Research Article

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Novel distribution records and molecular data for species of *Macrogyrodactylus* Malmberg, 1957 (Monogenea: Gyrodactylidae) from *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae) in southern Africa

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Abstract: The viviparous gyrodactylid genus *Macrogyrodactylus* Malmberg, 1957 is endemic to Africa, composed of nine species from hosts of four freshwater fish families, including catfishes (Siluriformes: Clariidae). Three species, *Macrogyrodactylus clarii* Gussev, 1961; *M. congolensis* (Prudhoe, 1957) and *M. karibae* Douëllou et Chishawa, 1995, are primarily known to parasitise the African sharp-tooth catfish *Clarias gariepinus* (Burchell) in various African countries. From November 2017 to September 2019, a total of 184 individuals of *C. gariepinus* were collected from selected localities in southern Africa and their skin, fins and gills were surveyed for monogeneans. Three species of *Macrogyrodactylus* (*M. clarii*, *M. congolensis* and *M. karibae*) were found parasitising *C. gariepinus* from five localities in South Africa and Zambia. Overall prevalence was 50% to 100% with intensities of up to 109 parasites per host individual. New locality records in southern Africa, morphological observations and additional molecular data on the complete Internal Transcriber Spacer (ITS-1-5.8S-ITS-2) regions of the rDNA gene for the three gyrodactylid species are presented in this study.

Keywords: fish parasites, gyrodactylids, endemic species, ITS region, phylogeny

Macrogyrodactylus Malmberg, 1957 (Gyrodactylidae) is one of the six endemic viviparous monogenean genera parasitising African freshwater fishes (Barson et al. 2010, Přikrylová et al. 2017, Řehulková et al. 2018). The genus comprises nine species parasitising hosts from the Anabantidae, Clariidae, Latidae and Polypteridae (Table 1). In addition to the nine species, a study on macrogyrodactylids from *Clarias gariepinus* (Burchell) in Kenya and Zimbabwe revealed a species possessing morphological features of *Macrogyrodactylus clarii* Gussev, 1961 and *Macrogyrodactylus heterobranchii* N'Douba et Lambert, 1999. Molecular characterisation of these specimens suggested a hybrid origin partially corresponding to the ribosomal 18S, Internal Transcribed Spacer (ITS), and mitochondrial (*cox1*) gene regions of *M. clarii* and *M. heterobranchii* (see Barson et al. 2010).

The majority of the literature on the species of *Macrogyrodactylus* originate from the Nilo-Sudanian, Abyssian Highlands and Zambezi ichthyofaunal provinces where *C. gariepinus* is highly valued in aquaculture and as food. Interest on this parasitic group was initially driven by its potential to exponentially increase in infection, first noted

on grey bichir, *Polypterus senegalus* Cuvier, in the Sudan infected with *Macrogyrodactylus polypteri* Malmberg, 1957, and on *C. gariepinus* and *Heterobranchius longifilis* Valenciennes reared in earthen ponds in Nigeria. These infections were characterised by increased mucous secretion, skin damage resulting in the formation of white patches on the skin, increased turbidity of the water, and in some cases death of the host (Amirthalingam 1965, Khalil 1964, 1970, Saoud and Mageed 1969, Obiekezie and Ajah 1994). Later in southern Africa, similar infections on the clariid host *C. gariepinus* were observed in natural conditions and greyish-white patches were noted on the skin of the infected hosts (Khalil and Mashego 1998).

Despite the small number of species in the genus, ample research has been published on the occurrence, morphology and ultrastructure, microhabitat and infection dynamics of some species in natural systems, earthen ponds and in aquaculture conditions (El-Naggar and Serag 1987, Cable et al. 1996, Khalil and Mashego 1998, Arafa 2000, El-Naggar et al. 2001a,b, 2004a, 2019, El-Naggar and Cable 2007, Madanire-Moyo et al. 2010, Arafa et al. 2012, 2013).

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Table 1. Species of *Macrogyrodactylus* Malmberg, 1957 from Africa with their host range.

	Type host	Type locality	Other hosts	Reference
<i>Macrogyrodactylus clarii</i> Gussev, 1961	<i>Clarias</i> sp.	Ghana	<i>C. anguillaris</i> (Linnaeus) <i>C. gariepinus</i>	Gusseu 1961, Paperna 1969, Shotter 1980, El-Naggar and Serag 1987
<i>M. congolensis</i> (Prudhoe, 1957)	<i>Clarias gariepinus</i> (Burchell)	Democratic Republic of the Congo	<i>C. anguillaris</i>	Prudhoe 1957, Thurston 1970, Paperna 1979, Příkrylová and Gelnar 2008
<i>M. heterobranchii</i> N'Douba et Lambert, 1999	<i>Heterobranchius longifilis</i> Valenciennes	Senegal	<i>C. anguillaris</i>	N'Douba and Lambert 1999, Příkrylová and Gelnar 2008
<i>M. karibae</i> Douëllou et Chishawa, 1995	<i>C. gariepinus</i>	Zimbabwe	-	Douëllou and Chishawa 1995, Khalil and Mashego 1998
<i>M. polypteri</i> Malmberg, 1957	<i>Polypterus senegalus</i> Cuvier	Gambia	<i>P. bichir</i> Lacépède	Malmberg 1957
<i>M. simentiensis</i> Příkrylová et Gelnar, 2008	<i>P. senegalus</i>	Senegal	-	Příkrylová and Gelnar 2008
Species inquirenda				
<i>M. anabantii</i> Paperna, 1973	<i>Ctenopoma muriei</i> Boulenger	Uganda	-	Paperna 1973
<i>M. ctenopomii</i> Paperna, 1973	<i>C. muriei</i> (Boulenger)	Uganda	-	Paperna 1973
<i>M. latesi</i> Paperna, 1969	<i>Lates niloticus</i> (Linnaeus)	Ghana	-	Paperna 1969

Table 2. Localities, number (N) of host individuals screened, host status and whether infected with *Macrogyrodactylus* spp. or not of 184 *Clarias gariepinus* (Burchell) collected from November 2017 to September 2019 in southern Africa.

Locality	Province	Host introduction status	Coordinates	N	Infected by one or more <i>Macrogyrodactylus</i> spp.
South Africa					
Riversonderend River	Western Cape	Translocated	34.0998S, 19.6656E	15	No
Great Fish River	Eastern Cape	Translocated	33.1543S, 26.8320E	11	No
Gariep Dam (Orange River)	Free State	Native	30.5682S, 25.5647E	15	No
Riet River	Free State	Native	29.5700S, 25.3042E	12	No
Sand River	Free State	Native	28.3232S, 27.3597E	15	No
Vaal River	North West	Native	26.8665S, 27.2944E	18	Yes
Boskop Dam (Mooi River)	North West	Native	26.5621S, 27.1181E	15	Yes
Ndumo Game Reserve	KwaZulu-Natal				
KuShokwe Pan		Native	26.8717S, 32.2141E	15	Yes
Nyamithi Pan		Native	26.8829S, 32.3113E	15	Yes
Phongolo River		Native	26.9297S, 32.3238E	4	No
Usuthu River		Native	26.8824S, 32.3116E	3	Yes
Zambia					
Kabompo River	Northern	Native	13.1025S, 24.6920E	2	Yes
Barotse floodplain	Northwestern	Native	15.1960S, 22.9647E	17	Yes

Increasing interest in the endemic species of gyrodactylids is the outcome of potential undiscovered high diversity, including morphological and molecular features that are suitable for advanced phylogenetic, evolutionary and parasite-host relationship studies (Bakke et al. 2002, Cribb et al. 2002, Poulin 2002, Boeger et al. 2003, Barson et al. 2010, Příkrylová et al. 2013). Although host specificity for all known species of *Macrogyrodactylus* has not been determined, specificity of *M. polypteri* towards bichirs has been confirmed, as only *Polypterus bichir* Lacépède and *P. senegalus* have been reported as hosts (Khalil 1964), and an affinity towards clariid hosts seems evident for three species of *Macrogyrodactylus*. Additionally, Barson et al. (2008) noted that *M. clarii*, *Macrogyrodactylus congolensis* (Prudhoe, 1957) and *Macrogyrodactylus karibae* Douëllou et Chishawa, 1995 exhibit a certain degree of specificity towards *C. gariepinus*.

In southern Africa, studies on macrogyrodactylids were initiated during the 1990's. Khalil and Mashego (1998) documented taxonomically significant features for the genus and provided a measurement scheme highlighting the sclerotised haptor structures and cirrus spines. It is only in the last two decades that studies on the systematics of *Macrogyrodactylus* and other genera of endemic gyrodac-

tylid species in Africa emerged (Matějusková et al. 2003, Barson et al. 2008, 2010, Příkrylová and Gelnar 2008, Vanhove et al. 2011, 2018, Příkrylová et al. 2013). The aforementioned authors emphasise the importance of studies of the morphological characters, distribution and genetics of these parasites.

This study presents novel distribution records for *M. clarii*, *M. congolensis* and *M. karibae* from *C. gariepinus* in southern Africa, as well as additional morphological observations and molecular data (ITS-1-5.8S-ITS-2 regions of the rDNA gene).

MATERIAL AND METHODS

Collection of hosts and parasites

A total of 184 individuals of *Clarias gariepinus* were collected from November 2017 to September 2019 at various localities in southern Africa (see Table 2 and Fig. 1A,B). Individual hosts were euthanised by percussive stunning and severance of the spinal cord (ethics Nos. NWU-00156-18-A5, NWU-00264-15-A5) and subjected to a full parasitological screening for metazoan parasites (see detailed procedures in Řehulková 2018). Herein, we report only on the infection with species of *Macrogyrodactylus* found on the skin and gills of the host. Whole specimens of

Fig. 1. Map illustrating the sampling localities of *Clarias gariepinus* (Burchell) during the present study. **A** – Zambia; **B** – South Africa.

Table 3. Information on the sequences of *Macrogyrodactylus* spp. (Monogenea: Gyrodactylidae) used in the phylogenetic analyses of the ITS1-5.8S-ITS2 region of the rRNA gene. *Laminiscus gussevi* (Bychowsky et Polyansky, 1953) was used as outgroup. Species sequenced in this study are in bold.

Species	Host	Locality	GenBank ID	Reference
<i>Macrogyrodactylus clarii</i> (Isolate 1)	<i>Clarias gariepinus</i>	Barotse floodplain, Zambia	MZ869846	Present study
<i>M. clarii</i> (Isolate 2)	<i>Clarias gariepinus</i>	Barotse floodplain, Zambia	MZ869850	Present study
<i>M. clarii</i> (Isolate 3)	<i>Clarias gariepinus</i>	Barotse floodplain, Zambia	MZ869847	Present study
<i>M. clarii</i> (Isolate 1)	<i>Clarias gariepinus</i>	Phongolo River floodplain, SA	MZ869849	Present study
<i>M. clarii</i>	<i>Clarias gariepinus</i>	Save River, Zimbabwe	GU252711	Barson et al. 2010
<i>M. clarii</i> × <i>heterobranchii</i>	<i>Clarias gariepinus</i>	Save River, Zimbabwe	GU252712	Barson et al. 2010
<i>M. clarii</i> × <i>heterobranchii</i>	<i>Clarias gariepinus</i>	Lake Turkana, Kenya	GU252713	Barson et al. 2010
<i>M. clarii</i> × <i>heterobranchii</i>	<i>Heterobranchius bidorsalis</i>	Lake Turkana, Kenya	HF548683	Přikrylová et al. 2013
<i>Macrogyrodactylus heterobranchii</i>	<i>Clarias anguillaris</i>	Gambia River, Senegal	GU252714	Barson et al. 2010
<i>Macrogyrodactylus heterolensis</i>	<i>Clarias anguillaris</i>	Lake Turkana, Kenya	GU252716	Barson et al. 2010
<i>M. congolensis</i>	<i>Clarias anguillaris</i>	Mare Simenti, Senegal	GU252717	Barson et al. 2010
<i>M. congolensis</i>	<i>Clarias gariepinus</i>	Vaal River, SA	MZ869848	Present study
<i>Macrogyrodactylus karibae</i>	<i>Clarias gariepinus</i>	Save River, Zimbabwe	GU252715	Barson et al. 2010
<i>M. karibae</i>	<i>Clarias gariepinus</i>	Barotse floodplain, Zambia	MZ869845	Present study
<i>M. karibae</i>	<i>Clarias gariepinus</i>	Phongolo River floodplain, SA	MZ869851	Present study
<i>Macrogyrodactylus polypteri</i>	<i>Polypterus senegalus</i>	—	AJ567672	Matějusková et al. 2003
<i>Macrogyrodactylus simentiensis</i>	<i>Polypterus senegalus</i>	Mare Simenti, Senegal	HF548681	Přikrylová et al. 2013
<i>Laminiscus gussevi</i>	<i>Mallotus villosus</i>	Iceland	HF548678	Přikrylová et al. 2013

Abbreviations: SA – South Africa.

Macrogyrodactylus spp. from parasitised fish were removed from the skin or gills using fine dissecting needles and cleared on a microscope slide in glycerine-ammonium picrate (GAP – Malmberg 1970) for later morphological identification, which were subsequently remounted in Canada Balsam, following the procedure described in Ergens (1969).

For molecular characterisation, specimens were fixed in 96% molecular-grade ethanol and processed as described below. The taxonomical relevant sclerites of individuals were measured using a Nikon Eclipse Ni compound microscope (Nikon Instruments, Tokyo, Japan) with DIC optics. Measurements were taken using NIS-Elements BR 4.60® software and line drawings of

Fig. 2. Micrographs showing the morphological features of *Macrogyrodactylus clarii* Gussev, 1961 (A–C), *M. congolensis* (Prudhoe, 1957) (D–F), and *M. karibae* Douëllou et Chishawa, 1995 (G, H). A, D, G – hamuli complex; B, E, H – sickle of marginal hook; C, F, I – cirrus with spines.

sclerotised structures were made with the aid of a camera lucida attached to the microscope and digitalised using a Wacom Intuos® Pro drawing tablet (Wacom, Saitana, Japan) and Adobe Illustrator™ software package.

Measurements of sclerotised structures were performed according to Khalil and Mashego (1998), N'Douba and Lambert (1999), and Příkrylová and Gelnar (2008). Morphometric data are presented as mean followed by range in parentheses. All measurements are in micrometres unless otherwise indicated.

Whole-mounted voucher specimens were deposited in the parasite collection of the National Museum, Bloemfontein (NMB), South Africa. Ecological descriptors for the monogenean parasites (prevalence and mean intensity) followed Bush et al. (1997).

DNA extraction, PCR amplification and sequence analyses

The specimens used for molecular analyses had the haptor mounted on slides with GAP to confirm morphological identity (see Řehulková 2018) and deposited as e-vouchers and molecular vouchers (hologenophore, see Pleijel et al. 2008 for terminology). Thereafter, the anterior section of the parasite was used for DNA extraction using PCR BIO Rapid Extraction® (ThermoFischer Scientific, Waltham, Massachusetts, USA) kit following the manufacturer's protocol. DNA amplifications were performed on the Internal Transcriber Spacer ITS1-5.8S-ITS2 regions of the rRNA gene using the primers ITS1A (5'-GTAACAAGGTTTCCGTAG-GTG-3') and ITS2 (5'-TCCTCCGCTTAGTGATA-3') (Matějusová et al. 2001). Each amplification reaction was performed with a total volume of 25 µl: 12.5 µl of DreamTaq PCR Master Mix (2X) (ThermoFischer Scientific, Waltham, Massachusetts, USA), 1 µl of 10 µM of each primer, 1 µl of DNA product and 9.5 µl of double distilled water. Thermocycling conditions followed Barson et al. (2010). PCR products were visually inspected on a 1% agarose gel electrophoresis and sent to Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa) for purification and sequencing. Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov>) was used to compare and identify species closely related to the new sequences.

Seven newly generated sequences of *Macrogyrodactylus* spp. were aligned with those of ten other congeneric species available in GenBank, and one sequence of *Laminiscus gussevi* (Bychowsky et Polyansky, 1953) used as outgroup (see Table 3 for detailed information on the sequences). The alignment was generated in Geneious v. 7.1.3 using implemented default parameters of MUSCLE (Kearse et al. 2012). The alignment was trimmed, resulting in the length of 810 bp. Evolutionary model selection was performed in MEGA 7 (Kumar et al. 2016). Phylogenetic analyses were run under Maximum Likelihood (ML) and Bayesian Inference (BI) criteria, applying the model of nucleotide evolution GTR + G. ML trees were generated in RAxML (Guindon and Gascuel 2003). The model parameters and bootstrap support values (1,000 repetitions) were estimated using RAxML. BI trees were generated in MrBayes (Ronquist et al. 2012), running two independent MCMC runs of four chains for 10 million generations and sampling tree topologies every 1,000 generations. Burn-in periods were set to the first 25,000 generations. ML and BI analyses were performed on the computational resource CIPRES (Miller et al. 2010). Genetic divergences were calculated using uncorrected p-distances model in MEGA 7 and are expressed as percentage (p-distances) and differences in number of nucleotides. Trees were visualised in FigTree v1.4.4 (Rambaut 2009).

RESULTS

In the present study, *Macrogyrodactylus clarii*, *M. congolensis* and *M. karibae* were found on the skin and gills of *Clarias gariepinus* from five localities in southern Africa (Table 2). Infection of *C. gariepinus* with species of *Macrogyrodactylus* are recorded for the first time from the Vaal

Fig. 3. Illustrations of the hamuli of *Macrogyrodactylus clarii* Gussev, 1961 (A); *M. congolensis* (Prudhoe, 1957) (B); *M. karibae* Douëllou et Chishawa, 1995 (C). **D** – sickle of marginal hooks of *M. clarii* (i), *M. congolensis* (ii), and *M. karibae* (iii). Abbreviations: Ham – hamuli, Vbar – ventral bar, Dbar – dorsal bar, R1 and R2 – ventral bar rod-like sclerites.

River (North West Province) and Ndumo Game Reserve (NGR) (KwaZulu-Natal Province), in South Africa (SA), and the Kabompo and Zambezi (Barotse floodplain) rivers in Zambia (ZM). The southernmost distribution records for *M. clarii* and *M. karibae* are recorded from NGR and for *M. congolensis* from the Vaal River (SA). All three species were present from sites within the NGR and Barotse floodplain, whereas no other species co-occurred with *M. congolensis* in the Vaal and Kabompo rivers.

Macrogyrodactylus congolensis was the most widely distributed species occurring at four of the five localities, followed by *M. karibae* (three localities), and *M. clarii* (two localities). The least prevalent and abundant species was

M. karibae, except in NGR where it was found to co-occur with *M. congolensis*. *Macrogyrodactylus clarii* was the most prevalent and abundant species even when in co-occurrence with the other two species found during this study in NGR and the Barotse floodplain. The highest intensity of infection was recorded from the Vaal River, with 109 individuals of *M. congolensis* found on a single host, and the same intensity of infection for an individual host was noted from the Barotse floodplain. However, no greyish-white mucous covering of the skin was observed. In the present study, the general morphology and morphometrics for *M. clarii*, *M. congolensis* and *M. karibae* corresponded with those from original descriptions and redescrptions for each species.

Table 4. Morphometric comparison of *Macrogyrodactylus clarii* Gussev, 1961 parasitising *Clarias gariepinus* (Burchell) in Africa. Measurements are presented in micrometres (except body length) as mean followed by range in parentheses.

	KuShokwe Pan SA n = 48 Present study	Barotse floodplain ZM n = 25 Present study	Lake Turkana & Lake Chivero KY / ZI n = 10 Barson et al. 2010	Nile Delta EG n = 10 El-Naggar and Serag 1987	Middle Letaba Dam SA – Khalil and Mashego 1998
Body					
length (mm)	3.39 (2.19–5.01)	3.17 (2.24–4.35)	–	2.33 (2.00–2.58)	1.80–2.60
Width	518 (221–788)	382 (278–584)	–	400 (360–440)	320–440
Haptor					
length	839 (544–1377)	680 (465–846)	–	460 (400–500)	–
width	600 (420–1053)	352 (306–583)	–	440 (400–480)	–
Hamuli					
total length	370 (193–465)	426 (378–497)	436 (374–475)	385 (376–392)	437–453
point length	113 (98–134)	153 (110–143)	120 (108–136)	120 (112–128)	131–140
shaft length	334 (281–411)	376 (340–440)	380 (350–457)	–	381–406
root length	171 (139–218)	197 (156–222)	191 (135–238)	171 (176–184)	193–203
Ventral bar					
anterior lateral arm	28 (17–38)	33 (23–43)	29 (17–38)	–	21–31
posterior central arm	63 (44–80)	74 (59–90)	69 (61–85)	–	46–55
total length	135 (110–166)	152 (122–175)	148 (137–172)	110 (104–116)	–
total width	111 (89–135)	112 (106–133)	138 (122–157)	106 (96–112)	–
ventral bar R1	250 (192–301)	272 (232–304)	191 (177–207)	–	–
ventral bar R2	137 (111–185)	152 (129–170)	147 (141–157)	–	–
Dorsal bar	divided	divided	divided	divided	divided
total length	18 (12–23)	18 (14–22)	16.5 (13.5–20)	–	15–18
total width	77 (61–93)	78 (68–92)	82.3 (72.5–92)	–	68–75
Marginal hooks					
total length	110 (92–128)	117 (100–130)	114 (103–132)	96 (91–101)	109–125
handle length	92 (72–108)	100 (84–112)	96 (87.5–112)	82	–
sickle length	17 (13–21)	18 (15–20)	18.5 (17–21)	14	–
sickle proximal width	12 (8–14)	13 (9–16)	13.8 (13–17)	–	–
Cirrus spines	13–15	13–15	–	12	12–13

Abbreviations: EG – Egypt; KY – Kenya; SA – South Africa; ZI – Zimbabwe; ZM – Zambia; n – number of specimens studied.

Gyrodactylidae Cobbold, 1864

Macrogyrodactylus Malmberg, 1957

Macrogyrodactylus clarii Gussev, 1961

Figs. 2A–C, 3A,D(i); Table 4

Type host: *Clarias* sp. (Siluriformes: Clariidae).

Type locality: Lake Awasa, Ethiopia.

Other records: *Clarias gariepinus*: Egypt – El-Naggar and Serag (1987), El-Naggar (1993), Hagra et al. (1995, 1999), El-Naggar et al. (1997, 2001a,b, 2004a,b, 2016, 2017, 2019, 2020), El-Naggar and Cable (2007), Arafa et al. (2009, 2012), Radwan et al. (2009), El-Seify et al. (2011), Mashalay et al. (2019); Ethiopia – Gussev (1961), Beletew et al. (2016); Ghana – Paperna (1969, 1979); Kenya – Barson et al. (2010); Nigeria – Obiekezie and Ajah (1994), Eyo et al. (2015); South Africa – Khalil and Mashego (1998), Olivier et al. (2009), Madanire-Moyo et al. (2010, 2012), Matla (2012), Mahlatji (2014); Uganda – Paperna (1979); Zimbabwe – Barson et al. (2008, 2010), Madanire-Moyo and Barson (2010). *Clarias anguillaris* (Linnaeus); Nigeria – Shotter (1980).

Localities (present study): South Africa: KuShokwe Pan, Ndumo Game Reserve, KwaZulu-Natal Province; Zambia: Barotse floodplain, Zambezi River, Northwestern Province.

Site of infection: Skin and gills.

Voucher material: Twenty-three voucher specimens deposited in NMB (Coll. Nos. P 800–809, 820–822).

Representative DNA sequences: 710–753 bp long

sequences of the ITS1-5.8S-ITS2 region of the rRNA gene of four isolates (GenBank accession numbers: MZ869846, MZ869850, MZ869847, MZ869849). Hologenophores: NMB (P 824–827).

Specimens studied: n = 48 (KuShokwe Pan, South Africa - SA); n = 25 (Barotse floodplain, Zambia - ZM).

Infection rate: KuShokwe Pan (SA): Prevalence (P) of 80% and intensity of infection (IF) 1–12; Barotse floodplain (ZM) P = 64%, IF = 1–4.

Morphological observations (measurements provided in Table 4): Morphology of haptor sclerites conform to description of Gussev (1961) and redescription of El-Naggar and Serag (1987). Individuals armed with 16 marginal hooks, two positioned in anterolateral lobes, 14 in posterior edge of haptor. Basal part of sickle has small indentation near anterior end of base, anterior end of sickle base thin, sharply rounded. Base of sickle rounded with slight curvature on distal side into slender sickle blade curving inward and tapering into a fine, sharp point that stops in line with anterior end of sickle base (see Figs. 2B, 3D(i)). Hamulus complex consisting of a pair of robust hamuli with curvature into point, sharp inward curved root, small dorsal bar consisting of two articulating sclerites, Y-shaped ventral bar with short anterolateral processes and long posterior central arm. Two narrow rod-like sclerites (R1 and R2) accompany ventral bar (Figs. 2A–C, 3A). Cirrus observed in 24 (SA) and 11 (ZM) specimens studied, each armed with 13–15 small spines and one large spine (Fig. 2C).

Table 5. Morphometric comparison of *Macrogyrodactylus congolensis* (Prudhoe, 1957) parasitising *Clarias gariepinus* (Burchell) in Africa. Measurements are presented in micrometres (except body length) as mean followed by range in parentheses.

	Vaal River SA n = 25 Present study	Usuthu River SA n = 2 Present study	Kabompo River & Barotse floodplain ZM n = 2 Present study	Lake Turkana KY n = 7 Barson et al. 2010	Mare Simenti SN n = 3 Přikrylová and Gelnar 2008	Middle Letaba Dam SA n = Khalil and Mashego 1998
Body						
length (mm)	3.74 (2.92–6.00)	4.75 (4.57–4.93)	3.55 (2.48–4.63)	–	–	1.70–2.80
width	464 (230–745)	643 (412–873)	374 (283–466)	–	–	220–580
Haptor						
length	666 (513–827)	849 (798–901)	618 (508–728)	–	–	–
width	552 (438–798)	760 (735–786)	546 (480–612)	–	–	–
Hamuli						
total length	456 (390–537)	399 (391–407)	430 (419–440)	421 (385–481)	383 (381–385)	490–530
point length	111 (94–131)	98 (95–102)	106 (105–107)	99 (93–103)	101 (99–103)	80–109
shaft length	368 (310–431)	325 (318–332)	339 (339)	334 (310–361)	306 (299–313)	296–312
root length	203 (166–272)	178 (177–179)	175 (165–186)	175 (153–203)	154 (145–165)	251–287
Ventral bar						
anterior lateral arm	93 (84–101)	85 (84–85)	85 (82–88)	90 (81.5–118)	65 (58–71)	84–93
posterior central arm	9 (5–13)	6 (5–7)	8 (7–10)	7 (5.5–8.5)	9 (8–10)	12–15
total length	156 (134–170)	147 (146–148)	147 (145–150)	143 (131–150)	129 (124–134)	140–162
total width	133 (110–153)	129 (126–133)	124 (122–125)	124 (118–129.5)	112 (109–114)	120–134
ventral bar R1	254 (222–306)	239 (230–248)	238 (237–239)	236 (217–254)	217 (211–224)	–
ventral bar R2	141 (118–179)	136 (134–137)	129 (118–140)	132 (126–141)	127 (120–137)	–
Dorsal bar	undivided	undivided	undivided	undivided	undivided	undivided
total length	22 (16–25)	20	20 (19–22)	17.5 (15.5–19.5)	14 (13–15)	15–18
total width	115 (97–140)	103	101	97.6 (93–102.5)	90 (88–94)	109–125
Marginal hooks						
total length	104 (96–124)	95 (91–100)	96 (91–101)	96.6 (94.5–100.5)	95	81–87
handle length	92 (83–114)	84 (80–87)	83 (77–89)	85.1 (83.5–89)	82.7 (78–86)	–
sickle length	12 (10–14)	11	5 (11)	11.4 (10.8–11.7)	10.7 (10–12)	–
sickle proximal width	11 (8–14)	9 (8–9)	5 (10–10)	11.2 (10.3–11.8)	10.3 (9–11)	–
Cirrus spines	18–20	–	19	–	–	14–15

Abbreviations: KY – Kenya; SN – Senegal; SA – South Africa; ZM – Zambia; n – number of specimens studied.

Remarks. The dimensions of the hamulus (total length and length of the shaft, root and point) from specimens studied from NGR (SA) and Barotse floodplain (ZM) (Table 4) are similar to those of specimens from the Middle Letaba Dam (SA) and localities in Kenya and Egypt (El-Naggar and Serag 1987, Khalil and Mashego 1998, Barson et al. 2010). Dimensions obtained for the dorsal bar in the present study do not deviate from the ranges found in other studies. Marginal hook size also conforms to previously recorded measurements. The marginal hook sickle morphology is identical to that illustrated for *M. clarii* specimens collected from Zimbabwe and *M. clarii* × *Macrogyrodactylus heterobranchii* from Kenya (see Barson et al. 2010). Gussev (1961) and El-Naggar and Serag (1987) reported 16 and 12 cirrus spines, with one large spine in their descriptions, respectively. Additionally, this study provides details on the morphology of the sickle, which was not described by Gussev (1961) and El-Naggar and Serag (1987). Comparative measurements are presented in Table 4.

***Macrogyrodactylus congolensis* (Prudhoe, 1957) Yamaguti, 1963**
Figs. 2D–F, 3B,D(ii); Table 5

Synonym: *Neogyrodactylus congolensis* Prudhoe, 1957

Type host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Type locality: Lake Upemba (Lualaba River), Democratic Republic of the Congo.

Other records: *Clarias gariepinus*: Egypt – El-Naggar et al. (1999, 2001a,b, 2004b, 2007, 2017, 2019), Hagraas et al. (1999), Arafat (2000, 2011), Arafat et al. (2003, 2013, 2014), Mashalay et al. (2019); Democratic Republic of the Congo – Prudhoe (1957), Vanhove et al. (2018); Kenya – Barson et al. (2010); South Africa – Khalil and Mashego (1998), Madani-re-Moyo et al. (2010, 2012); Uganda – Thurston (1970), Akoll et al. (2012). *Clarias anguillaris*: Senegal – Přikrylová and Gelnar (2008), Barson et al. (2010).

Localities (present study): South Africa: Vaal River, North West Province and Usuthu River, Ndumo Game Reserve (NGR), KwaZulu-Natal Province; Zambia: Barotse floodplain, Zambezi River, Northwestern Province, Kabompo River, Northern Province.

Site of infestation: Skin and gills.

Voucher material: Nineteen voucher specimens deposited in, NMB P 812–817, 823.

Representative DNA sequence: 696 bp long sequence of the ITS1-5.8S-ITS2 region of the rRNA gene of one isolate. GenBank accession number: MZ869848. Hologenophore, NMB P 828.

Specimens studied: n = 25 (Vaal River, SA), n = 2 (Usuthu River, SA), n = 1 (Barotse floodplain, ZM), n = 1 (Kabompo River, ZM).

Infection rate: Usuthu River (SA): P = 3%, IF = 1; Vaal River (SA): P = 33%, IF = 1–109; Barotse floodplain (ZM): P = 6%, IF = 1; Kabompo River (ZM): P = 50%, IF = 1.

Table 6. Morphometric comparison of *Macrogyrodactylus karibae* Douëllou et Chishawa, 1995 parasitising *Clarias gariepinus* (Burchell) in Africa. Measurements are presented in micrometres (except body length) as mean followed by range in parentheses.

	KuShokwe Pan & Usuthu River SA n = 9 Present study	Barotse floodplain ZM n = 2 Present study	Lake Chivero and Save River ZI n = 6 Barson et al. 2010	Lake Kariba ZI n = 14 Douëllou and Chishawa 1995	Middle Letaba dam SA – Khalil and Mashego 1998
Body					
length (mm)	3.31 (1.79–3.92)	2.56 (2.46–2.66)	–	2.30 (1.30–3.66)	1.20–3.95
width	643 (496–806)	339 (282–395)	–	530 (390–720)	390–680
Haptor					
length	622 (418–794)	420 (415–424)	–	–	–
width	545 (379–652)	389 (355–423)	–	–	–
Hamuli					
total length	298 (267–343)	306 (305–307)	312 (292–330)	289 (252–314)	296–375
point length	81 (73–87)	84	83 (74–90)	84 (69–101)	68–93
shaft length	249 (222–278)	262 (261–262)	259 (245–273)	–	251–296
root length	141 (122–164)	153 (149–158)	144 (114–160)	115 (106–128)	125–156
Ventral bar					
anterior lateral arm	23 (18–26)	22 (20–25)	18 (16.5–20)	20 (16–27)	21–31
posterior central arm	37 (26–42)	41 (40–43)	42 (37–47)	41 (34–49)	24–40
total length	98 (86–108)	101 (99–103)	104 (94–113)	94 (73–114)	84–111
total width	103 (96–107)	104 (103–105)	109 (99–120)	100 (93–110)	78–111
ventral bar R1	202 (182–231)	191 (190–192)	182 (177–189)	–	–
ventral bar R2	125 (106–166)	126 (126–127)	114 (101–125)	–	–
Dorsal bar	undivided	undivided	undivided	undivided	undivided
total length	19 (14–24)	17 (17–18)	16.8 (15–20)	16 (14–18)	15–21
total width	82 (76–90)	88 (87–90)	87.5 (80–92)	83 (78–93)	68–78
Marginal hooks					
total length	77 (72–92)	81 (80–83)	80 (72–84)	78 (71–88)	68–78
handle length	69 (65–97)	71 (70–73)	69 (61–74)	–	–
sickle length	11 (10–15)	11	10.9 (11–12)	–	–
sickle proximal width	10 (8–12)	9	9.3 (9–9.5)	–	–
Cirrus spines	15	11–13	–	–	14–15

Abbreviations: SA – South Africa; ZI – Zimbabwe; ZM – Zambia; n – number of specimens studied.

Morphological observations (measurements provided in Table 5): Morphology of haptor sclerites similar to illustrations provided in description of Prudhoe (1957). Hamulus complex of *M. congolensis* large in size, has slender hamuli less sharply curved into point and root, armed with 16 marginal hooks. Marginal hook sickle robust, with noticeable curvature in base near anterior end of sickle. Anterior end of sickle base rounded and convex, with indentation on distal side as it joins sickle blade that is short, curved inward tapering into a fine point and stops before the proximal end of sickle base (Figs. 2E, 3D(ii)). Hamuli complex has small uniform dorsal bar, wider than long, Y-shaped ventral bar with long anterolateral processes, and short posterior central arm. Two narrow rod-like sclerites accompany ventral bar (Figs. 2D–F, 3B). Cirrus observed in one specimen from the Usuthu River (SA) and Barotse floodplain (ZM), respectively, and 17 individuals from the Vaal River (SA), each armed with 18–20 small spines and one large spine (Fig. 2F).

Remarks. The dimensions of the hamulus (total length and the length of the shaft, root and point) from specimens studied from the Vaal and Usuthu rivers (SA) and the Kabompo River and Barotse floodplain (ZM) are similar in size to specimens from the Middle Letaba Dam and Lake Turkana in Kenya (Khalil and Mashego 1998, Barson et al. 2010). *Macrogyrodactylus congolensis* specimens from Senegal seems to have smaller hamuli compared to

the present and other studies (see Přikrylová and Gelnar 2008). Dimensions obtained for the undivided dorsal bar in the present study do not deviate from the ranges found in other studies. Marginal hook measurements are also in accordance with previously recorded measurements (see Table 5). The morphology of the marginal hook sickle conforms to those illustrated for *M. congolensis* from Kenya (see Barson et al. 2010). Prudhoe (1957) reported fewer cirrus spines (15), with one large spine in his description of *M. congolensis*. Details on the morphology of the marginal hooks were not provided in the description of Prudhoe (1957), which are added in the present study. Comparative measurements are presented in Table 5.

Macrogyrodactylus karibae Douëllou et Chishawa, 1995

Figs. 2G–I, 3C,D(iii); Table 6

Type host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Type locality: Lake Kariba, Zimbabwe.

Other records: *Clarias gariepinus*. South Africa – Khalil and Mashego (1998), Olivier et al. (2009), Matla (2012); Zimbabwe – Douëllou and Chishawa (1995), Barson et al. (2008, 2010).

Localities (present study): South Africa: KuShokwe Pan and Usuthu River, Ndumo Game Reserve, KwaZulu-Natal Province; Zambia: Barotse floodplain, Zambezi River, Northwestern Province.

Site of infection: Skin and gills.

Table 7. Nucleotide genetic divergence values among sequences of the ITS1-5.8S-ITS-2 regions of the rDNA gene of *Macrogyrodactylus* spp. and *Laminiscus gussevi* (Bychowsky et Polyanky, 1953) from the 810 bp long alignment. Values below the diagonal are expressed in percentage (p-distance) while values above the diagonal represent number of differences in nucleotides. Newly sequenced taxa are in bold.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 GU252711 <i>Macrogyrodactylus clarii</i> ZI		0	0	0	4	3	7	7	9	76	75	54	72	69	69	91	159	196
2 MZ869846 <i>Macrogyrodactylus clarii</i> isolate 1 ZM	0.0		0	0	4	3	6	6	8	68	68	54	68	67	67	79	113	153
3 MZ869850 <i>Macrogyrodactylus clarii</i> isolate 2 ZM	0.0	0.0		0	4	3	7	7	9	75	74	54	71	69	74	87	125	160
4 MZ869847 <i>Macrogyrodactylus clarii</i> isolate 3 ZM	0.0	0.0	0.0		4	3	7	7	9	76	75	54	71	69	74	88	126	162
5 GU252712 <i>M. clarii</i> x <i>heterobranchii</i> ZI	0.5	0.6	0.6	0.6		1	5	5	7	74	73	52	72	69	75	89	156	198
6 MZ869849 <i>Macrogyrodactylus clarii</i> isolate 1 SA	0.4	0.5	0.4	0.4	0.1		4	4	6	68	67	51	69	68	71	79	116	156
7 GU252713 <i>M. clarii</i> x <i>heterobranchii</i> KY	0.9	0.9	1.0	1.0	0.7	0.6		0	4	75	74	51	71	68	76	88	160	197
8 HF548683 <i>M. clarii</i> x <i>heterobranchii</i> SN	1.0	0.9	1.0	1.0	0.7	0.6	0.0		4	70	69	51	69	68	73	79	119	156
9 GU252714 <i>Macrogyrodactylus heterobranchii</i> SN	1.2	1.2	1.3	1.3	0.9	0.9	0.5	0.6		74	73	50	70	67	75	87	158	199
10 GU252716 <i>Macrogyrodactylus congolensis</i> KY	10.3	10.3	11.0	11.1	10.1	10.1	10.2	10.4	10.1		2	5	30	27	23	69	147	194
11 GU252717 <i>Macrogyrodactylus congolensis</i> SN	10.2	10.3	10.9	11.0	9.9	10	10.1	10.3	9.9	0.3		4	28	26	23	68	145	194
12 MZ869848 <i>Macrogyrodactylus congolensis</i> SA	8.6	8.6	8.6	8.6	8.3	8.1	8.1	8.1	7.9	0.8	0.6		11	12	10	51	81	139
13 GU252715 <i>Macrogyrodactylus karibae</i> ZI	9.8	10.3	10.4	10.4	9.8	10.3	9.7	10.3	9.5	4.1	3.8	1.7		1	14	75	144	197
14 MZ869845 <i>Macrogyrodactylus karibae</i> ZM	10.3	10.2	10.3	10.3	10.3	10.1	10.1	10.1	10.0	4.0	3.9	1.9	0.1		12	75	102	157
15 MZ869851 <i>Macrogyrodactylus karibae</i> SA	10.7	10.2	10.9	10.9	10.9	10.6	11	10.9	10.9	3.3	3.3	1.6	2.0	1.8		72	119	168
16 AJ567672 <i>Macrogyrodactylus polypteri</i>	12.7	12.0	12.8	12.9	12.4	11.8	12.3	11.8	12.1	9.6	9.5	8.1	10.5	10.3	10.5		134	194
17 HF548681 <i>Macrogyrodactylus simentiensis</i> SN	23.2	18.5	19.7	19.8	22.7	18.6	23.3	19.1	23	21.6	21.3	21.3	21.2	18.8	18.8	20.0		199
18 HF548678 <i>Laminiscus gussevi</i> IC	27.5	23.8	24.3	24.5	27.8	24.0	27.7	24.0	27.9	27.4	27.4	27.4	27.9	25.4	25.4	27.9	29.8	

Abbreviations: IC –Iceland, KY – Kenya, *M.* – *Macrogyrodactylus*, SN – Senegal, SA – South Africa, ZI – Zimbabwe, ZM – Zambia.

Voucher material: Four voucher specimens deposited in, NMB P 818–821.

Representative DNA sequences: 715–741 bp long sequence of the ITS1-5.8S-ITS2 region of the rRNA gene of two isolates. GenBank accession numbers: MZ869845, MZ869851. Hologenophore, NMB P 829.

Specimens studied: n = 8 (KuShokwe Pan, SA), n = 1 (Usuthu River, SA), n = 2 (Barotse floodplain, ZM).

Infection rate: KuShokwe Pan (SA): P = 20%, IF = 1–2; Usuthu River (SA): P = 66%, IF = 1; Barotse floodplain (ZM) P = 6%, IF = 1.

Morphological observations (measurements provided in Table 6): General morphology of the haptoral sclerites is similar to the description of Douëllou and Chishawa (1995). Marginal hook sickle blade slender and inwardly curved point, distal side slightly convex as it joins sickle blade. Base of sickle presents noticeable curvature toward anterior end of sickle, has a convex distal side with an indentation as it joins sickle blade that sharply curve inward, tapering into a sharp point that stops before proximal side of sickle base; proximal side of sickle base curved. Cirrus observed in two specimens from KuShokwe Pan (SA) and Barotse floodplain (ZM), respectively, armed with 15 small spines and one large spine (Fig. 2I).

Remarks. *Macrogyrodactylus karibae* can be easily distinguished from *M. congolensis* in having a ventral bar with short anterolateral processes and a short central arm. It can also be distinguished from *M. clarii* in having slender hamuli that is sharply curved into the point and root and in having a single uniform dorsal bar, wider than long (see Figs. 2G–H, 3C). Additionally, *M. karibae* presents a curved proximal side of sickle base, opposed to the absence of a curved appearance in *M. clarii* and *M. congolensis* (Fig. 2H and Fig. 3D(iii)).

Dimensions for the hamuli (total length, point, shaft and root length) for specimens in the present study from NGR (SA) and Barotse floodplain (ZM) are within the ranges

reported for the same species from other localities in southern Africa (Table 6) (Douëllou and Chishawa 1995, Khalil and Mashego 1998, Barson et al. 2010). The size of the dorsal and ventral bars is within the size ranges previously reported. The marginal hook size and morphology are comparable to previously reported data, specimens from NGR seem to have a slightly larger marginal hook sickle than previously reported. In the description and re-evaluation of *M. karibae*, Douëllou and Chishawa (1995) and Khalil and Mashego (1998) did not provide details on the morphology of the marginal hooks, which are provided in the present study. Comparative measurements are presented in Table 6.

Phylogenetic relationships

Seven sequences (696–753 bp long) of the ITS1-5.8S-ITS2 region of the rRNA gene of *Macrogyrodactylus* spp. from *C. gariepinus* were newly generated in the present study (see Table 3 for detailed information). Maximum likelihood and Bayesian inference analyses yielded trees with similar topology (Fig. 4). The genetic distances presented as p-distance (in %) and number of differences in nucleotides among *Macrogyrodactylus* spp. and the out-group are given in Table 7. The tree depicted a separated branch with *Macrogyrodactylus simentiensis* Příkrylová et Gelnar, 2008 from a polypterid, one single branch with *Macrogyrodactylus polypteri* also from a polypterid, and a clade grouping *Macrogyrodactylus* spp. from clariids that divided into two subclades. The first subclade shows that *M. clarii* isolates 1, 2 and 3 from ZM that clustered together with *M. clarii* from Zimbabwe (ZI) (GU252711) are all identical, whereas *M. clarii* isolate 1 from SA is more related to *M. clarii* x *heterobranchii* from ZI (GU252713) showing slight interspecific differences (p-distance 0.1–0.5%, number of differences in nucleotides 1–3); *M. clarii* x *heterobranchii* (GU252713 and HF548583) from Kenya (KY) and Senegal (SN) appeared closely related to

Fig 4. Maximum likelihood phylogram based on the ITS1-5.8S-ITS2 regions of the rDNA gene. GenBank accession number precedes species name. Species sequenced in the present study are in bold. Posterior probabilities and bootstrap support values are presented along branch nodes (posterior probability < 0.90 and bootstrap < 60 are not shown). *Laminiscus gussevi* (Bychowsky et Polyansky, 1953) was used as outgroup. Branch length scale bar indicates number of substitutions per site. Abbreviations: IC – Iceland; KY – Kenya; M. – *Macrogyrodactylus*; SA – South Africa; SN – Senegal; ZI – Zimbabwe; ZM – Zambia.

M. heterobranchii from SN (p-distance 0.5–0.6%, number of differences in nucleotides 4). The second subclade showed that *M. congolensis* and *M. karibae* are closely related. *Macrogyrodactylus congolensis* isolate from KY (GU252716) and from SN (GU252717) appeared more related (p-distance 0.3%, number of differences in nucleotides 2), whereas the isolate from SA showed interspecific divergence (p-distance 0.6–0.8%, number of differences in nucleotides 4–5); *M. karibae* from ZM appeared more related to *M. karibae* from ZI (GU252715) (p-distance 0.1%, number of differences in nucleotides 1) whereas *M. karibae* from SA showed more interspecific divergence (p-distance 1.8–2%, number of differences in nucleotides 12–14).

DISCUSSION

Novel distribution records for *Macrogyrodactylus clarii*, *M. congolensis* and *M. karibae* are confirmed in southern Africa. The presence of the three *Macrogyrodactylus* spp. in the Vaal River and NGR (SA), and from the Barotse floodplain and Kabompo River (ZM) reflect similar community composition of species as in other localities in southern Africa and Africa (Khalil and Mashego 1998, Barson et al. 2008, 2010, Olivier et al. 2009, Madanire-Moyo et al. 2012). The absence of *Macrogyrodactylus* species in the Eastern and Western Cape in the South Cape ichthyofaunal province confirms that *Macrogyrodactylus* was not co-introduced with *Clarias gariepinus* from the Zambezi and northern regions of the Southern Cape ichthyofaunal provinces, the geographical origin of these translocated *C. gariepinus* populations (see Van Steenberge et al. 2019 and references therein). This implies that *C. gariepinus* in the translocated regions initially did not harbour any species of *Macrogyrodactylus* and is supported by the absence of *Macrogyrodactylus* spp. in the Free State, the southernmost limit of the host's natural distribution. These results suggest that the parasite is either naturally absent from these systems, seasonally present or absent, or restricted by environmental barriers such as physicochemical variables and organic pollution (see Khan and Thulin 1991, Madanire-Moyo and Barson 2010, Madanire-Moyo et al. 2012, Gilbert and Avenant-Oldewage 2017, Sures et al. 2017).

The findings of the present study represent infection intensities in a single point in time and did not indicate high numbers or cause for concern as reported by Khalil and Mashego (1998). The host specimens from the Vaal River (SA) and Barotse floodplain (ZM) that had the highest worm burden (109 individuals of *Macrogyrodactylus* spp. each), did not present greyish-white patches on the skin, as previously recorded by the aforementioned authors in cases of severe infection in natural conditions. Infection of

C. gariepinus with *M. clarii*, *M. congolensis* and *M. karibae* found in this study is low if compared to reports from the Middle Letaba Dam (SA) where intensities of *M. clarii* peaked from 1 to 700 during summer months, and *M. karibae* numbers increased up to 150 (while *M. clarii* decreased) during the cooler months of autumn. The co-occurrence of *M. clarii* and *M. karibae*, the latter in fewer numbers, reported from the Middle Letaba Dam coincides with findings from KuShokwe Pan, South Africa (Khalil and Mashego 1998). From available data, it seems that *M. clarii* is an ever-present species occurring at all, but one locality studied in the southern African region (Khalil and Mashego 1998, Barson et al. 2008, Barson 2009, Madani-re-Moyo 2011).

The use of universal primers for the ITS region of the rDNA and its suitability as a diagnostic tool made it widely used within gyroductylid systematics, resulting in the availability of many sequences for this group (Matějusková et al. 2003, Přikrylová et al. 2013). The first study to ever sequence the ITS region of a *Macrogyrodactylus* species (*Macrogyrodactylus polypteri*) was conducted by Matějusková et al. (2003). These authors constructed a phylogeny including sequences of many species of *Gyrodactylus* von Nordmann, 1832, and a sequence of *Gyrdicotylus gallieni* (Vercammen Grandjean, 1960), and *Gyrodactyloides bychowsii* Albova, 1948. Their phylogenetic analyses recovered *M. polypteri* as the closest ancestor of *Gyrodactylus*, as suggested by Malmberg (1998) based on morphological characteristics, who also suggest that *Macrogyrodactylus* is supposed to be the earliest divergent gyroductylid lineage. However, Malmberg's hypothesis has recently been refuted by Vanhove et al. (2018), who analysed the near-complete mitogenome of *M. karibae* and compared it with other gyroductylid genera.

Barson et al. (2010) was the second study to include molecular analyses of the ITS region for *Macrogyrodactylus* spp., including for the first time *M. clarii*, *M. congolensis*, *M. heterobranchii* and *M. karibae* from African clariids. Sequences of the partial 18S rDNA, complete ITS and the mitochondrial gene *cox1*, together with morphology revealed a hybrid species that was intermediate between *M. clarii* and *M. heterobranchii*, which was confirmed by their phylogenetic analyses (Barson et al. 2010). Přikrylová et al. (2013) conducted a large-scale phylogenetic study on African gyroductylids, including for the first time sequences of the 18S and ITS rDNA of all (but one) genera occurring in the continent. Both ITS and 18S phylogenetic analyses of Přikrylová et al. (2013) recovered, with strong support, *Macrogyrodactylus* as a monophyletic group. Boeger et al. (2021) in their phylogenetic study based on 18S rDNA sequences of several gyroductylid genera also recovered *Macrogyrodactylus* as a monophyletic lineage related to *Diplogyrodactylus* Přikrylová, Matějusková, Musilová, Gelnar et Harris, 2009 and *Afrogyrodactylus* Paperna, 1968. In contrast, *Macrogyrodactylus* appeared related to *Paragyrodactylus* Gvozdev et Martechov, 1953 in their analyses of the mitochondrial gene *cox2*.

The clustering of *Macrogyrodactylus* from clariid hosts as two distinctive lineages in the phylogenetic analyses in

this study reflects their morphological synapomorphies. *Macrogyrodactylus clarii* and *M. heterobranchii* share the following morphological characteristics: 1) hamuli with a strong inward curvature in the root, 2) a divided dorsal bar with two articulating sections, 3) a ventral bar with two short anterolateral arms and a long central posterior arm, and, 4) slender marginal hook sickle and slender sickle blade tapering into a sharp, thin point ending in line with the anterior end of the sickle base.

Macrogyrodactylus congolensis and *M. karibae* share the following morphological characteristics: 1) hamuli with slight inward curvature of the root, 2) single undivided dorsal bar, and 3) robust marginal hook sickle and short sickle blade tapering into a fine point that end in line with the proximal end of the sickle base. However, the relationships of *Macrogyrodactylus* from polypterids remained inconclusive in the present study.

The phylogenetic analyses showed that the ITS-1-5.8S-ITS-2 sequences of *M. clarii* from South Africa differed slightly from those of *M. clarii* from Zambia and Zimbabwe; similarly, *M. congolensis* from South Africa differed slightly from *M. congolensis* from Kenya and Senegal (see Fig. 4 and Table 7 for genetic distances). The ITS-1-5.8S-ITS-2 sequences of *M. karibae* from South Africa also differed from those of *M. karibae* from Zambia and Zimbabwe (Fig. 4, Table 7). Such differences can be related to the geographical separation of the hosts among the African ichthyofaunal provinces of Zambezi and Southern (Cape) as proposed by Roberts (1975), Teugels (1986) and Van Steenberge et al. (2019) and references therein. *Clarias gariepinus* inhabiting the Southern Cape retains similar haplotypes as those from the central and eastern Zambezi Province, thus these catfish occurred sympatrically before a recent separation into the Southern Cape and brought along their parasites (Barson et al. 2010, Van Steenberge et al. 2019).

There is still disproportionate availability of data on freshwater fish parasite diversity from Africa. The continent holds ~25% of the world's freshwater fishes (with the second highest species richness on the globe), and some of the most widely distributed endemic freshwater species (Scholz et al. 2018). It is also evident that there is disparity in distribution and diversity records throughout the continent, even from a widely distributed fish host such as *C. gariepinus*. No data on its parasitic communities exist from at least 3/4 of African countries, where this fish is either native or translocated, and highly utilised for aquaculture. Therefore, more studies encompassing occurrence, environmental or seasonal variation, morphology and genetics of *Macrogyrodactylus* spp. are necessary to better understand their distribution and irradiation into the African continent.

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