Parasite hybridization in African *Macrogyrodactylus* spp. (Monogenea, Platyhelminthes) signals historical host distribution

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**SUMMARY**

*Macrogyrodactylus* spp. from the gills of *Clarias gariepinus* in Zimbabwe and Kenya, and *C. anguillaris* in Senegal were identified using haptoral sclerite morphology and by sequencing the nuclear ribosomal DNA internal transcribed spacers (ITS) 1 and 2, partial 18S and the complete 5.8S rRNA gene. A molecular phylogeny was constructed using all sequenced *Macrogyrodactylus* species to date. Based on morphology, *Macrogyrodactylus congoensis, M. heterobranchii*, *M. clarii*, and *M. karibae* were identified, with one specimen from Zimbabwe displaying morphological features that were intermediate between *M. heterobranchii* and *M. clarii*. In the intermediate form, the partial 18S and ITS1 sequence was identical to that of *M. clarii* while the remaining ITS1 and complete ITS2 region was almost identical to *M. heterobranchii* as was the partial coxl fragment, thus strongly suggesting a hybrid origin. At present, the catfish host of *M. heterobranchii* and *M. clarii* do not co-occur in southern Zimbabwe; this hybridization event is therefore proof of historical sympatry of both fish species.

Key words: introgression, hybridization, catfish, Clariidae, phylogeography, Africa, host tags.

**INTRODUCTION**

With a worldwide focus on northern latitudes, the bulk of fish parasitic species are yet to be discovered and described in Africa. The ectoparasitic monogenean flatworms have generated most of the volume of research done on fish parasites in Africa (Paperna, 1979, 1996; Khalil and Polling, 1997). Research undertaken on monogenean parasites of fishes in Africa has mainly focussed on taxonomy of tilapiine parasites such as *Cichlidogyrus* Paperna, 1960 (Pariselle and Euzet, 1998, 2003) because cichlids comprise a major source of food. A small number of studies have also looked at systematics and host-parasite coevolution (Pouyaud et al. 2006), pathology (Arafa et al. 2009) and faunistics (Khalil and Polling, 1997; Barson et al. 2008).

Viviparous monogeneans of the genus *Macrogyrodactylus* Malmberg, 1957 are common skin and gill parasites of African fishes. Currently 9 species are recognized from 4 fish families (Khalil and Polling, 1997; Příkrylaová and Gelnar, 2008). The taxonomy of species belonging to the genus *Macrogyrodactylus* Malmberg, 1957 was previously based on haptoral morphology (Paperna, 1979), host and geographical range (Khalil and Polling, 1997), and structure of the excretory system (Malmberg, 1998). The recent development of molecular markers for gyrodactylds (Matějusová et al. 2001) resulted in the first and only sequence of the ITS region of the rDNA of *Macrogyrodactylus polypterti* Malmberg, 1957 from the bichir, *Polypterus senegalus* Cuvier, 1829 (Matějusová et al. 2003). DNA sequence data on *Macrogyrodactylus* species, and African flatworm species in general, are still very rare. By virtue of their monoxenic life cycle and host specificity, monogenean parasites are excellent candidates for host-parasite coevolutionary studies. In cases where co-speciation occurs, the parasite can provide extra information on the evolution of the host (Pouyaud et al. 2006; Šimková and Morand, 2008).

The purpose of this study was to identify the species of *Macrogyrodactylus* infesting African clariid catfishes using molecular and morphological characteristics, and to compare the morphology and genetics of these species in southern Africa (Zimbabwe), eastern Africa (Kenya) and western Africa has mainly focussed on taxonomy of tilapiine parasites such as *Cichlidogyrus* Paperna, 1960 (Pariselle and Euzet, 1998, 2003) because cichlids comprise a major source of food. A small number of studies have also looked at systematics and host-parasite coevolution (Pouyaud et al. 2006), pathology (Arafa et al. 2009) and faunistics (Khalil and Polling, 1997; Barson et al. 2008).

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Table 1. Localities, collection period and number of Macrogyrodactylus spp. analysed in the present study (N = number of specimens in morphometrical study, n = number of specimens analysed by molecular methods.)

<table>
<thead>
<tr>
<th>Country</th>
<th>Locality</th>
<th>Host species</th>
<th>Date of collection</th>
<th>N/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zimbabwe</td>
<td>Save River (20°21'07&quot; S, 32°15'58&quot; E)</td>
<td>C. gariepinus</td>
<td>March 2006 and February 2008</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>Lake Chivero, Harare</td>
<td>C. gariepinus</td>
<td>March 2008</td>
<td>8/6</td>
</tr>
<tr>
<td></td>
<td>Mukuvisi River, Harare</td>
<td>C. gariepinus</td>
<td>December 2008</td>
<td>4/0</td>
</tr>
<tr>
<td>Senegal</td>
<td>Niokolo Koba National Park, Mare Simenti (13°01'8&quot; N, 13°17'07&quot; W)</td>
<td>C. anguillaris</td>
<td>March 2006 and 2007</td>
<td>0/3</td>
</tr>
<tr>
<td>Kenya</td>
<td>Lake Turkana, Kalokol–Longech (03°33'17&quot; N, 35°56'30&quot; E)</td>
<td>C. gariepinus</td>
<td>September 2008</td>
<td>4/3</td>
</tr>
<tr>
<td></td>
<td>Lake Turkana, Todenyang village (04°27'10&quot; N, 35°56'36&quot; E)</td>
<td>C. gariepinus</td>
<td>September 2008</td>
<td>9/5</td>
</tr>
</tbody>
</table>

Morphometric analysis

The sclerites were observed under an Olympus BX51 phase-contrast microscope, and measurements of the different parts were taken according to the method of Příkrýlová and Gelnar (2008). The marginal hooks on the posterior part of the haptor were selected for measuring. Species identification was based on Douellou and Chishawa (1995), Khalil and Mashego (1998), and Příkrýlová and Gelnar (2008). Drawings were made with the aid of a drawing tube and also by comparison with the drawings and measurements carried out on type material obtained from the Natural History Museum London (NHM) (M. congolensis – 3 paratypes, no. 1960.5.27.1-15; M. clarii – specimen no. 2006.9.1.1 and M. karibaee – no. 2006.9.1.3), from the Museum National d’Histoire Naturelle Paris (MNHN) (M. heterobranchii – paratype no. MNHN 574 HF, slide Tg 91 and M. congolensis karibaee – paratype no. MNHN 143, slides Tg 14) and from the Swedish Museum of Natural History (M. polypteri – paratype no. 6976, 6977). Voucher specimens of each species will be deposited in the Natural History Museum, London.

Parasite collection

Catfish (Clarias gariepinus (Burchell, 1822) Valenciennes, 1840) were collected from the Mukuvisi River and Lake Chivero near Harare, Zimbabwe in March 2008, and from the Msaize River, a tributary of the Save River, southeastern Zimbabwe in March 2006 and February 2008 (Barson et al. 2008). Monogenean specimens were also collected from C. anguillaris L., 1758 in the Niokolo Koba National Park, Senegal (West Africa) and from C. gariepinus in Lake Turkana, Kenya (East Africa) (Table 1). Monogenean parasites, all of them Macrogyrodactylus spp., were removed from the gills of the fish and stored in 96% ethanol. In the laboratory, the opisthaptor was separated from the rest of the body enabling simultaneous morphological and molecular analyses to be carried out. The body was placed in 5 µl of milli-Q water and stored at –20 °C, while the opisthaptor was digested with proteinase K solution (30 mg/ml) for 5 min at 65 °C. The morphology of Macrogyrodactylus spp. collected in Senegal has already been published (Příkrýlová and Gelnar, 2008) and thus only the material for molecular analysis was included.

MATERIALS AND METHODS

DNA extraction and amplification

DNA was extracted from remnants of the bodies using the NucleoSpin ® Tissue kit (Macherey-Nagel, Germany, 2007) according to the manufacturer’s protocol. The DNA was eluted in 60 µl. The Internal Transcribed Spacers (ITS) 1 and 2 were amplified using the primers ITS1A (5'-GTAAAGGGTTTCCGTAGGTG-3') and ITS2 (5'-TCCTCCGCTTATGATA-3') (Matějusová et al. 2001), and partial 18S was amplified using S1 (ATTCCGATAACGAGCAGACT) that anneals in the terminal region of the 18S gene (Sinnappah et al. 2001) and ITS3A (GAGCCGAGTGATC-CACC) annealing in the 5.8S gene (Matějusová et al. 2001), in a GeneAmp thermocycler (Applied Biosystems). Each amplification reaction contained 5 µl of DNA, 2.5 µl of 10× PCR buffer (Eurogentec), 2.5 µl of 25 mM MgCl₂, 1 µl of 2.5 mM dNTPs, 0.1 µl of 10 µM forward primer, 0.1 µl of 10 µM reverse primer, 0.2 µl of 5 U/µl Taq polymerase (Promega). The temperature program for the amplification reactions was 94 °C for 3 min, 35 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, with a final extension step of 72 °C for 10 min.

Morphometric analysis

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Parasite hybridization signals historical host distribution

2·5 μl of 2 μM dNTPs (Amersham Pharmacia Biotech), 1 μl of each primer (20 μM) (Eurogentec), 1 mM MgCl₂ (Eurogentec), 0·2 μl of Taq polymerase (5 U/μl) and milli-Q water to a total volume of 25 μl. The reaction cycle was as follows: 3 min at 96°C followed by 36 cycles of 50 s at 95°C, 50 s at 50°C, and 50 s at 72°C; a final elongation time of 7 min at 72°C and finally cooling down at 4°C. The PCR products were visualized on an ethidium bromide-stained agarose gel (1%). The amplified product was approximately 865 base pairs long. Part of the cytochrome c oxidase I gene (cox1) was amplified (in 25 μl reaction volumes) using the newly developed primers Mono_cox1L and Mono_cox1H (unpublished), spanning a region of 705 bp. The PCR solution and cycle conditions were as above, except for the annealing temperature which was 54°C.

Nucleotide sequencing and phylogenetic analysis

The PCR products were purified using the GFX purification system (Healthcare). Both DNA strands were sequenced using the same PCR primers and Big Dye Chemistry Cycle Sequencing Kit (version 3.1) in a 3130 DNA Analyzer (Applied Biosystems). Sequences were submitted to GenBank (Accession numbers: GU252711–GU252721). Comparative sequences of *M. polypteri* were obtained from GenBank (Accession number AJ567672). The sequences were visually inspected and assembled in Sequencher™ 4.5 (GeneCodes Corporation) and aligned in MacClade 4.08 (Maddison and Maddison, 2008). Phylogenetic analyses were conducted using PAUP* v. 4.01b (Swofford, 2003) and MEGA v. 4 (Tamura et al. 2007). The GenBank sequence of *M. polypteri* was assigned as outgroup. ModelTest v. 3.06 (Posada and Crandall, 1998) was used to estimate the optimal model of evolution in a likelihood-testing framework. The optimal model was the General Time Reversible model with gamma-distributed rates. Neighbour-joining (NJ) and maximum likelihood (ML) searches were conducted using the selected model and the trees were statistically tested using bootstrap searches (1000 pseudo-replicates). Maximum parsimony (MP) trees were inferred with the branch and bound algorithm; gaps were treated as missing data and all sites were equally weighted.

RESULTS

Morphological and morphometric analysis

*Clarias gariepinus* from Lake Chivero and the Save River, Zimbabwe, were infested by *M. kariba* and *M. clarii*, and a specimen morphologically resembling both *M. heterobranchii* and *M. clarii* was additionally recorded from the Save River. The same host from Kenya was infested by *M. congolensis* and *M. clarii*, which are new records of these parasites in East Africa, as well as 2 specimens bearing intermediate features between *M. heterobranchii* and *M. clarii*. *Clarias anguillaris* from Senegal were infested by *M. congolensis* and *M. heterobranchii*. None of the recovered specimens resembled *M. polypteri*.

The size and shape of haptoral sclerites were used to delineate species (Table 2; Figs 1–3). The haptors of *M. clarii* from Zimbabwe were the largest of all species, and also larger than *M. clarii* specimens from the other regions. The shape of the haptors of *M. clarii*, *M. kariba* and *M. congolensis* were quite distinct (cf. Fig. 2 A and B), while *M. clarii × heterobranchii* (Ken & Zim) showed characters intermediate between those of *M. clarii* and *M. heterobranchii*. *Macrogryrodactylus heterobranchii* is smaller and its marginal hook sickle is more open than in *M. clarii* where the curve is more rounded and a little bit more closed (Fig. 3 A and B).

Molecular analysis and link with morphological results

All phylogenetic methods produced the same tree; all clades are supported by high bootstrap values although the position of *M. clarii × heterobranchii* from Kenya remains unresolved (Fig. 4). There are 2 main groups, the first comprising *M. kariba* and *M. congolensis*, and the other group consists of *M. heterobranchii*, *M. clarii*, and the species that are intermediate between these 2 species both in genotype and in morphotype.

The pairwise genetic distances are summarized in Table 3. The distance between the 2 groups (*M. clarii + M. heterobranchii versus M. kariba + M. congolensis*) is about 12%, slightly less than the distance observed between the outgroup *M. polypteri* and all other species. *M. polypteri* is more closely related to the clade *M. kariba + M. congolensis* (distance of about 12.5%) than to the *M. clarii + M. heterobranchii* clade (distance of about 15.5%). The pairwise difference between *M. clarii* and *M. heterobranchii* is only 1-4%, while that between *M. kariba* and *M. congolensis* is 4%. The intra-specific/geographical differences range from 0-2% to 0-5%.

The first 260 bp of the ITS1 sequence denoted in the tree as *M. clarii × heterobranchii* Zim is identical to *M. clarii* while the remainder of ITS1 and ITS2 is identical to *M. heterobranchii* with the exception of a single T/A substitution. This strongly suggests a hybrid origin of this sequence. The mutations in the sequence denoted as *M. clarii × heterobranchii* Ken are either shared with *M. clarii* (4 mutations) or with *M. heterobranchii* (6 mutations), with only a single unique T/A substitution, and are therefore also suggestive of an (albeit older) hybrid origin. These hypotheses are supported by the partial cox1...
Table 2. Morphometrics of haptoral sclerites of *Macrogryrodactylus* species from Zimbabwe, Kenya and Senegal

(All measurements are in micrometers.)

<table>
<thead>
<tr>
<th></th>
<th><em>M. clarii</em> (n=10)</th>
<th><em>M. kariba</em> (n=6)</th>
<th><em>M. congolensis</em> (n=7)</th>
<th><em>M. heterobranchii</em> (n=2)</th>
<th><em>M. clarii × heterobranchii</em> (hybrid)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hamulus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamulus total length</td>
<td>436 (374–475)</td>
<td>312 (292–330)</td>
<td>421 (385–481)</td>
<td>325 (322–328)</td>
<td>468</td>
</tr>
<tr>
<td>Hamulus point length</td>
<td>120 (108–136)</td>
<td>83 (74–90)</td>
<td>99 (93–103)</td>
<td>93 (92–94)</td>
<td>128</td>
</tr>
<tr>
<td>Hamulus shaft length</td>
<td>380 (350–457)</td>
<td>259 (245–273)</td>
<td>334 (310–361)</td>
<td>278</td>
<td>390</td>
</tr>
<tr>
<td>Hamulus root length</td>
<td>191 (135–238)</td>
<td>144 (114–160)</td>
<td>175 (153–203)</td>
<td>147.5 (140–155)</td>
<td>202</td>
</tr>
<tr>
<td><strong>Ventral bar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of ant. lateral arm</td>
<td>29 (17–38)</td>
<td>18 (16.5–20)</td>
<td>90 (81.5–118)</td>
<td>–</td>
<td>17</td>
</tr>
<tr>
<td>Length of post. central arm</td>
<td>69 (61–85)</td>
<td>42 (37–47)</td>
<td>7 (5.5–8.5)</td>
<td>–</td>
<td>70</td>
</tr>
<tr>
<td>Total length of ventral bar</td>
<td>148 (137–172)</td>
<td>104 (94–113)</td>
<td>143 (131–150)</td>
<td>–</td>
<td>139</td>
</tr>
<tr>
<td>Width of ventral bar</td>
<td>138 (122–157)</td>
<td>109 (99–120)</td>
<td>124 (118–129.5)</td>
<td>–</td>
<td>142</td>
</tr>
<tr>
<td><strong>Dorsal bar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of dorsal bar</td>
<td>16.5 (13.5–20)</td>
<td>16.8 (14.5–19.5)</td>
<td>17.5 (15.5–19.5)</td>
<td>14.5 (13.5–15.5)</td>
<td>18</td>
</tr>
<tr>
<td>Width of dorsal bar</td>
<td>82.3 (72.5–92)</td>
<td>87.5 (80–92)</td>
<td>97.6 (93–102.5)</td>
<td>60.5</td>
<td>73</td>
</tr>
<tr>
<td><strong>Ventral bar rod</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of R1</td>
<td>191 (177–207)</td>
<td>182 (177–189)</td>
<td>236 (217–254)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Length of R2</td>
<td>147 (141–157)</td>
<td>114 (101–125)</td>
<td>132 (126–141)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Marginal hook</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>114.5 (103.5–132)</td>
<td>80 (72–83.5)</td>
<td>96.6 (94.5–100.5)</td>
<td>101</td>
<td>128</td>
</tr>
<tr>
<td>Length of handle</td>
<td>96 (87.5–112)</td>
<td>68.6 (61–73.5)</td>
<td>85.1 (83.5–89)</td>
<td>84.5 (83–86)</td>
<td>109</td>
</tr>
<tr>
<td>Length of sickle</td>
<td>18.5 (17–21)</td>
<td>10.9 (10.5–11.5)</td>
<td>11.4 (10.8–11.7)</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Proximal width of sickle</td>
<td>13.8 (13–17)</td>
<td>9.3 (9–9.5)</td>
<td>11.2 (10.3–11.8)</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

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sequence. The cox1 sequence of *M. clarii* and *M. clarii × heterobranchii* Zim is identical with the exception of 2 transitions in a 705bp fragment (0.3% Kimura two parameter (K2P) distances, Kimura, 1980), while the distance between these 2 species and *M. heterobranchii* is 5.4% (K2P distances). The sequence of *M. clarii × heterobranchii* Ken is more closely related to *M. heterobranchii* (2.1% K2P distance), while the distance with *M. clarii* is about 5.5%.

**DISCUSSION**

Prior to this study, *M. polypteri* was the only species of *Macrogyrodactylus* that had been identified using molecular tools (Matejusova et al. 2003), while the other 8 species described on the African continent had been identified by morphology alone (Paperna, 1979; Douellou and Chishawa, 1995; Khalil and Mashego, 1998; Přikrylová and Gelnar, 2008). This study therefore provides the first ITS1 and ITS2 rDNA and partial cox1 sequences of the species infesting the gills of clarid catfishes. In total, 15 specimens belonging to 4 *Macrogyrodactylus* species and 2 hybrids collected from 3 different African countries have been sequenced.

**Hybridization**

Parasites can provide much information about the hosts in which they live, such as the type of food they eat (Bush et al. 2001), their migration (Combes, 2001), and also about the ecosystem in which they live. In this study, *Macrogyrodactylus* parasites provided evidence for historical distribution ranges of the catfish hosts. We showed that *M. heterobranchii* and *M. clarii* are very closely related sister taxa, differing only by 1.2–1.4% in the ITS rDNA region and 5.4% in the cox1 mtDNA fragment. Morphologically, they can be clearly differentiated from each other by the shape and size of the ventral bar and the shape of the marginal hook sickles. However, our sequence data suggest that they are able to interbreed, with specimens *M. clarii × heterobranchii* from Zimbabwe and Kenya being possible outcomes of such hybridization events.

The nuclear ribosomal RNA genes and the ITS region are very suitable markers to detect hybridization, because they can retain both parental copies for several generations before they are homogenized by concerted evolution (Sang et al. 1995). The rate or extent of homogenization after hybridization can, however, differ among species.
(Waters and Schaal, 1996). Biased homogenization towards one of the parental sequences can already occur in F2 hybrids or backcross generations, such that the signal of hybridization is blurred (Fuertes Aguilar et al. 1999). In such cases, mitochondrial DNA can help to identify hybridization events, as this is a purely maternally inherited marker.

The signature of hybridization is still very clear in the specimen from Zimbabwe, where partial 18S rRNA and the beginning of the ITS region were identical to one parental sequence and the remaining part identical to the other parental species (except for 1 bp) as a result of crossing over. This together with the cox1 fragment that is nearly identical to the parental M. clarii, suggest it to be a ‘younger’ hybrid compared to the specimens from Kenya. However, the ITS and cox1 fragments already accumulated respectively 1 and 2 unique mutations in comparison to the parental species, suggesting it to be a stable hybrid. Parental backcrossing will complicate time estimations, as it will erase new mutations. Applying a standard molecular clock of 2% per million years (Avise, 1994) for the cox1 fragment will therefore result in a timing of hybridization of at least 75 000 years ago, but it is probably much older. The specimens from Kenya appear to be the result of the reverse cross, with M. heterobranchii as the original female parent because these specimens had a cox1 sequence most similar to M. heterobranchii. The ITS profile was intermediate between both parental species, with alternating fixation of parental mutations. This pattern and the unique mutations in the cox1 fragment, suggest that these 2 specimens are the result of an older hybridization event, of at least 500 000 years according to the same calibration as above.

A prerequisite for hybridization to occur is sympatry. Normally M. heterobranchii is supposed to be specific to the vundu Heterobranchus longifilis Valenciennes, 1840 (N’douba and Lambert, 1999), while M. clarii is described on Clarias gariepinus (Khalil and Polling, 1997). Since Heterobranchus and

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**Fig. 2. Ventral bars of Macrogryrodactylus spp. from clariid catfish. (A) M. heterobranchii (Zim). (B) M. clarii (Zim). (C) M. clarii (Ken). (D) M. clarii × heterobranchii (Zim). (E) M. kariba (Zim). (F) M. congolensis (Ken). Scale bars = 50 μm.**
Clarias are very closely related catfish genera with a very similar biology (Agne`se et al. 1997; Rognon et al. 1998; Agne`se and Teugels, 2005), host switching between both fish hosts might be facilitated. However, the current southern-most range of Heterobranchus longifilis is the Zambezi River system (Skelton, 1994), whose tributaries drain the northern parts of Zimbabwe (including Lake Chivero) (Marshall, 1999). One of the hybrids was found in the Save River System, a geographically distinct basin in southern Zimbabwe. One explanation for the presence of the parasite in this system could be that the Zambezi and Save systems used to be connected, but when they separated, Heterobranchus was restricted to the Zambezi system, while C. gariepinus remained in both systems, together with monogenean parasites contracted from the Zambezi. This is supported by the fact that the current geomorphological erosion surfaces of the Save-Runde system in Zimbabwe are connected to the Limpopo River basin in the south (Moore et al. 2009), which in turn has been historically linked to the upper Zambezi system until the beginning of the Paleocene (Stankiewicz and de Wit, 2006). Our results might suggest a more recent exchange, which can possibly be linked with the Pleistocene cycling (Trauth et al. 2009); see below. The occurrence of the hybrids found in Lake Turkana (Kenya) could be explained by the fact that although only C. gariepinus is predominant in the lake, fossils of Heterobranchus sp. have been found (Trapani, 2008), which proves a historical connection with the Nile system (Goudie, 2005) where the latter fish species still occurs today (see also below).

Species complex

The observed hybridization events might lead to a species complex of closely related taxa that are difficult to identify based on morphology alone. Similar
to this, the anisakid nematode *Contracaecum rudolphi* exists as a species complex comprising sibling species in different geographical regions (Shamsi et al. 2009). Future studies should focus on obtaining more molecular data on *Macrogyrodactylus* species and propose a sibling classification system of the *M. clarii-heterobranchii* complex, and our results pave the way for this possibility. It might well be that, with the current advances in molecular genotyping, previously described species-complexes turn out to be complexes of hybrid species. In that case, the biological species concept (Mayr, 1963) cannot be applied (no reproductive isolation) and the difficulties in species identification are not the result of incomplete lineage sorting but a result of the mixed, hybrid origin.

**Host and parasite phylogeography**

As suggested by Pariselle (personal communication; and also by Agnès and Teugels, 2005), monogeneans can provide information on the evolution and zoogeography of fishes. This is because they have a close relationship with their host and they are mostly host specific (Kearn, 1998). Klassen and Beverly-Burton (1988) showed that the ecological association between ancyrocephalid monogeneans and their centrarchid fish host in North America could be attributed to the combined effects of host hybridization and the differential geographical distribution of hosts and parasites in response to Pleistocene glaciation events. Where hybridization between sympatric hosts takes place, changes in host ethology and ecology may be reflected by changes in their monogenean parasite specificity, prevalence and intensity (Le Brun et al. 1992).

If we compare the 4 basins from where *Macrogyrodactylus* species were collected (Senegal River, Lake Turkana, Zambezi and Save Rivers), they seem distinctly separated spatially, but the parasite fauna suggests that they were once in sufficiently close contact to be able to exchange parasites. The above example of hybridization, together with the fact that *M. karibae* collected from *C. gariepinus* in the Save River basin in southern Zimbabwe (Barson et al. 2008) were morphologically identical to those collected from Lake Chivero (Zambezi system, this study), point to a historical connection between the two systems. Additionally, the close relationship between *M. karibae* from the Zambezi system and *M. congolensis*, which is absent from the Zambezi based on current data, was here confirmed phylogenetically, and might explain why Douèllo and Chishawa (1995) initially termed *M. karibae* as *M. congolensis-karibae*, although it has been found in South Africa (Khalil and Mashego, 1998). This close relationship can be a result of the historical connection between the Congo and Zambezi River basins (ichthyofaunal regions 3 and 5), in which catfishes could have migrated across the wetland headwaters of these basins (Bell-Cross, 1965; Skelton, 1994). Geological evidence actually shows that a current tributary of the Zambezi River, the Luangwa River, used to be part of the Congo River before the east African rifting events in the Pliocene-Pleistocene periods (about 1–5 million years ago; see Goudie, 2005; Stankiewicz and de Wit, 2006).

There was a marked distinction (12.5% distance) between the *M. congolensis + M. karibae* clade and the *M. heterobranchii + M. clarii* clade although some catfish individuals were infected by representatives of the 2 clades, e.g. *C. gariepinus* in the Save River was infested by both *M. clarii* and *M. karibae*. This can probably be explained by the fact that this catfish has one of the widest geographical ranges (Rognon et al. 1998; Giddelø et al. 2002; Agnès and Teugels, 2005; Jansen et al. 2006). The parasite *M. congolensis* shows a similarly wide distribution. Even though we
currently have no sequence data of *M. congolensis* in southern African systems*, its presence has been confirmed by morphology in the Limpopo system (Khalil and Mashego, 1998; K. Christison, personal communication). Here we show that specimens from Senegal and Kenya (Lake Turkana) are very similar genetically (0-2% distance) in spite of the large geographical separation. Such a close relationship is also found for *Clarias gariepinus* populations from both regions and can be explained by the historical connection of the Nile system and West African basins which is currently lost because of climate changes (Trapani, 2008). This shows a common phylogeographical pattern for host and parasites, consistent with the savanna belt formation around the central African tropical forest shaped by Pleistocene glaciations (Hewitt, 2004), which influenced many of Africa’s fauna and flora (Trauth *et al*. 2009).

This study has shown that parasites can hold clues to the history of their hosts. Essential to this, is the simultaneous use of nuclear and mitochondrial markers, otherwise most hybridization events remain unnoticed (Huyse *et al*. 2009). Parasite hybridization is the ultimate proof of (historical) close connection between host species, especially in case of host-specific parasites. Future ichthyological field studies of (African) fishes should therefore be matched by intensive parasitological studies.

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* Please note that *M. congolensis* reported erroneously by Barson *et al*. (2008) from the Save River, Zimbabwe, has actually been confirmed to be *M. karibae*.

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**Table 3. Genetic distances (Tamura Nei – gamma model) between the *Macrogyrodactylus* specimens sequenced in this study**

| Specimen 1 | Specimen 2 | Specimen 3 | Specimen 4 | Specimen 5 | Specimen 6 | Specimen 7 | Specimen 8 | Specimen 9 | Specimen 10 | Specimen 11 | Specimen 12 | Specimen 13 | Specimen 14 | Specimen 15 | Specimen 16 |
|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| M. congolensis Ken | 0.000 | 0.002 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| M. congolensis Sen | 0.000 | 0.002 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| M. karibae Zim | 0.000 | 0.002 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| M. clarii × heterobranchii Ken | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| M. polypodi × heterobranchii Sen | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| M. clarii × heterobranchii Sen | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| M. clarii × heterobranchii Zim | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
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