Journal of Natural History, 2007; 41(9–12): 647–708

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Allopatric differentiation in the *Marcusenius macrolepidotus* species complex in southern and eastern Africa: the resurrection of *M. pongolensis* and *M. angolensis*, and the description of two new species (Mormyridae, Teleostei)

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(*Accepted 18 January 2007)*

Abstract
We critically compared local populations of the bulldog fish, *Marcusenius macrolepidotus* (Peters 1852), from different watersheds, from the furthest south (28° South, South Africa) to the Equator in Kenya. We ascertained allopatric differentiation from topotypical *M. macrolepidotus* from the Lower Zambezi River (Mozambique) in morphology, electric organ discharges, and molecular genetics for: (1) samples from the Okavango and Upper Zambezi Systems (Botswana and Namibia), (2) samples from South Africa's rivers draining into the Indian Ocean, and (3) samples from the East African Tana River (Kenya). Significant genetic distances in the mitochondrial cytochrome b gene and differing ISSR-PCR profiles corroborate differentiation between the four taxa. We resurrect *M. pongolensis* (Fowler, 1934) for South Africa (sample 2), and *M. angolensis* (Boulenger, 1905) for the Quanza River/Angola. We recognize *M. altisambesi* sp. n. for the Upper Zambezi/Okavango specimens (sample 1), and *M. devosi* sp. n. for those from Kenya (sample 3).

Keywords: *Teleostei, Mormyridae,* Marcusenius, *systematics, morphometrics, electric organ discharges, molecular genetics, southern Africa*

Introduction

Marcusenius Gill, 1862 is the largest genus of the Mormyridae, comprising 33 species in the catalogue of fishes (Eschmeyer 2006), distributed throughout tropical central, western, eastern, and north-eastern Africa (Nile System). In southern Africa only a single species, *M. macrolepidotus* (Peters, 1852), has been recognized, known commonly as the bulldog

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ISSN 0022-2933 print/ISSN 1464-5262 online
DOI: 10.1080/00222930701250987

fish, a name which refers to this fish's well-developed mental lobe on the lower jaw that is also present in other *Marcusenius* species (Figure 1). *Marcusenius macrolepidotus* ranges widely in southern and East Africa, occurring in most rivers of East Africa from the equatorial Tana River in Kenya southwards (Seegers 1996, p. 76) to the Mhlatuze River in South Africa's KwaZulu-Natal province (almost 29° S; Skelton 1993, 2001). It also occurs in the Upper Zaire (Congo River), and is widespread and common in the western Cunene, Okavango and Upper Zambezi Systems.

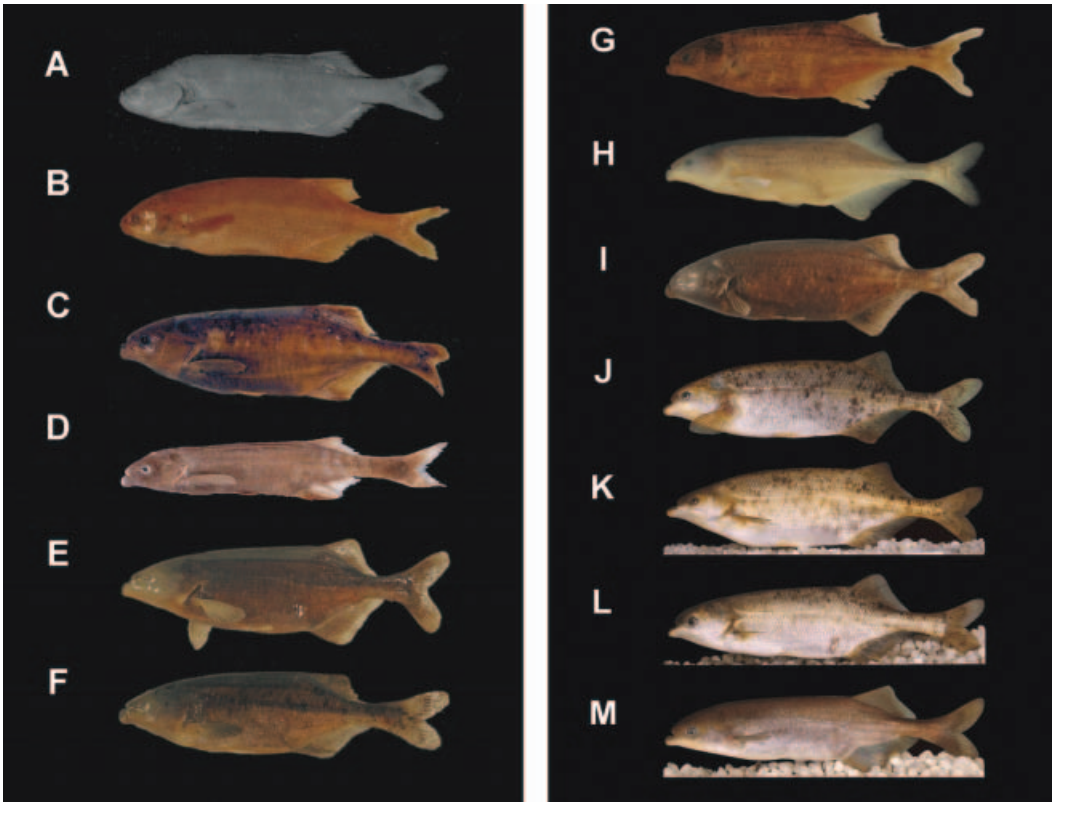

Figure 1. All species and forms of the *Marcusenius macrolepidotus* species complex, as studied in the present paper. (A) *M. macrolepidotus* (Peters, 1852), ZMB 3678 (lectotype L. Seegers; photo: L. Seegers). (B) *Gnathonemus angolensis* Boulenger 1905, BMNH 1905.5.29.64 (holotype). (C) *Gnathonemus moeruensis* Boulenger 1915, MRAC 14137 (holotype). (D) *Gnathonemus pongolensis* Fowler 1934, ANSP 54950 (holotype). (E) *M. macrolepidotus* (Peters 1852), SAIAB 060847, coll. R. Bills 1 Aug.1999, Lower Zambezi. (F) *M. macrolepidotus* (Peters 1852), SAIAB 060947, coll. R. Bills 15 Aug.1999, lower Pungwe River System. (G) *M. devosi* sp. n., coll. L. de Vos and B. Kramer 3/6 Sept. 2001, Lower Tana River/Kenya. (H) *M. macrolepidotus* (Peters, 1852), SAIAB 73790 (largest specimen), coll. R. Bills 14 Aug. 2003, Rovuma System. (I) *M. macrolepidotus* (Peters 1852), SAIAB 055874, coll. R. Bills 20 July 1997, Mulela River/Mozambique. (J) *M. altisambesi* sp. n., coll. F.H. van der Bank and B. Kramer, 11/12 August 2004, Okavango River, live fish of SL 13 cm photographed 20 April 2006. (K) *M. altisambesi* sp. nov, coll. F. H. van der Bank and B. Kramer, 21 August 1999, Upper Zambezi, Kalimbeza, live specimen of 16.5 cm SL photographed 3 July 2003. (L) *M. macrolepidotus* (Peters 1852), sampled together with SAIAB 67369, coll. R. Bills 29 Sept. 2002, Buzi River System, specimen of 13 cm SL photographed alive 22 February 2005. (M) *M. pongolensis* (Fowler, 1934), resurrected species, specimen of 11 cm SL reared in captivity from parents caught in Crocodile River, Incomati System, in February 1997, photographed alive 15 March 2005.

As in many other fish of wide southern and even east African distribution, a critical comparison of allopatric populations has not been made, with the notable exceptions of the large-scaled *Marcusenius* species of west-central and central Africa by Boden et al. (1998), none of which occur in the area we were able to visit, and Malawi *Marcusenius* species (Tweddle and Willoughby 1982). While conducting an analysis of electric organ discharges (EODs) on mormyrids from southern Africa, usually regarded as *Marcusenius macrolepidotus*, specimens from the Incomati System (South Africa) emitted distinctly different EODs compared to bulldogs from the Upper Zambezi in Namibia (for Upper Zambezi bulldogs, see Kramer 1997a, 1997b). Bulldogs from other southern African and East African origins revealed still more differentiation on which we report here.

DNA markers, such as sequences of mitochondrial DNA (especially cytochrome b) have been widely employed to reconstruct the molecular phylogeny of some members of the Mormyridae in comparison to their corresponding morphology and electrophysiology (Alves-Gomes and Hopkins 1997; Sullivan et al. 2000, 2002; Lavoué et al. 2000; Lavoué and Sullivan 2004, Kramer et al. 2003, 2004). We have chosen the cyt b gene to reconstruct the phylogeny of the *M. macrolepidotus* complex. Since mtDNA is inherited maternally, hybridizations can distort the mtDNA phylogeny. In order to corroborate the findings from cyt b analysis we therefore used genomic fingerprinting with ISSR-PCR to analyse variation in the nuclear genome. The ISSR (Inter-simple-sequence-repeat) method has recently been added to the growing list of molecular tools. ISSR analysis is useful for testing genomic instability (Leroy et al. 2000), genetic diversity (Kantety et al. 1995), cultivar identification (Charters et al. 1996), molecular mapping (Ratnaparkhe et al. 1998), in forensic DNA profiling (Kumar et al. 2001) in plants, as well as for sexing in birds (Wink et al. 1998), or detecting hybrids in birds and reptiles (Wink et al. 2000). This PCR-based method uses primers annealing to microsatellite repeats to amplify the regions between adjacent, inversely orientated SSRs, provided they are close enough to allow exponential multiplication. The method targets inversions, insertions, deletions, and mutational events of microsatellites at multiple loci in the genome. Individuals of the same species usually show few to no differences between their ISSR patterns, whereas closely related taxa, such as subspecies and species, give a specific banding profile that can be used to study phylogenetic questions.

EODs are a communication signal in mormyrid fishes that are also used for active location of objects (for reviews, see Kramer 1990, 1996; Bastian 1994; Moller 1995; Hopkins 1999). EODs play a key role in pair formation, mating, and social attraction in the bulldog fish (Werneyer and Kramer 2002, 2005; Hanika and Kramer 2005). EODs of sympatric mormyrids of the Upper Zambezi are species-specific (Kramer 1996) and have been used for phylogenetic analysis (Van der Bank and Kramer 1996; Kramer and Van der Bank 2000; Kramer et al. 2003, 2004). In addition to anatomical and genetic data, we utilize EOD as a taxonomic tool for analysing some of the populations forming a complex of allopatric species for *M. macrolepidotus* in southern and East Africa.

Material and methods

Electrical and morphological studies

A total of 414 specimens was examined and at least 15 measurements (see Figure 2) and at least three meristic characters taken. The following abbreviations were used: PDL, predorsal length: distance tip of snout (excluding mental lobe or chin) to dorsal fin origin. PAL, distance

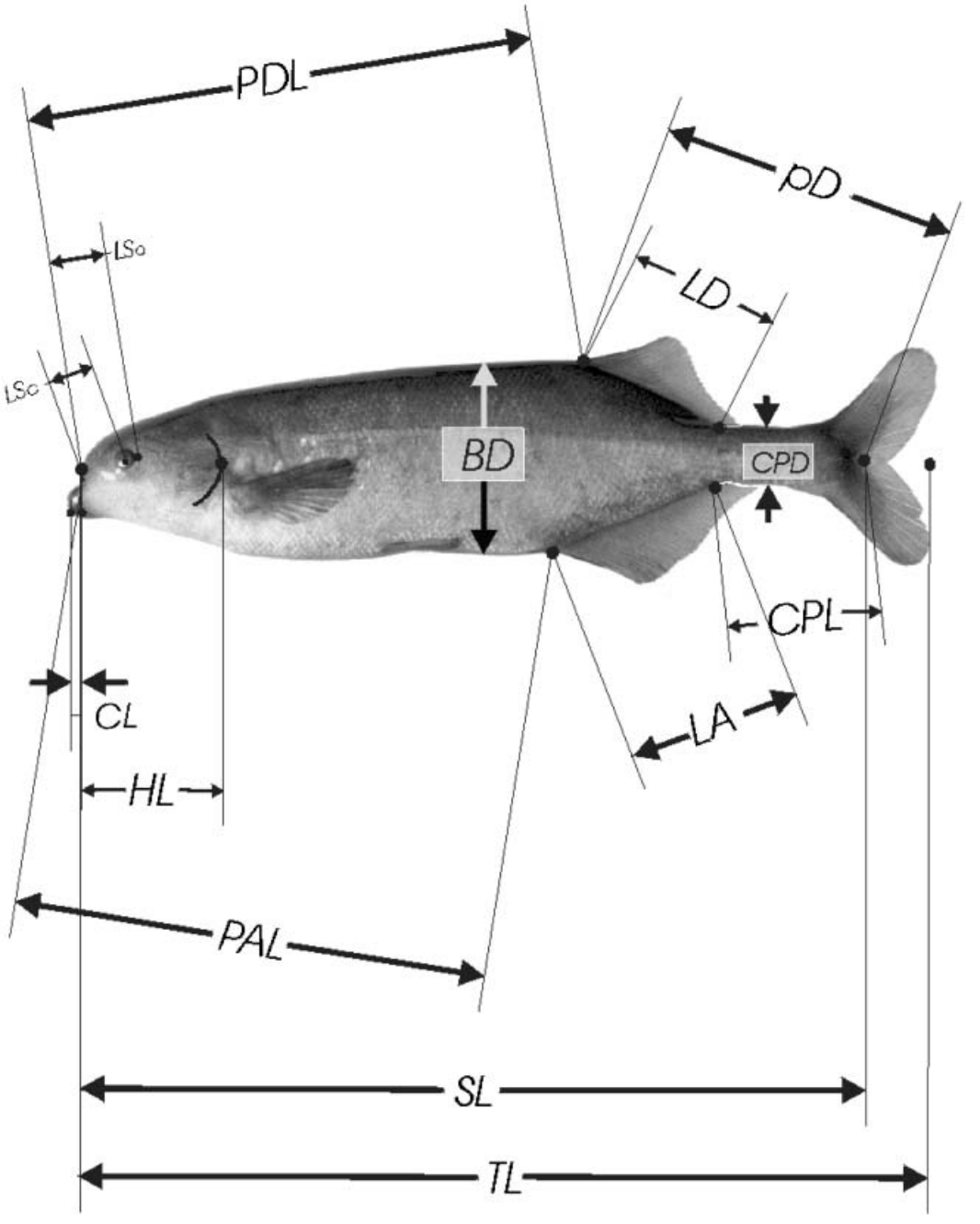

Figure 2. Morphological measures used in the present study. For explanation of abbreviations, see Material and methods.

tip of snout to anal fin origin. LD, dorsal fin length. LA, anal fin length. pD, distance dorsal fin origin to end of caudal peduncle. CPL, length of caudal peduncle (end of anal fin base to midbase caudal fin). CPD, depth of caudal peduncle: the least vertical distance across the

caudal peduncle. LS, length of snout: distance tip of snout to posterior orbital rim of eye (LS_o), centre of eye (LS_c). HL, head length: distance tip of the snout to furthest bony edge of the operculum. Na, distance between the pair of nares of one side (from centre to centre). ED, eye diameter: defined by orbital rims. LPF, length of pectoral fins. SL, standard length: distance tip of snout to midbase caudal fin. BD, body depth: the greatest vertical distance across the body. TL, total length: distance tip of snout to end caudal fin. CL, mental lobe or chin length. nD, number of dorsal fin rays. nA, number of anal fin rays. SPc, number of scales around caudal peduncle. SLS, number of scales in linear series along the lateral line row, as detailed in Skelton (2001, p. 67). SLS range of accuracy, ± 2 counts.

Abbreviations used to represent institutions and collections cited follow Leviton et al. (1985), with the exception of RUSI having since been replaced by SAIAB (South African Institute of Aquatic Biodiversity, Grahamstown, South Africa). Specimens examined were initially identified using dichotomous keys in Bell-Cross and Minshull (1988) and Skelton (1993, 2001), which are considered effective for fish populations occurring in southern Africa.

EODs of 269 fish were recorded in the field (exceptions, below) immediately after capture in a 37-litre plastic aquarium filled with river water where the fish was collected, and was maintained for the duration of the analysis, which was accomplished at a field laboratory, set up nearby. Conductivity changes possibly affecting EOD were excluded. Fish of the second South African sample (Sabie River) were recorded in this manner both in the field and in the (European, Regensburg) laboratory, and identity of results established after a suitable waiting period following transport (two weeks, see below). Specimens of the third South African sample (from the Crocodile River), the second Upper Zambezi sample, and from the Buzi River were only recorded in the laboratory (see Material examined, below).

Temperature ($\pm 0.1^{\circ}$C) and water conductivity ($\pm 1\,\mu S\,cm^{-1}$) were constantly monitored using an electronic apparatus (LF92 by WTW, Germany). Fish were placed between a pair of carbon rod electrodes that were connected to a differential amplifier with a variable gain (up to $\times 10$; 0.2 Hz … 100 kHz; filter slopes, -3 dB per octave; electronics workshop, Biology Department, University of Regensburg). Amplifier output was recorded with a digital storage oscilloscope (up to at least 10 MHz conversion rate, amplitude resolution 8 bit, 512 points per trace in the field, replaced by a 9 bit/10 000 points oscilloscope from 2003 on; 13 bit/5000 points in the laboratory), and data were numerically transferred onto the hard disk of a computer via digital interface. Usually eight traces per fish were recorded. Field equipment was battery-operated.

Custom-designed computer programs were used for analysis of EODs (programmed using a software package for signal analysis, Famos v3.1 to v4). When necessary, EOD duration was corrected to 25°C using a Q_{10} value of 1.5 (Kramer and Westby 1985) before data analysis.

Definition of EOD waveform variables (compare with Figure 3): *P*amp, peak amplitude of positive *P* phase (i.e. from baseline to peak, which is equal to 1 by definition); Namp, peak amplitude of negative *N* phase of EOD re: Pamp = 1; Pdur, Ndur, durations of *P* phase and *N* phase; PNsep, separation (or interval) between the peaks of the *P* and *N* phases; Parea, Narea, areas under the *P* and *N* phases. Durations in microseconds; amplitudes in relative volts (re: *P*-phase amplitude = 1). Area measures, dimension (V $\times$ microseconds). Because of the asymptotic start and termination of an EOD, Pdur started at +5% of Pamp, and Ndur ended at -5% of Pamp. This threshold criterion was also used for Parea and Narea estimations.

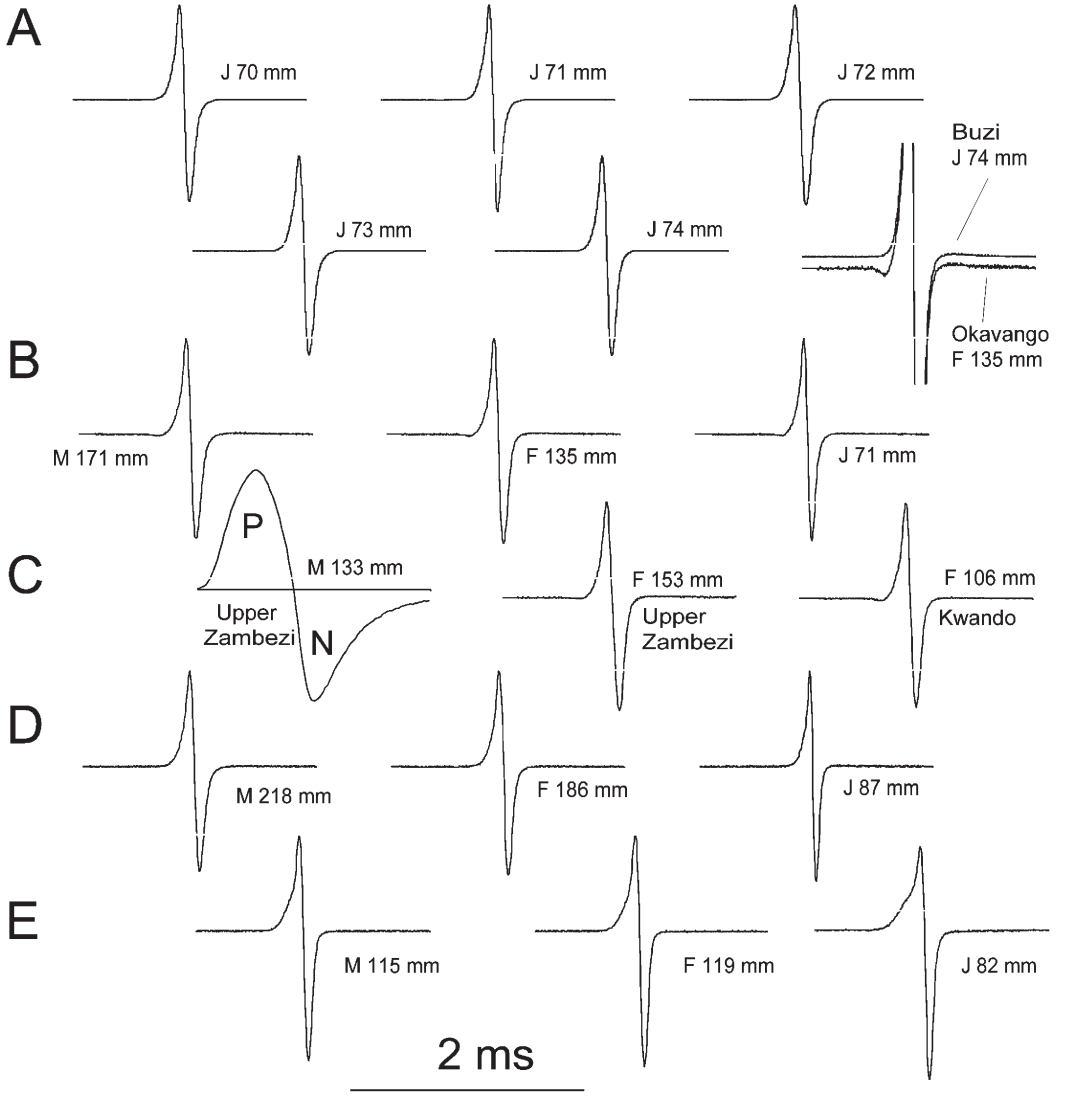

Figure 3. Oscillograms of the waveforms of electric organ discharges (EODs) in the *M. macrolepidotus* species complex. (A) specimens from the Buzi River (Mozambique), (B) from the Okavango River (Botswana), (C) from the Upper Zambezi and Kwando Rivers (Namibia) as indicated, (D) from the Incomati System (South Africa), (E) from the Tana River (Kenya). J, juveniles; F, females; M, males, all with standard length in mm. (C) Superimposed baseline shown in male discharge of long duration. Head-positivity is upwards. Abscissa, see time bar (identical for all). All waveforms normalized to 25°C. (A, lower right) Head-negative pre-potential present in specimens from the Upper Zambezi/Okavango Systems, not from the Buzi River nor any other origin (amplified $\times 3$).

Subsequent to EOD recording, fish were either killed by an overdose of the anaesthetic 2-phenoxy-ethanol, SL determined using vernier callipers, and fixed in 10% formalin for morphological studies (Figure 2), or else transported live usually to Johannesburg, by road (with the water permanently oxygenated by a battery-powered air bubbler). After a recovery period of 3–5 days in the aquaria of University of Johannesburg, fish were put on an overnight flight direct to Germany. They were packed using medical oxygen-inflated plastic bags and

temperature-insulated boxes from the aquarium trade. Fish were sexed by using (1) the kink criterion of the anal fin base for orientation (kink absent in females), (2) dissection of the gonad, and (3) histology of the gonad (which was the decisive criterion in cases of conflict). Paraffin-embedded 7 µm slices of the gonads were stained with Azan (Romeis 1989).

Principal component analyses (PCA) on correlations among anatomical characters were used to test differences in body shape among populations because it does not require *a priori* assumptions about taxonomic groups. Analyses of variance (ANOVA) were performed to test hypotheses of no difference between samples for each character individually. Multivariate analyses of variance (MANOVA) were required in order not to overestimate differentiation when examining the hypothesis of no morphological difference between fish from different origins by inferential statistics (McGarigal et al. 2000). *P* values are two-tailed unless otherwise stated. For interpreting the principal components in terms of the anatomical characters, we determined the component loadings, i.e. the principal component structure (see McGarigal et al. 2000). For assessing the significance of component loadings we followed Tabachnick and Fidell (1989), as suggested by McGarigal et al. (2000). These authors recognise five levels of significance: loadings >0.32 or <-0.32 are poor, >0.45 or <-0.45 fair, >0.55 or <-0.55 good, >0.63 or <-0.63 very good, and >0.71 or <-0.71 excellent. These benchmarks account for 10%, 20%, 30%, 40% and 50% of the variance in the component, respectively. The software used was JMP IN v. 5.1 (SAS Institute, 2003).

Genetic studies

DNA isolation. DNA was isolated from muscle tissue, which was preserved in ethanol, using the "proteinase K method" (Kocher et al. 1989).

PCR- and DNA sequencing. Our target sequence was the mitochondrial cytochrome b gene (cyt b), which is phylogenetically highly informative in mormyrids. Primer pairs used for PCR (modified from Kocher et al. 1989; Pääbo 1990) were L-14841 (5′-CCA TCC AAC ATC TCA GCA TGA TGA AA-3′) [positions refer to the gene of *Gallus*; Desjardins and Morais 1990]: and mt-F (H-15917; 5′- TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3′): or L-14724 (5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′) and Mt-E (H-15713; 5′-AAT AGG AAG TAT CAT TCG GGT TTG T-3′). For amplification, 0.5 µg of total DNA was used as a template, plus 20 pmol each of the two corresponding PCR primers, 1.5 mM $MgCl_2$, 0.1 mM of each dNTPs, 5 µl 10 × amplification buffer (100 mM Tris HCl, pH 8.5, 500 mM KCl, 5% Triton x-100) and 0.5 units Taq-Polymerase (Pharmacia, Freiburg) in a total volume of 50 µl. After an initial denaturation (4 min at 94°C), 30 cycles of 45 s at 94°C , of 60 s at 52°C, and of 120 s at 72°C were performed on a Biometra thermocycler. After 30 cycles the reaction temperature was maintained at 72°C for 10 min and then lowered to 4°C for further storage.

PCR products were purified by precipitation under the following conditions: 1 vol PCR-product (30 µl), 1 vol 4M NH_4Ac (30 µl) and 12 vol EtOH (100%; 360 µl). DNA was pelleted by centrifugation (15 min at 13 000 rpm) and the pellet washed with 70% ethanol. The pellet was dissolved in 20 µl H_20. Sequencing was carried out under the following conditions: sequencing primers: Mt-CCy 5′-CTA/C CCA TGA GGA/C CAA ATA/C TC-3′, Mt-LECy 5′-TCA AAC CCG AAT GAT AC/TT TCC TAT T-3′; Mt-ECy 5′-AAT AGG AAA/G TAT CAT TCT GGT TTG A-3′, and S-Mt-BCy 5′-TCA AAA TGA TAT TTG TCC TC-3′. Sequencing solution: 1 µl primer (5 pM/µl), 1–3 µl PCR-product, 1 µl premix and H_20 to give a final volume of 5 µl. The premix was supplied by DYEnamic

ET Terminator Cycle Sequencing Kit (Amersham Biosciences). PCR cycle: 26 × 20 s at 95°C, 15 s at 50°C, and 2 min at 60°C. Sequencing products were purified on Sephadex G50 columns in Multiscreen MAHVN 4510 well plates (Millipore). Purified sequencing products were sequenced with a MegaBace 1000 instrument (Amersham Biosciences) equipped with 96 capillaries filled with MegaBace Long Read Matrix (Amersham Biosciences). Raw data were analysed with Sequence Analyzer Version 3.0 (Professional Edition; Amersham Biosciences).

Alternatively, a cycle sequencing reaction (final volume 10 µl) was carried out after the initial PCR. Reaction buffer consisted of: 2 µl reaction mix with BigDye terminators (according to the BigDye Terminator Protocol; ABI Applied Biosystems), 10 pmole sequencing. The cycle sequencing was carried out over 25 cycles at 96°C for 10 s, at 52°C for 5 s, and at 60°C for 4 min. Sequencing products were purified by precipitation: 1 vol reaction mix, 1/10 vol. 3 M NaAcetate (pH 4.6), 2.5 vol ethanol. After centrifugation for 15 min at 13 000 rpm, DNA pellets were washed in 70% ethanol and taken up in 20 µl distilled water. The purified DNA was diluted 1:5 in water and applied to a 16 column automatic capillary sequencer (ABI 3100) using 50 cm capillaries and POP6 as a polymer.

Sequence data have been deposited in the Sequence Library of GenBank and can also be obtained from wink@uni-hd.de. Phylogenetic trees were reconstructed by the character state method Maximum Parsimony (MP), the distance matrix method Neighbour Joining (NJ) and Maximum Likelihood analysis (ML) using PAUP version 4.0b10 (Swofford 2001). In Neighbour Joining analyses, genetic distances were calculated based on uncorrected p-distance. Bootstrap analyses were performed to support confidence estimates for each furcation. In MP a Branch and Bound exact search (addition sequence furthest; swapping algorithm: TBR; MULPARS OPTION) was carried out. ML conditions corresponded to the GTR model (number of substitution types 6, nucleotide frequencies: $A=0.272$, $C=0.324$, $G=0.147$, and $T=0.256$). Results can be illustrated as clado- or phylograms (in phylograms, branch lengths are proportional to the number of inferred changes or evolutionary distances).

For ISSR-amplification: 15 ng of total DNA and 3 pmol primer (GACA)4 was used in a total volume of 12.5 µl: 1.5 mM $MgCl_2$, 0.1 mM of dGTP, dCTP, and dTTP, 0.075 mM dATP, 1 µCi [α-^{33}P]-dATP, 1.25 µl of 10x amplification buffer (100 mM Tris-HCl, pH 8.5, 500 mM KCl, 5% Triton X-100) and 0.4 units Taq polymerase (Amersham Pharmacia Biotech) were used. After an initial denaturation (120 s at 94°C), 33 cycles of 60 s at 94°C, 120 s at 55°C, and 120 s at 72°C were performed on a Biometra thermocycler; then at 72°C for 4 min, followed by 4°C for storage. After electrophoresis on 0.2 mm denaturing polyacrylamide gels at 65 W for 3 h (size 45 × 30 cm), the gel was exposed to Kodak Hyperfilm for several hours. Alterations of fragment patterns were detected as gains and losses of individual bands and combined into a 1/0 matrix. Faint bands or those that could not unequivocally be scored as present or absent were not taken into account. The matrix was used to calculate an UPGMA distance tree in PAUP version 4.0b10 (Swofford 2001).

The methods used for the allozyme studies were reported in Van der Bank (1996).

Results

Morphological comparisons

Gnathonemus angolensis was synonymized with *M. macrolepidotus* as the subspecies *M. m. angolensis* by Poll and Gosse (1963), perhaps because of a prevailing sentiment of

relatedness of the fishes of the upper Quanza with those of the Zambezi basin (Trewavas 1973), although from the geographical detail on the Ansorge collection given in Boulenger (1910), the type specimen seems to originate from the Lower Quanza. The origins of *G. angolensis* and also *G. moeruensis* are outside our study area, and we were unable to sample these places. We confirm Boulenger (1915, p. 163) who stated that *G. moeruensis* was ''Très voisin de *G. angolensis*''. These two species' combination of meristic characters (nA and nD highest, SPc lowest; Table XI) sets the two nominal species *G. angolensis* and *G. moeruensis* well apart from all other bulldog samples we were able to study (including those from the Upper Zambezi). We confirm that among 89 bulldog fish sampled from the Upper Zambezi, and 32 fish from the Okavango system, not a single specimen anatomically referable to *G. angolensis* Boulenger, 1905 was found, and therefore resurrect *M. m. angolensis* as a valid species, *M. angolensis* (Boulenger, 1905).

Considering the comparison of *M. macrolepidotus* populations, only three of six specimens from the lecto- and paralectotype material (Seegers 1996) are fully useful. Therefore, only visual inspection of means and ranges (Table XI) and geographical considerations remained for addressing the question of whether any one of our present samples represent the historical types.

Our geographically closest morphology samples are those labelled ''Lower Zambezi'' (Tables I and XI) from the wet edge of the southern delta region ($N=81$, locality 17 in Figure 4), which is adjacent to and interconnected with the south-western Pungwe River- ($N=10$, locality 16) and Buzi River Systems (locality 15). These specimens, as well as those from the Mulela System (locality 18 in the north of the delta), showed a median SPc count of 16 as observed in the three type material fish where these scales are still present (Table XI). Medians for nA and nD were slightly lower than in the types; however, ranges are overlapping. Comparisons of means and ranges yielded acceptable agreement between the Lower Zambezi sample and the types for most mensural characters, only in CPD/CPL the upper limit in types exceeded anything seen in any other sample of the present study. This may be related to the big difference in body size (mean SL 23.5 ± 1.4 cm for the five non-cut-up type specimens vs. 8.36 ± 0.26 cm for our 81 Lower Zambezi fish), and the possibility of allometric growth. In conclusion, based on an inspection of anatomical characters, the Lower Zambezi (also Pungwe, Buzi and Mulela) samples represent the type material quite well.

The first three Principal Components (PC1–PC3) accounted for almost two-thirds (64.3%; Table XIV) of the variation in the whole data set (as summarized in Table XI, with the Type material and the characters Na, LS_o and Chin, excluded; see below). This shows that there was considerable redundancy in the data set, and PCA was quite successful. Therefore, a multivariate analysis of variance (MANOVA) was required (McGarigal et al. 2000). For statistical reasons, we excluded all samples with less than 25 specimens from the analysis, using the specimens from the Lower Zambezi as the standard rather than the type material. As in PCA (see above), we excluded the characters Na and Chin length from all samples. Na was excluded because of the danger of measurement error for this extremely small measure, and chin length because in type material specimens, chin appendages were no longer apparent (for technical reasons of museum storage). In addition, truly standardized, objective chin length measurement appeared difficult for anatomical reasons, hence potentially unreliable. LS_o was excluded for its redundancy with LS_c.

The hypothesis of no morphological overall difference between bulldog samples from six different origins was clearly rejected by MANOVA ($F_{\geqslant 13,275} \geqslant 19.542$, $P<0.0001$; Table I). Subsequent univariate ANOVAs, testing the hypothesis of no difference between samples

Table I. Comparison of anatomical characters in the *Marcusenius macrolepidotus* species complex for samples from different origins. Multivariate analysis of variance (MANOVA). For sample sizes, see Table XI.

	PDL/SL	PAL/SL	LD/SL	LA/SL	pD/SL	CPL/SL	CPD/CPL	LS_c/HL	HL/SL	BD/SL	nD	nA	SPc
MANOVA							**<0.0001**						
ANOVA	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
post tests													
L Zambezi, Incomati	**<0.01**	**<0.01**		**<0.01**		**<0.01**	**<0.01**	**<0.01**		**<0.01**			**<0.01**
L Zambezi, U Zambezi	**<0.01**	**<0.01**		<0.05				**<0.01**	**<0.01**	<0.05	**<0.01**	**<0.01**	**<0.01**
L Zambezi, Okavango			<0.05				**<0.01**	<0.05	**<0.01**	<0.05	<0.05	**<0.01**	**<0.01**
L Zambezi, Tana	**<0.01**	**<0.01**			**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**		
Incomati, U Zambezi	**<0.01**	**<0.01**	<0.05	**<0.01**		**<0.01**	**<0.01**	**<0.01**		**<0.01**	<0.05		**<0.01**
Incomati, Okavango	**<0.01**	**<0.01**	**<0.01**	**<0.01**		**<0.01**		**<0.01**		**<0.01**			**<0.01**
Incomati, Tana	**<0.01**	**<0.01**		**<0.01**	**<0.01**		**<0.01**	**<0.01**		**<0.01**			**<0.01**
Okavango, Tana	**<0.01**	**<0.01**	**<0.01**		**<0.01**	**<0.01**				**<0.01**			**<0.01**
Okavango, U Zambezi				<0.05			**<0.01**	**<0.01**					
Tana, U Zambezi	**<0.01**	**<0.01**		**<0.01**	**<0.01**	**<0.01**	**<0.01**		**<0.01**	**<0.01**		<0.05	**<0.01**
Buzi, L Zambezi		<0.05		**<0.01**	<0.05		<0.05		**<0.01**				
Buzi, Incomati			<0.05	**<0.01**		**<0.01**				**<0.01**			**<0.01**
Buzi, Tana	**<0.01**	<0.05			**<0.01**	**<0.01**		**<0.01**		**<0.01**			
Buzi, U Zambezi	**<0.01**	**<0.01**		**<0.01**	**<0.01**		**<0.01**	**<0.01**					**<0.01**
Buzi, Okavango		**<0.01**						**<0.01**					**<0.01**

Abbreviations of anatomical characters, Material and methods. L/U Zambezi, Lower and Upper Zambezi, respectively; MANOVA *P* value: same for Wilk's Lambda, Roy's Greatest Root, Hotelling-Lawley Trace, and Pillai Trace tests. Post tests followed the Games/Howell procedure. *P* values smaller than 0.01 are displayed in bold, those smaller than 0.05 are also given to indicate possible borderline cases.

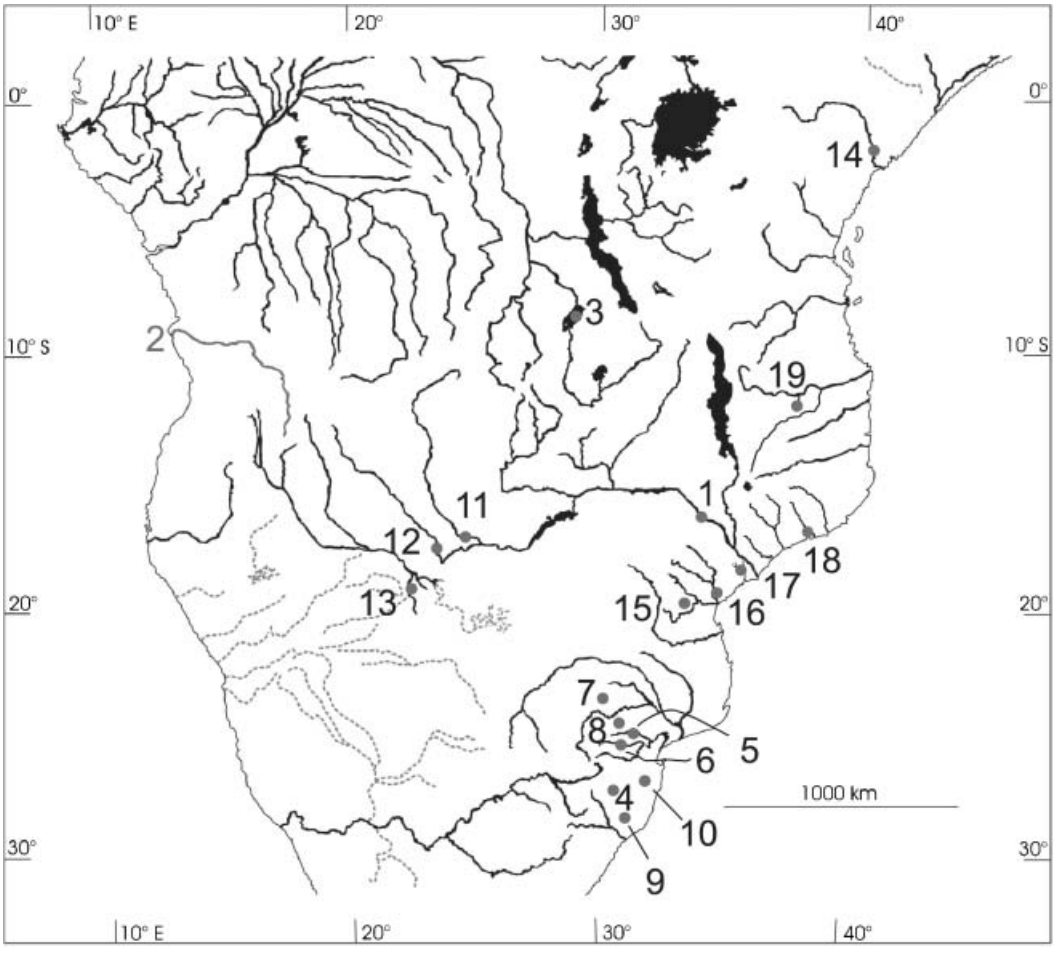

Figure 4. Partial map of southern Africa showing the sampling localities for *Marcusenius* sp. Only the larger rivers are shown. Numbers code for origins of samples: (1) Tete on the Lower Zambezi (*M. macrolepidotus*, as for types); (2) Quanza River *(G. angolensis*, type; exact sampling site unknown); (3) Lake Mweru (*G. moeruensis*, type); (4) Pongola River near Paulpietersburg (*G. pongolensis*, type); (5) Sabie River; (6) Crocodile River; (7) Groot Letaba River (Limpopo System); (8) Blyde River (Limpopo System; (9) KwaMaZulu stream, Mhlatuze System (not shown); (10) Pongola River, more downstream compared to (4); (11) Lisikili, Upper Zambezi; (12) Kwando River, (13) Okavango System; (14) Tana River; (15) Buzi River System; (16) Pungwe River; (17) Lower Zambezi, delta region; (18) Mulela River; (19) Rovuma River System.

for each character individually, yielded a similar result for all 13 characters studied: not in a single character was origin irrelevant, and significant geographical differentiation was found in each ($F_{5,283} \geqslant 5.659$, $P < 0.0001$).

PCA identified the main characters responsible for this differentiation. PC1 captured 31.5% (Table XIV) of the variation in the data set and represented a gradient for ''trunk length and depth vs tail length'', and was loaded strongest with BD, PAL, PDL, CPL, and CPD, classified as ''excellent'', and SPc as ''very good''. PC2 captured an additional 22.6% of the variation, representing a gradient for characteristics of ''fish rear section development or size''. ''Excellent'' were the loadings by pD and LD, whereas those by nD, LA, and nA were ''good''. PC3 captured an additional 10.2% of the variation, and

represented various characteristics of the fish rear section but also snout length. "Fair" were the loadings on PC3 by SPc and LA, whereas those by LS_c and CPL were only "poor". HL was the only character not significantly loading on PC1-PC3, but did so on PC4, "fair", and PC5, "excellent". As PC4 and PC5 account for 7.8 and 6.9% of the variation, respectively, HL does not seem to contribute significantly to any dominant morphological gradient present in the data sample set.

Post-hoc tests to MANOVA identified the sources of difference by comparing specific pairs of samples (Games-Howell, Table I). With so many comparisons as performed in Table I, the significance level alpha was set at a strict value of 0.01 (although P values $\leqslant 0.05$ are also shown in Table I to indicate possible borderline cases). Samples from the Buzi System differed from Lower Zambezi samples in two characters only, LA and HL (that loaded strongest on PC3 and PC5, respectively); therefore, we consider samples from the Buzi System, and also the samples from the Pungwe and Mulela Systems which are anatomically similar, as also representing *M. macrolepidotus* (confirmed by mt-DNA sequencing analysis and ISSR-PCR for Buzi River fish, see below).

With significant differences in seven characters, three of which load strongly on PC1 (PAL, PDL, SPc), Upper Zambezi samples (N=89) differed markedly from Lower Zambezi samples (N=81; Table I). Both populations show a distinctive distribution in a plot of the first three principal components (Figure 5). Therefore, morphology supports Upper Zambezi samples as representing a taxon distinct from *M. macrolepidotus* (the new species, *M. altisambesi*). Because Upper Zambezi samples differed significantly from those of the adjacent Okavango in only two characters, CPD and LS, we consider Okavango

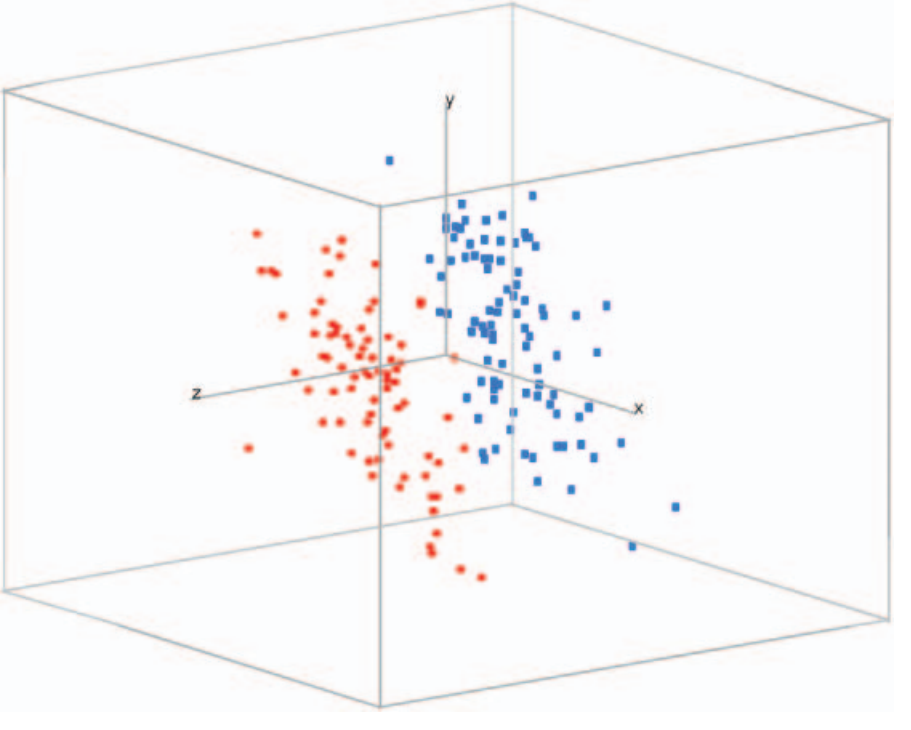

Figure 5. Principal component analysis for anatomy of Lower (red dots) and Upper (blue squares) Zambezi bulldog fish. X, Y, Z axes for PC1, PC2 and PC3, respectively. [Colour online.]

samples as, anatomically, also representing *M. altisambesi* (*M. altisambesi* confirmed by mt-DNA sequencing analysis and ISSR-PCR, see below).

Between samples from the East African Tana River (Kenya) and from the Lower Zambezi River there were nine significant anatomical differences, five of which load strongest on PC1 (PAL, PDL, CPL, CPD, BD). The first three Principal Components separated the populations well, with a small region of overlap remaining. When the character SLS was added as a variable it was found to be loading strongly on PC1, and separation between origins was complete (Figure 6). Together with the clear (M)ANOVA result (Table I), we recognize this as anatomical support for a new species, *M. devosi* (*M. devosi* was also confirmed by mt-DNA sequencing analysis and ISSR-PCR, and EOD analysis, see below). Tana samples also differed significantly from Upper Zambezi samples in nine, and from Incomati samples in eight characters, confirming their independent status (Table I).

The present status of the South African nominal species *G. pongolensis* Fowler 1934 is that of a relegated but available species (relegated by synonymisation with *M. macrolepidotus*, Crass 1960). The first three principal components separated the Pongola specimens from the Lower Zambezi specimens (with the two specimens from the nearby, about 44 km, Nkanini stream included; Figure 7). Using MANOVA we also tested the justification of the synonymization, using the Incomati System sample of bulldogs for its greater sample size of $N=32$. The Incomati is the immediate northern neighbour of the

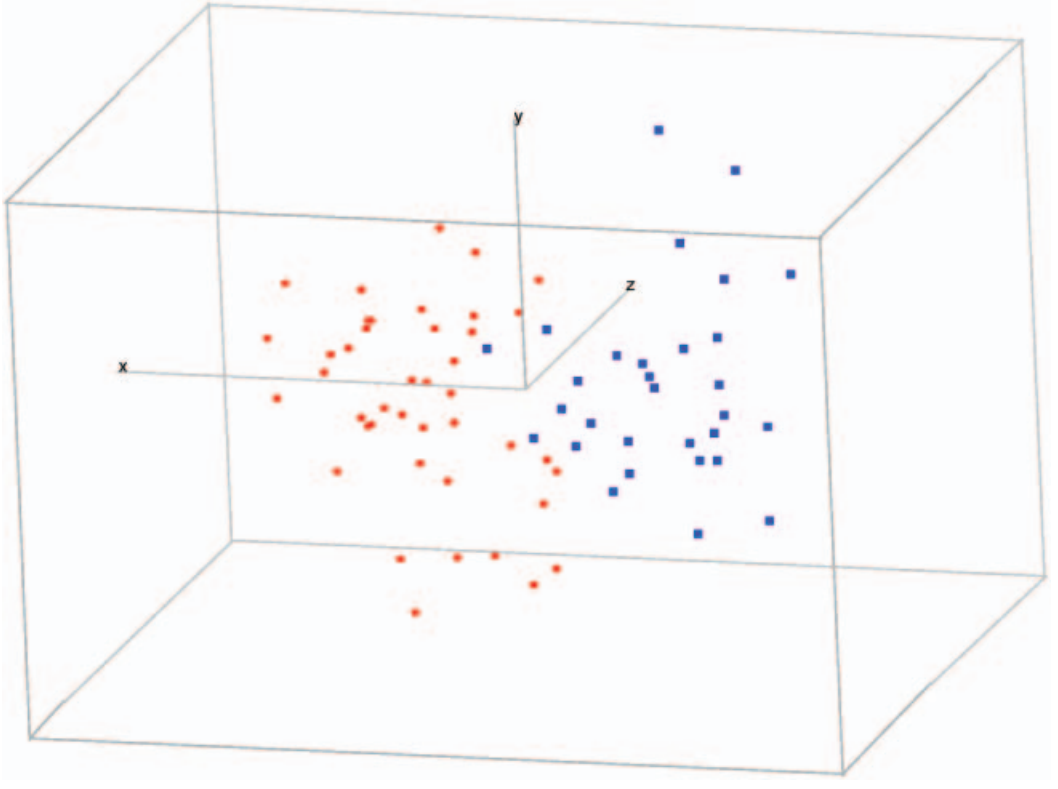

Figure 6. Principal component analysis as for Figure 5, but for Lower Zambezi (red dots, $N=42$) and Tana River (blue squares, $N=30$) specimens, and SLS included as a character. [Colour online.]

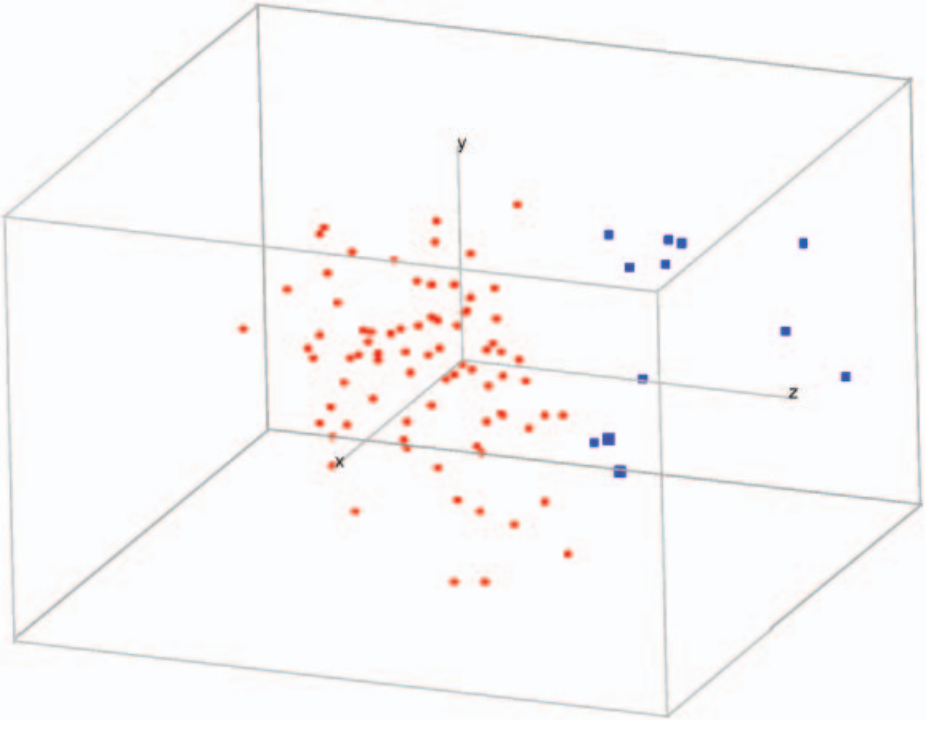

Figure 7. Principle component analysis as for Figure 5, but for Lower Zambezi (red dots) and Pongola River (blue squares) bulldog fish. The two emphasized blue squares are two fish from the Nkanini stream, independent from but close to the Pongola. [Colour online.]

Pongola (Figure 8), and the two samples are not separated by PCA (not shown). Eight significant differences between samples from the Lower Zambezi and the Incomati demonstrated clear anatomical differentiation, supporting a South African taxon distinct from *M. macrolepidotus* (Table I). This is also corroborated by eight significant differences from Upper Zambezi (as well as Okavango) samples. Similar differentiation from Lower Zambezi specimens should also hold for the systems to the south of the Pongola, such as the Mhlatuze (which is the extreme, southernmost locality for bulldogs, and for which three-dimensional PC1–PC3 plots revealed complete separation from Lower Zambezi specimens; not shown). These systems are still more distant and isolated from the type locality of *M. macrolepidotus*, the Lower Zambezi, than the Incomati (Figure 8). However, for the systems north of the Incomati that are closer to the Lower Zambezi, such as the major and independent Limpopo System, differentiation might be lacking.

Therefore, we tested by MANOVA whether or not the other South African samples (from the Limpopo, Pongola, and Mhlatuze rivers) corresponded to the Incomati samples. Because our South African sample sizes (except that from the Incomati) were rather small we ran a separate analysis exclusively for the South African samples to keep sample size differences at a more similar level.

The overall MANOVA result rejected the hypothesis of no differentiation among origins ($F_{\geqslant 13,45} \geqslant 6.866$, $P < 0.0001$), and this was supported by significant univariate ANOVA results for nine characters ($F_{3,55} \geqslant 5.869$, $P \leqslant 0.0015$; Table II). Pairwise comparisons between origins identified differentiation in two or three characters for all pairs, except for

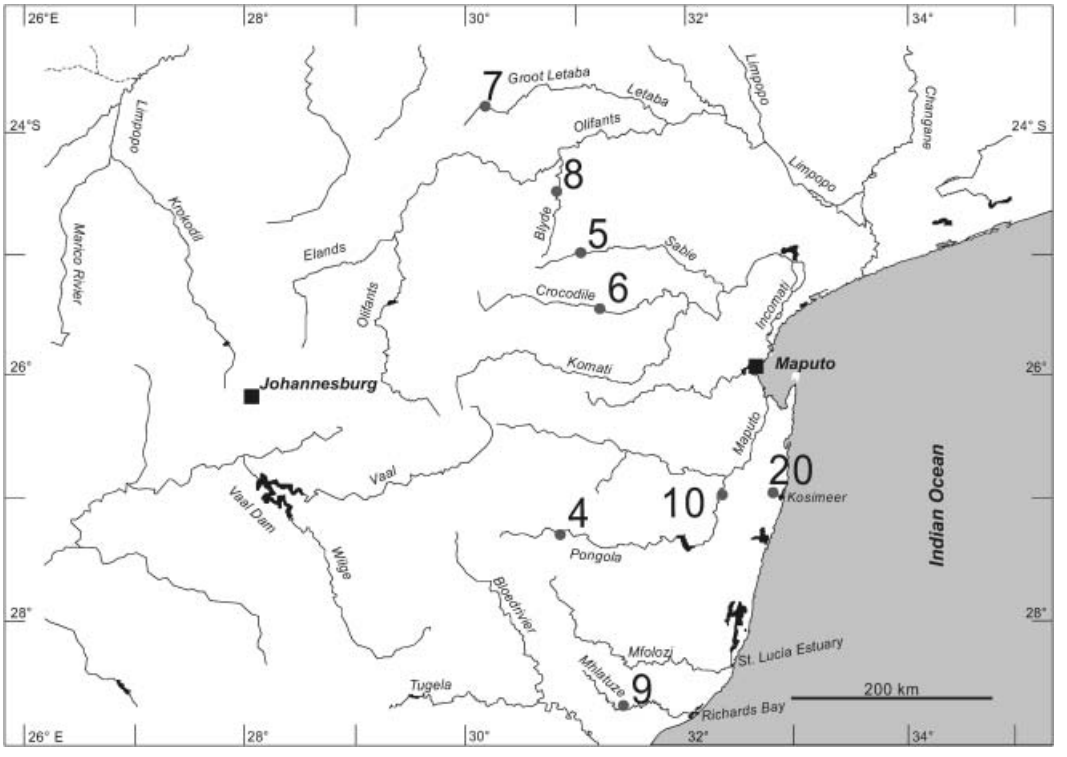

Figure 8. Map of sampling locations in South Africa. Numbers explained in Figure 3, except no. (20) which represents two samples from the Nswananzi River: Nkanini stream, KwaZulu-Natal.

the pair Pongola/Limpopo which differed in five characters (LA, CPD, BD, nD and nA). This rather strong contrast between Pongola and Limpopo was confirmed by PCA that separated the two samples into non-overlapping spaces when plotting PC1–PC3 (however, sample sizes were small). The difference between these two samples was bridged by the geographically intermediate Incomati samples which differed from the Pongola samples in two characters (CPD and BD), and from the Limpopo samples in three other characters (CPL, nA, and SPc; three-dimensional PCA separation not successful, in spite of a tendency for segregation of points). Therefore, with the possible exception of the Limpopo samples, for all other South African samples morphology supports infrasubspecific, allopatric variation within a single species that is distinct from and south to *M. macrolepidotus*, the resurrected species *M. pongolensis* (*M. pongolensis* supported by ISSR-PCR including Mhlatuze, Incomati and Pongola samples, see below). The Limpopo samples differed clearly from Lower Zambezi samples in LD, LA, CPD, and BD, all of which are lower, and CPL and SPc which are higher. In a three-dimensional plot of PC1–PC3 the clouds of points did not overlap (not shown). To clearly ascertain the status of Limpopo samples more specimens are needed.

Samples from the Rovuma River System, which is geographically intermediate between the Tana River and the Lower Zambezi, are anatomically close to *M. devosi* (Table XI). Rovuma samples differed significantly ($P<0.01$) from Lower Zambezi samples in seven characters (PD, PAL, pD, CPL, CPD, nD, nA) but only in two characters from Tana samples (CPD, BD), as revealed by a separate MANOVA (not shown). Three-dimensional

Table II. Comparison of anatomical characters in the *Marcusenius pongolensis* complex for samples from different South African origins. Multivariate analysis of variance (MANOVA). *P* values in the body of the table not shown when >0.01. For sample sizes, see Table XI.

	PDL/SL	PAL/SL	LD/SL	LA/SL	pD/SL	CPL/SL	CPD/CPL	LS_c/HL	HL/SL	BD/SL	nD	nA	SPc
MANOVA							**<0.0001**						
ANOVA			**0.0015**	**<0.0001**		**0.0003**	**<0.0001**	**<0.0001**		**<0.0001**	**0.0006**	**<0.0001**	**<0.0001**
Post tests													
Pongola, Limpopo				**<0.01**			**<0.01**			**<0.01**	**<0.01**	**<0.01**	
Pongola, Incomati							**<0.01**			**<0.01**			
Pongola, KwaMaZulu				**<0.01**				**<0.01**		**<0.01**			
Limpopo, Incomati						**<0.01**						**<0.01**	**<0.01**
Limpopo, KwaMaZulu						**<0.01**		**<0.01**				**<0.01**	
Incomati, KwaMazulu								**<0.01**					**<0.01**

Abbreviations of anatomical characters, Material and methods. MANOVA *P* value: same for Wilk's Lambda, Roy's Greatest Root, Hotelling-Lawley Trace, and Pillai Trace tests. Post tests followed the Games/Howell procedure. Bold values indicate.

PCA plots did not achieve complete separation for neither comparison with Rovuma specimens. (ISSR-PCR revealed an independent, intermediate position for Rovuma fish, see below.)

We estimated the number of scales in lateral series (SLS) in some groups but not always for all individuals (Table XI). In a comparison of specimens from the Pongola, Tana, Upper Zambezi, Okavango and Lower Zambezi Rivers, SLS depended significantly on origin (ANOVA $F_{4,200} = 211$, $P<0.0001$). Pongola specimens ($N=12$, the two Nswananzi individuals included) showed the highest number (a median of 73), and Upper Zambezi and Okavango specimens the lowest (54 and 53, respectively). (Note the big difference in scale size on photographs, Figures 1K,J vs. M). Nine out of the 10 pairwise comparisons that were possible yielded significant differences (all $P<0.01$, Student's t; except Upper Zambezi vs Lower Zambezi: $P<0.05$), with variances not significantly different. Not significant was the comparison of Okavango with Upper Zambezi. There was no dependency of the number of scales on SL (least-squares regressions, all not significant). SLS of Tana individuals was intermediate between the extremes (62.5). SLS counts in specimens from the other South African locations (Mhlatuze/KwaMaZulu, Incomati and Limpopo) were similar to those in Pongola specimens.

After finishing the study, the questions of allopatric differentiation of eye diameter and pectoral fin length were brought up, and samples of about 10 specimens from different origins compared for orientation; unfortunately, the Lower Zambezi samples were no longer available for study (Table XI). Both eye diameter ED/HL and pectoral fin length PFL/HL depended significantly on origin when Pongola ($N=10$), Okavango ($N=10$), Upper Zambezi ($N=10$) and Tana ($N=11$) samples were compared (ED/HL: ANOVA $F_{3,37} = 4.926$, $P=0.0056$; PFL/HL: $F_{3,37} = 20.80$, $P<0.001$). *Post hoc* tests revealed that Tana samples had the longest pectoral fins, significantly longer than those of Pongola and Okavango samples ($P<0.01$, Games/Howell test) and Upper Zambezi samples ($P<0.05$), but the smallest eye diameter, significantly smaller than Pongola samples ($P<0.01$) and Upper Zambezi samples ($P<0.05$). Surprisingly, pectoral fins of Okavango samples were shorter than those of Upper Zambezi samples ($P<0.05$).

Electric organ discharges

All members of the genus *Marcusenius* that have been studied, including those from distant West Africa, display an EOD of simple waveform consisting of two phases, a head-positive *P* phase that is followed by a head-negative *N* phase (e.g. Scheffel and Kramer 1997). This was also recorded in all members of the *Marcusenius* species complex that we have been able to study (Figure 3).

At present we have not been able to record EODs from bulldogs sampled from the type locality, the Lower Zambezi River. The closest are EODs from five live specimens from the Buzi River (Figure 3) which our anatomical comparison (Table I), confirmed by molecular DNA sequence analyses (Figures 9 and 10), have identified as *M. macrolepidotus* (Peters, 1852). We, therefore, use the Buzi sample EODs as the standard against which to compare the other samples.

Female and juvenile EODs. When they arrived at our laboratory, the five live Buzi bulldogs studied were juveniles of SL 7.9–8.8 cm. We compared them with juveniles and females from the other samples in the present study (for all bulldogs studied up to now, female and juvenile EODs go together).

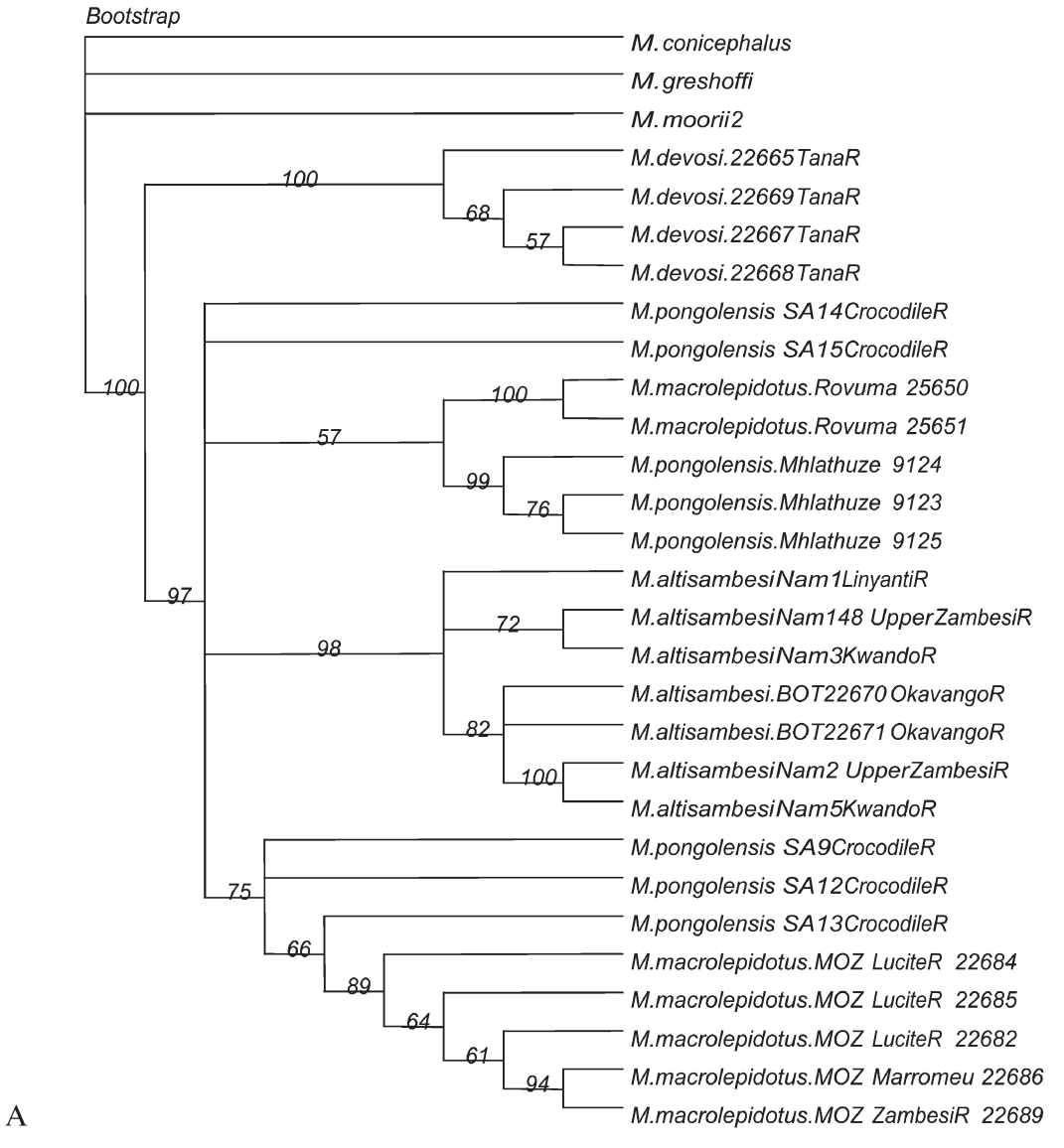

Figure 9. Reconstruction of the molecular phylogeny of the *M. macrolepidotus* complex based on cyt b sequences using *Marcusenius conicephalus, M. greshoffi and M. moorii* as outgroups. (A) Neighbour Joining (NJ) analysis: numbers at each furcation in the cladogram are bootstrap values (10 000 replicates) using uncorrected Kimura-2 distances. (B) Maximum Parsimony (MP) analysis. Strict consensus cladogram of 610 most parsimonious trees (length 457 steps, CI=0.781, HI=0.219, RI=0.802). (C) Maximum Likelihood (ML) analysis.

Means for EOD variables such as Namp, Pdur, Ndur etc. varied between samples of different origins (Table III). Female and juvenile bulldogs originating from the Upper Zambezi and the Okavango Systems all showed an initial EOD head-negativity of miniature amplitude that was not present in the EOD of any of the Buzi, South African, or Tana River

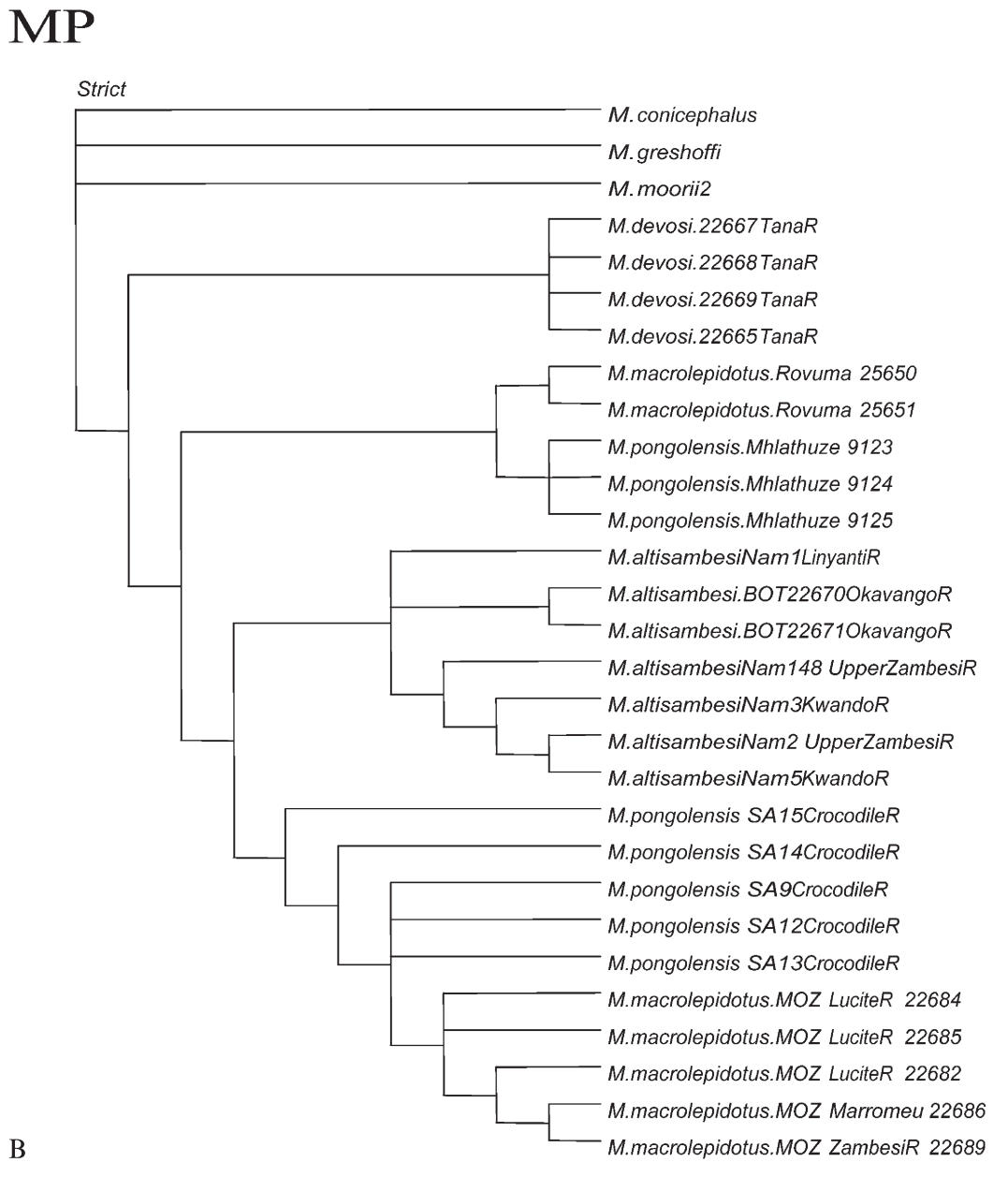

Figure 9. (*Continued.*)

bulldogs (Figure 3). This supports the suspicion that bulldogs from the Upper Zambezi/Okavango Systems represent a taxon distinct from *M. macrolepidotus*. Buzi EODs differed from their southern Incomati neighbours' EODs by their much longer Ndur, and from their northern Tana neighbours' EODs additionally in all other EOD variables except Narea. In samples from the Incomati, two EOD variables depended on SL (Namp decreased and PNsep increased with SL). Pdur was fairly similar in all samples except those from Tana whose highest values were the only ones to depend on SL (an increase). In order

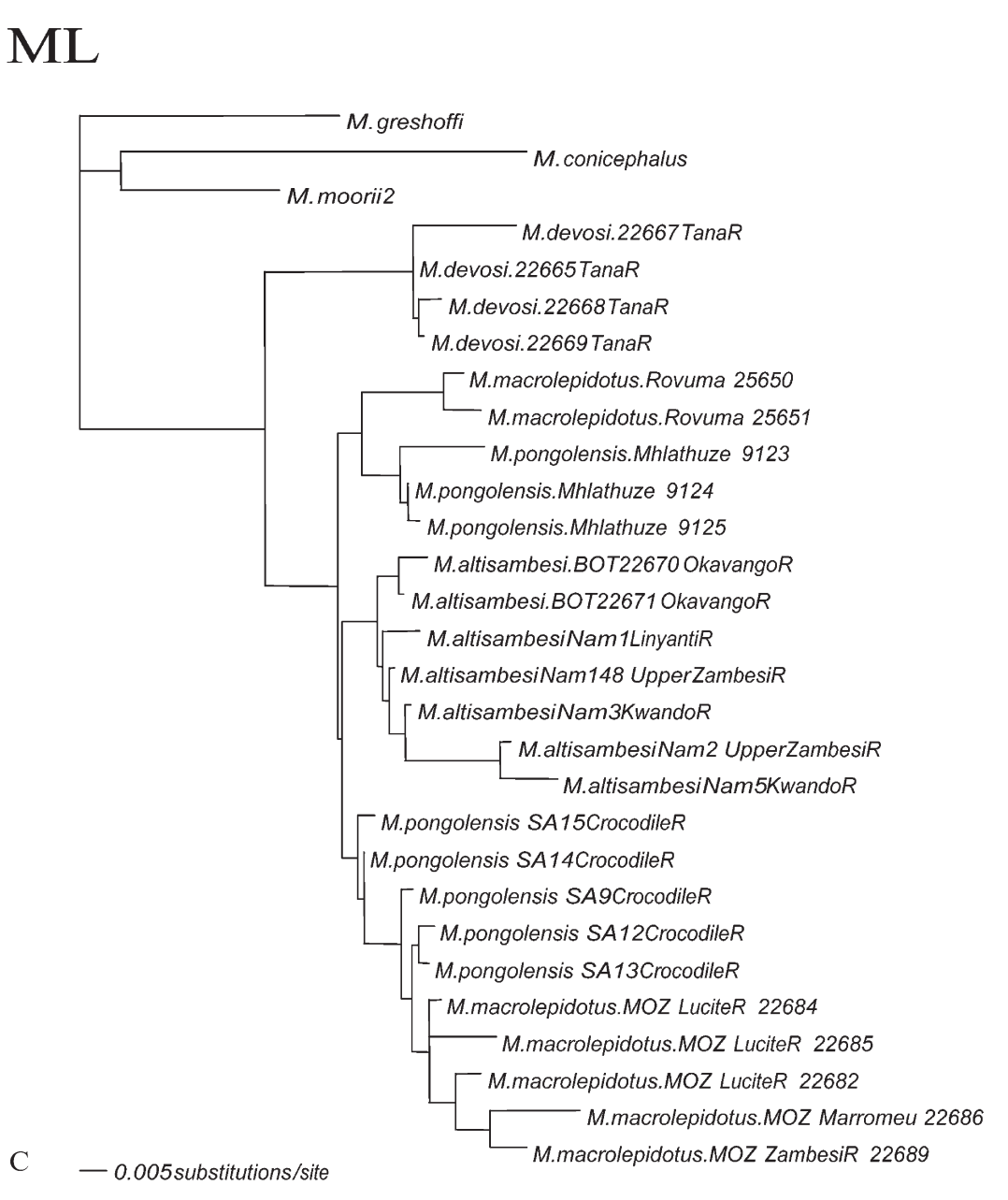

Figure 9. (*Continued.*)

to better ascertain the differentiation in EOD waveform characteristics between our samples from different origins, we used a multivariate analysis of covariance that included SL as a regressor (or covariate), and origin as a factor (MANCOVA). We excluded Pdur since it caused significant interaction between origin and SL.

The null hypothesis of no difference between EOD waveforms (as characterized by the remaining five dependent variables taken together; Table IV) from samples of different origins was clearly rejected by MANCOVA ($F_{\geqslant 5,147} \geqslant 7.684$, $P < 0.0001$). Subsequent

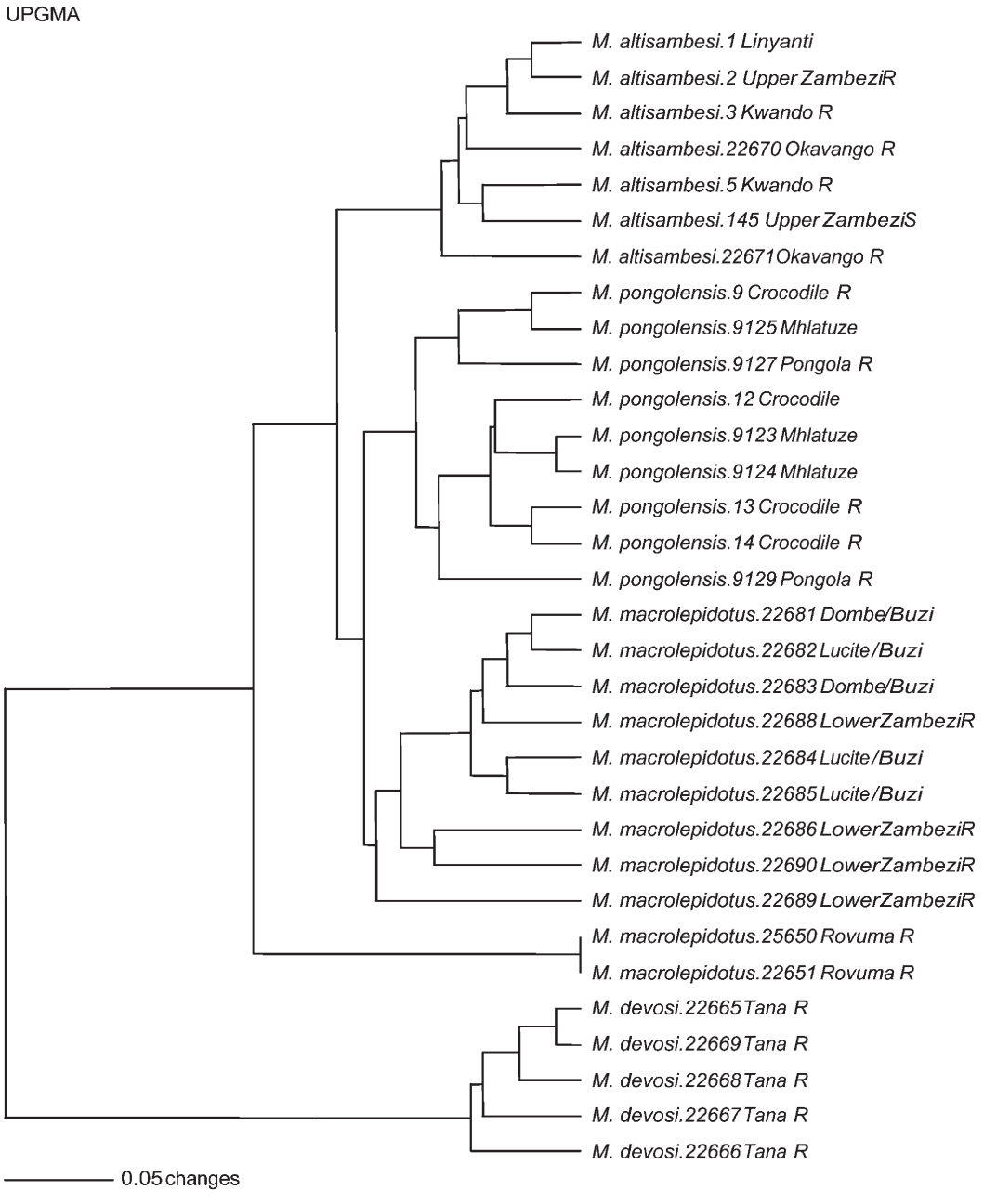

Figure 10. Analysis of genomic fingerprinting (ISSR PCR) in the *M. macrolepidotus* complex. Illustration as a UPGMA cladogram. Sample nos 9127 and 9129 from the Pongola River, type river for *M. pongolensis* (a third specimen's DNA was degraded too much for analysis).

univariate ANCOVAs pointed out specific variables in which the null hypothesis of no difference between samples was rejected; this was the case for all five variables ($F_{4,148} \geqslant 3.427$, $P \leqslant 0.0104$). *Post hoc* tests identified which pairwise between-sample comparisons for specific variables were causing significant ANCOVA results.

Table III. EOD waveform characters in females and juveniles of the *Marcusenius macrolepidotus* species complex for samples from different origins. Descriptive statistics and least-squares regression of EOD waveform variables with fish standard length. Parameters for regression only shown where significant.

Origin of samples	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)	SL (cm)
Buzi (N=5)							
Mean/Median*	−1.097	182.78	173.76	86.38	80.84	90.18	8.11*
SE/SIQ*	0.019	2.629	4.869	3.512	1.743	2.333	0.44*
size range							7.9–8.8
Incomati (N=36)							
Mean/Median*	−1.174	177.82	140.27	78.93	73.99	83.52	10.41*
SE/SIQ*	0.012	2.533	2.746	1.594	0.971	1.234	2.68*
r	0.656			0.345			
slope	0.012			0.848			
SE	2			0.396			
Y-icpt	−1.296			70.332			
SE	0.026			4.29			
P(slope)	<0.0001			0.0394			4.9–20
Size range							
Upper Zambezi: Summer, N=47 (Winter, N=22)							
Mean/Median*	−1.091	183.03	203.843	96.049	81.809	102.828	12* (11.3)
	(1.149)	(179.3)	(160.9)	(78.5)	(78.7)	(85.54)	
SE/SIQ*	0.022	3.585	14.761	3.828	2.56	4.155	0.79*
	(0.02)	(2.727)	(4.419)	(1.752)	(1.15)	(1.283)	(1.9)
Size range							10.1–15.7
							(7.2–13.1)
Okavango (N=28)							
Mean/Median*	−1.192	184.104	165.9	90.004	81.386	99.121	9.96*
SE/SIQ*	0.018	1.727	5.62	2.969	0.906	2.234	0.57*
Size range							7.1–16.9
Tana (N= 16)							
Mean/Median*	−1.368	235.219	141.119	75.9	88.75	90.925	11.13*
SE/SIQ*	0.018	5.125	5.101	2.279	1.705	2.344	0.57*
r		0.589					
Slope		19.507					
SE		7.145					
Y-icpt		17.494					
SE		79.857					
P(slope)		0.0163					
Size range							10.1–12

Abbreviations of EOD waveform characters, Material and methods. P(slope) given where least-squares regression of waveform variable with SL significant; Y-icpt, Y-intercept of a regression line. SE, standard error; r, Pearson correlation coefficient. SIQ, semi-inter-quartile range. For Upper Zambezi, winter sample N=22 in parentheses (different individuals). * Median and SIQ (semi-interquartiles) for SL only.

In spite of an extremely low N of only five for Buzi samples, Tana samples showed significantly (P<0.01) stronger Namp scores (a mean 1.37, strongest observed for all samples of the present study, vs. 1.1), and shorter Ndur (together with Incomati shortest, 141 μs vs. 174 μs). Two additional between-samples comparisons suggest that the degree of differentiation thus determined would increase with Buzi sample size (the comparisons of Buzi with Incomati, P<0.01 for Ndur, and Buzi with Okavango samples, P<0.05, for Namp).

The question remains to what extent are the four non-Buzi samples differentiated amongst each other. With sample sizes ranging from N=16 (Tana) to N=69 (Upper

Table IV. Comparison of EOD waveform characters in females and juveniles of the *Marcusenius macrolepidotus* species complex for samples from different origins. Multivariate analysis of covariance (MANCOVA).

	Namp (V)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)
MANCOVA			**<0.0001**		
ANCOVA	**<0.0001**	**0.0036**	**0.0104**	**0.0006**	**0.008**
post tests					
Buzi, Incomati		**<0.01**			
Buzi, U Zambezi					
Buzi, Okavango	<0.05				
Buzi, Tana	**<0.01**	**<0.01**		<0.05	
Incomati, U Zambezi	<0.05	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Incomati, Okavango		**<0.01**	<0.05	**<0.01**	**<0.01**
U Zambezi, Okavango	**<0.01**				
Incomati, Tana	**<0.01**			**<0.01**	
U Zambezi, Tana	**<0.01**	**<0.01**	**<0.01**	<0.05	
Okavango, Tana	**<0.01**	<0.05	**<0.01**	**<0.01**	

Abbreviations of EOD waveform characters, Material and methods. MANCOVA *P* value: same for Wilk's Lambda, Roy's Greatest Root, Hotelling-Lawley Trace, and Pillai Trace tests. Post tests followed the Games/Howell procedure. *P* values smaller than 0.01 are displayed in bold, those smaller than 0.05 are also given to indicate possible borderline cases.

Zambezi), there is little doubt about strong differentiation between (1) both Okavango and Upper Zambezi samples from Tana samples (three variables), (2) Incomati from Tana samples (two variables, plus greatest difference in Pdur, see above), (3) Incomati from both Upper Zambezi and Okavango samples (in all of which $P \leqslant 0.01$ in 3–4 EOD variables, sometimes plus one or two for which $P \leqslant 0.05$). Okavango samples differed from Upper Zambezi samples in a single variable, Namp (a mean 1.19 vs. 1.09), with no additional differences at a weaker significance level. This difference vanished in fish from the Upper Zambezi in the dry and cool "winter" season (1.149, $N=22$). The higher "summer" average in Upper Zambezi fish was due to a few outliers (Figure 11B); apparently, the seasonal release of sexual hormones affected the EOD waveform of certain females although the majority of individuals showed very little difference between seasons. EOD variables for Upper Zambezi females did not differ significantly between "summer" and "winter" (Table III; MANOVA test for samples of $N=47$, "summer", against $N=22$, "winter", not significant; not shown).

In conclusion, none of the present samples corresponds to the standard represented by Buzi fish; therefore, all non-Buzi samples appear to represent taxa different from *M. macrolepidotus* (Peters, 1852). In addition, differentiation as determined here confirms separation east (*M. macrolepidotus*) from west (*M. altisambesi*); differentiation also increases with distance along the coastline of the Indian Ocean, as apparent from three distinct taxa between Kenya and South Africa, corresponding to and supporting *M. devosi*, *M. macrolepidotus*, and *M. pongolensis*.

Male EODs. Males recognisable by a clear kink in the anal fin were present among our Upper Zambezi, South African, Okavango and Tana samples. The sex of some of these males was confirmed by gonad histology.

The four samples of males clearly differed from each other in EOD waveform. Male EODs from South Africa, the Okavango and Tana samples resembled their female/juvenile EODs, respectively (Figure 3). For example, the EODs of male Okavango bulldogs showed a leading head-negativity of miniature amplitude, whereas South African and Tana males,

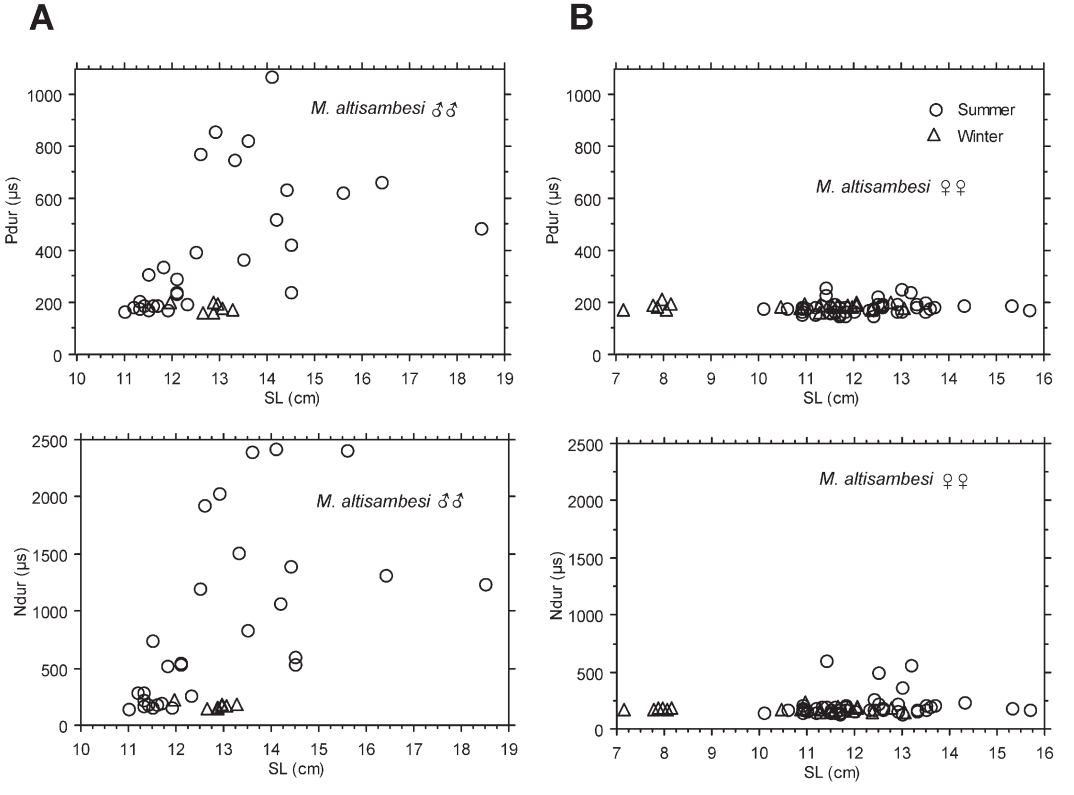

Figure 11. EOD waveform variables Pdur and Ndur for *M. altisambesi* as a function of SL. (A) males, (B) females. Each point is one fish. Dots for fish caught in ''summer'', triangles in ''winter''.

just like their respective females, did not. Bulldogs from the Upper Zambezi and Kwando Rivers (collectively referred to as Upper Zambezi System bulldogs because there was no difference between them) showed a spectacular sexual dimorphism (Kramer 1997a, 1997b) that was, however, limited to the reproductive season. Each one of the eight males of sufficient size for sexual maturity that were sampled in ''winter'' showed a discharge of short, female duration (Table V, Figure 11), in addition featuring the leading head-negative potential of miniature amplitude that was not present in the male ''reproductive discharge'' of long duration (Figure 3).

Between the sexes, the EOD waveforms of Okavango bulldogs did not show any substantial difference (MANCOVA, as well as univariate ANCOVAS, not significant; not shown) and very little tendency for change with SL (Tables III and V). There was no seasonal growth in EOD duration in the male sex (little difference between nine ''summer'' males >14.1 cm SL and one ''winter'' male of 13.5 cm SL). This is in sharp contrast to Upper Zambezi ''summer'' bulldogs the males of which showed spectacular, sigmoidal growth with SL for most variables on turning sexually mature at about 12.5 cm SL (except for a linear decrease of Namp with SL, also starting at maturity; Kramer 1997b).

In Incomati samples both female and male EODs showed a very consistent decrease with SL for Namp, and weak increase for PNsep in females (Table III) and Ndur in males (Table V). Incomati males showed consistently higher values than females for Pdur, Ndur and their corresponding ''area'' measures (MANCOVA and the four ANCOVAS, all significant; Table VI). After excluding two male outliers (that ''spoiled'' regression statistics

Table V. EOD waveform characters in males of the *Marcusenius macrolepidotus* species complex for samples from different origins. Basic statistics and least-squares regression of EOD waveform parameters with fish standard length. Parameters for regression not shown when not significant, or nonlinear.

Origin of samples	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)	SL (cm)
Incomati (N=33)							
Mean/Median*	−1.052 (1.061)	216.1 (207.39)	214.01 (198.11)	107.74 (99.37)	97.3 (92.1)	105.07 (99.765)	18.3*
SE/SIQ	0.021 (0.022)	7.27 (4.19)	14.27 (9.601)	7.13 (4.36)	4.44 (2.68)	4.49 (2.705)	5.65 (5.81)*
r	0.677 (0.757)	−0.623	0.376	−0.73	−0.77	−0.711	
Slope	0.012		4.609				
SE	0.002		2.037				
Y-icpt	−1.273		131.7				
SE	0.046		38.78				
P(slope)	<0.0001	−0.0002	0.0308 (<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	
Size range							5.5–27.5
Upper Zambezi: Summer, N=30 (Winter, N=8)							
Mean/Median*	−0.866 (1.11)	402.4 (176.04)	867.31 (158.1)	286.43 (78.1)	221.6 (78)	295.03 (82.3)	12.2 (13)*
SE/SIQ*	0.036 (0.024)	47.04 (5.7)	136.68(9.4)	40.354 (1.63)	31.21 (1.9)	45.07 (1.94)	1.3 (0.125)*
r	0.377	Summer: sigm	Summer: sigm	Summer: sigm	Summer:sigm	Summer: sigm sigmoidal	
Slope	0.042						
SE	0.019						
Y-icpt	−1.406						
SE	0.253						
P(slope)	0.0401						
Size range							11–18.5(12–13.3)
Okavango (N=10)							
Mean/Median*	−1.06	187.61	216.16	111.9	83.5	109.82	14.45*
SE/SIQ*	0.069	4.08	20.75	11	2.753	6.45	1.2*
Size range							13.5–18.1
Tana (N=5)							
Mean/Median*	−1.385	231.88	140.9	75.68	89.98	93.04	10.7*
SE/SIQ*	0.014	6.54	4.36	2.591	2.56	3.9	0.31*
r	0.953						
Slope	0.069						
SE	0.013						
Y-icpt	−2.136						
SE	0.139						
P(slope)	0.0123						
Size range							10.4–11.5

Abbreviations of EOD waveform characters, Material and methods. *P*(slope) given where least-squares regression of waveform variable with SL significant; Y-icpt, Y-intercept of a regression line. SE, standard error; r, Pearson correlation coefficient. SIQ, semi-inter-quartile range. Incomati sample: values in parentheses, for a sample size of N=31 (after exclusion of two outlier specimens, 8Sabi SL=12.3 cm, and 12Sabi SL=17.1 cm). For Upper Zambezi, winter sample N=8 in parentheses (different individuals). *Median and SIQ (semi-interquartiles) for SL only.

Table VI. Comparison of EOD waveform characters in males vs. females and juveniles of *Marcusenius pongolensis* for samples of Incomati origin. Multivariate analysis of covariance (MANCOVA). For sample sizes and other statistics, see Tables III and V. *P* values in the body of the table not shown when >0.05.

	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)
MANCOVA				0.0003		
ANCOVA		0.001	0.0072		0.0033	0.0059

Abbreviations of EOD waveform characters, Material and methods. MANCOVA $F_{6,61}=5.008$, *P* value: same for Wilk's Lambda, Roy's Greatest Root, Hotelling-Lawley Trace, and Pillai Trace tests. ANCOVA $F_{1,66}\geqslant 7.687$.

by their exceedingly high values compared to the group, shown in Figure 12), consistent and strong increase with SL in all duration and "area" measures emerged for males (correlation coefficients >0.7 except for Pdur where $r=0.62$, given in parentheses in Table V; full detail, see Table X). In two males breeding spontaneously and successfully in our laboratory, with no experimental treatment whatsoever (such as hormone administration, slow decrease of water conductivity, etc.), we observed a marked increase of EOD duration at the time of spawning that receded when spawning was over (Werneyer and Kramer 2005, their Figure 6, which was the second such observation in our laboratory). In both cases young were reared successfully. For its great behavioural and systematic significance the first observation is given in Appendix C in some detail.

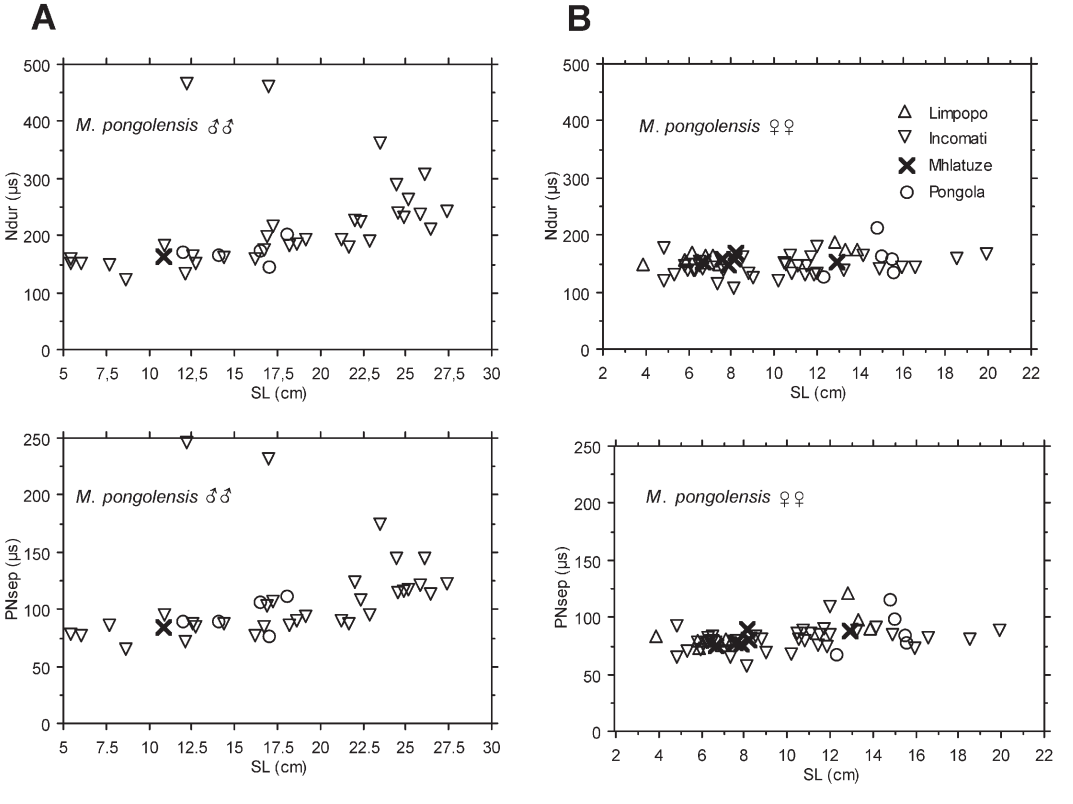

Figure 12. EOD waveform variables Ndur and PNsep for *M. pongolensis* of different origins, as a function of SL. (A) males, (B) females. Each point is one fish (shown are also the two male outliers that were excluded from regression analysis). Note rising tendency with SL and higher values in males, and weak contrasts between origins in both sexes.

There were only five recognizable Tana males; their EODs were similar to their female/juveniles' EODs, except that in males there was a significant decrease of Namp with SL whereas in females Pdur increased with SL. These differences between the sexes in Tana fish, in spite of each being statistically significant ($P<0.02$), should not be considered definitive because of the small size range and small number of males studied.

A univariate ANCOVA for Namp (with SL as a regressor and origin as a factor; not shown) rejected the null hypothesis of no difference between the four samples of male bulldogs (Incomati, Upper Zambezi in ''summer'', Okavango, and Tana; $F_{3,73} = 22.129$, $P<0.0001$). Among the six possible pairwise comparisons for origin, only two did not reveal a significant ($P<0.01$) difference (for Okavango samples compared with Upper Zambezi samples, and Incomati samples with Okavango samples; $P>0.05$, Games/Howell *post hoc* tests). For a multivariate study of the differentiation between all male waveform variables combined (MANCOVA), the Upper Zambezi ''summer'' males had to be excluded because they caused significant interaction of the regressor SL with the factor origin for all EOD parameters except Namp. With only Upper Zambezi ''winter'' males included, preconditions for MANCOVA were met.

The hypothesis that between Incomati, Okavango, Upper Zambezi and Tana bulldog males there are no differences in EOD waveform variables was rejected by MANCOVA ($F_{\geqslant 6,48} \geqslant 8.453$, $P<0.0001$; Table VII). This result was due to significant differences between origins for Namp but also Pdur (ANCOVAs, $F_{3,51} \geqslant 4.625$, $P \leqslant 0.0062$, Table VII). For all pairwise comparisons that were possible, one or even both variables differed significantly between origins ($P<0.01$, Games/Howell *post hoc* test; Table VII), except for the comparison between Upper Zambezi and Okavango males where there was no difference. These results support *M. altisambesi*, *M. devosi*, and *M. pongolensis* as distinct taxa. The difference between Upper Zambezi and Okavango ''summer'' males regarding the presence or absence of a sexual EOD dimorphism remains astounding but may be due to an only short expression of the exaggerated trait when reproduction readiness peaks; hence difficulty to sample at the right time.

Table VII. Comparison of EOD waveform characters in males of the *Marcusenius macrolepidotus* species complex for samples from different origins, with ''summer'' males from the Upper Zambezi excluded. Multivariate analysis of covariance (MANCOVA). For sample sizes and other statistics, see Table V. *P* values in the body of the table not shown when >0.05.

	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)
MANCOVA				<0.0001		
ANCOVA	0.0011	0.0062				
Post tests						
Incomati, Okavango		<0.01				
Incomati, Tana	<0.01					
Okavango, Tana	<0.01	<0.01				
Upper Zambezi, Okavango						
Upper Zambezi, Incomati		<0.01				
Upper Zambezi, Tana	<0.01	<0.01				

Abbreviations of EOD waveform characters, Material and methods. *P* value: same for Wilk's Lambda, Roy's Greatest Root, Hotelling-Lawley Trace, and Pillai Trace tests. Post tests followed the Games/Howell procedure.

Comparisons within South African fish

Females and juveniles. Differentiation within *M. pongolensis* EODs from different South African origins is clearly present. Female and juvenile EODs from Incomati and Limpopo both decreased in Namp and increased in PNsep with SL; in addition Ndur increased with SL in Limpopo samples (Table VIII). Not a single EOD variable was correlated with SL in Pongola and Mhlatuze samples, an observation that may change with a bigger sample size. A MANCOVA analysis still confirmed that (1) significant differentiation among the four South African origins was present in females and juveniles, (2) that differentiation regarded the variables Ndur, Parea and Narea, (3) that Limpopo samples differed significantly from Incomati samples in these three variables, whereas Mhlatuze samples showed longer Ndur scores than Incomati samples (Table IX).

Males. Comparisons among South African bulldog males was seriously limited by the absence of recognizable males in our Limpopo sample, by the presence of only one male in the Mhlatuze sample, and only five males in the Pongola sample (Table X). Only the

Table VIII. EOD waveform characters in females and juveniles of *Marcusenius pongolensis* samples from different origins. Descriptive statistics and least-squares regression of EOD waveform variables with fish standard length SL. Parameters for regression only shown where significant.

Origin of samples	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)	SL (cm)
Incomati (N=36)							
Mean/Median*	−1.174	177.82	140.27	78.93	73.99	83.52	10.41*
SE/SIQ*	0.012	2.533	2.746	1.594	0.971	1.234	2.68*
r	0.656			0.345			
Slope	0.012			0.848			
SE	0.002			0.396			
Y-icpt	−1.296			70.33			
SE	0.026			4.29			
P(slope)	<0.0001			0.0394			
Size range							4.9–20
Limpopo (N=11)							
Mean/Median*	−1.193	187.37	160.09	83.94	82.34	92.32	7*
SE/SIQ*	0.034	3.981	3.833	4.171	1.574	0.884	2.77*
r	0.857		0.783	0.739			
Slope	0.028		2.885	2.961			
SE	0.006		0.763	0.9			
Y-icpt	−1.426		136.28	59.5			
SE	0.05		6.78	8			
P(slope)	0.0008		0.0043	0.0094			
Size range							4.0–14.0
Mhlatuze (N=6)							
Mean/Median*	−1.141	182.5	157.17	81.62	79.3	88.52	7.98*
SE/SIQ*	0.021	3.627	2.86	2.457	1.604	1.74	0.31*
Size range							6.6–12.9
Pongola (N=5)							
Mean/Median*	−1.078	191.32	159.6	88.92	80.16	86.1	15*
SE/SIQ*	0.02	8.61	14.975	8.47	4.355	5.803	0.65*
Size range							12.3–15.5

Abbreviations of EOD waveform characters, Material and methods. *P(slope)* given where least-squares regression of waveform variable with SL significant; Y-icpt, Y-intercept of a regression line. SE, standard error; r, Pearson correlation coefficient. *Median and SIQ (semi-interquartiles) for SL only.

Table IX. Comparison of EOD waveform characters in females and juveniles of *Marcusenius pongolensis* for samples from different origins. Multivariate analysis of covariance (MANCOVA). For sample sizes and other statistics, see Table VIII. *P* values in the body of the table not shown when >0.05.

	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)
MANCOVA				<0.0001		
ANCOVA			0.0006		0.0006	0.0097
Post tests						
Limpopo, Incomati			<0.01		<0.01	<0.01
Limpopo, Mhlatuze						
Limpopo, Pongola						
Incomati, Mhlatuze			<0.01			
Incomati, Pongola						
Mhlatuze, Pongola						

Abbreviations of EOD waveform characters, Material and methods. MANCOVA $F_{\geqslant 6,50} \geqslant 3.671$, *P* value: same for Wilk's Lambda, Roy's Greatest Root, Hotelling-Lawley Trace, and Pillai Trace tests. ANCOVAs, $F_{3,53} \geqslant 4.197$. Post tests followed the Games/Howell procedure.

Incomati sample allowed meaningful regression analysis with $N=33$ males (two of which were outliers in all variables except Namp, and were excluded from the regression study, Table X). With this precaution, Incomati males showed significant increase with SL in all variables (a decrease for Namp). For Namp and PNsep, males therefore resembled their females which, in contrast to the males, did not show any other significant correlations with SL. Also, the males showed generally higher EOD-duration related values than the females. The inspection of scatter plots (examples, Figure 12) did not reveal any substantial differences among males from different South African origins. The single Mhlatuze male and the five Pongola males fell right among the Incomati males in all six variables.

Table X. EOD waveform characters in males of *Marcusenius pongolensis* samples from different origins. Descriptive statistics and least-squares regression of EOD waveform variables with fish standard length. Parameters for regression only shown where significant.

Origin of samples	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)	SL (cm)
Incomati ($N=31$[1])							
Mean/Median⋆	−1.061	207.39	198.11	99.37	92.15	99.765	18.7⋆
SE/SIQ⋆	0.022	4.19	9.601	4.36	2.682	2.705	5.81⋆
r	0.757	0.623	0.756	0.73	0.77	0.711	
Slope	0.013	2.125	5.91	2.588	1.682	1.567	
SE	0.002	0.496	0.951	0.45	0.259	0.288	
Y-icpt	−1.303	169.01	91.366	52.62	61.77	71.461	
SE	0.041	9.558	38.78	8.681	4.988	5.544	
P(slope)	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	
Size range							5.5–27.5
Mhlatuze ($N=1$)							
Mean	−1.072	183.6	162.9	84.3	80.4	86.8	10.3
Pongola ($N=5$)							
Mean/Median⋆	−1.06	204.42	172.16	95.08	85.48	90.28	16.43⋆
SE/SIQ⋆	0.023	3.94	9.22	6.39	2.91	3.526	1.85⋆
Size range							11.6–17.6

Abbreviations of EOD waveform characters, Material and methods. P(slope) given where least-squares regression of waveform variable with SL significant; Y-icpt, Y-intercept of a regression line. SE, standard error; r, Pearson correlation coefficient. [1]) Two outliers of double duration values excluded. ⋆ Median and SIQ (semi-interquartiles) for SL only.

Gonad histology

First sample of South African bulldogs. The first sample is from the Sabie, and fish were collected in the dry ''winter'' season (22–25 September 1993); accordingly, the histology revealed reproductively inactive ovaries, composed of oocytes of stage III (Takashima and Hibiya 1995) even in the biggest females ($N=15$). Five females were of sufficient size (112, 118, 119, 142, 150 mm SL) to become sexually mature, that is, close to or beyond 40% of the maximum species size (confirmed for *M. altisambesi*; Kramer 1997b). A few females had additional larger oocytes, but these were of irregular shape and atretic (in various states of decomposition or resorption).

For the males ($N=11$) an almost uniform picture of reproductive inactivity emerged: although there was clearly spermiogenesis occurring in some of the larger males (as documented by the presence of cysts containing cells of identical stage), there was no sperm present and almost no lumen to the canals (stage 0, similar to the juveniles among *M. altisambesi*; Kramer 1997b). The only exception was a male of 12.8 cm (our largest fish of the first sample had 17.1 cm standard length) that had a well-developed testis full of sperm. The two outlier males had completely regressed testes; their long-duration EODs (Figure 12) were, therefore, not due to reproductive readiness.

The sex of four small fish could not be determined because the gonads were too small for histology, or were not found during dissection (size range, 49–64 mm SL). These fish were included in the category ''females and juveniles Sabie 1993'' for the statistical analyses.

Second sample of South African bulldogs. The second sample was collected after the reproductive period was over in the Sabie (end of March 1996), and transported to Germany alive after EOD recordings had been taken in the field. These fish were not killed for gonad histology. Thirty-nine *Petrocephalus wesselsi* Kramer and Van der Bank 2000, the single other mormyrid of this region, were collected at the same time and place, and killed within one day of collection. Their reproductive state, as determined from gonad histology, was inactive (similar to the one described above; Kramer and Van der Bank 2000).

Third sample of South African bulldogs. Collected at the peak of the ''summer'' reproductive season, 14 February 1997; arrival in Germany, 18 February 1997. From 29 fish, five males and two females were killed on 5 March 1997. Gonad histology revealed fully reproductive status in all fish except one male (see below). The huge ovaries of the two females were ripe with eggs of the last stage (VI) of approx. 1.2 mm diameter. The testes of four males showed massive sperm and a wide lumen of their testicular canals (stage III), whereas one male (of 252 mm SL) had only little sperm and the testicular canals were almost closed. The big testis of this fish consisted largely of cysts of cells of identical stage, a clear indicator of ongoing spermiogenesis (stage II).

Molecular genetics

DNA data. DNA sequencing of southern African electric fishes was performed using seven specimens of *M. altisambesi* (five from the Upper Zambezi System and two from the Okavango), nine *M. pongolensis* (four from the Incomati, two from the Pongola, three from the Mhlatuze), 11 *M. macrolepidotus* (four from the Lower Zambezi River and five from the middle Buzi River System), and five specimens of *M. devosi.* One specimen each of the Central African congeners *M. greshoffi*, *M. moorii* and *M. conicephalus* served as outgroup (Genbank accession numbers AF201594, AF201595, and AF201593, respectively;

Sullivan et al. 2000). The alignment of nucleotides with variable and parsimony informative characters are shown in Table XII. The evaluation of 10 000 random trees (excluding the outgroup; using PAUP*) shows a substantial skewness of tree-length distribution and provides a g1 value of −3.16 indicating that the data set contains a significant ($P<0.05$) phylogenetic signal (Hillis and Huelsenbeck 1992).

The underlying phylogeny was reconstructed employing MP (strict consensus of 100 equally parsimonious trees), NJ (bootstrap consensus of 10 000 trees), and ML (phylogram) (Figure 9 A,B,C). The topology of the resulting trees was almost identical for MP and ML reconstructions. A small difference was seen in NJ reconstruction: two individuals of *M. pongolensis* (SA 14 and SA15) clustered unresolved at the base of the *M. macrolepidotus* complex.

Marcusenius altisambesi and *M. devosi* form well recognized clades in MP, ML and NJ that are supported by bootstrap values of 98 or 100% (NJ). *Marcusenius devosi* is basal to the other taxa in all reconstructions (including ISSR analysis; Figure 10). Members of the *M. macrolepidotus* clade cluster are monophyletic (89% bootstrap support), except the two individuals from Rovuma. They appear to be more isolated (and might even represent a new taxon) which can also be seen from ISSR data where they assume an intermediate position between *M. devosi* and *M. macrolepidotus* (Figure 10; anatomically, they are close to *M. devosi*, see above). All *M. macrolepidotus* specimens from the Lower Zambezi and from the Buzi River form a single clade in all reconstructions (including ISSR analysis). *Marcusenius pongolensis* is genetically heterogeneous; they do not form a consistent clade in cyt-b derived trees (Figure 9A,B,C).

The ISSR fingerprinting analysis (Figure 10) recovers five major clades, which represent the four taxa *devosi*, *pongolensis*, *altisambesi* and *macrolepidotus*. As mentioned before, the two specimens from Rovuma take an independent position. In ISSR trees, all specimens classified as *pongolensis* form a clade, which is a sister to *macrolepidotus*. *Marcusenius pongolensis* and *M. macrolepidotus* are closely related according to cyt b data (Figure 9); the inconsistent phylogeny pattern of *M. pongolensis* in cyt b trees might be due to former hybridizations with *M. macrolepidotus*, since mtDNA is inherited maternally. The ISSR method that recovers genomic DNA indicates that *M. pongolensis* represents a genetic clade of its own, also reflecting the morphological and EOD data.

Allozyme data. Allozyme comparisons of the allopatric *M. altisambesi* and *M. pongolensis* (from the Sabie River) showed no distinct genetic differences at 26 protein coding loci to indicate the existence of congeneric species (Van der Bank 1996). Subsequent to this study, these results were confirmed by H.M. Beker (Agricultural Research Counsel, Animal Improvement Institute, Irene, South Africa), using polyacrylamide gel-electrophoresis, non-specific protein dye (coomassie blue) and the same samples analysed by Van der Bank (1996).

Comparative material and species descriptions

For *Material examined*, see Appendix A.

Marcusenius macrolepidotus (Peters, 1852)
(Figures 1A, H)

Nominal species in boldface.
Mormyrus macrolepidotus Peters, 1852a, p. 275.

Mormyrops macrolepidotus: Marcusen 1864, p. 142.
Gnathonemus macrolepidotus: Boulenger 1898, p. 804.
Gnathonemus macrolepidotus macrolepidotus: Poll and Gosse 1963, p. 93.
Marcusenius macrolepidotus: Taverne 1971a, p. 103.
Gnathonemus okavangensis Pappenheim 1907, p. 354, nomen dubium.
Gnathonemus moeruensis Boulenger, 1915, p. 163; Jackson 1961, p. 32 (most probably a subspecies of *Gnathonemus macrolepidotus*) (type locality, no. 3, Figure 4).
Marcusenius moeruensis: Taverne 1971a, p. 102.
Gnathonemus graeverti Steindachner, 1914, p. 536 (in full Steindachner 1916); Matthes 1967, p. 4 (synonymy with *Gnathonemus macrolepidotus*).
Marcusenius graeverti: Taverne 1971a, p. 103.

Type specimens. ZMB 3678 (lectotype L. Seegers 13.04.1993) from Tete, Lower Zambezi, Mozambique, with paralectotypes ZMB 6730 (one specimen), 3677 (one specimen), and 32043 (three specimens; Seegers 1996).

Type locality. Mozambique: Lower Zambezi: Tete (no. 1, Figure 4), and tributaries to the Lower Zambezi, also Licuare River (Zambezi delta region).

For a type locality, Peters (1852) gives no more detail than ''riverine fishes from Mozambique'' (as per title). In his more detailed 1868 publication, Peters specifies the ''Zambezi and its tributaries'' (''… in dem Zambeze und seinen Nebenflüssen''), and on p. 83 the township of ''Tette'' (modern spelling Tete; locality 1 on Figure 4) on the Lower Zambezi, 400 km from the Indian Ocean (''Tette'' is also marked outside and inside the jars holding the type material). As an additional origin Peters (1868) gives the Licuare River which is a small coastal river flowing into the Indian Ocean at the township of Quelimane, at the northern range of the Zambezi Delta.

Since Taverne's (1971b) major revision of several genera of the Mormyridae, there are no members of *Gnathonemus* in southern Africa. Presently, two subspecies of *M. macrolepidotus* are recognized (Poll and Gosse 1963): *M. m. macrolepidotus* and *M. m. angolensis*, listed by Gosse (1984) and Eschmeyer (2006). Based on morphology (Table XI), we resurrect the latter subspecies as a valid species, and in consequence drop the former subspecies. The origin of *G. moeruensis* Boulenger 1915, a single specimen from Lake Mweru in the Democratic Republic of Congo (Zaïre)/Zambia, west of Lake Tanganyika (locality 3 on Figure 4), forms part of the Lualaba/Congo drainage system, and from morphology the synonymization appears questionable to the present authors (Table XI). Also synonymized with *G. macrolepidotus* was *G. graeverti* Steindachner 1914 whose origin is the Ruaha, an east-flowing river south of Daressalaam, Tanzania; the unique holotype is lost (from its SPc of 12 and an SLS of 69–70 as given in the original description the synonymization is questionable, and also the geographically closer *M. devosi* and Rovuma samples do not match). *Gnathonemus pongolensis* Fowler 1934 is still another relegated species based on a single specimen from the Pongola River in KwaZulu-Natal/South Africa, a species that is resurrected below (locality 4 on Figures 4 and 8). *Gnathonemus okavangensis* Pappenheim 1907 is ''not available'' (see below under the heading *M. altisambesi*).

Diagnosis. Marcusenius macrolepidotus is best distinguished from *M. altisambesi* by its median SPc of 16 (12–18) vs. 12 (12–14), and EOD lacking an initial head-negativity; from *M. devosi* by a median SLS of 55.5 (52–62) vs. 62.5 (56–66), shorter CPL (mean 18.8% vs.

20.2% of SL), and weaker Namp of its EOD (mean $-1.1 \pm$SE 0.019 normalized volts vs. $-1.39 \pm$SE 0.023 V; from *M. pongolensis* by a median SLS of 55.5 (52–62) vs. 73 (70–76), a median SPc of 16 (12–18) vs. 18.5 (16–20), greater BD of 26.5% vs. 24.7% of SL, longer Ndur of its EOD in females and juveniles ($174 \pm$SE 5 µs vs $140 \pm$SE 3 µs); from *M. angolensis* by its lower nA ($\leqslant 29$ vs. 33) and nD ($\leqslant 24$ vs. 26), and higher SPc (a median of 16 vs. 12).

Description. For a detailed description, see Peters (1868, pp. 79–83). Since several similar species have now been recognized that have routinely been considered *M. macrolepidotus*, additional description seems necessary. Head with terminal mouth well in front of eye, mental lobe on lower jaw protruding beyond upper jaw. Head and body dorsolaterally compressed. Dorsal fin situated about two thirds of standard length from snout, obliquely orientated, anteriorly higher and posteriorly lower, proximal tip rounded, distal tip sharply pointed, distal margin sometimes only slightly crescentic with anterior two or three rays longer than posterior rays, number of rays 20 ($N=6$), 21 ($N=27$), 22 ($N=37$), 23 ($N=13$), 24 ($N=3$); anal fin opposite dorsal fin with distinctly more anterior origin, obliquely orientated, anteriorly lower and posteriorly higher, anterior rays longer than posterior ones, especially in males where they also appear stronger and often darkened, distal margin crescentic (in males only posterior to rounded, elongated anterior part of fin), number of rays 26 ($N=6$), 27 ($N=30$), 28 ($N=37$), 29 ($N=10$), 30 ($N=2$), 31 ($N=1$). Scales cycloid with reticulate striae, scales extending anteriorly to operculum and pectoral fins (beyond pelvics). Scales on caudal peduncle circumference, 12 ($N=2$), 13 ($N=2$), 14 ($N=15$), 15 ($N=11$), 16 ($N=52$), 17 ($N=1$), 18 ($N=1$). Caudal peduncle relatively deep, subcylindrical entire length, usually 19% (17–21%) in SL. Colour in life (determined from five fish originating from the Buzi River): back and sides grey-silver, dark on the back and lighter on the sides, to very fair on the underside, gold-olive or purple shimmer depending on angle of light incidence, dark-grey blotches on sides and on unpaired fins, especially on the tail, but absent on head and belly, area next to first ray of anal fin darker, less distinct in females. Electric organ discharge biphasic lacking a weak pre-potential, Pdur around 183 µs (at 25°C and "5% threshold criterion"), Ndur around 174 µs, Pamp and Namp of similar strength. No sexual dimorphism in EOD recognized. Males approaching sexual maturity develop a kink in the base of the anal fin which is absent in juveniles and females where the anal fin base is straight.

Colour in preservation. Blackish-brown on back, fading into ochre on lower body parts, light ochre on underside, blotches seen in life less distinct in preservation.

Ecology. The Zambezi at Tete is a wide and shallow river with prominent sand islands where people punt their boats (at least in July and August when water flow is low). River banks are not very high. Extensive reed beds offer day-time hiding for a great many mormyrids, and apparently also foraging grounds when active during night (mormyrids identified: *Cyphomyrus discorhynchus*, *Mormyrops anguilloides*, *Mormyrus longirostris*). There is very little riparian vegetation in the lower reaches of Cahora Bassa Dam and also very few mormyrids. Water conductivity at Cahora Bassa Dam and Tete was rather high for an African river (133–140 µS/cm in July and August 2002 and 2003, at 22–23°C; Kramer, pers. obs.), but it was even higher by at least one order of magnitude in the delta region, at places considered freshwater sites and at times of the year when water flow was low (mean conductivity of 3150 µS/cm around Marromeu area, July 1999; R. Bills unpubl.). Values

reported from the Buzi River were 127 µS/cm at 23.5°C (early October 2002; R. Bills, pers. comm.)

Distribution. Lower Zambezi River from the delta upstream to at least Tete, if not Victoria Falls; also present in the coastal Pungwe and Buzi systems that are associated with the Zambezi in its south. *Marcusenius macrolepidotus* is also found in the coastal Mulela system north of the delta. Considered by R. Bills (unpubl.) the most widespread mormyrid in the delta region, with habitats varying from small acidic streams draining the edges of the delta, swamps in the delta, and also the main channel to small pools along the Pungwe–Zambezi divide. Its common presence in the Lower Shire River is confirmed by Tweddle and Willoughby (1982) who also noted more distant records from slow-flowing streams, lagoons and sheltered estuaries of or near Lakes Malawi, Chiuta and Chilwa (the taxonomic identity of these records of *M. macrolepidotus* needs to be confirmed with regard to the present revision).

Relationships. Considered most closely related to neighbouring species, such as *M. altisambesi* in the Upper Zambezi River, and also *M. pongolensis* and *M. devosi*, due to the confusion of their identities. Also closely related seems to be *M. livingstonii* (Boulenger 1989) which occurs in parts of the Malawi-Shire system (Tweddle and Willoughby 1982).

Etymology. Mormyrus macrolepidotus Peters, 1852, the large-scaled mormyrid, apparently was an exceptionally large-scaled mormyrid when discovered but no longer holds the record in this regard. *M. altisambesi* and still more so several *Marcusenius* species from Cameroon are larger-scaled (Boden et al. 1998).

***Marcusenius angolensis* (Boulenger, 1905)**
(Figure 1B)

Gnathonemus angolensis Boulenger, 1905, p. 458. Origin, Quanza River, Angola.
Gnathonemus macrolepidotus angolensis: Poll and Gosse 1963, p. 93.
Marcusenius angolensis: Taverne 1971a, p. 103; Taverne 1971b, p. 134; Taverne 1972, p. 166.
Marcusenius macrolepidotus angolensis: Gosse 1984, p. 86. ''Distribution: Angola (Zaïre basin and Upper Zambesi), Zaïre, (Upper basin)''. In consequence, Skelton et al. (1985) state that *Marcusenius macrolepidotus angolensis* applies to the Upper Zambezi form.

Type specimens. Holotype (unique): BMNH 1905.5.29.64.

Type locality. Angola: Quanza River (no. 2, Figure 4).

The present subspecies *M. macrolepidotus angolensis* (Poll and Gosse 1963) refers to the single specimen of *G. angolensis* Boulenger 1905 from the Quanza River in Angola. The fishes of the upper Quanza appear to be related to the fishes of the Zambezi basin and to the Cunene (Trewavas 1973; P. Skelton, unpublished), and *G. angolensis* was therefore included in our anatomical comparisons.

Diagnosis. Together with *M. moeruensis*, most extreme nA (33) count for all specimens of the present study, maximum nD (26) count for whole study, longest LA, long LD, low CPL/SL (documented Table XI), shorter chin appendix than *M. moeruensis*.

Description. See Boulenger (1905, p. 458). *Gnathonemus angolensis* ($N=1$) exceeded even outliers in LA, nD and nA, and the 90th percentile of LD in *M. altisambesi* ($N=89$). With nA=33, well beyond the maximum observed in large sample of *M. altisambesi* ($nA_{max}=30$; Table XI).

Colour in preservation. A homogeneous brown-ochre, but Boulenger (1905) noted ''brown above, silvery white beneath; a few irregular dark brown blotches on the body; fins dark brown''.

Ecology. Boulenger (1905) is no more specific than ''… from the Quanza River''. However, Boulenger (1910) cites Dr. J. W. Ansorge as reporting that he made his collection from rivers, lakes and swamps produced from overflows of these rivers during the rainy season (see next paragraph).

Distribution. ''… from the Quanza River'' (locality not given in Boulenger 1905). Boulenger (1910) states that Dr. Ansorge's ''collection was made from three rivers (the Quanza, the Luculla, and the Bengo), …'', and further below, ''Exploration of the Quanza River at Cunga, Dondo, and Cambambe gave also valuable results''. The description in Boulenger (1910) refers to the terminal section of the Quanza in or near the coastal lowland of northern Angola; therefore, this region is the likely origin of *G. angolensis* Boulenger, 1905.

Relationships. Poll and Gosse (1963, p. 93) have synonymized *Gnathonemus angolensis* Boulenger, 1905 with *M. macrolepidotus* as the subspecies *M. m. angolensis* (''*Gnathonemus angolensis* est une espèce qui doit être considérée comme sous-espèce de *Gnathonemus macrolepidotus* et la référence d'*angolensis* est par conséquent comprise dans celle de *macrolepidotus*''), a status which we do not confirm. Its combination of meristic characters (nA and nD highest, SPc lowest; Table XI) sets *G. angolensis* (and also *G. moeruensis*, of which Boulenger 1915 states that it is ''très voisin'' to *G. angolensis*) well apart from all other bulldog samples we were able to study. ''Allied to *G. senegalensis*, Stdr; distinguished by larger scales'' (Boulenger 1905). *Gnathonemus senegalensis* is the West-African species *Marcusenius senegalensis* (Steindachner, 1870). Boulenger did not state why he recognised *G. moeruensis* as distinct from *Gnathonemus angolensis* when both are so similar. However, he did note an only feeble mental swelling in *G. angolensis* (Boulenger 1905) and ''un renflement sphérique très developpé au menton'' in *G. moeruensis* (Boulenger 1915).

***Marcusenius altisambesi*, sp. n.**

(Figure 1 K)

Gnathonemus okavangensis Pappenheim 1907. ''Appeared as a form of *Gnathonemus macrolepidotus* Peters from the Okavango R., Damaraland, Africa; regarded as infrasubspecific and not available'' (Eschmeyer 2006). ''Nomen dubium'' according to Gosse (1984) and Seegers (1996, p. 73).

Gnathonemus macrolepidotus: Gilchrist and Thompson 1913, pp. 330–331.

Type specimens. Holotype: SAIAB 79135 (specimen L39isi), Namibia: Caprivi Strip: Lisikili on Upper Zambezi River. Paratypes: SAIAB 79136 (6), SAIAB 79137 (3), ZSM 35086 (5), ZSM 35085 (2), ZSM 35097 (1), ZSM 35082 (2).

Type locality. Upper Zambezi in East Caprivi, specifically Upper Zambezi River comprised between Lisikili and Kalimbeza (or Kalambesa, 17°33′S, 24°29′E to 17°32′27.3″S, 24°31′26.2″E; 22–26 km straight line downstream from Katima Mulilo; no. 11, Figure 4).

The first record of *M. macrolepidotus* for the Upper Zambezi is that of Gilchrist and Thompson (1917, p. 562; then termed *Gnathonemus macrolepidotus*), specifying Lialui, Barotseland as the origin. For a description, the authors refer to Gilchrist and Thompson (1913, p. 330), a description of South African specimens. The presence of *G. macrolepidotus* in the Upper Zambezi System was confirmed by Jubb (1958). We disagree with Gilchrist and Thompson's view (1917, p. 562) that their description of a South African specimen of *Gnathonemus macrolepidotus* (in Gilchrist and Thompson 1913) closely matches that of *G. macrolepidotus* from the Upper Zambezi.

Another possible synonym for our new species would be *G. okavangensis* if it were available. ''*Gnathonemus okavangensis*'' (apostrophes by Pappenheim) is not available because, first, Pappenheim (1907) stated that he would *perhaps* suggest that name *if* certain conditions were met (such as more material, better conservation status, sufficient differentiation). Second, there is neither a formal description nor a fish body deposited at a Museum with an accession number. Third, the origin ''Okavango-Fluß (Damaraland, D.-S.-W.-Afrika)'' of the single specimen sent to the Berlin Museum by Oberleutnant Volkmann in 1904 is somewhat mysterious since Damaraland is centred on Windhoek (confirmed by consulting a German map from the time in question), a dry region that is far off the Namibian, or any other, part of the Okavango River. In our opinion ''*G. okavangensis*'' should be removed from the list of nominal species for *M. macrolepidotus*; it has not even been relegated simply because it never existed.

Diagnosis. A median SPc of 12 (12–14; Okavango,12–12), median SLS of 54 (49–60; Okavango, 51 – 57 – 57), median 29 (26–30) anal fin rays, median 23 (20–25; Okavango, 20 – 23 – 26) dorsal fin rays, deep-bodied with mean BD 27.5% (20.1–32.8% of SL; Okavango, 21.8% – 28.1% – 32.5%), other mensural body proportions summarized in Table XI; EOD with weak head-negative pre-potential in juveniles and females, sexual dimorphism in EOD waveform present in ''summer'' in the form of greatly increased EOD duration in sexually mature males of greater than about 12.5 cm SL, but dimorphism not confirmed for Okavango specimens.

Description. Head with terminal mouth well in front of eye, mental lobe on lower jaw protruding beyond upper jaw. Head and body dorsolaterally compressed. Dorsal fin situated about two thirds of standard length from snout, obliquely orientated, anteriorly higher and posteriorly lower, distal margin sometimes only slightly crescentic with anterior two or three rays longer than posterior rays, number of rays 20 (N=1), 21 (N=6), 22 (N=30), 23 (N=35), 24 (N=16), 25 (N=1) [Okavango specimens, similar distribution]; anal fin opposite dorsal fin with distinctly more anterior origin, obliquely orientated, anteriorly lower and posteriorly higher, anterior rays longer than posterior ones, especially in males where they also appear stronger and often darkened, distal margin crescentic (in males only posterior to rounded, elongated anterior part of fin), number of rays 26 (N=4), 27 (N=7), 28 (N=32), 29 (N=27), 30 (N=19) [Okavango specimens, similar distribution but mode at 29 rather than 28]. Scales cycloid with reticulate striae, scales extending anteriorly to operculum and pectoral fins (beyond pelvics). Scales on caudal peduncle

circumference, 12 (N=83), 13 (N=3), 14 (N=3) [Okavango, 12 in all specimens]. Caudal peduncle relatively deep, subcylindrical entire length, usually 19% (16–22%) in SL (Table XI). Electric organ discharge biphasic with weak pre-potential in juveniles and females (Figure 3). In sexually mature males greater than about 12.5 cm SL, sexual dimorphism in the form of greatly increased EOD duration in "summer" (up to $\times$ 11), not confirmed for specimens from Okavango. Males approaching sexual maturity develop a kink in the base of the anal fin (e.g. Figure 1K) which is absent in juveniles and females where the anal fin base is straight. Colour in life: beige-grey, head yellow-gold with greenish hue, paired fins yellow, dark brown blotches except on head, fewer on belly, purple shimmer depending on the angle of light incidence. Okavango specimens: similar to Upper Zambezi fish but darker, brownish grey with many distinct dark-brown blotches, less on belly, paired fins beige rather than yellow.

Colour in preservation. Specimens from the Upper Zambezi: medium brown with darker, irregular blotches on the sides. Narrow dark zone on the back not seen from the side. Belly same colour as body sides. Darkness of head reduced by opaque mormyrid skin (carrying electroreceptor organs, absent on the body sides). Specimens from the Okavango: similar to those from the Upper Zambezi, however, clearly darker, including the fins. Increasingly darker from belly to back.

Ecology. The Upper Zambezi River at Caprivi (Namibia) is a free ranging, major system with regular flooding of a vast savannah plain at high altitude (>900 m above sea level) that is covered by fine sand. The Zambezi in this region is characterized by major side channels giving rise to secondary and tertiary arms with a weaker current. It is a permanent river although the side channels of higher order, and especially peripheral pans, may be temporary or seasonal. At low water level, steep river banks may rise 10 m high. The tributary of the Upper Zambezi, the Kwando River, lacks high banks at least in Caprivi, and is more like the major Okavango River in its West except for its much smaller size, and by also dying in the savannah (however, without forming a vast inland delta). The Kwando River is occasionally flooded by the Upper Zambezi via Chobe, Linyanti, and Lake Liambezi. Lake Liambezi dried up completely in 1985 (except for brief periods of flooding by the Zambezi, e.g. in October 2001 when it was 28% full). During the day, bulldogs are found in rooted reed areas in main channels as well as inside floating reed mats in side channels like at Lisikili. These mats may be 2 m deep, and fish leave cover only at night. Huge numbers of small juveniles may be found in grassy pans on the periphery of major river arms or side channels in the dry season. The Okavango ecology is similar, but differing by an extensive system of lake-like lagoons, where water chestnut, water lilies and dense papyrus and reed beds are common.

Distribution. Occurs in the Upper Zambezi River system in Namibia's Caprivi Strip, including the lower Kwando River (including its terminal parts named Linyanti River). Because these rivers are international borders, this system is bordered by Zambia in its north, Botswana south, Zambia and Zimbabwe east. The species' downstream (south-eastern) limit is assumed to be the nearby Victoria Falls (Zimbabwe and Zambia), as with many other Upper Zambezi fish species. Northern limits in Zambia and Angola are not yet explored. The species also occurs in the western Okavango System (Botswana, Namibia, Angola). Limits still further to the west, e.g. the Cunene River, have not been sufficiently explored.

Relationships. *Marcusenius altisambesi* is considered closest to *M. macrolepidotus* based largely on the confusion of the identity of both species, and the occurrence in the same river, the Zambezi. *Marcusenius altisambesi* is distinguished most easily by the circumferential caudal peduncle scale count of 12 vs. 16, whereas medians 23 vs. 22 for dorsal ray counts, and 29 vs. 28 for anal ray counts, are less distinct. In *M. altisambesi* when compared to *M. macrolepidotus* the anterior body part is relatively longer and body depth higher, as shown by high PDL, PAL, and BD measurements; by contrast, head length HL is shorter. The mental lobe is longer in *M. altisambesi* than in *M. macrolepidotus*. In female and juvenile EOD small head-negative prepotential is present in *M. altisambesi* but not *M. macrolepidotus* (from Buzi River; Figure 3); Upper Zambezi bulldog males have a sexually dimorphic EOD of long duration when in the breeding condition. Another close relationship is *G. moeruensis* of which Boulenger (1915) states that it is ''très voisin'' to *G. angolensis*. However, with nA = 33, both *G. angolensis* and *G. moeruensis* are beyond the maximum observed in our large sample of *M. altisambesi* ($nA_{max}=30$). *G. moeruensis* also differs by HL which is greater than the 90th percentile observed in *M. altisambesi* (Table XI).

Etymology. *Marcusenius altisambesi* refers to the Upper Zambezi River, that is, the section of the Zambezi that ends at Victoria Falls (with ''sambesi'' as a noun in apposition).

Marcusenius pongolensis **(Fowler, 1934)**
(Figures 1D, 1M)

Gnathonemus macrolepidotus: Gilchrist and Thompson 1913, pp. 330–331.
Gnathonemus pongolensis **Fowler, 1934** p. 419, his Figure 6; Crass 1960, p. 416 (synonymy with *Gnathonemus macrolepidotus*).
Marcusenius pongolensis: Taverne 1971a, p. 103.

Type specimens. Holotype (unique): ANSP 54950 (Figure 1D).

Type locality. South Africa: KwaZulu-Natal Province: Pongola River at Paulpietersburg district (no. 4, Figures 4 and 8).

The first South African record is that of three specimens of *Gnathonemus macrolepidotus* (Peters, 1852), one each from the Waterberge District (Limpopo System), the Crocodile River and the Sabie River (the latter two rivers form part of the Incomati System) by Gilchrist and Thompson (1913, pp. 330–331). Without reference to Gilchrist and Thompson (1913, 1917), Fowler (1934) described a new species of bulldog, *Gnathonemus pongolensis*, from the Pongola River in KwaZulu-Natal (South Africa), a river south of the Incomati System. *Gnathonemus pongolensis* was synonymized with *G. macrolepidotus* (Peters, 1852) by Crass (1960, p. 416): ''Fowler's *pongolensis* was described from a single specimen taken in the upper Pongola, near Paulpietersburg. It appears to differ from typical *macrolepidotus* only in its more slender shape. Other species such as *Barbus aureus*, are commonly more slender in headwaters than farther downstream, and there seems no reason to regard *pongolensis* as a valid species''. *Gnathonemus pongolensis* is resurrected here, as *Marcusenius pongolensis* (Fowler, 1934).

Diagnosis. A median 73 (70–76) scales in lateral series, median 18.5 (15–20) scales around caudal peduncle, median 23 (21–24) dorsal fin rays, median 28.5 (27–29) anal fin rays, mean BD 22.6% (19.1–26.2%) of SL, EOD lacking a weak head-negative pre-potential, in

females and juveniles Ndur of short duration (typically, 140 ± S.E. 2.8 µs at 25°C and "5% threshold criterion"), no striking sexual dimorphism in EOD waveform present, but longer EOD duration and statistically significant increase with SL in males.

Description. Head with terminal mouth well in front of eye, mental lobe on lower jaw protruding beyond upper jaw. Head and body dorsolaterally compressed. Dorsal fin situated about three fourths of standard length from snout, obliquely orientated, anteriorly higher and posteriorly lower, distal margin sometimes only slightly crescentic with anterior two or three rays longer than posterior rays, number of rays 18 (N=2), 19 (N=1), 20 (N=1), 21 (N=16), 22 (N=24), 23 (N=13), 24 (N=2); anal fin opposite dorsal fin with distinctly more anterior origin, obliquely orientated, anteriorly lower and posteriorly higher, anterior rays longer than posterior ones, especially in males where they also appear stronger and often darkened, distal margin crescentic (in males only posterior to rounded, elongated anterior part of fin), number of rays 24 (N=1), 25 (N=1), 26 (N=6), 27 (N=15), 28 (N=23), 29 (N=12), 30 (N=1). Scales cycloid with reticulate striae, scales extending anteriorly to operculum and pectoral fins (beyond pelvics). Scales on caudal peduncle circumference, 15 (N=1), 16 (N=19), 17 (N=7), 18 (N=10), 19 (N=11), 20 (N=11). Caudal peduncle subcylindrical entire length, usually 20% (18–22%) in SL. Electric organ discharge biphasic lacking a weak pre-potential, in females and juveniles Ndur of short duration (140 ± 2.8 µs at 25°C and "5% threshold criterion"). In large males, statistically longer EOD duration and significant tendency for increase of both Pdur and Ndur with SL, however, no sexual dimorphism when compared to Upper Zambezi males (effect not seasonal, and no sudden growth spurt at sexual maturity; however, some plasticity observed when spawning). Males approaching sexual maturity develop a striking kink in the base of the anal fin, with several of its first rays longer, stronger and often curved backwards compared to females and juveniles, where the anal fin base is straight and its rays are similar amongst each other. The kink is distinctly curving inward in many specimens. Colour in life ranging from an almost homogeneous medium brown to grey-brown with yellow-golden shimmer, sometimes going into purple, underside lighter, a few strongly faded, darker blotches on sides detectable, fins yellowish.

Colour in preservation. Light to medium brown; fins whitish; irregular fair spots on body sides (from aggression?).

Ecology. Although transformed by some river impoundment, the Sabie and the Crocodile rivers in the Lowveld are fast-flowing, major, perennial rivers, bordered by dense, subtropical or tropical vegetation. Water conductivity appears to be raised by human settlements and activities, both inside and especially outside the Kruger National Park. Similar for the Pongola in the Lowveld, where the water conductivity is very high. During the day *M. pongolensis* is often encountered below undercut river banks, especially in dense networks of tree roots; places where there is a countercurrent seem to be especially attractive. In Swaziland *M. pongolensis* has been observed high up in river systems with rapid water flow and rocky substrate (R. Bills, pers. comm.).

Distribution. Marcusenius pongolensis occurs in coastal rivers draining into the Indian Ocean, ranging from the Incomati system in the north (Mpumalanga Province of South Africa, and Mozambique) to the Mhlatuze System in KwaZulu-Natal (>28°S) as its southern limit, including the Pongola which is the type river. *Marcusenius pongolensis* is the mormyrid

ranging farthest south for the whole of Africa. The form of bulldog occurring in the Limpopo River System could not be determined with certainty.

Relationships. *Marcusenius pongolensis* is considered closest to *M. macrolepidotus* based largely on the confusion of the identity of both species. *Marcusenius pongolensis* is distinguished by a higher median count of SLS (70–73 vs. 55.5), of SPc of 16–20 (range, 15–20) vs. 16 (range, 12–18 for *M. macrolepidotus*). Although there is considerable geographic variation, *M. pongolensis* when compared to *M. macrolepidotus* is more slender (elongated, lower body depth) with PAL shorter (anal fin origin situated closer to head), pD longer, CPL longer, head length shorter, and chin length greater. Female and juvenile EODs with Ndur of shorter duration than in *M. macrolepidotus* (represented by Buzi fish); the EOD of *M. pongolensis* does not exhibit striking sexual dimorphism but a clear sex difference in pulse duration that increases with SL in the male sex.

Fowler (1934) described a single specimen from the Pongola River in KwaZulu-Natal, the type specimen (his Figure 6). The morphology of this fish is in good agreement with the present sample of 10 Pongola bulldogs. An anal fin ray count of only 24 by Fowler as opposed to a minimum of 27 ($N=4$) in the present fish from the Pongola was incorrect, as shown by a recount of nA by one of us (P.S.) that yielded 27 in Fowler's specimen. A pericaudal scale count of 16 in Fowler's fish is identical to the lower limit in our fish ($N=2$), likewise the number of 21 rays of the dorsal fin ($N=2$). HL/Na in Fowler's fish is greater than the maximum of the present sample, but this is not of great concern because of the Na measurement being very small and, hence, less reliable. CPL is slightly greater and LA slightly lower than the range limits of our sample; this should not be considered a reason of much concern because our sample comprises only 10 fish, and then we could not compare the type specimen with our own specimens side by side. The type specimen was kindly measured by Museum personnel and not by one of us (except for nA, see above, unconnected with the present study), in order to avoid the potentially damaging shipping of the valuable type specimen, or else overseas travel. The identity of Limpopo system specimens could not be established with certainty and needs more research.

***Marcusenius devosi*, sp. n.**

(Figure 1 G)

Type specimens. Holotype: SAIAB 79138 (specimen Ta13na), Kenya: Tana River. Paratypes: SAIAB 79139 (14), ZSM 35091 (3), ZSM 35092 (1), ZSM 35093 (4), ZSM 35094 (7).

Type locality. Kenya: Lower Tana River near village Wenje: 1°52′38.1″S, 40°8′22.5″E (no. 14, Figure 4).

The presence of *G. macrolepidotus,* or a form of this species, in the equatorial Tana River of East Africa was suggested by Whitehead and Greenwood (1959) and Whitehead (1959, 1962); for *M. macrolepidotus* adopted by Seegers (1996, p. 76). However, a critical comparison with *M. macrolepidotus* has not been made.

Diagnosis. Longest mean pD of 41% (39.5–43.4%) of SL, shortest mean PAL of 59.1% (57–61%) of SL, shortest mean PDL of 62.3% (59.8–64%), long mean CPL of 20.2% (19.2–22.1%), median SLS of 62.5 (56–66) vs. 55.5 (52–62) in *M. macrolepidotus*, a median 22 (21–24) dorsal fin rays, 28 (26–30) anal fin rays, 16 (14–18) scales around

caudal peduncle; EOD lacking weak head-negative pre-potential, strongest Namp relative to Pamp among samples from all origins (typically 137%), but short Ndur (about 140 µs at 25°C and "5% threshold criterion"), Pdur of long duration (typically greater than 230 µs), brief PNsep (typically 76 µs). Fish from Rovuma River: anal and dorsal fin ray counts, one ray more each, SPc identical, CPD/CPL ratio significantly lower, LD and HL higher.

Description. Head with terminal mouth well in front of eye, mental lobe on lower jaw protruding beyond upper jaw. Head and body dorsolaterally compressed. Dorsal fin situated about two thirds of standard length from snout, obliquely orientated, anteriorly higher and posteriorly lower, distal margin sometimes only slightly crescentic with anterior two or three rays longer than posterior rays, number of rays 21 (N=3), 22 (N=18), 23 (N=5), 24 (N=4); anal fin opposite dorsal fin with distinctly more anterior origin, obliquely orientated, anteriorly lower and posteriorly higher, anterior rays longer than posterior ones, especially in males where they also appear stronger and often darkened, distal margin crescentic (in males only posterior to rounded, elongated anterior part of fin), number of rays 26 (N=1), 27 (N=9), 28 (N=14), 29 (N=4), 30 (N=2). Scales cycloid with reticulate striae, scales extending anteriorly to operculum and pectoral fins (beyond pelvics). Scales on caudal peduncle circumference, 14 (N=2), 15 (N=1), 16 (N=26), 17 (N=0), 18 (N=1). Caudal peduncle relatively deep, subcylindrical entire length, usually 20% (19–21%) in SL. Electric organ discharge biphasic lacking a weak pre-potential, Pamp of relatively long duration (around 230–235 µs at 25°C and "5% threshold criterion"), increasing with SL at least in females and juveniles, Ndur of very short duration (usually around 140 µs), but very strong Namp (137% of Pamp). No sexual dimorphism recognized. Males approaching sexual maturity develop a kink in the base of the anal fin which is absent in juveniles and females where the anal fin base is straight.

Colour in preservation. Head and back when seen from above, dark. Body sides light brown to light ochre, the ochre found especially on lower parts and underside. Dorsal fin usually darker than anal fin. Homogeneous coloration without any blotches, increasingly lighter from back to belly.

Ecology. The Tana River is a major, perennial river of about 700 km length that originates from the equatorial Mt. Kenya and flows into the Indian Ocean. In its final part close to the sea the river is bordered by gallery forest, surrounded by dry savannah on both sides. In August the water was murky and brown, and visibility very low. River borders are high and steep and difficult to climb up or down. Even though the water level was low the current was strong.

Distribution. Presently only known from the Tana River but range extension both to the north and south likely. Samples from the Rovuma River System more than 1000 km to the south are more similar to *M. devosi* than *M. macrolepidotus* in anatomical characters but take an intermediate, independent position in ISSR-PCR genetic analysis.

Relationships. Marcusenius devosi is considered closest to *M. macrolepidotus* based largely on the confusion of the identity of both species. *Marcusenius devosi* is distinguished most easily by its longer posterior body part relative to the anterior body part, as evidenced by higher lengths for the caudal peduncle and for pD, and lower lengths for PDL, PAL, and HL. BD was also lower in *M. devosi*. *Marcusenius devosi* when compared to *M.*

macrolepidotus carries a longer mental lobe on the lower jaw, and has an EOD of shorter N phase duration and much greater strength relative to P phase (than Buzi specimens); the EOD of *M. devosi* shows no evidence for sexual dimorphism. There is also very little affinity with *G. moeruensis* whose morphological parameters are outside the range observed in the Tana River sample for: PDL, PAL, LD, pD, CPL, CPD, BD, nA, and SPc.

Etymology. *Marcusenius devosi* is named in honour of Dr. Luc De Vos, late curator of fishes at Nairobi Museum (born 8 December 1957 at Sint-Niklaas/Belgium, deceased 14 June 2003 at Nairobi/Kenya), for his contributions to African ichthyology and promotion of ichthyology in East Africa (see obituary by J. Snoeks).

Discussion

The present study has discovered allo- or parapatric differentiation in what was hitherto believed to represent a single species, *M. macrolepidotus* (Peters, 1852). Based on the study of characters from morphology, molecular genetics, and electrical signalling behaviour, we recognize at least four species for our study area, and an additional one outside (the resurrected species *M. angolensis*). One of the four is the resurrected nominal species *M. pongolensis* (Fowler, 1934) for South Africa, and two are the new species *M. altisambesi* for the Upper Zambezi/Okavango Systems, and *M. devosi* for the Tana River in Kenya. The cytochrome b sequences and ISSR data (Figures 9 and 10) clearly define *M. altisambesi* and *M. devosi* as monophyletic units that are separated from the other two taxa by genetic distances between 2.8 and 5.7%. Also *M. macrolepidotus*, except the two individuals from Rovuma, form a well-defined clade, whereas *M. pongolensis* shows a higher heterogeneity at the sequence level, indicating that it might contain even more taxa that are presently not recognized. A more extensive sampling is necessary to get a more precise picture.

The populations from different origins differed markedly in all three fields of characters studied. Differentiation in morphology seems to reflect adaptations to ecology for different lifestyles. There was a clear gradient for a deep-bodied, compressed form vs. a streamlined, fusiform shape, representing agile fish vs. active swimmers, respectively. These differences might arise from the presence or absence of strong currents, of rapids and waterfalls. Good examples are the streamlined, small-scaled *M. pongolensis*, which inhabit relatively short, steep, and seasonally cold rivers dropping from the Drakensberg Mountains to the Indian Ocean, compared to the deep-bodied, large-scaled *M. altisambesi* inhabiting the floodplains of the more tropical Upper Zambezi (Zambia and Namibia). Different kinds of food, species of predators, places for breeding, and type of climate are all known to affect such adaptations.

The prime communication signal of mormyrid fish, the EOD waveform, showed wide variation with origin. Upper Zambezi males of sufficient size exhibited a spectacular sexual dimorphism in EOD duration that occurred only in the ''summer'' season (to the best of our knowledge, this is the first such record for a mormyrid; Kramer 1997a, 1997b). No such dimorphism has yet been observed in Okavango males; otherwise and in the ''winter'' season, bulldogs from the two rivers showed very little difference in EOD waveform. A possible further differentiation within our study area would be the Rovuma fish that differed markedly from *M. macrolepidotus* both genetically (with their independent position on a deep branch that is basal to all except *devosi*) and anatomically (no EODs available at present). Limpopo fish differed substantially not only from *M. macrolepidotus* to their north

but also from their southern *M. pongolensis* neighbours, both in morphology and EOD characters; all requiring more research.

Many questions regarding the biology and behaviour of bulldogs remain, all of which are potential areas for discovering still more differentiation. The presence of a miniature head-negativity that precedes the first EOD main phase (of positive polarity) is regarded by some as a distinctive character of phylogenetic relevance among *Marcusenius* (Boden et al. 1998) or even mormyrid species (Hopkins 1999) that indicates electrocytes with penetrating stalks and anterior innervation (as opposed to nonpenetrating stalks with posterior innervation; Bennett 1971). However, in the EODs of Upper Zambezi bulldog males *(M. altisambesi)* an initial head-negativity was present or not according to season. It is not clear why the males of *M. pongolensis* show a much weaker increase in EOD duration when in reproductive condition (less than twofold) than the males of *M. altisambesi* (10-fold, or even more) (Appendix C, and Werneyer and Kramer 2005). In South Africa, clariid predators are apparently unsuccessful in preying on bulldogs (Bruton 1979), presumably because of the much shorter EOD pulse duration than in sexually mature *M. altisambesi* males, the long-duration EODs of which are easily detected by electroreceptive catfish (Hanika and Kramer 2000). Whereas in *M. altisambesi* runaway selection for still longer-duration EODs (by female choice) seems blocked by predatory catfish (Merron 1993), so efficiently so that out of season the difference between the sexes vanishes completely, in *M. pongolensis* a weaker sex difference is permanently present (albeit accentuated in reproductive condition), and at least in part, due to intrasexual selection between competing males (Hanika and Kramer 2005).

A male *M. pongolensis* neither overtly courts nor attacks a female visiting his territory, he does not construct a nest, and the offspring receive no parental care (Werneyer and Kramer 2005), all in marked contrast to the West African mormyrid *Pollimyrus adspersus* (Kirschbaum 1987; Bratton and Kramer 1989), and its southern African congeners *P. castelnaui* (Boulenger, 1911) and *P. mariannne* Kramer et al. 2003 (Lamml and Kramer 2005, 2006). The larvae of the three *Pollimyrus* species and of *M. pongolensis* all generate head-positive larval EODs of long duration and almost identical waveforms, but only those of the three *Pollimyrus* species that have been studied are functionally replaced by a distinct adult discharge of short duration, reversed polarity, and following each larval EOD at an interval of about 0.7 ms during a short transition period after which the larval electric organ degenerates (Westby and Kirschbaum 1978; Baier et al. 2006, Werneyer and Kramer 2006). The monopolar larval EOD of *M. pongolensis*, however, is gradually transformed into the bipolar adult EOD (Werneyer and Kramer, 2006), with no ''electric'' evidence for two distinct, subsequently active electric organs.

Another potential area for differentiation between bulldog populations is acoustic signalling in territory defence and courtship, which appears no longer limited to members of the ''strongly acoustic'' genus *Pollimyrus* (Crawford et al. 1997; Lamml and Kramer 2005, 2006), but also includes bulldog fish, the Growl and Hoot sounds of which were found to be differentiated among *M. macrolepidotus*, *M. altisambesi* and *M. pongolensis* (Lamml and Kramer, 2007).

In cottoid fish of Lake Baikal divergence between related species was between 0.7 and 3.8% (Slobodyanyuk et al. 1995), which is also in the same range as in the *altisambesi*/*devosi*/*pongolensis* species pairs. Distances between well established and unrelated genera, e.g. between *Marcusenius* and *Pollimyrus, Schilbe* or *Eutropius* are much higher (i.e. 12.2–22.6%), indicating that speciation of *Marcusenius* must be more recent. In mammals a crude molecular clock equates 2% nucleotide differences with one million years

divergence time (Brown et al. 1979; Wilson et al. 1987). It has been suggested that the molecular clock shows an even slower rate in fishes than in mammals (Cantatore et al. 1994; Slobodyanyuk et al. 1995). Even if this calibration is very crude it suggests that the taxa within the *Macrolepidotus* complex that differ by genetic distances between 1.5 and 8.5% must have separated more than one to four million years ago which would be long enough to develop into separate species. Since, in addition to genetic differentiation, the animals of the present study show clear differences in electrophysiology, morphology, and geographical distribution we believe it is justified to speak of differentiation at the species level.

A separation time of at least one million years is in agreement with geological data. According to the scenario considered most likely at present (Skelton 1994), the Zambezi discharged to the Indian Ocean through the Limpopo valley until about 2 mya, and *M. pongolensis* and *M. altisambesi* are descendants of the same *Marcusenius* stock. When the encroaching Lower and Middle Zambezi captured and diverted the Upper Zambezi to its present-day northern destination, the two populations became isolated and diverged. A huge ancient lake at the approximate position of the Makgadikgadi salt pan in Botswana, of which the Okavango delta is a northern remnant, has recently been suggested as an evolution centre for haplochromine cichlid fish that seeded all major river systems of southern Africa (Joyce et al. 2005). It may have been for other fish as well, including mormyrids.

It is clear enough that the traditional taxonomy grossly under-represents the degree of differentiation in bulldog fish. Mormyrid fish need to be better known for their importance in ethological, neuroethological and other research. The comparisons of allo- and parapatric populations offer an unequalled research opportunity to study the driving forces for the evolution of communication systems in vertebrates. Management and conservation of fish communities, of which mormyrids form an important part, would be impossible without knowing the species that do exist.

Acknowledgements

For acting as generous hosts and making available base camps, as well as their efficient help during the collection of fish, we wish to thank Dez Weeks (then Zoology Department, Rhodes University, South Africa), as well as A.R. Deacon (who also gave permission) and P.J. Kotze (both from the Research Department, Kruger National Park). Johan Engelbrecht (Mpumalanga Parks Board) gave permission for, and assisted in, the sampling of fish from the Crocodile and Blyde Rivers. Wynand Vlok (University of the North, now Limpopo) gave logistical support and also assisted in catching fish. Johan Engelbrecht is thanked for organizing a successful sampling expedition to KwaZulu-Natal (Mhlatuze and Pongola Rivers), and his assistance in the Okavango and Caprivi. For confirming electrophoretic results, we thank H.M. Beker (Animal Improvement Institute, Irene, South Africa). For two specimens from Nswananzi River, Ofer Gon (SAIAB). For taking anatomical measurements, we thank S. Singer and I. Kaunzinger who also recorded the reproductive activity of a male *M. pongolensis* in aquarium; for gonad histology, B. Otto. For additional measurements, Kathrin Kahler, Ellen Fröhlich, Peter Machnik, Henriette Seichert, Sabine Hartl, Birgit Blaul, Lena Dietz. For helping with EOD recordings on imported *M. pongolensis* of the third sample, we thank S. Hanika, S. Paintner and A. Scheffel, and for fish photographs, Stefan Buchhauser, Michael Lamml, Ellen Fröhlich and Peter Machnik. We thank Hedi Sauer-Gürth (Heidelberg University) for carrying out the sequence determinations. We thank Roger Bills of SAIAB for sampling and making available Lower Zambezi

bulldogs (both alive and preserved), and advice on systematic procedure; Dr. P. Bartsch, ZMB Berlin, for permission and support with studying the type material; Dr. L. Seegers, for photographs; Dr. A. Gill and Mr. James MacLaine, BMNH; the late Dr. G. Teugels, MRAC; Dr. J. Lundberg, ANSP; the late Dr. L. De Vos, Nairobi Museum, Kenya, organized an expedition to Tana River and provided also other support; Dr. K. Heine, E. Ardelean and E. Fröhlich for support with Figures 4 and 8; Dr. F. Fajen, Classical Studies, for advice with Latin species names, B. Markowski for proof-reading an earlier version of the manuscript. Roger Bills, Ernst Swartz and Luis da Costa for critically reviewing the manuscript. Supported by travel grants and funding by Deutsche Forschungsgemeinschaft (grants SFB4/H1, and Kr 446/10-1 through Kr 446/11-3 to B.K.).

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Appendix A: Material examined

Morphometrics and EOD

Marcusenius macrolepidotus (Peters, 1852)

- *Mormyrus macrolepidotus* Peters, 1852. ZMB 3678 (lectotype L. Seegers 13.04.1993) from Tete, Lower Zambezi, Mozambique, with paralectotypes ZMB 6730 (one specimen), 3677 (one specimen), and 32043 (three specimens; Seegers 1996), locality 1 on Figure 4,

- *Gnathonemus moeruensis* Boulenger, 1915. Origin: Lake Moeru (Lake Mweru), Zaire/ Zambia. Holotype (unique): MRAC 14137, locality 3 on Figure 4,

- Lower Zambezi System: SAIAB 060847 coll: Bills R. 01.08.1999, tributary of the Lower Zambezi in the delta region, SE of Marromeu, 18°33′54″S, 35°39′46″E, 81 specimens, size range 52–104 mm SL, locality 17 on Figure 4,

- Pungwe System: SAIAB 060947 coll: Bills R. 15.08.1999, one of the tributaries of the lower Pungwe floodplain, on the road going north from Beira to Tete but close to Beira, Dondo Site near Dondo, 19°34′12″S, 34°43′30″E, 10 specimens, size range 89–106 mm SL, locality 16 on Figure 4,

- Buzi System: SAIAB 67369 coll: Bills R. 29.9.2002, first tributary of the Lucite River leaving Dombe Nyangapwa Stream which is part of the middle Buzi River System, on the road east of Dombe, Manica Province, 19°58′00″S, 33°24′52″E, 25 specimens, size range 70–143 mm SL, locality 15 on Figure 4,

- five live specimens from the Buzi System, same location, R. Bills coll., arrived 8 October 2002 in Regensburg, Germany, studied for EOD and meristic characters, size range 79–88 mm SL, Figure 1 L, still alive,

- Mulela River: SAIAB 055874 coll: Bills R. 20.7.1997, Mulele Village, 16°53′42″S, 38°17′27″E, five specimens, size range 98.4–134.3 mm SL, locality 18 on Figure 4,

- SAIAB 73790, four specimens, Rovuma River System, Mozambique: Nakajambo stream, at Mbatamila-Matondovela Road, Niassa Reserve, Nakajambo, 12°07′45″S, 037°21′41″E, locality 19 on Figure 4, coll. R. Bills, 14 August 2003,

- SAIAB 73884, eight specimens, Rovuma River System, Mozambique: Lucombe stream, at Nyati Road, Niassa Reserve, 12°05′15″S, 037°33′38″E, locality 19 on Figure 4, coll. R. Bills, 22 August 2003.

Marcusenius angolensis (Boulenger, 1905)

Gnathonemus angolensis Boulenger, 1905 p 458. Holotype (unique): BMNH 1905.5.29.64. Origin, Quanza River, Angola, no. 2 on Figure 4 (no more specific origin is given, but the Lower Quanza is likely from Boulenger (1910)).

Marcusenius altisambesi, sp.n.

Holotype. SAIAB 79135 (specimen L39isi), SL 15.3 cm, total length 17.5 cm, Namibia: Caprivi Strip: Lisikili on side arm of Upper Zambezi River, 17°33′S, 24°29′E, 7 March 1994, male, coll.: F.H. van der Bank and B. Kramer, collected within 2 m depth from surface.

Paratypes. SAIAB 79136 (6) [L01isi, L06isi, L10isi, L19isi, L20isi, L26isi], ZSM 35085 (2) [L41isi, L40isi], ZSM 35086 (5) [L02isi, L04isi, L05isi, L12isi, L16isi], 5–7 March 1994, same origin and collectors as holotype, SAIAB 79137 (3) [B5, ID62, ID69], ZSM 35097 (1) [ID30], also from a site at Lisikili, 17°32′31.9″S, 24°26′17.7″E, coll.: F.H. van der Bank and B. Kramer, 5–6 August 2004, water conductivity and temperature, 75–76 μS cm^{-1}, 18.2–20.0°C , ZSM 35082 (2) [ID86, ID87)], paratypes, two specimens as in preceding paragraph but from nearby locality Kalimbeza, 17°32′27.3″S, 24°31′26.2″E, 7 August 2004, similar water conductivity and temperature,

- 104 specimens from the Upper Zambezi River System, East Caprivi, Namibia, size range 35–195 mm SL, smallest gravid female 113 mm SL (juveniles could not be sexed), some specimens from Kalimbeza presently alive in Aquarium:

- SMF 28264 (22 specimens, voucher references: LXXisi, XX ranging from 01 to 50) from the Zambezi River, Lisikili backwater, 17°33′S, 24°29′E (type locality), coll.: F.H. van der Bank and B. Kramer, 5–7 March 1994, water conductivity and temperature, 56.1 μS cm^{-1}, 26.8°C, 11 gravid females,

- SMF 28264 (45 specimens, voucher references: NXXka or NXXXk (XX or XXX ranging from 54 to 126), ZSM 35084 (1) (specimen N123ka), from the Kwando River, Nakatwa, 18°06′S, 23°23′E, in Mudumu National Park, coll.: B. Kramer, 9–15 March 1994, water conductivity and temperature, 130 μS cm^{-1}, 24.9 °C, eight gravid females, locality 12 on Figure 4,

- SMF 28264 (two specimens, voucher references: ''7fish'', 195 mm SL, female, and ''11fish'', 73 mm SL), from Kwando River, Nkasa Island (18°27′S, 23°42′E) in Mamili National Park, close to locality 12 on Figure 4, coll.: F.H. van der Bank and B. Kramer, 9–10 September 1993, water conductivity and temperature, 108 μS cm^{-1}, 18–19°C, not gravid,

- 31 specimens, about 500 m from opposite Kalimbeza fishing camp, at downstream tip of small island between Lisikili side channel and main channel, coll.: F.H. van der Bank and B. Kramer, caught 21 August 1999, water conductivity and temperature, 84 μS cm^{-1}, 22°C, size range 7.2–13.3 cm SL, arrival live in Regensburg 2 September 1999, EOD recording 28 September–7 October 1999 at 100 μS cm^{-1} water conductivity and 21 °C (EOD recording in Germany for quicker transport in Africa), Figure 1K, presently alive,

-1 specimen from Kwando River, Kongola Bridge, 17°47′26.7″S, 23°20′40.0″E, 24 January 2001, coll.: F.H. van der Bank & B. Kramer, ZSM 35083 (1) (specimen Kon08g),

- 63 specimens from the Okavango River, Botswana, totalling at least 10 males, male size range 110–181 mm SL, juvenile/female size range 54–169 mm SL:

- SAIAB 79140 (9), ZSM 35079 (1), ZSM 35080 (3), ZSM 35081 (6) from the Okavango River, Makwena Lodge, near the township of Etsha no. 6, 19°07′30″S 22°22′E, coll.: F.H. van der Bank, J. Engelbrecht and B. Kramer, 20–22 January 2001, water conductivity and temperature, 37 μS cm^{-1}, 29.9–30.6°C, voucher references: OkaXXv (XX integer numbers ranging from 04 to 52), at least nine males as determined by the presence of a kink in anal fin, locality 13 on Figure 4,

- SAIAB 79143 (6), ZSM 35096 (5), and 24 specimens presently alive in aquarium, from the Okavango River at Guma Lagoon, 18°57′46.6″S, 22°22′25.3″E, coll.: F.H. van der Bank and B. Kramer, 10–12 August 2004, water conductivity and temperature 38 μS cm^{-1}

and 21.4°C, voucher references: Guma00XX (X for integer numbers ranging from 4 to 64), one male, close to locality 13 on map of Figure 4, Figure 1J,

- SAIAB 79141 (1), ZSM 35095 (1), and seven specimens presently alive in aquarium, details as in preceding paragraph, except for locality at Makwena, 19°03′13.85″S,22°22′42.6″E, 12 August 2004, voucher references Makw00XX (X ranging from 0 to 28).

Marcusenius pongolensis (Fowler, 1934)

Material examined, all from South Africa.
- *Gnathonemus pongolensis* Fowler, 1934. Holotype (unique): ANSP 54950. Origin, Pongola River, Paulpietersburg (locality no. 4, Figures 4 and 8; Figure 1D),

- SAIAB 54445–54448: 30 specimens from the Sabie River System in or close to the Kruger National Park, totalling at least nine males, at least 14 females, size range 49 – 171 mm SL, largest male 171 mm SL, largest female 150 mm SL, no gravid material, coll.: P. Skelton and B. Kramer, 23–25 September 1993,

- SAIAB 54445, one specimen from the Sand River (tributary of the Sabie) at Londolozi, site 14, 24°47′31″S, 31°31′32″E, standing pool, muddy water of 250 $\mu S\,cm^{-1}$ and 26°C temperature, male, SL 87 mm,

- SAIAB 54446, 11 specimens from the Sabie River above Hazy View, at least seven of which are male, two female, size range 49–171 mm SL 25°02′S, 31°00′E, water conductivity and temperature, 120 $\mu S\,cm^{-1}$, 21.1°C,

- SAIAB 54447, six specimens from the Sabie River above Skukuza just below the weir, all female, size range 74–150 mm SL, site 8, 24°58′35″S, 31°35′05″E, water conductivity and temperature, 160 $\mu S\,cm^{-1}$, 26°C,

- SAIAB 54448, 12 specimens at least one of which male, six female, size range 49–118 mm SL, Sabie River immediately above Hazy View (municipal picnic site), site 5, 25°01′48″S, 31°01′21″E, water temperature and conductivity, 125 $\mu S\,cm^{-1}$, 24.4°C,

- 14 specimens from the Sabie River, bridge near Lower Sabie tourist camp (25°07′S, 31°55′E), water conductivity and temperature, 139 $\mu S\,cm^{-1}$, 25.1°C, coll.: F.H. van der Bank and B. Kramer, 29–30 March 1996, size range 103–173 mm SL, largest male 173 mm SL, largest female 133 mm SL, no gravid material, imported live,

- SAIAB 79144 (1), one specimen from Sabie River, Sekurekwane (24°59′S, 31°11′E), water conductivity and temperature, 106 $\mu S\,cm^{-1}$, 23.3 °C, coll.: F.H. van der Bank and B. Kramer, 27 March 1996, 82 mm SL,

- SAIAB 79145 (1), one specimen from the anastomosing reaches of the Sabie/Sand confluence at 24°57′22.7″S, 31°42′38.7″E, water conductivity and temperature, 129 $\mu S\,cm^{-1}$ and 24.3 °C, coll.: F.H. van der Bank and B. Kramer, 28 March 1996, 8.6 cm SL, all of the above near locality 5 on Figures 4 and 8,

- 29 specimens (23 for EOD) from the Crocodile River (Incomati System), Stentor Estates (town: Kaapmuiden, Province Mpumalanga), 25°30′35″S, 31°11′58″E, altitude 432 m, coll.: F.H. van der Bank and J. Engelbrecht, 14 February 1997, water conductivity and temperature, 230 $\mu S\,cm^{-1}$ at 25°C, 23 males, six females, size range 148–275 mm SL, largest male 275 mm SL, largest female 200 mm SL (in the Crocodile River region it had

rained a lot, the river was flowing very strong and the water was not clear), locality 6 on Figures 4 and 8, Figure 1M, imported live,

- ZSM 35089 (1), one specimen from the Groot Letaba River just below Tzaneen Dam (Northern Province), 23°49′00″S, 30°10′00″E, coll.: W. Vlok and B. Kramer, 22 September 1998, water conductivity and temperature, 114 µS cm^{-1}, 21.4°C, 69 mm SL, locality 7 on Figures 4 and 8, field no. Lim03,

- SAIAB 79147 (6), ZSM 35088 (4), 10 specimens from Blyde River just below Blyderivierspoort Dam (Mpumalanga), 24°32′11″S, 30°47′52″E, coll.: J. Engelbrecht, W. Vlok and B. Kramer, 25/26 September 1998, water conductivity and temperature, 154 µS cm^{-1}, 16.7°C, size range 40–140 mm SL (both the Blyde and Letaba rivers are tributaries of the Olifants River that forms part of the Limpopo System), locality 8 on Figures 4 and 8,

- SAIAB 79149 (4), ZSM 35090 (3), seven specimens from the KwaMaZulu stream close to where it flows into Goedertrouw Dam, part of the Mhlatuze System in KwaZulu-Natal (approx. 28° 25′ 30″S, 31° 1′ 30″E), water conductivity and temperature, 134 µS cm^{-1} and 17.1°C, coll.: J. Engelbrecht and B. Kramer, 12 August 1999, locality 9 on Figures 4 and 8,

- SAIAB 79148 (5), ZSM 35087 (5), 10 specimens from the Pongola River in KwaZulu-Natal (27° 01′ 15″S, 32° 18′E), bridge at road connecting Ndumo with Kosibay, coll.: J. Engelbrecht and B. Kramer 14 August 1999, water conductivity and temperature, 600 µS cm^{-1}, 22°C, locality 10 on Figures 4 and 8,

- SAIAB 77296 (2), two specimens from the Nswananzi River: Nkanini stream in KwaZulu-Natal, Kosibay, below bridge just outside Manguzi town on road to Mozambique, 26°56′24″S 032°44′39″E, coll: O. Gon, H. Larson, V. Mthombeni and S. Kyle, 13 November 2005, fresh and clear water, sandy to silt bottom with rocks on the banks (no. 20 on Figure 8).

Further downstream the Mhlatuze, mormyrids (only possibility: bulldogs) were also demonstrated (but not caught) using an electronic fishfinder (making EODs audible through headphones) at Lake Mzingazi near Richards Bay, in water of 496 µS cm^{-1} and 21.8°C on August 12, 1999. The Mhlatuze System is the southernmost record for bulldogs and mormyrids in general (J. Engelbrecht, pers. comm.).

Additional material studied for gonad histology. Of the above specimens from the Crocodile River, five males (178, 183, 245, 250 and 252 mm SL), two females (148 and 153 mm SL), plus six additional fish from the Crocodile River (same collection dates), size range 93–141 mm SL, one male of 137 mm SL, five females, largest female 141 mm SL.

***Marcusenius devosi*, sp. n.**

Thirty specimens

Holotype. SAIAB 79138 (specimen Ta13na), SL 10.2 cm, total length 11.6 cm, Kenya: Tana River: Tana Primate Research Centre, 1°52′38.1″S, 40°08′22.5″E, south of village Wenje, east of road B8, 48 m above sea level, locality 14 on Figure 4, 3 September 2001, female, coll. by L. De Vos and B. Kramer at $\leqslant$1.5 m depth, water conductivity and temperature, 185 µS cm^{-1} and 26°C.

Paratypes. SAIAB 79139 (14), ZSM 35091 (3), ZSM 35092 (1), ZSM 35093 (4), ZSM 35094 (7) (specimens Ta10na, TA11na, Ta14na, Ta18na, Ta20na, Ta21na, Ta22na,

Ta25na, Ta27na, Ta28na, Ta29na, Ta30na, Ta31na, Ta32na, Ta33na, Ta34na, Ta47na, SinEOD1, SinEOD4, SinEOD7, ID1–ID9), same origin as holotype, 3–6 September 2001, size range 79–121cm SL.

Material examined for DNA

Four specimens of *M. macrolepidotus* from the Lower Zambezi River, Mozambique (macrolepidotus 22688, 12 August 1999, 18°17′S 35°57′E, near village Marromeu; macrolepidotus 22689, 22690, 13 August 1999, 18°19′S35°55′E, SE from Marromeu; macrolepidotus 22686, Marromeu fish market, 11 August 1999, 18°17′S 35°57′E; numbers are institutional accession numbers, University of Heidelberg, M. Wink) [GenBank accession numbers, M.macrolepidotus.MOZ DQ863663, M.macrolepidotus.MOZ DQ863664], five specimens of *M. macrolepidotus* from the middle Buzi River System (macrolepidotus 22681, 22682, 22683, 22684, 22685; see Material examined); [GenBank accession numbers, M.macrolepidotus.MOZ DQ863662, M.macrolepidotus.MOZ DQ863665, M.macrolepidotus.MOZ DQ863669, M.macrolepidotus.MOZ DQ863671], two specimens of *M. macrolepidotus* from the Rovuma River (origin and date as given in Material examined) [GenBank, M.macrolepidotus.Rov DQ863673, M.macrolepidotus.Rov DQ863674]. Five specimens of *M. pongolensis* from the Crocodile River, Stentor Estates, Kaapmuiden, Mpumalanga, South Africa (25°30′35″S, 31°11′58″E), 14 February 1997, ecological data as given above, two female (112 and 141 mm SL), two male (134 and 137 mm SL), one juvenile (109 mm SL) [GenBank, M.pongolensisSA14.C DQ863650, M.pongolensisSA15.C DQ863651, M.pongolensisSA9.Cr DQ863657, M.pongolensisSA12.C DQ863658, M.pongolensisSA13.C DQ863659]; two specimens from the Pongola River, South Africa, 14 August 1999, SL 15, 16.9 cm [M. pongolensis.9127, M. pongolensis.9129]; three specimens from the Mhlatuze System, KwaMazulu stream, South Africa, 12 August 1999, SL 7.6, 8.1 and 12.9 cm, the latter of which a male [GenBank, M.pongolensis.Mhlat DQ863647, M.pongolensis.Mhlat DQ863648, M.pongolensis.Mhlat DQ863649]. Five specimens of *M. altisambesi* from the Upper Zambezi System, East Caprivi, Namibia: NAM1 (18°04′53″S, 24°02′35″E) from the Linyanti River, 22 February 1997, NAM3 and NAM5 (18°09′24″S, 23°23′09″E) from the Kwando River, NAM2 (23 February 1997; 125 mm SL, 24 g, SAIAB 74735) and NAM148 (7 April 1996, 81 mm SL, juvenile) from the Upper Zambezi River (17°32′24″S, 24°31′25″E), altisambesi.145 from Upper Zambezi System (Upper Zambezi River at same location, or lower Kwando River) [GenBank, M.altisambesi.NAM2.U DQ863652, M.altisambesi.NAM5.K DQ863653, M.altisambesi.NAM1.L DQ863654, M.altisambesi.NAM148 DQ863655, M.altisambesi.NAM3.K DQ863656]. Two specimens of *M. altisambesi*, BOT22670 and BOT 22671, from the Okavango, Thoage River, Makwena, Botswana (19°07′30″S, 22°22′E), coll. F.H. van der Bank, 21 January 2001 [GenBank M.altisambesi.BOT226 DQ863660, M.altisambesi.BOT226 DQ863661]. Included as a confamilial outgroup are two *Pollimyrus marianne* Kramer et al. 2003 from the Upper Zambezi System, East Caprivi, Namibia, Linyanti River, 18°04′53″S, 24°02′35″E, 22 February 1997 [GenBank: AY234099], and one specimen each of *Hippopotamyrus ansorgii* (Kwando River, Kongola bridge, 24 January 2001) [GenBank: AY236995] and *H. szaboi* (Upper Zambezi River, Wenela at Katima Mulilo, 27 August 1999) [GenBank: AY236983]. As a more distant outgroup (Siluriformes) served one *Schilbe intermedius* Rüppell, 1832 (SAIAB 056106) sampled from the Upper Zambezi River, Katima Mulilo, Namibia (17°32′S, 24°31′E), 8 May 1997 [GenBank: DQ863646].

Appendix B: Morphological differences and genetic distances between samples

Table XI. Morphological measures for the types of *Marcusenius macrolepidotus* (Peters, 1852), and other bulldog fish from various origins. For abbreviation of morphological characters, see Material and methods.

Types (Peters 1852)	PDL/SL	PAL/SL	LD/SL	LA/SL	pD/SL	CPL/SL	CPD/CPL	LS_c /HL	LS_o /HL	HL/SL	HL/Na	BD/SL	CL/ SL	nD	nA	SPc	SLS	ED/HL	PFL/HL	SL(cm)
Mean/Median[1])	0.6377	0.6127	0.1854	0.2407	0.3955	0.1893	0.4377	0.3564	0.4318	0.1966	14.29	0.2688	–[2])	23	30	16				23.2
Min	0.6273	0.5946	0.1759	0.2233	0.3778	0.1692	0.4025	0.3402	0.4114	0.1891	12.88	0.2516	–[2])	22	27	16				18.9
Max	0.6500	0.6312	0.1913	0.2511	0.4120	0.2022	0.5000	0.3977	0.4788	0.2043	15.74	0.2883	–[2])	24	31	16				27.4
SE/SIQ	0.0045	0.0076	0.0029	0.0048	0.0069	0.0061	0.0179	0.0112	0.0122	0.0028	0.57	0.0059	–[2])	0.625	1.25	0				1.39
N	5	5	5	5	5	5	5	5	5	5	5	5	–[2])	5	5	3[3])				5
Lower Zambezi																				
Mean/Median[1])	0.6478	0.6149	0.1805	0.2337	0.3884	0.1879	0.3870	0.3755	0.4555	0.2257	14.04	0.2655	0.0275	22	28	16	55.5			8.3
Min	0.6148	0.5900	0.1549	0.2072	0.3622	0.1693	0.3343	0.3412	0.4038	0.2052	11.17	0.2214	0.0113	20	26	12	52			5.1
Max	0.6866	0.6575	0.1992	0.2738	0.4143	0.2089	0.4572	0.4215	0.5223	0.2538	18.07	0.3222	0.0440	24	29	18	62			16.9
SE/SIQ	0.0015	0.0014	0.0009	0.0011	0.0012	0.0011	0.0028	0.0016	0.0038	0.0011	0.1637	0.0021	0.0008	0.5	0.5	0.5	1.5			0.254
N	81	81	81	81	81	81	81	81	81	81	81	81	81	81	81	81	42			81
Pungwe																				
Mean/Median[1])	0.6457	0.6080	0.1869	0.2433	0.3984	0.1845	0.3802	0.3701	0.4332	0.2182	14.23	0.2846	0.0359	21.5	28	16	57			9.8
Min	0.6245	0.5859	0.1703	0.2242	0.3822	0.1736	0.3172	0.3584	0.4203	0.2063	13.08	0.2679	0.0273	21	26	13	53			8.9
Max	0.6677	0.6439	0.2017	0.2594	0.4117	0.2061	0.4264	0.3865	0.4584	0.2385	15.94	0.3206	0.0411	23	29	16	60			10.6
SE/SIQ	0.0041	0.0054	0.0031	0.0036	0.0032	0.0029	0.0102	0.0031	0.0035	0.0029	0.34	0.0046	0.0011	0.5	0.5	1	2.5			0.21
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10			10
Gnathonemus angolensis Boulenger 1905																				
holotype (unique)	0.6742	0.6141	0.1963	0.2577	0.3909	0.1707	0.3808	0.3720	0.4536	0.2028	15.96	0.2659	0.0071	26	33	12	58*)			12.75
Gnathonemus moeruensis Boulenger 1915																				
holotype (unique)	0.6488	0.6262	0.1961	0.2504	0.3937	0.1693	0.4277	0.3634	0.4638	0.2263	15.22	0.2928	0.024	25	33	12	57*)			12.71
Gnathonemus pongolensis Fowler 1934																				
holotype (unique)	0.6336	0.5936	0.1693	0.2058	0.4017	0.2029	0.38	–	0.4667	0.1913	17.37	0.1971	–	21	27	16				17.25
South Africa: pooled																				
Mean/Median[1])	0.6403	0.6033	0.1752	0.2199	0.3886	0.198	0.3529	0.3918	0.4695	0.2143	13.79	0.2257	0.0293	22	28	18				9.4
Min	0.6131	0.5838	0.1547	0.1873	0.3619	0.1761	0.2770	0.3525	0.4180	0.1863	10.57	0.1909	0.0079	18	24	15				4.8
Max	0.6632	0.6256	0.2015	0.2488	0.4108	0.2226	0.4317	0.4730	0.5541	0.2425	16.66	0.2617	0.0435	24	30	20				17.7
SE/SIQ	0.0013	0.0013	0.0012	0.0014	0.0014	0.0013	0.0047	0.0029	0.0036	0.0018	0.21	0.0020	0.0012	1	0.875	1.5				0.47
N	59	59	59	59	59	59	59	59	59	59	59	59	54	59	59	59				59
South Africa: Pongola																				
Mean/Median[1])	0.6402	0.6022	0.1790	0.2295	0.3966	0.1983	0.3892	0.3796	0.5012	0.2076	15.64	0.2471	0.0383	23	28.5	18.5	73	0.2273	0.8103	14.32
Min	0.6312	0.5838	0.1655	0.2192	0.3743	0.1835	0.3471	0.3616	0.4861	0.1982	14.04	0.2304	0.023	21	27	16	70	0.1999	0.7455	11.5
Max	0.6556	0.6213	0.2015	0.2412	0.4054	0.2093	0.4304	0.3950	0.5288	0.2168	16.66	0.2617	0.043	24	29	20	78	0.2540	0.9002	17.6
SE/SIQ	0.0025	0.0031	0.0032	0.0021	0.0029	0.0028	0.0085	0.0036	0.0038	0.0018	0.27	0.0028	0.002	0.5	0.5	1	1.5	0.0063	0.0136	0.60
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
South Africa: KwaMaZulu																				
Mean/Median[1])	0.6492	0.6047	0.1708	0.2145	0.3815	0.1897	0.3771	0.4344	0.5030	0.2066	11.83	0.2199	0.0331	21	28	20	70			8.25
Min	0.6372	0.5922	0.1602	0.2073	0.3726	0.1780	0.3364	0.4150	0.4800	0.1946	10.57	0.2082	0.0294	18	27	19	65			6.03
Max	0.6583	0.6148	0.1841	0.2228	0.3930	0.2013	0.4317	0.4730	0.5541	0.2289	12.75	0.2339	0.0350	22	28	20	75			11.44
SE/SIQ	0.0029	0.0036	0.0033	0.0023	0.0031	0.0034	0.0154	0.0084	0.011	0.0049	0.30	0.0034	0.0007	1.125	0.375	0.5	2.875			0.72
N	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7			7

Table XI. (*Continued.*)

Types (Peters 1852)	PDL/SL	PAL/SL	LD/SL	LA/SL	pD/SL	CPL/SL	CPD/CPL	LS_c /HL	LS_o /HL	HL/SL	HL/Na	BD/SL	CL/ SL	nD	nA	SPc	SLS	ED/HL	PFL/HL	SL(cm)
South Africa: Incomati																				
Mean/Median[1])	0.6391	0.6042	0.1775	0.2214	0.3884	0.1964	0.3438	0.3878	0.4571	0.2177	13.89	0.2248	0.0249	22	28	16	71			8.56
Min	0.6131	0.5888	0.1547	0.2007	0.3619	0.1761	0.2931	0.3561	0.4206	0.1863	11.3	0.1936	0.0079	18	25	15	65			4.83
Max	0.6632	0.6256	0.1938	0.2488	0.4108	0.2157	0.3962	0.4118	0.4923	0.2400	16.47	0.2489	0.0432	24	30	20	74			16.8
SE/SIQ	0.0019	0.0018	0.0013	0.0016	0.0020	0.0017	0.0049	0.025	0.0032	0.0025	0.24	0.0020	0.0014	0.75	0.5	0.75	1.5			0.56
N	32	32	32	32	32	32	32	32	32	32	32	32	28	32	32	32	23			32
South Africa: Limpopo																				
Mean/Median[1])	0.6382	0.6006	0.1667	0.2089	0.3862	0.2089	0.3289	0.3871	0.4541	0.2156	13.00	0.2114	0.0303	21.5	26.5	19	70			7.95
Min	0.6263	0.5906	0.1579	0.1873	0.3727	0.1992	0.2770	0.3525	0.4180	0.1984	11.45	0.1909	0.0218	20	24	16	61			5.36
Max	0.6530	0.6157	0.1828	0.2235	0.4026	0.2226	0.3868	0.4115	0.4748	0.2425	14.39	0.2344	0.0426	22	27	20	73			12.75
SE/SIQ	0.0024	0.0028	0.0027	0.0039	0.0034	0.0022	0.0107	0.0061	0.0054	0.0044	0.33	0.0044	0.0024	0.5	0.5		1.75			0.95
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	8			10
Upper Zambezi																				
Mean/Median[1])	0.6566	0.6249	0.1829	0.2287	0.3872	0.1876	0.3985	0.3536	0.4299	0.2108	14.56	0.2754	0.0509	23	29	12	54	0.2288	0.8645	11.89
Min	0.6202	0.5927	0.1610	0.1997	0.3609	0.1618	0.3216	0.3167	0.3965	0.1955	11.23	0.2011	0.0195	20	26	12	49	0.1892	0.7852	4.4
Max	0.6865	0.6591	0.2011	0.2537	0.4180	0.2232	0.4824	0.3923	0.5021	0.2758	17.25	0.3280	0.0670	25	30	14	60	0.2638	0.9468	19.8
SE/SIQ	0.0014	0.0014	0.0010	0.0011	0.0013	0.0013	0.0034	0.0015	0.0019	0.0011	0.11	0.0021	0.0010	0.5	0.5	0	2	0.0084	0.0143	0.247
N	89	89	89	89	89	89	89	89	89	89	89	89	83	89	89	89	89	10	10	89
Okavango																				
Mean/Median[1])	0.6497	0.6189	0.1867	0.2364	0.3926	0.1879	0.3521	0.3660	0.4456	0.2116	13.75	0.2807	0.0370	23	29	12	53	0.2137	0.8039	11.56
Min	0.6244	0.5958	0.1625	0.1994	0.3731	0.1755	0.2902	0.3373	0.4053	0.1966	11.62	0.2184	0.0184	20	26	12	49	0.1868	0.7393	5.3
Max	0.6702	0.6418	0.2102	0.2563	0.4153	0.2170	0.4190	0.3897	0.5000	0.2452	15.95	0.3255	0.0460	26	30	12	58	0.2769	0.8441	17.8
SE/SIQ	0.0021	0.0019	0.0018	0.0020	0.0018	0.0015	0.0065	0.0025	0.0039	0.0019	0.184	0.0043	0.0011	0.5	0.5	0	2.5	0.0095	0.0119	0.60
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	10	10	32
Tana																				
Mean/Median[1])	0.6228	0.5909	0.1779	0.2379	0.4094	0.2018	0.3690	0.3596	0.4456	0.2168	15.47	0.2436	0.0174	22	28	16	62.5	0.1954	0.9228	10.4
Min	0.5984	0.5701	0.1598	0.2229	0.3954	0.1918	0.3272	0.3405	0.4219	0.2078	12.61	0.2000	0.0076	21	26	14	58	0.1831	0.8565	7.9
Max	0.6397	0.6098	0.1943	0.2542	0.4339	0.2206	0.3972	0.3880	0.4659	0.2305	18.98	0.2802	0.0245	24	30	18	66	0.2170	0.9648	12.1
SE/SIQ	0.0018	0.0020	0.0014	0.0016	0.0018	0.0011	0.0035	0.0025	0.0025	0.0011	0.25	0.0034	0.0007	0.5	0.5	0	0.5	0.0031	0.0094	0.18
N	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	11	11	30
Buzi [4])																				
Mean/Median[1])	0.6433	0.6037	0.1837	0.2410	0.3964	0.1849	0.3638	0.3822	0.4807	0.2154	13.65	0.2692	0.0239	22 (22)	28 (27)	16 (16)				10 (8.3)
Min	0.6173	0.5692	0.1687	0.2249	0.3823	0.1704	0.3125	0.3675	0.4596	0.2000	12.36	0.2433	0.012	20 (21)	26 (25)	13 (14)				7 (7.9)
Max	0.6594	0.6417	0.2043	0.2604	0.4182	0.1974	0.4340	0.4121	0.5195	0.2385	15.27	0.2968	0.0399	25 (24)	31 (29)	17 (16)				14.3 (8.8)
SE/SIQ	0.0021	0.0029	0.0016	0.0016	0.0021	0.0013	0.0058	0.0020	0.0023	0.018	0.18	0.0025	0.0013	0.5 (0.5)	0.5 (1)	0.5 (0)				0.38 (2.01)
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25 (5)	25 (5)	25 (5)				25 (5)
Mulela																				
Mean/Median[1])	0.6719	0.6285	0.1765	0.2316	0.3716	0.1780	0.4056	0.3912	0.4773	0.2096	14.39	0.2818	0.0221	22	27	16				11.5
Min	0.6597	0.6218	0.1727	0.2199	0.3621	0.1740	0.3675	0.3815	0.4559	0.2028	12.83	0.2665	0.0168	21	27	14				9.8
Max	0.6867	0.6322	0.1791	0.2373	0.3791	0.1842	0.4386	0.4007	0.4834	0.2169	15.24	0.2972	0.0316	22	28	17				13.4
SE/SIQ	0.0044	0.002	0.0014	0.0031	0.0028	0.0019	0.0134	0.0037	0.0054	0.0023	0.4095	0.058	0.026	0.5	0.125	0.375				0.683
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5				5

Table XI. (*Continued.*)

Types (Peters 1852)	PDL/SL	PAL/SL	LD/SL	LA/SL	pD/SL	CPL/SL	CPD/CPL	LS_c /HL	LS_o /HL	HL/SL	HL/Na	BD/SL	CL/ SL	nD	nA	SPc	SLS	ED/HL	PFL/HL	SL(cm)
Rovuma																				
Mean/Median[1])	0.6293	0.5896	0.1887	0.2409	0.4087	0.1995	0.3163	0.3653	0.4695	0.2291	14.35	0.2644	0.022	23	29	16				6.96
Min	0.6158	0.5779	0.1736	0.2255	0.3985	0.1894	0.2954	0.3293	0.4207	0.2054	13.1	0.2432	0.0131	22	27	14				5.1
Max	0.6414	0.6008	0.2004	0.2617	0.4204	0.2105	0.3426	0.3994	0.5008	0.2439	16.5	0.2794	0.031	24	30	16				11.96
SE/SIQ	0.0021	0.0022	0.0029	0.0032	0.0022	0.002	0.0037	0.0061	0.0082	0.0036	0.3177	0.0032	0.0016	0.25	0.5	0				0.5028
N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12				12
Natal/Nswananzi																				
Mean/Median[1])	0.6474	0.6139	0.1657	0.2053	0.3901	0.2167	0.3764	0.4002	0.5140	0.2341	13.94	0.2513	0.0160	22	27	16	70			9.42
Min	0.6433	0.6020	0.1652	0.2039	0.3798	0.2154	0.3714	0.3983	0.5061	0.2219	13.01	0.2459	0.0140	22	26	16	70			6.71
Max	0.6156	0.6258	0.1662	0.2067	0.4005	0.2181	0.3814	0.4021	0.5218	0.2462	14.87	0.2567	0.0179	22	28	16	70			12.13
N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2			2

[1]) Median and SIQ (semi-interquartiles) for count measures only (nD, nA, SPc). [2]) Chin flattened by ''head-stand'' museum storage (type material). [3]) These scales gone in two specimens of the type material. [4]) Meristic characters also determined on five live fish under light anaesthesia (in parentheses). *) Boulenger 1905.

Table XII. Variable positions in the cytochrome b data set. Identical characters to those in the first line are indicated by ".".

#Marc_conicephalus	TCTACTACAT	TCGCGAGGCA	CCACTCACTT	AACCCCTGCT	GACCCATACC	ACTTCCTGGT	CTTGGTCCTA	CCACCACATC	TCATCCCAAA	ATACCTCGAA	TAGTACCAAC	TTCCTACGCA	CCACTCCTCC	CCCCATAACA	GCTCCCG
#Marc_greshoffi	.....C.T..	C.ATA....C	.A..CT..C.	....TT..T.	....TC.G.G	G.C.A.CC..	.CCC..TTCG	T.T.A...C.	....TTAGGG	.CC..C...G	.G..CTT...	.C.T....T.	..GTCT.CTA	.T..CC....	..C.T.A
#Marc_moorii_2	.....C....	CTA.AG....	T...C...C.	.....T....	.....C..T.	..C.G.CC..	..CT...T..	..T.A...C.	.T..T.A...	.C...C..C.	.C..C.TG..	.C.T.G.CT.	A.G.C..CTA	..T.CC.G.C	..C.TTA
#M.devosi.22667_TanaR	.T..AC...A	CTATA...GC	..G..T..C.	........T.	C.TT.C....	..C.G.CC..	T.CT.AT.C.	TATTA...T	.A..T.A..G	CCC.TCT...	...GC.T..T	.CT.CT..TG	AAG.C..C.A	..T.TC....	..C...A
#M.devosi.22668_TanaR	.T..AC....	CTATA...GC	..G..T..C.	........TG	..TT.C....	..CGG.CC..	T.CT....C.	TATTA...T	.A..T.A..G	CCC.TCT...	....C.T..T	.C..CT..T.	TAG.C..C.A	..T.TC....	..C...A
#M.devosi.22669_TanaR	.T..AC....	CTATA...GC	..G..T..C.	........T.	..TT.C....	..C.G.CC..	T.CT....C.	TATTA...T	.A..T.A..G	CCC.TCT...	....C.T..T	.C..CT..T.	TAG.C..C.A	..T.TC....	..C...A
#M.devosi.22665_TanaR	.T..AC....	CTATA...GC	..G..T..C.	........T.	..TT.C....	..C.G.CC..	T.CT....C.	TATTA...T	.A..T.A..G	CCC.TCT...	....C.T..T	.C..CT..T.	TAG.C..C.A	..T.TC....	..C...A
#M.altisambesi_Nam2_Upper_ZambesiR	...TAC....	CTATA....C	..G.CT..C.	..TT....T.	..TT.C....	.TC.GT.C..	T.CTA..TC.	.AT.AGA..T	.AG.T.A...	CCC..CT...	..A.C.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	ATC...A
#M.altisambesi_Nam5_KwandoR	.T.TTCC.G.	C.A.a....c	...ACAC.C.	..TT....T.	..TT.C....	.TC.GT.C..	T.CTA..TC.	.AT.AGA..T	.AG.T.A...	CCC..CT...	..A.C.T.GT	.C.....T.	TAG.C..C.A	..T.T.T..	ATC...A
#M.altisambesi_Nam148_Upper_ZambesiR	.T..AC....	CTATA....C	..G.CT.GC.	..TT....T.	..TT.C....	.TC.GT.C..	T.CTA..TC.	.AT.AGA..T	.AG.T.A...	CCC..CT...	..A.C.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	ATC...A
#M.altisambesi_Nam1_LinyandiR	.T..AC....	CTATA.CAGC	..GACT..C.	..TT....T.	..TT.C....	.TC.GT.C..	T.CTA..TC.	.AT.AGA..T	.AG.T.A...	CCC..CT...	..A.C.T.GT	.C.....T.	GAG.C..C.A	..T.G.....	ATC...A
#M.altisambesi_Nam3_KwandoR	.T.TAC....	CTATA....C	..G.CT.TC.	..TT....T.	..TT.C....	.TC.GT.C..	T.CTA..TC.	.AT.AGA..T	.AG.T.A...	CCC..CT...	..A.C.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	ATC...A
#M.altisambesi.BOT22670_OkavangoR	.T..AC....	CTATA....C	..G.CT..C.	..TT....TG	..TT.CG...	.TC.GTGC..	T.CTA..TC.	.AT.AGAG.T	.AG.T.A...	CCC..CT...	..A.C.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	ATC...A
#M.altisambesi.BOT22671_OkavangoR	.T..AC....	CTATA....C	..G.CT..C.	..TT....T.	..TT.C....	.TC.GTGC..	T.CTA..TC.	.AT.AGA..T	.AG.T.A...	CCC..CT...	..A.C.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	ATC...A
#M.pongolensis.Mhlathuze_9123	.T..AC....	CTATA....C	..G.CT..C.	...T...AT.	..TT.C....	.TC.GT.CC.	T.CT...TC.	.AT.AG..CT	....T.A...	CCCT.CT...	....T.T.GT	AC.....T.	TAG.C..C.A	..T.T.....	.TC...A
#M.pongolensis.Mhlathuze_9124	.T..AC....	CTATA....C	..G.CT..C.	...T...AT.	..TT.C....	.TC.GT.CC.	T.CT...TC.	.AT.AG...T	.AG.T.A...	CCCT.CT...	....T.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	.TC...A
#M.pongolensis.Mhlathuze_9125	.T..AC....	CTATA....C	..G.CT..C.	...T...AT.	..TT.C....	.TC.GT.CC.	T.CT...TC.	.AT.AG...T	.AG.T.A...	CCCT.CT...	....T.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	.TC...A
#M.pongolensis_SA9_CrocodileR	.T..AC....	CTATA....C	..G.CT..C.	...T....T.	..TT.C....	..C.GT.C..	..CT...TC.	.ATTAT...T	.AG.T.A...	CCCTACT...	....C.T.GT	.C.....T.	TAG.C..C.A	..T.T.....	.TC...A
#M.pongolensis_SA12_CrocodileR	.T..AC....	CTATA....C	..T.CT..C.	...T....T.	..TT.C....	.TC.GT.C..	..CT...TC.	.AT.AG...T	.AG.T.A...	CCCTACT...	....C.T.GT	.C.....T.	TAG.C..C.G	..T.T...A.	.TC...A
#M.pongolensis_SA13_CrocodileR	.T..AC....	CTATA....C	....CT..C.	...T....T.	..TT.C....	..C.GT.C..	..CT...TC.	.ATTAG...T	.AG.T.A...	CCCTACT...	....C.T.GT	.C.....T.	TAG.C..C.G	..T.T...A.	.TC...A
#M.pongolensis_SA14_CrocodileR	.T..AC....	CTATA....C	..G.CT..C.	...T....T.	..TT.C....	.TC.GT.C..	T.CT...TC.	.AT.AG...T	.AG.T.A...	CCCTACT...	....C.T.GT	.C.....T.	TAG.C..C.A	..T.T...A.	.TC...A
#M.pongolensis_SA15_CrocodileR	.T..AC....	CTATA....C	..C.CT..C.	...T....T.	..TT.C....	.TC.GT.C..	T.CT...TC.	.AT.AG...T	.AG.T.A...	CCC.ACT...	....C.T.GT	.C.....T.	TAG.C..C.G	..T.T...A.	.TC...A
#M.macrolepidotus.Rovuma_25650	GT..AC....	CTATA....C	..G.CT..C.	...T....T.	..TT.C....	.TC.G..C..	T.CT...TC.	.ATTAG...T	.AG.T.A...	CCC..CT...	....T.T.GT	.C....T.T.	TAG.C..C.A	..TGT.....	.TCT..A
#M.macrolepidotus.Rovuma_25651	.T..AC....	CTATA....C	..G.CT..C.	...T....T.	..TT.C....	.TC.G..C..	T.CT...TC.	.AT.AG...T	.AG.T.....	CCC..CT...	....T.T.GT	.C....T.T.	TAG.C.TC.A	T.TGT.....	.TCT..A
#M.macrolepidotus.MOZ_LuciteR_22682	.T..AC....	CTATA....C	....CT..C.	...T....TG	..TT.CG...	..CGGTGC..	...CT...TC.	.ATTAG...G	.AG.T.A...	CCCTACT...	....C.T.GT	.C.....T.	TAG.C..C.A	..T.T...A.	.TC...A
#M.macrolepidotus.MOZ_LuciteR_22684	.T..AC....	CTATA....C	....CT..C.	...T....T.	..TT.C....	..C.GT.C..	..CT...TC.	.ATTAG...T	.AG.T.A...	CCCTACT...	....C.T.GT	.C.....T.	TAG.C..C.A	..T.T...A.	.TC...A
#M.macrolepidotus.MOZ_LuciteR_22685	.T..AC....	CTATA....C	....CT..C.	...T....T.	..TT.C....	..C.GT.C..	..CT.G.TC.	.ATTAG...T	.AG.TGA...	CCCTACT...	....C.T.GT	.C.....T.	AAG.C..C.A	..T.T...A.	.TC...A
#M.macrolepidotus.MOZ_Marromeu_22686	.TA.AC....	CTATA....C	....CT..CC	GG.G..G.T.	..TT.CG...	..CGGT.C.C	..CT.C.TC.	.ATTAG...T	.AGCT.A...	CCCTACTT..	....C.T.GT	.C.....T.	GAG.C..C.A	..T.T...A.	.TC...A
#M.macrolepidotus.MOZ_ZambesiR_22689	.TA.AC....	CTATA....C	....CT..C.	...G....TG	.TTT.CG...	..CGGTGC..	..CT.C.TC.	.ATTAG...T	CAG.T.A...	CCCTACT...	G...C.T.GT	.C.....T.	GAG.C..C.A	..T.T...A.	.TC...A

Appendix C: Reproductive plasticity of electric organ discharge

Second sample Sabie fish

Among the 15 fish from the Lower Sabie that were caught in March 1996 at the end of the local breeding season and recorded from in the field, 11 were female or juvenile; they agreed well with juveniles and females of the September population in all EOD waveform parameters (slopes and *Y*-intercepts of regression lines not significantly different). Four fish could clearly be recognized as males from the presence of a kink in their anal fin base; however, only the largest one (SL, 17.3 cm) had slightly higher values for duration and area-under-curve parameters of its EOD waveform than females and juveniles. The EODs of 13 fish (including the large male) were also recorded in the European laboratory, three months after both field recordings and arrival. Compared to the field recordings, small statistical differences in EOD waveform parameters of individually known fish ($N=13$) were all not significant; similar result for subsequent EOD recordings, 6 and 8 months after the field recordings (Kramer, pers. obs.; Lang, unpublished thesis 1997). One second-sample male *M. pongolensis* reproduced repeatedly in a communal aquarium where it was kept with four females, from July to December 1997. This group of fish were well fed, had an aquarium with many plants and good cover under a natural light/dark regime but were left undisturbed otherwise, and water conductiviy had swung upwards (about 400 $\mu S\,cm^{-1}$).

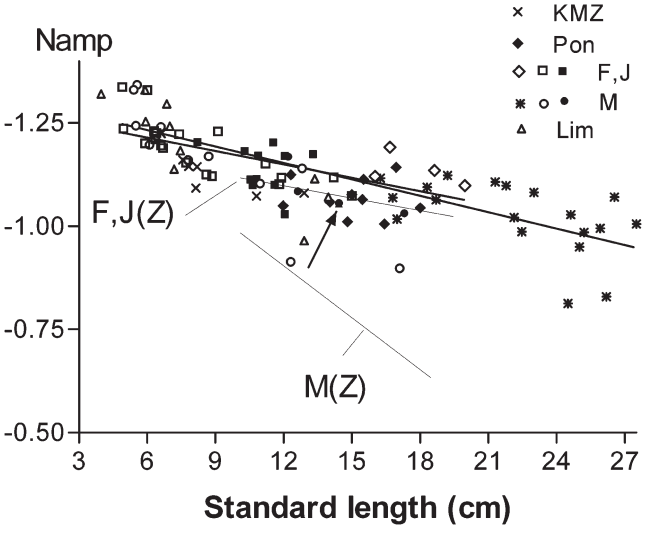


Figure 13. The amplitude of the head-negative phase of an EOD (Namp re: Pamp=1; dimension: Volt) decreases with fish size in *M. pongolensis* (heavy lines) according to a least-squares regression line. The lines for males (M) and females (including some small juveniles the sex of which could not be determined; F,J) are similar; they differ neither in slope nor in elevation ($P>0.05$). First sample: □, 11 males; □, 15 females (including four juveniles of indeterminate sex). Second sample: ●, four males; ■, 11 females and juveniles. Third sample: ✶, 19 males; ◇, four females. Hairline regressions, for female (including juvenile, F,J(Z)) and male *M. altisambesi* (M(Z)). No data points shown for *M. altisambesi*. A male *M. pongolensis* (●) of 14.4 cm SL is also shown 18 months after capture, when reproducing in aquarium (▲, arrow, not included in regression analyses). Open triangles, *M. pongolensis* from the Limpopo System (Lim; $N=11$). ×, *M. pongolensis* from the KwaMaZulu stream (KMZ; $N=7$) of the Mhlatuze System. □, *M. pongolensis* from the Pongola River (Pon; $N=10$).

Table XIII. Change of EOD waveform characters of a male *M. pongolensis* (no. 10) during reproduction in captivity.

Months	Namp (V)	Pdur (μs)	Ndur (μs)	PNsep (μs)	Parea (V × μs)	Narea (V × μs)
0	−1.056	295	216	85.9	82.2	87.1
3	−1.03	339	217	92	76.9	84.4
6	−1.025	327	208	92	76.5	83.6
8	−1.001	411	275	100	87.9	93.1
18	−1.054	442	452	132	142.5	147.3

Abbreviations of EOD waveform characters, Material and methods. ''Zero months'' designates measurements taken in the field immediately after capture. Reproduction at 18 months following import to European laboratory.

No reproduction-correlated change was observed for the amplitude of this fish's *N* phase throughout the whole observation period (Table XIII; Figure 13, ▲, covered by ●, arrow). However, during its reproductively active period (July–November 1997), the male's EOD increased markedly in duration, and so did the interval between the peaks (Table XIII). In December EOD duration receded somewhat back to a more ''normal'' value for this fish, and reproduction was over (Figure 14). Disregarding the two outliers from the first-sample fish (main text, Figure 11), the EOD of the reproducing male showed the highest *P* and *N* area values among all *M. pongolensis* of this study, an increase of around 70% compared to this fish's EOD as recorded in the field (in the non-reproductive season). Duration values (*P* and *N* phases, PNsep), as measured during reproduction, were very high for a fish of this size (Figure 15, ▲). These observations were our first evidence of a temporary, reversible EOD waveform change during ongoing reproduction in a mormyrid fish. It occurred spontaneously and was not provoked by any coercive measure like hormone treatment. Figure 15 shows that even in this reproductively active male a clear difference separates *M. pongolensis* from *M. altisambesi* male EODs.

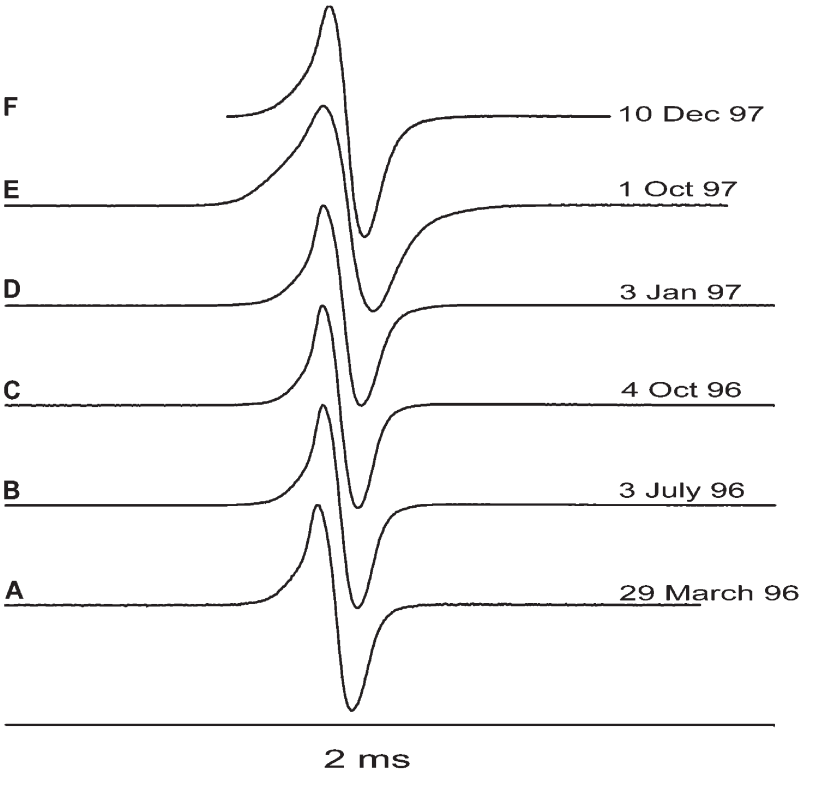
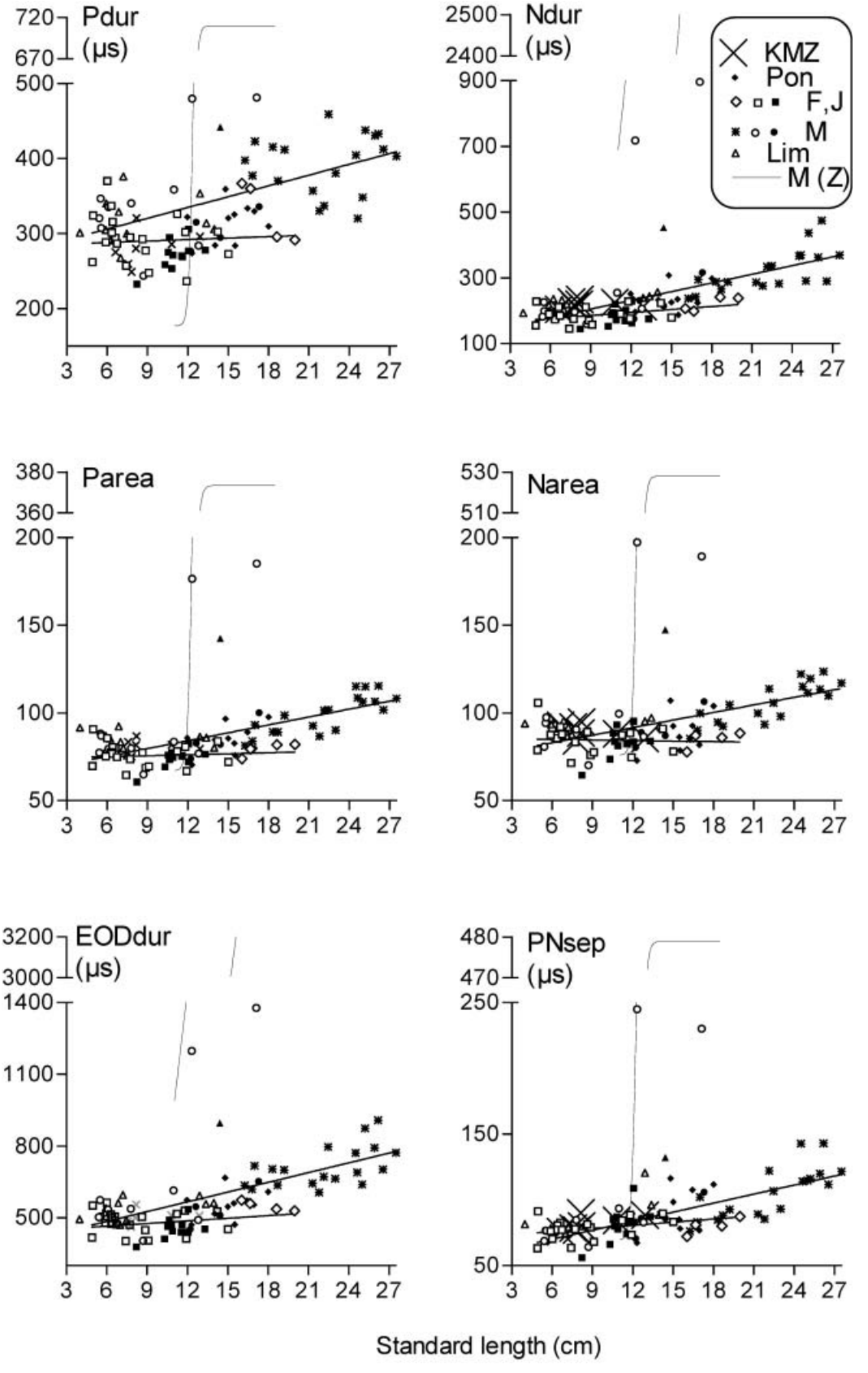


Figure 14. Oscillograms of EODs of *M. pongolensis* no. 10, a male of the second sample. (A) Recording in the field; (B–F) in the laboratory. (B–D) No reproductive activity; (E) during successful reproduction with eggs on the tank floor (reared into adulthood); (F) post-reproduction. (A) Recorded at 23.3°C, normalized to 25°C. (B–D) Recorded at 25°C; (E, F) at 25.5±0.5°C.

Figure 15. Relationship of electric organ discharges (EODs) in *M. pongolensis* with fish size (standard length), as studied by regression analysis (symbols for the four fish samples, see Figure 13). Abbreviations of EOD parameters: Parea, Narea, areas under the *P* and the *N* phases, respectively (with the peak amplitude of the *P* phase normalized to 1; dimension, V×µs); Pdur, Ndur, durations of the *P* and *N* phases in µs, respectively; PNsep, interval, or separation, between the peaks of the P and N phases in µs; EODdur, duration of an EOD (that is, *P* plus *N* phase duration) in µs. In contrast to females (including small juveniles of unknown sex), in males there is a statistically significant (Table V) increase in each of these parameters with fish size (for females, also in Ndur). These sex differences are only weak when compared to those observed in *M. altisambesi* that represent a sexual dimorphism occurring at sexual maturity, around 12.5 cm SL (superimposed hairline curves of sigmoidal shape for males, M(Z); Kramer 1997b). A male *M. pongolensis* (●) of 14.4 cm SL is also shown 18 months after capture, when reproducing in aquarium (▲, not included in regression analyses). Open triangles, *M. pongolensis* from the Limpopo System (Lim; *N*=11). ×, *M. pongolensis* from the KwaMaZulu stream (KMZ; *N*=7). □, *M. pongolensis* from the Pongola River (KMZ; *N*=10). Size of ×s increased where almost totally covered by other symbols.


Pdur
(µs)
720
670
500
400
300
200
Ndur
(µs)
2500
2400
900
700
500
300
100
KMZ
Pon
F,J
M
Lim
M (Z)
Parea
380
360
200
150
100
50
Narea
530
510
200
150
100
50
EODdur
(µs)
3200
3000
1400
1100
800
500
PNsep
(µs)
480
470
250
150
50
3 6 9 12 15 18 21 24 27
Standard length (cm)

Appendix D: Principal components analysis on morphology

Table XIV. Principal Components Analysis on correlations for morphological characters from bulldog fish from various origins (N=352)

Eigenvalue	4.096	2.942	1.325	1.009	0.8982	0.6401	0.5209
Percent	31.504	22.628	10.195	7.762	6.91	4.924	4.007
Cum Percent	31.504	54.132	64.328	72.089	78.999	83.923	87.93
Eigenvectors							
PDL/SL	0.3719	−0.2736	−0.0262	−0.0470	0.0969	0.2483	0.0774
PAL/SL	0.3816	−0.2630	−0.1794	−0.0295	−0.0032	0.0764	0.1051
LD/SL	0.1876	0.4287	0.1772	0.0847	0.1278	0.1007	0.2936
LA/SL	0.1579	0.3568	0.4559	0.2884	0.0157	0.0129	−0.3539
pD/SL	−0.2133	0.4416	0.0069	0.2065	−0.0811	0.1419	0.2596
CPL/SL	−0.3709	0.0468	−0.3827	0.0964	−0.1857	0.2861	0.1623
CPD/CPL	0.3604	−0.1144	0.2168	0.0161	−0.0080	−0.4198	0.3934
LSc/HL	−0.2057	−0.2132	0.3888	−0.3889	0.3255	0.5481	0.0103
HL/SL	−0.10001	−0.1153	−0.2706	0.4932	0.7915	−0.1108	−0.0707
BD/SL	0.3879	0.0899	0.0228	0.278	0.0209	0.5349	0.0492
nD	0.1077	0.3666	−0.2760	−0.4033	0.2828	−0.0265	0.4439
nA	0.148	0.3475	−0.1451	−0.4667	0.2589	−0.1106	−0.4846
SPc	−0.3266	−0.1130	0.4606	−0.047	0.2305	−0.1795	0.2892
Component loadings							
PDL/SL	0.7527	−0.4692	−0.0301	−0.0472	0.0918	0.1986	0.0558
PAL/SL	0.7722	−0.4511	−0.2065	−0.0296	−0.0030	0.0611	0.0759
LD/SL	0.3797	0.7352	0.2041	0.0851	0.1211	0.0806	0.2119
LA/SL	0.3196	0.6119	0.5248	0.2897	0.0149	0.0103	−0.2554
pD/SL	−0.4316	0.7573	0.0079	0.2074	−0.0768	0.1136	0.1874
CPL/SL	−0.7506	0.0802	−0.4406	0.0969	−0.176	0.2289	0.1172
CPD/CPL	0.7294	−0.1962	0.2496	0.0162	−0.0076	−0.3359	0.284
LSc/HL	−0.4162	−0.3656	0.4476	−0.3907	0.3085	0.4386	0.0075
HL/SL	−0.2025	−0.1977	−0.3116	0.4954	0.7501	−0.0887	−0.0510
BD/SL	0.7851	0.1542	0.0262	0.2792	0.0198	0.4279	0.0355
nD	0.218	0.6287	−0.3178	−0.4051	0.2680	−0.0212	0.3204
nA	0.2995	0.596	−0.1670	−0.4688	0.2453	−0.0885	−0.3497
SPc	−0.6609	−0.1938	0.5303	−0.0472	0.2184	−0.1436	0.2087