6.5 μm respectively, and *G. nyongensis* 17 μm/11.6 μm (measured during the re-examination of type material). In contrast, in the species from the first morphotype, the proportion MHHL/MHSL is substantially higher: mean values 28 μm and 4.2 μm for *G. rysavyi* and 29 μm and 4.3 μm for *G. gelnari* n. sp.

Molecular analyses

The first genetic data on *Gyrodactylus* parasitising African catfishes are presented, and phylogenetic tree reconstruction was performed. The molecular data concur with species distinctions suggested on a morphological basis. Over the region comprising ITS-1 and -2 and 5.8S rDNA, the overall gamma-corrected pairwise genetic distance is 1.2% between Senegalese and Mozambican *G. rysavyi*. This approaches the value of 1% suggested to concur with species delineation by Ziętara and Lumme (2002). However,

the same authors state that a variety of other criteria would be needed to ascertain species distinction (Zięta ra and Lumme 2003), e.g. regarding host specificity and consistent morphological differences. As these do not seem to be fulfilled for the two *G. rysavyi* populations (both infest *C. gariepinus*, and morphometrics showed an overlap between sampled ichthyogeographical regions), we see no reason to rise any of them to species status. The genetic and morphological differences observed are, in view of their distant geographic origin, most likely a result of incipient speciation because of isolation-by-distance.

Phylogenetic analyses consistently revealed the existence of three lineages in the catfish *Gyrodactylus* spp.: (1) a clade of both *Synodontis* parasites included, (2) *G. alekosi* n. sp. and (3) the Sudanese *Gyrodactylus* sp. together with *G. rysavyi*. Several authors suggest the 5.8S rDNA gene to be useful in distinguishing between *Gyrodactylus* subgenera as defined, based on the excretory system, by Malmberg (1970), (Zięta ra et al. 2002; Huyse et al. 2003). Hence, it is applied to give a clue about subgenus affiliation or the recognition of new subgenera (García-Vásquez et al. 2007; Vanhove et al. 2011a). Remarkably, the 5.8S sequence of all genetically studied catfish *Gyrodactylus* spp. is identical, or, in the case of *G. alekosi* n. sp., near-identical (differing in one deletion and one transversion only). As previously mentioned, BLAST searches using the entire amplified fragment did not reveal any close hits. When specifically comparing the 5.8S portion to a range of gyrodactylids (spanning the various *Gyrodactylus* subgenera, all African and South-American species for which molecular data are available, and other gyrodactylid genera: see Vanhove et al. 2011a; results not shown), no identical genes were retrieved. This could imply that the *Gyrodactylus* parasites of African siluriforms belong to a hitherto unknown subgenus. As morphological investigation of soft body parts is also highly informative in *Gyrodactylus* systematics (Pugachev et al. 2009), one cannot at this stage formally identify or describe the "subgenus" the newly described species belong to. The relationship between the three clades of catfish parasites mentioned above are unresolved in the analyses. However, despite the slight difference in 5.8S, the overall similarity in the ITS regions is highest between *G. alekosi* n. sp. and its other congeners parasitising clariids. Although sampling efforts are currently far too low for definitive conclusions, a certain co-evolutionary effect cannot be excluded here. Indeed, the affiliation between the catfish parasites, and within those, between species hosted by mochokids and clariids, respectively, might suggest an influence of host phylogeny on parasite speciation, or, in view of limited host range studied, might point to traces of within-host speciation events.

While *G. turkanaensis* n. sp. seems to be its most morphologically similar congener, the yet undescribed *Gyrodactylus* sp. seems to be genetically close to *G. rysavyi*. Of course, molecular analysis of the other *Gyrodactylus* species mentioned is necessary to investigate whether the haptor-based phenotypic affinities are mirrored by genetic data, and whether the two morphotypes of *Gyrodactylus* parasites of catfish, suggested above, hold. Both (clariid) catfishes and their gyrodactylid parasites often display a wide geographic range (cfr. the occurrence of *G. rysavyi* in areas and drainage basins as far apart as Senegal and Mozambique). In this respect, additional molecular characterisation can also fruitfully assist in inferring the (common) phylogeographic history of host and parasite. This was exemplified in a study by Barson et al. (2010) on *Macrogyrodactylus* Malmberg, 1957 of clariid catfishes, revealing ancient connections between presently distant host populations.

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References

Barson M, Přikrylová I, Vanhove MPM, Huyse T (2010) Parasite hybridization in African *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution. Parasitol 137:1585–1595

Cable J, van Oosterhout C, Barson N, Harris PD (2005) *Gyrodactylus pictae* n. sp. (Monogenea: Gyrodactylidae) from the Trinidadian swamp guppy *Poecilia picta* Regan, with a discussion on species of *Gyrodactylus* von Nordmann, 1832 and their poecilid hosts. Syst Parasitol 60(3):159–164

Calendini F, Martin JF (2005) PaupUp v1.0.3.1. A free graphical frontend for Paup* Dos software. Available from http://www.agromontpellier.fr/sppe/Recherche/JFM/PaupUp/.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25(15):1972–1973

Chocha Manda A (2010) Evolutionary genetics of the catfish *Clarias gariepinus* (Burchell, 1822) in the Congo Basin, PhD Thesis, FUNDP Namur, Namur, K.U.Leuven, Leuven and Université de Lubumbashi, Lubumbashi, 115 pp

Christison KW, Shinn AP, van As JG (2005) *Gyrodactylus thlapi* n. sp. (Monogenea) from *Pseudocrenilabrus philander philender* (Weber) (Cichlidae) in the Okawango Delta, Botswana. Syst Parasitol 60 (3):165–173

Cunningham CO, McGillivray DM, MacKenzie K (1995) Identification of *Gyrodactylus* (Monogenea) species parasitising salmonid fish using DNA probes. J Fish Dis 18:539–544

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32 (5):1792–1797

Ergens R (1973) Two new species of *Gyrodactylus* from *Clarias lazera*. Revue de Zoologie et de Botanique africaines 87(1):77–80

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle. Available from http://evolution.genetics.washington.edu/phylip.html

García-Vásquez A, Hansen H, Shinn AP (2007) A revised description of *Gyrodactylus cichlidarum* Paperna, 1968 (Gyrodactylidae) from the Nile tilapia, *Oreochromis niloticus niloticus* (Cichlidae), and its synonymy with *G. niloticus* Cone, Arthur et Bondad-Reantaso, 1995. Folia Parasit 54:129–140

García-Vásquez A, Hansen H, Christison KW, Bron JE, Shinn AP (2011) Description of three new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) parasitising *Oreochromis niloticus niloticus* (L.) and *O. mossambicus* (Peters) (Cichlidae). Acta Parasitol 52(1):20–33

Gillardin C, Vanhove MPM, Pariselle A, Huyse T, Volckaert FAM (2011) Ancyrocephalidae (Monogenea) of Lake Tanganyika: II: description of the first *Cichlidogyrus* spp. parasites from Tropheini fish hosts (Teleostei, Cichlidae). Parasitol Res. doi:10.1007/s00436-011-2490-5

Glez-Peña D, Gómez-Blanco D, Reboiro-Jato M, Fdez-Riverola F, Posada D (2010) ALTER: program-oriented conversion of DNA and protein alignments. Nucleic Acids Res 38(suppl 2):W14–W18

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst Biol 52:696–704

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol Electron 4(1), 9 pp

Harris PD, Shinn AP, Cable J, Bakke TA (2004) Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. Syst Parasitol 59:1–27

Haylor GS (1993) Controlled hatchery production of African catfish, *Clarias gariepinus* (Burchell): an overview. Aquac Res 24 (2):245–252

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Hurvich CM, Tsai CL (1989) Regression and time series model selection in small samples. Biometrika 76(2):297–307

Huyse T, Audenaert V, Volckaert FAM (2003) Speciation and host-parasite relationships in the parasite genus *Gyrodactylus* (Monogenea, Platyhelminthes) infecting gobies of the genus *Pomatoschistus* (Gobiidae, Teleostei). Int J Parasitol 33:1679–1689

Huyse T, Malmberg G (2004) Molecular and morphological comparisons between *Gyrodactylus ostendicus* n. sp. (Monogenea: Gyrodactylidae) on *Pomatoschistus microps* (Krøyer) and *G. harengi* Malmberg, 1957 on *Clupea harengus membras* L. Syst Parasitol 58:105–113

Kabata Z (1985) Parasites and diseases of fish cultured in the tropics. Taylor and Francis, London and Philadelphia, 318 pp

Khalil FL, Polling L (1997) Checklist of the helminth parasites of African freshwater fishes. University of the North, Pietersburg, 185 pp

Ky H (1968) New species of monogeneans from freshwater fishes of North Vietnam. I. Parazitologiya 2:297–301, In Russian, English Summary

Lévêque C (1997) Biodiversity dynamics and conservation: the freshwater fish of tropical Africa. Cambridge University Press, Cambridge, 438 pp

Lundberg JG, Kottelat M, Smith GR, Stiassny M, Gill T (2000) So many fishes, so little time: an overview of recent ichthyological discoveries in fresh waters. Ann Missouri Bot Gard 87:26–62

Lundberg JG, Sullivan JP, Rodiles-Hernández R, Hendrickson DA (2007) Discovery of African roots for the Mesoamerican Chiapas catfish, *Lacantunia enigmatica*, requires an ancient intercontinental passage. P Acad Nat Sci Phila 156:39–53

Luus-Powell WJ, Mashego SN, Khalil LF (2003) *Mormyrogyrodactylus gemini* gen. et sp. n. (Monogenea: Gyrodactylidae), a new gyrodactylid from *Marcusenius macrolepidotus* (Mormyridae) from South Africa. Folia Parasit 50:49–55

Malmberg G (1970) The excretory systems and marginal hooks as basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). Ark Zool 2:1–235

Matějusová I, Gelnar M, McBeath AJA, Collins CM, Cunningham CO (2001) Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). Int J Parasitol 31:738–745

Mullen AJ, Cone DK, Easy R, Burt MDB (2010) Taxonomy and host-specificity of *Gyrodactylus aideni* n. sp and *G. pleuronecti* (Monogenea: Gyrodactylidae) from *Pseudopleuronectes americanus* (Walbaum) in Passamaquoddy Bay, New Brunswick, Canada. Syst Parasitol 77(3):233–239

Nack J, Bilong Bilong CF, Euzet L (2005) Monogenean parasites from Clariidae (Teleostei, Siluriformes) in Cameroon: I. Description of two new species of *Gyrodactylus* from the Nyong Bassin. Parasite J Soc Fr Parasitol 12:213–220

Obiekezie AI, Taege M (1991) Mortality in hatchery reared fry of the African catfish *Clarias gariepinus* (Burchell) caused by *Gyrodactylus groschafti* Ergens, 1973. Bull Eur Ass Fish Pathol 11:82–85

Paperna I (1973) New species of Monogenea (Vermes) from African freshwater fish. A preliminary report. Revue de Zoologie et de Botanique africaines 87(3):505–518

Paperna I (1996) Parasites, infections and diseases of fishes in Africa. An update. CIFA Technical Paper 31, Food and Agriculture Organization, Rome, 220 pp

Pariselle A, Morand S, Deveney M, Pouyaud L (2003) Parasite species richness of closely related hosts: historical scenario and "genetic" hypothesis. In C Combes, J Jourdan, Hommage à Louis Euzet—Taxonomie, écologie et évolution des métazoaires parasites. Taxonomy, ecology and evolution of metazoan parasites, Presses Universitaires de Perpignan, Perpignan, p. 147–166

Pariselle A, Euzet L (2009) Systematic revision of dactylogyridean parasites (Monogenea) from cichlid fishes in Africa, the Levant and Madagascar. Zoosystema 31(4):849–898

Posada D (2003) Using Modeltest and PAUP* to select a model of nucleotide substitution. In AD Baxevanis, DB Davison, RDM Page, GA Petsko, LD Stein, GD Stormo, Current Protocols in Bioinformatics, Wiley, New York, p. 6.5.1–6.5.14

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Prudhoe S, Hussey CG (1977) Some parasitic worms in freshwater fishes and fish-predators from the Transvaal, South Africa. Zool Afr 12(1):113–147

Přikrylová I, Matějusová I, Jarkovský J, Gelnar M (2008) Morphometric comparison of three members of the *Gyrodactylus nemachili*-like species group (Monogenea: Gyrodactylidae) on *Barbatula barbatula* L. in the Czech Republic, with a reinstatement of *G. papernai* Ergens and Bychowsky, 1967. Syst Parasitol 69:33–44

Přikrylová I, Matějusová I, Musilová N, Gelnar M, Harris PD (2009a) A new gyrodactylid (Monogenea) genus on gray bichir, *Polypterus senegalus* (Polypteridae) frem Senegal (West Africa). J Parasitol 95(3):555–560

Přikrylová I, Matějusová I, Musilová N, Gelnar M (2009b) *Gyrodactylus* species (Monogenea: Gyrodactylidae) on the cichlid fishes of Senegal, with the description of *Gyrodactylus ergensi* n. sp from Mango tilapia, *Sarotherodon galilaeus* L. (Teleostei: Cichilidae). Parasitol Res 106:1–6

Pugachev ON, Gerasev PI, Gussev AV, Ergens R, Khotenowsky I (O. N. Pugachev, P. Galli, and D. Kritsky (Eds.)) (2009) Guide to Monogenoidea of freshwater fish of Palaearctic and Amur regions, Ledizione-LediPublishing, Milan, 564 pp

Rodriguez F, Oliver JL, Marin A, Medina JR (1990) The general stochastic model of nucleotide substitution. J Theor Biol 142:485–501

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Rokicka M, Lumme J, Ziętara M (2007) Identification of *Gyrodactylus* ectoparasites in Polish salmonid farms by PCR-RFLP of the nuclear ITS segment of ribosomal DNA (Monogenea, Gyrodactylidae). Acta Parasitol 52(3):185–195

Rokicka M, Lumme J, Ziętara MS (2009) Two new Antarctic *Gyrodactylus* species (Monogenoidea): description and phylogenetic characterization. J Parasitol 95:1112–1119

Sabaj MH, Armbruster JW, Ferraris Jr. CJ, Friel JP, Lundberg JG, Page LM, editors. The All Catfish Species Inventory [homepage on the Internet]. 2003–2006 [updated 2009 Feb 19; cited 2011 Jun 6]. Available from: http://silurus.acnatsci.org/.

Swofford DL (2001) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts.

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. In RM Miura, Some mathematical questions in biology—DNA sequence analysis, American Mathematical Society, Providence, Rhode Island, p. 57–86

Teugels GG (1986) A systematic revision of the African species of the genus *Clarias* (Pisces; Clariidae). Ann Mus R Afr Centr, Sci Zool 247:1–199

Vanhove MPM, Snoeks J, Volckaert FAM, Huyse T (2011a) First description of monogenean parasites in Lake Tanganyika: the cichlid *Simochromis diagramma* (Teleostei, Cichlidae) harbours a high diversity of *Gyrodactylus* species (Platyhelminthes, Monogenea). Parasitology 138(3):364–380

Vanhove MPM, Volckaert FAM, Pariselle A (2011b) Ancyrocephalidae (Monogenea) of Lake Tanganyika: I: Four new species of *Cichlidogyrus* from *Ophthalmotilapia ventralis* (Teleostei, Cichlidae), the first record of this family in the basin. Zoologia (Curitiba, Impr) 28(2):253–263

Vaughan DB, Christison KW, Hansen H, Shinn AP (2010) *Gyrodactylus eyipayipi* sp. n (Monogenea: Gyrodactylidae) from *Syngnathus acus* (Syngnathidae) from South Africa. Folia Parasit 57(1):11–15

Zhang Z, Schwarts S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7(1–2):203–214

Ziętara MS, Lumme J (2002) Speciation by hostswitching and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). Evolution 56:2445–2458

Ziętara MS, Huyse T, Lumme J, Volckaert FAM (2002) Deep divergence among subgenera of *Gyrodactylus* inferred from rDNA ITS region. Parasitology 124:39–52

Ziętara MS, Lumme J (2003) The crossroads of molecular, typological and biological species concepts: two new species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). Syst Parasitol 55:39–52