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A new species of Slender Stonebasher within the *Hippopotamyrus ansorgii* complex from the Cunene River in southern Africa (Teleostei: Mormyriformes)

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Previous work has revealed that *Hippopotamyrus ansorgii* (Boulenger, 1905) is a species complex, with specimens from the Upper Zambezi system (Caprivi Strip in Namibia) that are well differentiated from the Angolan type specimens. Here, we sampled the Cunene River, one of several possible type rivers for *H. ansorgii*, on the border between Namibia and Angola. The specimens are morphologically differentiated from the six other known morphs within the *H. ansorgii* species complex, including the types and *H. szaboi*. The electric organ discharge of the Cunene specimens has a different pulse waveform and associated amplitude spectra to that of the three Upper Zambezi system morphs of the species complex. The Cunene specimens are genetically distinct, but relatively closely related to other taxa of the *H. ansorgii* complex, such as the Upper Zambezi system morphs. We recognize the Lower Cunene morph as the new species, *Hippopotamyrus longilateralis* sp. nov.

Keywords: Mormyridae; allopatric speciation; electric organ discharge; molecular genetics; vicariance

Introduction

The mormyrid fish genus *Hippopotamyrus* is defined on certain skeletal characters (Taverne 1971b) and comprises about 16 species in tropical and subtropical Africa. The southern African species of this genus, called *Hippopotamyrus ansorgii* (Boulenger, 1905) or the slender stonebasher, has been recorded in the Cuanza, Cunene, Okavango, Upper Zambezi, Buzi and Pungwe River systems and in Malawi (Skelton 2001). Despite this widespread distribution, populations are fragmented. The type locality for *H. ansorgii* was only defined as "between Benguella and Bihé" (Boulenger 1909: 74), which probably refers to the Angolan highlands in the current provinces of Benguela, Huambo and Bié. The mountains in this region give rise to at least five major river systems, one of them being the Cunene River. The latter could therefore be the type river for *H. ansorgii*. A critical comparison among the populations isolated in different river systems is further complicated, because only two syntype specimens and no paratypes were designated for *H. ansorgii*.

A study of the electric organ discharge (EOD) and the genetics of *H. ansorgii* in the Upper Zambezi River system revealed the presence of a species complex rather than a single species (Figure 1), and one morph was described as the new species

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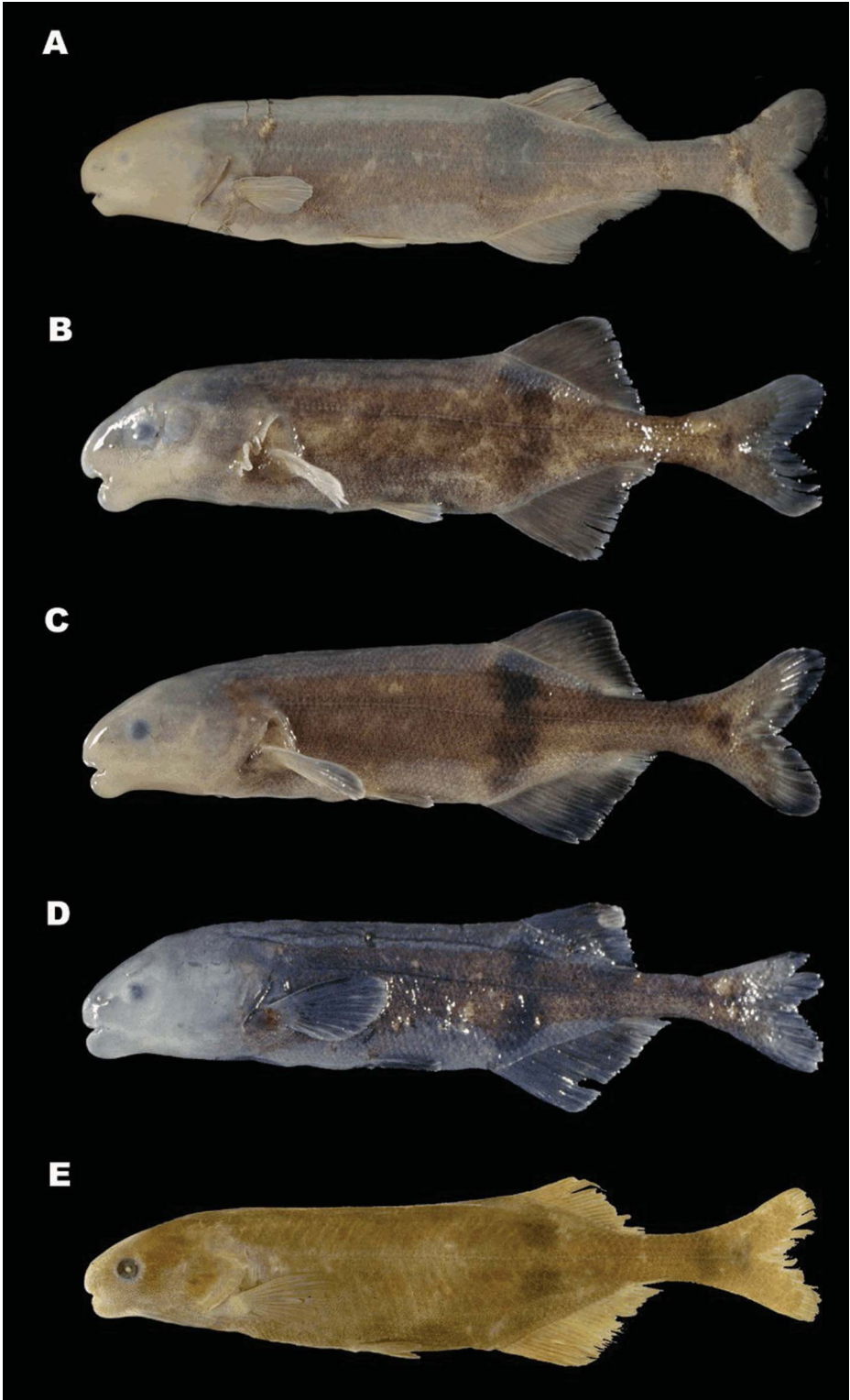
Hippopotamyrus szaboi (Kramer et al. 2004). The other two morphs will be referred to as the Upper Zambezi and the Kwando morphs, based on their original collection localities. The specific status of the latter two morphs remains unresolved, because of insufficient knowledge of the taxonomic diversity within *H. ansorgii*. There are even two morphs from the general type region that are distinct from the types (the Rio Cuchi and the upper Cunene Cukimaala morphs). Although the Upper Zambezi study revealed clear differentiation between sympatric morphs, the question of whether speciation among the geographically isolated populations has occurred remains largely unanswered.

When studying slender stonebashers from the lower Cunene River, it became clear that they belonged to yet another morph of *H. ansorgii*. We compared the EODs, morphology and mitochondrial DNA sequences with all available morphs within the *H. ansorgii* species complex, to determine their taxonomic status. Previous studies of other southern African mormyrid species have been successful using this approach (Kramer et al. 2003, 2004, 2007; Kramer and van der Bank 2000). For more tropical mormyrid species, the usefulness of genetic studies, often in combination with morphological or electrical techniques, was shown by Sullivan et al. (2000, 2002, 2004), Lavoué et al. (2000, 2004), Lavoué and Sullivan (2004), Agnèse and Bigorne (1992), Arnegard and Hopkins (2003), Arnegard et al. (2006) and Feulner et al. (2006, 2007).

In the present study, we compare the new sample from the lower Cunene River with the types and with the five other samples. We tested the hypothesis that the Cunene specimens belong to a different allopatric species within the *H. ansorgii* complex, therefore showing a clear divergence in EODs, morphology and mitochondrial DNA sequences compared with other morphs.

Materials and methods

Sampling

Hippopotamyrus specimens were caught with monofilament gill nets and anaesthetized with 2-phenoxy-ethanol after recording EODs from the live specimens. Muscle samples were placed in 98% ethanol and deposited in the Biomaterial Bank of the South African Institute for Aquatic Biodiversity (SAIAB, Grahamstown). The source specimens and additional voucher specimens were fixed in 10% formalin and deposited in the ichthyological collection of the same institution and in the Bavarian State Collection, Munich (ZSM, Zoologische Staatssammlung München, Germany). The specimens were collected with permits from the Namibian Ministry of Fisheries and Marine Resources and the ethics of the collecting methods were approved by SAIAB.

Morphology

In total, 29 specimens from the Cunene River were compared to 97 specimens from Angola and the Namibian part of the Upper Zambezi system (geography, Figure 2). Fifteen anatomical measurements (Figure 3) and four counts were recorded: PDL,

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Figure 1. Specimens of the *Hippopotamyrus ansorgii* species complex. (A) *Hippopotamyrus longilateralis* (specimen Kune20); (B) *Hippopotamyrus szaboi*; (C) Upper Zambezi *H. ansorgii* morph; (D) Kwando *H. ansorgii* morph; (E) *H. ansorgii* Type specimen.

Figure 2. Geography of the study area, with the sites Epupa Falls and Ruacana Falls on the Cunene River (lower Cunene morph), the Wenela site on the Upper Zambezi River (Upper Zambezi morph and *Hippopotamyrus szaboi*) and the Kongola site on the Kwando River (Kwando morph).

predorsal length (distance from the tip of the snout to the dorsal fin origin); PAL, distance from tip of snout to anal fin origin; LD, dorsal fin length; LA, anal fin length; pD, distance from dorsal fin origin to end of caudal peduncle; CPL, length of caudal peduncle (end of anal fin base to midbase of the caudal fin); CPD, depth of caudal peduncle (the least vertical distance across the caudal peduncle); LSo, length of snout (distance from the tip of the snout to the posterior orbital rim of the eye); LSc, length of snout (distance from the tip of the snout to the centre of the eye – measured but not

Figure 3. Morphological characters and how they were measured on specimens of the *Hippopotamyrus ansorgii* species complex. For abbreviations, see the Material and methods section.

used for redundancy with LSo); HL, head length (distance from the tip of the snout to the furthest bony edge of the operculum); Na, distance between the pair of nares on one side (from centre to centre); OD, eye diameter defined by the orbital rims; LPF, length of the pectoral fins; PPF, distance from the origin of the pelvic fin to the origin of the anal fin (some specimens); SL, standard length (distance between the tip of the snout to the midbase of the caudal fin); BD, body depth (the greatest vertical distance across the body); TL, total length (distance from the tip of the snout to the end of the caudal fin); nD, number of dorsal fin rays; nA, number of anal fin rays; SPc, number of scales around the caudal peduncle; SLS, number of scales in a linear series along the lateral line row, as detailed in Skelton (2001: 67); SLS, range of accuracy ± 2 counts.

Abbreviations used to represent institutions and collections cited follow Leviton et al. (1985), with the exception of the acronym RUSI which has 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 (2001), which are considered effective for fish populations occurring in southern Africa. Abbreviations for species and morphs of the *H. ansorgii* species complex: Hal, Upper Zambezi morph; Ha2, *Hippopotamyrus szaboi*; Ha3, Kwando morph; Ha4, lower Cunene morph; Ha5, upper Cunene-Cukimaala morph; Ha6, Rio Cuchi morph.

The male sex was inferred when the anal fin base showed a kink, a character also present in many other species of mormyrids.

Electric organ discharges

The EODs of live fish were recorded in a field laboratory immediately after capture. Measurements were made in a 37-litre plastic aquarium filled with water from the river where the fish was collected. Conditions in the aquarium were kept constant throughout the analysis to exclude the possibility that water quality (especially conductivity) could affect EOD measurements.

Temperature (± 0.1°C) and water conductivity (± 1 µS/cm) were constantly monitored using an electronic apparatus (LF318 WTW, Weilheim, Germany). Fish were placed between a pair of carbon rod electrodes that were connected to a differential amplifier with a variable gain (up to × 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 (100 MHz/9 bit/10 000 points per sweep) and data were numerically transferred onto disk via digital interface. Usually eight traces per fish were recorded. All the field equipment was battery operated.

Custom-designed computer programs were used for analysis of EODs (programmed using a software package for signal analysis, FAMOS v5). When necessary, EOD duration was corrected to 25°C using a Q_{10} value of 1.5 (Kramer and Westby 1985) before data analysis.

The EOD waveform variables can be defined as follows (compare with Figure 4): P1amp, peak amplitude of positive P1 phase (i.e. from baseline to peak, which is equal to 1 V by definition); P0amp and P2amp, positive peak amplitudes of pre- and post-potentials, respectively; N0amp and N1amp, negative peak amplitudes of N0 and N1 phases of EOD re: P1amp = 1; N0dur, P1dur and N1dur, durations of respective
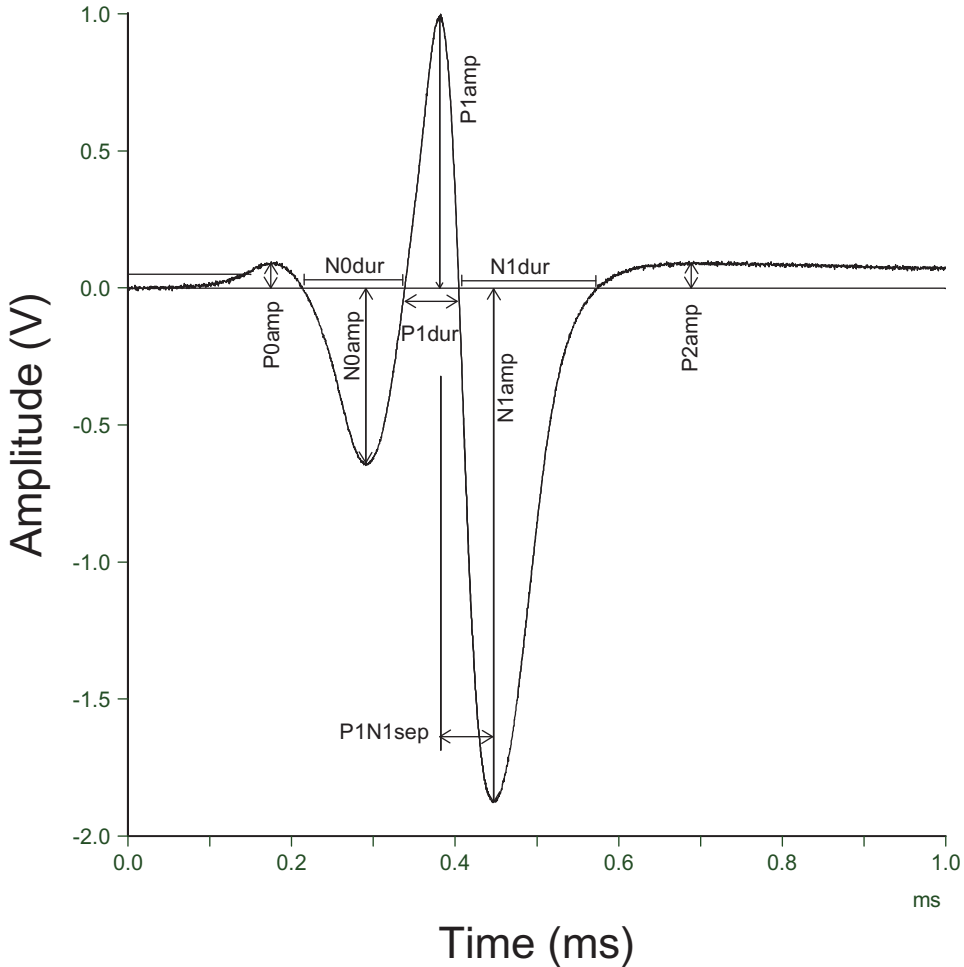
Figure 4. Characters of an electric organ discharge (EOD) pulse from the lower Cunene *Hippopotamyrus* morph, recognized as *Hippopotamyrus longilateralis* sp. nov., as measured in the present paper. By definition, the positive peak amplitude (P1amp) equals 1 V.

phases; P1N1sep, separation (or interval) between the peaks of the P1 and N1 phases; P1area and N1area, areas under the P1 and N1 phases. Durations in microseconds; amplitudes in relative Volts (re: P1-phase amplitude = 1). Area-under-curve measures, dimension (V × microseconds). The start of the initial P0 phase was determined at a threshold level of 5% of P1amp; other phases start and end between zero-crossings.

A fast Fourier transform (FFT) routine provided by FAMOS performed amplitude spectra of single EOD pulses. A/D sampling rates were reduced to between 100 and 125 kHz. The number of data points for FFT analysis was 2^{19}, obtained by extending baseline with zeros such that a single EOD per record was centred. Frequency resolution was < 0.24 Hz.

Subsequent to EOD recording, fish were killed with an overdose of the anaesthetic 2-phenoxy-ethanol. SL was measured with vernier callipers before fixing the specimen in 10% formalin for morphological studies.

Statistical analysis

Principal component analyses on correlations among anatomical characters were used to test differences in body shape among populations, because PCA does not require *a priori* assumptions about taxonomic groups. Analyses of variance (ANOVA) were performed to test the hypotheses of no difference between samples for each character separately. Multivariate analyses of variance (MANOVA; Table 1) were used to prevent overestimating differentiation in testing the hypothesis of no morphological difference between fish from different origins (McGarigal et al. 2000). Values of *p* were two-tailed unless otherwise stated. We determined the component loadings, i.e. the principal component structure, for interpreting the principal components in terms of the anatomical characters (Appendix 1, Table 3; see McGarigal et al. 2000). We followed Tabachnick and Fidell (2000) to interpret the significance of component loadings. These authors recognize five levels of significance: loadings >0.32 or < – 0.32 are poor, >0.45 or < – 0.45 are fair, >0.55 or < – 0.55 are good, >0.63 or < – 0.63 are very good, and >0.71 or < – 0.71 are excellent. These benchmarks account for 10%, 20%, 30%, 40% and 50% of the variance in the component. We also performed a discriminant analysis to find the best separation among the lower Cunene, the Upper Zambezi, and the Kwando morphs and *H. szaboi* in multidimensional space (Figure 5B), using JMP v. 7.0.1 software (SAS Institute, Cary, NC, USA, 2007).

DNA extraction, amplification and sequencing

Genomic DNA was isolated from the ethanol-preserved tissues using a purification kit (Promega). Amplification by polymerase chain reaction (PCR) of the mitochondrial cytochrome *c* oxidase I (MT-COI) gene was achieved with the primers VF2-T1 5′ TGT AAA ACG ACG GCC AGT CAA CCA ACC ACA AAG ACA TTG GCA C 3′ and VR1-T1 5′ CAG GAA ACA GCT ATG ACT AGA CTT CTG GGT GGC CAA AGA ATC A 3′ (Ivanova et al. 2007) and for mitochondrial cytochrome *b* with the primers GluF 5′ AAC CAC CGT TGT ATT CAA CTA CAA 3′ (Machordom and Doadrio 2001) and H15917 5′ TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT 3′ (Kramer et al. 2003). Amplification was performed in 25-μl volumes containing 1 × buffer, 2.5 mM $MgCl_2$, 0.2 mM of each of the four nucleotides (ABGENE, Epsom, UK), 1 μM of each primer, 1 U of Super-Therm DNA polymerase (ABGENE) and 100–200 ng template DNA. Conditions for the amplification of cytochrome oxidase I (COI) were 3 minutes at 94°C, then 38 cycles of 30 seconds at 94°C, 30 seconds at 54°C and 50 seconds at 72°C, finishing with 7 minutes at 72°C. For cytochrome *b* the PCR conditions were 4 minutes at 94°C with 31 cycles of 45 seconds at 94°C, 1 minute at 52°C and 2 minutes at 72°C, finishing with 5 minutes at 72°C. PCR products were sent to Macrogen Inc. (Seoul, Korea) for purification and sequencing. Sequences were deposited in GenBank under accession numbers GU453680–GU453688 and HM065027–HM065034.

Table 1. Morphological comparisons among four electrical phenotypes of the *Hippopotamyrus ansorgii* species complex, *Hippopotamyrus longilateralis* sp. nov. (Ha4), *Hippopotamyrus szaboi* (Ha2), the Upper Zambezi morph (Ha1), and the Kwando morph (Ha3).

	PDL/SL	PAL/SL	LD/SL	LA/SL	pD/SL	CPL/SL	CPD/CPL	LSo/HL	HL/SL	BD/SL	nD	nA	SPc	SLS
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	<0.0001
Post-tests*														
Ha1,Ha2		<0.05				<0.05	<0.05		<0.01				<0.01	
Ha1,Ha3	<0.05		<0.01	<0.01		<0.05	<0.05	<0.01	<0.05	<0.01				
Ha1,Ha4	<0.01				<0.01	<0.05	<0.01		<0.01		<0.01	<0.01	<0.01	<0.01
Ha2,Ha3			<0.01	<0.01	<0.01	<0.01	<0.01	<0.05		<0.01	<0.05		<0.01	
Ha2,Ha4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01	<0.01	<0.01		<0.01	<0.05	<0.01
Ha3,Ha4	<0.01	<0.05	<0.01	<0.01	<0.01		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Notes: Multivariate analysis of variance (MANOVA), followed by ANOVAS for individual morphological characters, and pairwise *post-hoc* tests. Means, SEs, etc., see Table 2. Abbreviations of morphological characters are given in Material and methods.

P values > 0.05 are not given.

MANOVA P value: same for Wilk's lambda, Roy's Greatest Root, Hotelling–Lawley Trace, and Pillai Trace tests ($F_{\ge 14,67} \ge 12.468$).

ANOVAS: $F_{3,78} \ge 8.785$. Ha1, $N = 21$; Ha2, $N = 12$; Ha3, $N = 20$; Ha4, $N = 29$.
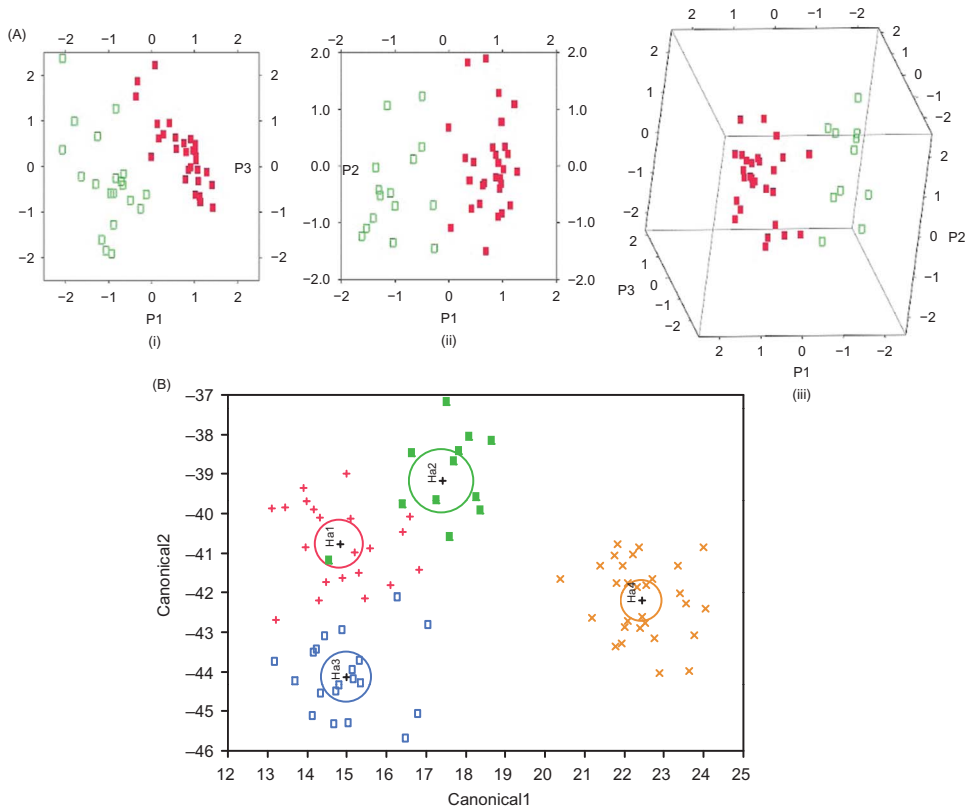
*Games–Howell test.

Figure 5. (A) Principal components analysis on 14 morphological characters for *Hippopot-amyrus szaboi* and the lower Cunene, Upper Zambezi and Kwando morphs of the *Hippopot-amyrus ansorgii* species complex. Lower Cunene morph (*n* = 29, red filled squares) plotted against (i) the Upper Zambezi morph (*n* = 21, with SLS, green open squares); (ii) Kwando morph (*n* = 20, with SLS, green open squares); (iii) *H. szaboi* (*n* = 12, with SLS, green open squares). P1–P3, principal components 1 to 3. (B) Discriminant function analysis on 14 mor-phological characters for *H. szaboi* and the lower Cunene, Upper Zambezi and Kwando morphs of the *H. ansorgii* species complex. Lower Cunene morph specimens (orange crosses, *n* = 29); Kwando morph specimens (blue open squares, *n* = 20); *H.* szaboi from Upper Zambezi (green solid squares, *n* = 12); Upper Zambezi morph specimens (red plus signs, *n* = 21). Circles indicate 95% confidence limits. [This figure can be viewed in colour online].

Phylogenetic analyses

Only the cytochrome *b* sequences were used in phylogenetic analysis because of the avail-ability of outgroups in GenBank. A model of nucleotide substitution that best fitted the sequence data was selected from 56 such models with an Akaike test in MODELTEST ver-sion 3.06 (Posada and Crandall 1998). These parameters were used in a maximum likeli-hood (Felsenstein 1981) estimation of phylogenetic relationships in the program PAUP* (Swofford 2002) to assess the phylogenetic relationships among the mormyrid species. Starting trees for the maximum likelihood analysis were obtained through the neighbour-joining method (Saitou and Nei 1987). The optimal tree was obtained through a heuristic search with 10 random sequence additions. A parsimony analysis (Hennig 1966) was also

performed in PAUP* to assess branch support with 10000 non-parametric bootstrap replicates (Felsenstein 1985). Each bootstrap replicate was performed with 10 replicates of random taxon addition. The parsimony analysis was performed using heuristic searches, tree bisection–reconnection branch swapping and 1000 random additions of taxa.

Material examined

Hippopotamyrus ansorgii *(Boulenger, 1905)*

Marcusenius ansorgii Boulenger, 1905. Syntypes: BMNH 1905.5.29.62–63 (2) from "Betweeen Benguella and Bihé", Angola.

   Marcusenius ansorgii Boulenger, 1905. ZMH 1817. ZMH 1817, upper Cunene Cukimaala morph (also 'morph Ha5'), two specimens, Rio Cukimaala near north Lisboa (which has been renamed Huambo after 1975), a small tributary (headwater) of the Cunene in Angola, leg. Machado, 12 May 1961. The collecting site was at 12°45′ S, 15°46′ E according to P. Skelton (personal communication), based on Penrith (1982), at 1700 m altitude.

   Marcusenius ansorgii Boulenger, 1905. ZMH 1790, Rio Cuchi morph (also 'morph Ha 6'), two specimens, Rio Cuchi in Angola, a tributary of the Kubango (see map of Figure 2), no specific place given, leg. Ladiges and Voelker, 9 October 1961.

   Hippopotamyrus ansorgii, Upper Zambezi morph (also 'morph Ha1'). Thirty-four specimens from the Upper Zambezi River, East Caprivi, Namibia,(ZSM 29790, 29792–29807, 17 specimens; and SAIAB 67459, 67461–67464, 67467–67469, 17 specimens): (1) four specimens from Zambezi River, Katima Mulilo, approx. 17°29′30″ S, 24°16′18″ E, 11 September 1993; (2) eight specimens from Zambezi River, rapids at Wenela just upstream of Katima Mulilo (border post to Zambia; 17°29′21.5″ S, 24°15′33″ E), 3–5 April 1996; (3) five specimens from Zambezi River, Wenela, 9 September 1997; (4) six specimens from Zambezi River, Wenela, 23–27 August 1999; (5) 11 specimens from Zambezi River, Wenela, 25 January 2001.

   Hippopotamyrus ansorgii, Kwando morph (also 'morph Ha3'). Thirty-six specimens of what is considered a different species of *H. ansorgii* from the Kwando River, Caprivi Strip, Kongola (at bridge of Golden Highway, B8, crossing the Kwando), 17°47′33″ S, 23°20′33″ E, (ZSM 29773–29789, 29791, 18 specimens; and SAIAB 67460, 67465, 67466, 18 specimens): (1) four specimens, 9 April 1996; (2) 20 specimens, 26 August 1999; (3) 12 specimens, 24 January 2001.

Hippopotamyrus szaboi *Kramer et al. 2004*

Hippopotamyrus szaboi Kramer et al. 2004, also referred to as 'morph Ha2' in earlier papers and present crowded Tables. Holotype SAIAB 67143, paratypes SAIAB 67144 (1), SAIAB 67145 (4), ZSM 29765–70 (6), SAIAB 67146 (4), SAIAB 67147 (1), ZSM 29771 (1), ZSM 29772 (1), from Upper Zambezi River, Katima Mulilo, rocks in middle of river, 17°29′30″ S, 24°16′18″ E, and Wenela, close by upstream, 17°29′21.5″ S, 24°15′33″ E.

Hippopotamyrus longilateralis *sp. nov.*

Hippopotamyrus longilateralis, sp. nov., also referred to as 'morph Ha4'. Holotype SAIAB78793 (field code, Kune20), 25 paratypes: SAIAB79502 (Kune01), SAIAB 79503 (Kune02), SAIAB78778 (Kune03), SAIAB78779 (Kune05, Kune07, Kune08, Kune09, Kune12), SAIAB78793 (Kune22, Kune25), SAIAB78783 (Kune32, Kune34), ZSM38561–ZSM38567 (13 specimens: Kune04, Kune13, Kune15, Kune17, Kune19,

Kune21, Kune23, Kune26, Kune27, Kune29, Kune30, Kune31, Kune33). From above Epupa Falls on the Namibian/Angolan border, 17°00′07″ S, 013°14′57″ E, altitude about 600 m. Additional material from an upstream location just below Ruacana Falls, 17°24′24″ S, 014°13′01″ E, about 800 m altitude: SAIAB78787 (three specimens: Ruac04, Ruac05, Ruac08). A total of 22 specimens for EOD, two from Ruacana.

Additional material from which DNA was extracted

Hippopotamyrus sp. SAIAB 67420: Country: Mozambique Province: Manica Area: Chimanimani Transfrontier Conservation Area System: Buzi River Branch: Mussapa Grande River: Mudzira River Locality: Mudzira River below Mahate 19°40′29″ S, 33°11′26″ E.

   Hippopotamyrus wilverthi SAIAB 79865: Country: Zambia Province: Northern Area: Sumbu System: Lake Tanganyika Water body: Lake Tanganyika Locality: Cape Kashese Harbour 8°29.29′ S, 30°28.47′ E.

   Cyphomyrus discorhynchus from Sullivan et al. (2000), CU 79743 from Lake Malawi.

   Cyphomyrus psittacus from Sullivan et al. (2000), MNHN 199-610 from the Niger River in Mali.

   Marcusenius altisambesi from Kramer et al. (2007), Upper Zambezi (17°32′24″ S, 24°32′25″ E).

   Pollimyrus castelnaui from Kramer et al. (2003), from the Okavango Delta in Botswana, close to Makwena River Camp near locality Etsha 10, 19°03′16.2″ S, 22°22′51.3″ E, 20–21 January 2001.

Results

Anatomical comparisons

*Hippopotamyru*s *ansorgii* (Boulenger, 1905) is morphologically different to the sample from the lower Cunene (Appendix 1, Table 2). The two syntypes do not overlap with the lower Cunene morph ($n = 29$) in their lower SPc (16 versus median 20, range 19–22), lower SLS (max. 72 versus median 76, range 74–78), and lower LSo; they overlap only marginally in their lower BD and lower pD (maximum scores for both characters less than the 10th percentiles of the lower Cunene morph).

   Hippopotamyrus szaboi and all the other morphs of the *H. ansorgii* species complex also differ morphologically from the lower Cunene morph. The specimens from the upper Cunene in the Cukimaala tributary ($n = 2$) have a greater PAL and Na, and lower scores for pD, CPL, nD and nA. These samples are therefore well differentiated from the lower Cunene morph, despite being from the same river system. The Cuchi River specimens ($n = 2$) differ in having lower SPc counts (16 versus median 20, range 19–22). The 90th percentiles of the lower Cunene morph scores were less than the lower limits of the Cuchi morph specimens in PDL, PAL and Na, whereas the upper limits of the Cuchi morph samples were less than the 10th percentiles of the lower Cunene morph samples in CPD, nD and nA. The Upper Zambezi morph ($n = 29$) also had lower SPc counts (median 16, range 16–18 versus median 20, range 19–22). It also had lower SLS counts (median 66, range 58–71, versus median 76, range 74–78). In the lower Cunene morph 25th percentile scores were greater than and did not overlap with the 75th percentiles for the Upper Zambezi morph in pD, CPL, nD and nA, whereas the reverse relationship held for HL. The Kwando morph ($n = 37$) differed in a number

of characters from the lower Cunene morph. The 90th percentile levels for the Kwando morph sample were less than the 10th percentiles for the lower Cunene morph in SPc and SLS. Less extreme differences were found where the 25th percentiles for the lower Cunene morph were greater than and did not overlap with the 75th percentiles for the Kwando morph, such as in characters LD, LA, pD, LSo, nD and nA. The reverse relationship (25th percentiles of the Kwando morph were greater than the 75th percentiles of the lower Cunene morph) was found for PDL, HL and Na.

The species *H. szaboi* from the Upper Zambezi River ($n = 19$), showed greater HL scores than the lower Cunene morph samples with no overlap, whereas the 10th percentile for PAL of *H. szaboi* did not overlap the smaller 90th percentile in the lower Cunene morph. Also PDL and CPD were greater in *H. szaboi* than in the lower Cunene morph, since the 25th percentiles of *H. szaboi* did not overlap with the 75th percentiles in the lower Cunene morph For pD the same, but reversed, relationship was found.

A MANOVA on 14 morphological characters rejected the hypothesis of no difference among the lower Cunene, Upper Zambezi and Kwando morphs and *H. szaboi* (Table 1). As shown by subsequent ANOVAs followed by *post-hoc* tests, the lower Cunene morph differed significantly from *H. szaboi* in seven characters, the Upper Zambezi morph in eight characters and from the Kwando morph (the geographically closest population) in 13 characters.

Principal components analysis of correlations on the same 14 morphological characters for the same individuals showed that more than 71% of the variance among the four samples was explained by the first three components (P1–P3). The analysis therefore successfully reduced redundancy (dimensionality) in the dataset (Appendix 1, Table 3). P1 appears to be a gradient for the length or importance of the rear section of a fish, starting at about the origin of the unpaired fins. Characters that positively loaded on P1 were LA, LD, pD, SLS, SPc (significance all "excellent"), nD ("very good"), nA, BD ("good"), LSo, CPD ("fair"). Negatively correlated with P1 were characters for more frontal body parts such as HL and PDL (significance both "excellent"), and PAL ("poor"). Correspondingly, the characters that correlated positively with P2 were mainly those relating to length or depth of frontal body parts, such as PAL, PDL, HL and BD, but also CPD and LD. The characters that negatively correlated with P2 were CPL and pD. Characters loading significantly on P3 did so "poorly", but their main effect was on P1. The only exception was that nA loaded almost as strongly on P1 as it did on P3 (significance "good" in both cases).

Scatter plots in principal component co-ordinates showed separation of data points in different regions of multidimensional space for the lower Cunene morph compared with the Upper Zambezi and Kwando morphs and *H. szaboi* (Figure 5A). These plots show that the lower Cunene morph tends to have more positive values for P1 than the other three morphs, reflecting the more slender body form of the lower Cunene morph.

The discriminant analysis gave similar results to the principal components analysis (Figure 5B). Among 119 specimens from the lower Cunene, Upper Zambezi and Kwando morphs and *H. szaboi*, only three were misclassified (3.7%), none of which were from the lower Cunene morph. One sample each of the Upper Zambezi and Kwando morphs and *H. szaboi* was misclassified as one of the two others, but none were misclassified as the lower Cunene morph.

The specimens from the Namibian section of the Cunene River (between below Ruacana Falls and above Epupa Falls) are therefore morphologically distinct from the six other samples, including the Types.

EOD comparisons

Among the members of the *H. ansorgii* species complex studied so far, the lower Cunene form is the only one with a bipolar pulse waveform, as opposed to monopolar, head-positive pulse waveforms for all other forms (Upper Zambezi and Kwando morphs and *H. szaboi*; Kramer et al. 2004). Whereas the latter forms displayed only weak negative pre- or post-potentials, the EOD of the lower Cunene form was distinctly bipolar and pentaphasic. In addition, to a strong head-positive phase, it had an even stronger head-negative main phase and weaker pre- and post-potentials (Figure 6).

The amplitude spectra of EODs from Cunene specimens therefore had greater band-width and higher frequency than all three morphs from the Upper Zambezi River system, the latter with almost pure DC pulses and energy rapidly dropping off with frequency. EODs from the Cunene samples had amplitude spectra that were distinctly bi-lobed, with a low-frequency peak between 520 and 590 Hz and a second high-frequency peak of sim-ilar strength between 4.33 and 5.05 kHz. The high-frequency cut-off at –10 dB was between 7.45 and 8.89 kHz, much higher than in EODs from Kwando morph specimens, whose EODs were the shortest (that is, the highest frequency: 4.6–6 kHz at –10 dB) among the morphs of the Upper Zambezi River system (Figure 7). The strong low-fre-quency content in Cunene EODs is probably the result of the long duration of the trailing P2 phase, which ends only about 5 milliseconds after the start of the P0 phase.

In the females there was significant correlation with SL in two characters, not observed in males. This negative result for males could be because they are larger (> 15.5 cm), except for one specimen. The median was 16.2 cm for males and 15.2 cm for females and the size range was 13.1–17.0 cm ($n = 11$) for males and 11.9–17.4 ($n = 11$) for females. This difference between the sexes cannot be considered reliable with the current dataset and a more evenly distributed size range of males should be investigated to test this phenomenon in future. In females, P1N1sep and N0dur increased with SL (P1N1sep: $y = 0.045 + 0.002x$, $R^2 = 0.386$, $F_{1,9} = 5.664$, $p = 0.0412$; N0dur: $y = 0.017 + 0.009x$, $R^2 = 0.797$, $F_{1,9} = 35.34$, $p = 0.0002$). There was no reliable difference between the sexes (Appendix 2, Table 5).

DNA sequence analysis

In choosing appropriate outgroups, BLAST searches in GenBank and subsequent phy-logenetic analyses confirmed that taxa of the mormyrid genera *Cyphomyrus*, *Genyomyrus*, *Gnathonemus*, *Ivindomyrus*, *Marcusenius*, *Mormyrops*, *Mormyrus*, *Paramormyrops*, *Petrocephalus* and *Pollimyrus* are genetically only distantly related to the lower Cunene *Hippopotamyrus* morph. Apart from including all available

Figure 6. Electric organ discharge (EOD) pulse waveforms for four specimens representing four morphs of the *Hippopotamyrus ansorgii* species complex. Note that (D), the lower Cunene morph, has a pentaphasic waveform with strong positive and negative phases, whereas the morphs from the Upper Zambezi River system (A–C) all show essentially monopolar, head-positive EOD pulses (resulting in a strong DC component). (A) *Hippopotamyrus szaboi*; (B) Upper Zambezi morph of *H. ansorgii*; (C) Kwando River morph of *H. ansorgii*. To the left of each panel, the longest EOD pulse of the whole sample of specimens, respectively, is shown; right, shortest EOD; middle, an EOD of a specimen with near average duration.
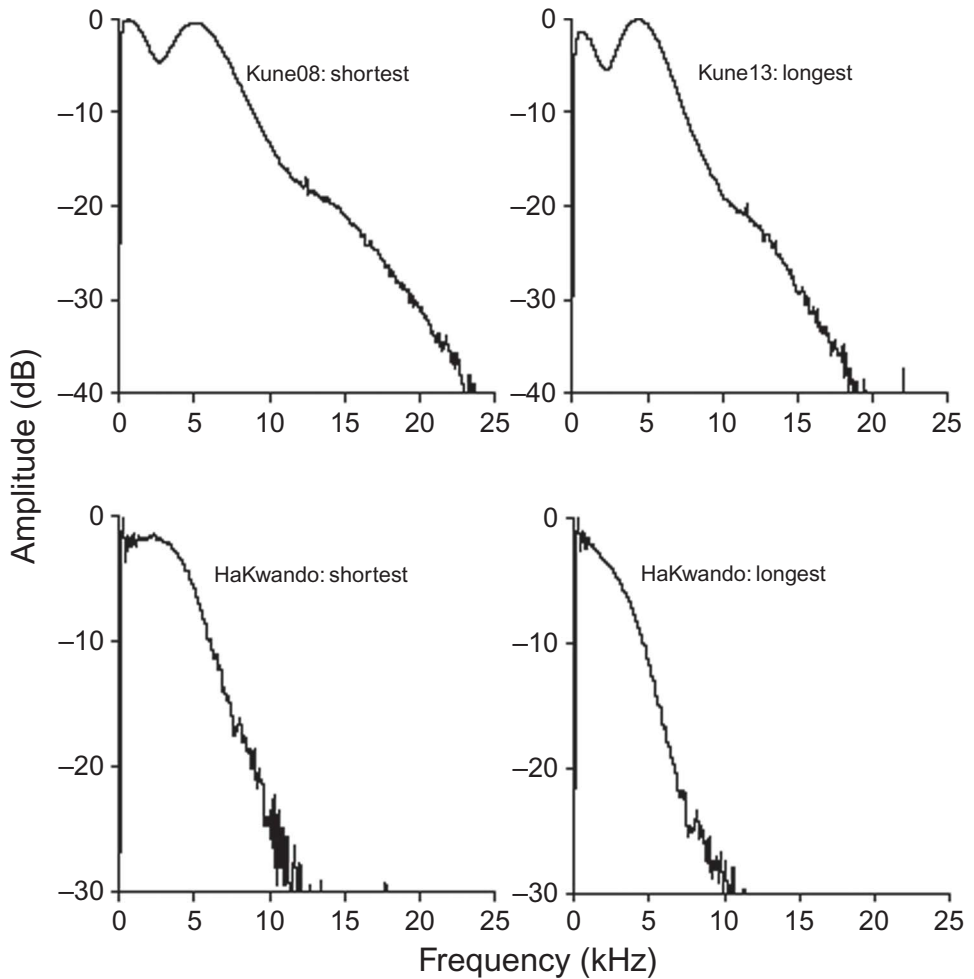
10 ms
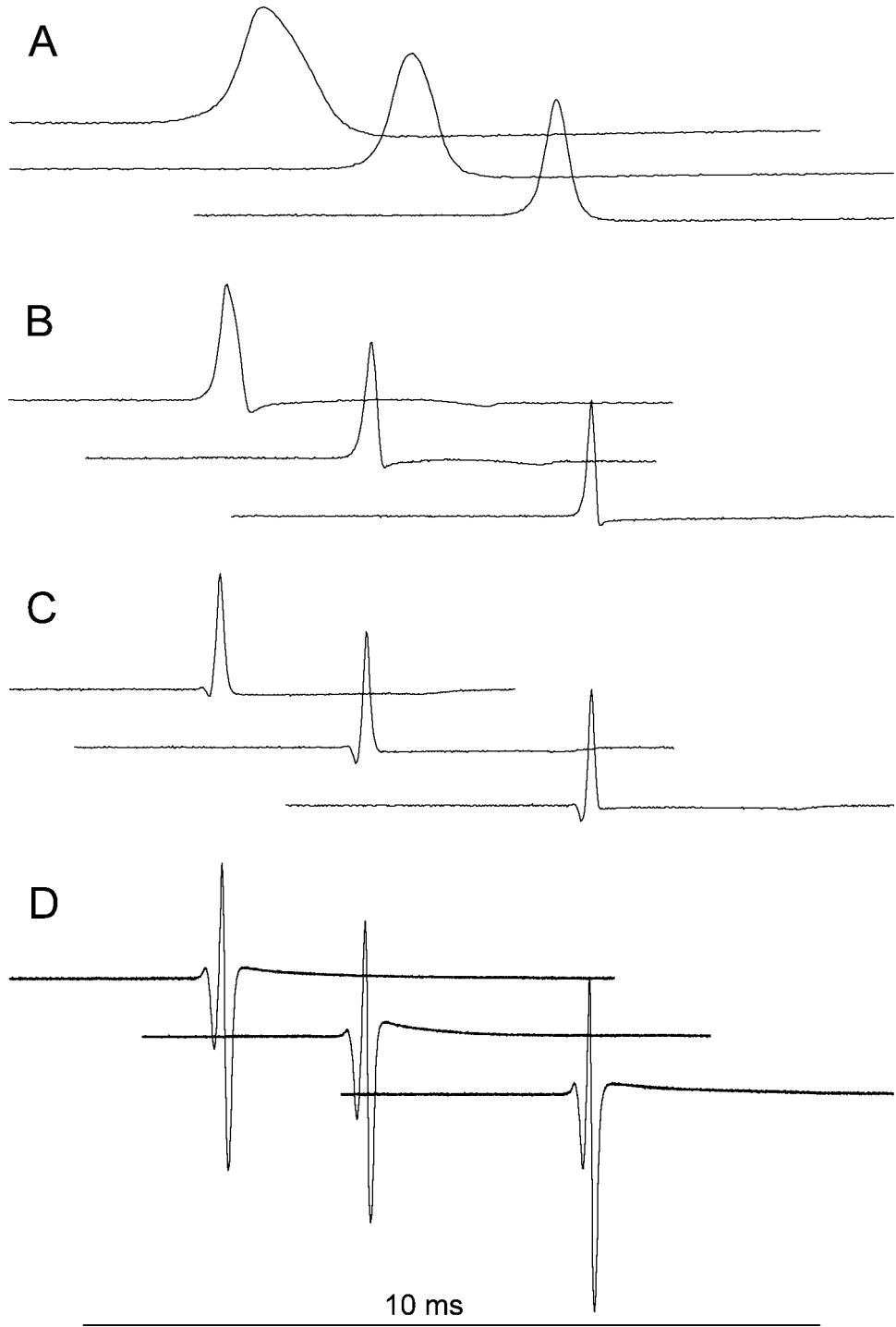
Figure 7. Amplitude spectra for electric organ discharge (EOD) pulses. Top panels, *Hippopotamyrus longilateralis* (the lower Cunene morph); bottom panels, *Hippopotamyrus ansorgii* from the Kwando River. Left hand panels, shortest EOD pulses of a sample of specimens, respectively; right hand panels, longest pulses of a sample. The spectra for the Upper Zambezi and Kwando morphs and *Hippopotamyrus szaboi* all reveal essentially monopolar DC pulses, like those shown on the bottom panels, whereas the spectra for the lower Cunene morph reveal a second peak of higher frequency, resulting from a bipolar, pentaphasic pulse waveform.

Hippopotamyrus specimens and GenBank sequences, it was therefore decided to only include additional sequences of recently studied individuals that were sampled close to their type localities and for which multiple datasets were available. *Cyphomyrus psittacus* from the Niger River in Mali (Lavoué et al. 2000), *Cyphomyrus wilverthi* from the Ubangi River in Central African Republic and *Cyphomyrus discorhynchus* from Lake Malawi (Sullivan et al. 2000), were included as in-group taxa, because they have been regarded as belonging to *Hippopotamyrus*. *Marcusenius altisambesi* was included because it may occur in the same river as the lower Cunene *Hippopotamyrus* morph.
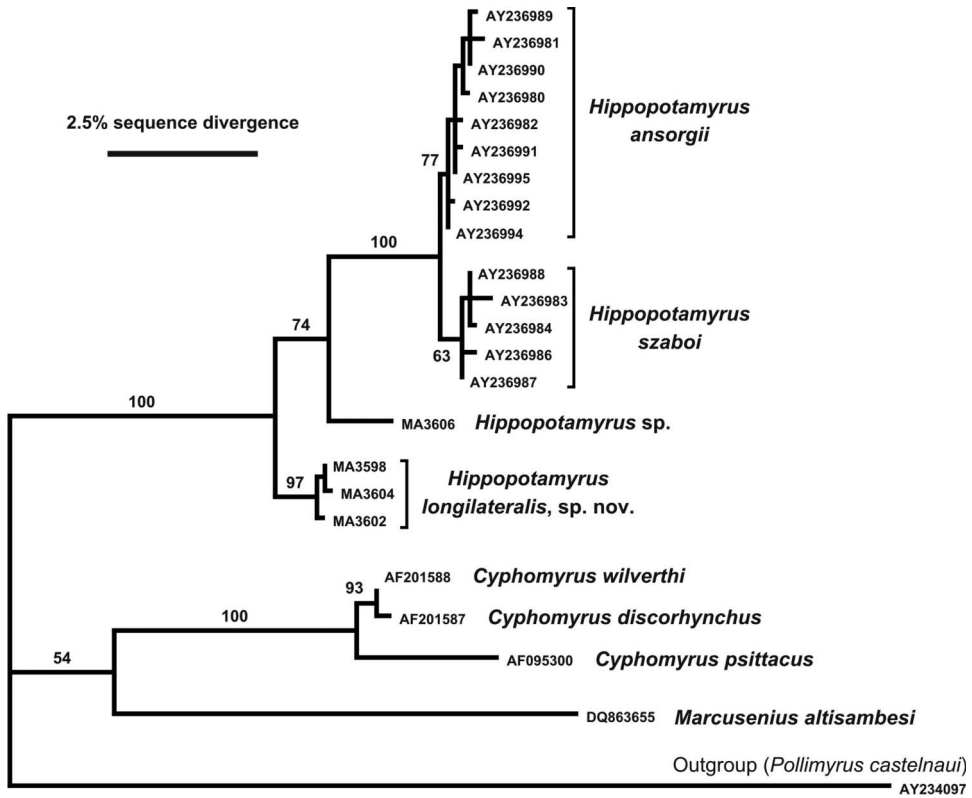
Figure 8. Maximum likelihood phylogram based on mitochondrial cytochrome *b* sequences from the present study and GenBank, showing the phylogenetic relationships of the lower Cunene *Hippopotamyrus* morph. Values above the branches are bootstrap support based on 10 000 parsimony bootstrap replicates.

The maximum likelihood analysis and the genetic distances among the different taxa were based on the transversional model (TVM) model with a gamma distribution (α value 0.1745) found in MODELTEST. The phylogenetic analysis revealed three deep lineages within *Hippopotamyrus* between a unique *Hippopotamyrus* lineage from the Buzi River system in Mozambique, the Upper Zambezi River system species and morphs (*H. szaboi* and the Upper Zambezi and Kwando *Hippopotamyrus* morphs) and the lower Cunene morph (Figure 8). There was strong bootstrap support for the *Hippopotamyrus* taxa as a monophyletic group. The lower Cunene *Hippopotamyrus* morph is therefore closely related but distinct from the other taxa in the *H. ansorgii* complex. All the other species representing the genera *Cyphomyrus*, *Marcusenius* and *Pollimyrus* were only distantly related. The level of divergence between the lower Cunene *Hippopotamyrus* morph and other taxa of the *H. ansorgii* complex is comparable with species-level differences in other groups of organisms, with very little intraspecific differentiation (0.1–0.4%) compared with interspecific divergence from *Hippopotamyrus* sp. from the Buzi River system (2.4–2.6%), Upper Zambezi and Kwando *Hippopotamyrus* morphs (3.2–3.7%) and *H. szaboi* (3.3–4.0%)

(Appendix 1, Table 4). The major sequence divergences between the lower Cunene *Hippopotamyrus* morph and the *Cyphomyrus* species (7.8–11.4%), *Marcusenius altisambesi* (9.5–9.6%) and *Pollimyrus castelnaui* (11.9–12.1%), supports their classification as different genera.

Systematics

Genus *Hippopotamyrus* Pappenheim, 1906

Diagnosis (translation of Taverne 1971a)

Body moderately elongated to elongated; snout shorter than postorbital segment of skull, with upper jaw high, curved downward and somewhat longer than lower jaw; caudal peduncle 2–$3^3/_4$ times longer than high; 16–41 dorsal fin rays; 22–35 anal fin rays; 10–12 pectoral fin rays; 45–93 lateral line scales; 10–21/14–23 scales transversely to body; 9–19/9–19 scales transversely between dorsal and anal fins; 12–16 scales around caudal peduncle; 3–7/6–10 bicuspid teeth; lateral ethmoid present and well developed; mesethmoid big and curved backwards; five circumorbital bones; preorbital, and first infraorbital fused; five hypural bones; 41 to 48 vertebrae.

Type species
Hippopotamyrus castor Pappenheim, 1906

Included species (from Eschmeyer and Fricke 2010)

aelsbroecki, *Gnathonemus* Poll 1945. Current status: valid as *Hippopotamyrus aelsbroecki* (Poll 1945)

aequipinnis, *Paramyomyrus* Pellegrin 1927. Current status: synonym of *Hippopotamyrus castor* Pappenheim 1906

ansorgii, *Marcusenius* Boulenger 1905. Current status: valid as *Hippopotamyrus ansorgii* (Boulenger 1905)

castor, *Hippopotamyrus* Pappenheim 1906. Current status: valid as *Hippopotamyrus castor* Pappenheim 1906

cubangoensis, *Marcusenius* Pellegrin 1936. Current status: synonym of *Hippopotamyrus discorhynchus* (Peters 1852)

grahami, *Marcusenius* Norman 1928. Current status: valid as *Hippopotamyrus grahami* (Norman 1928)

harringtoni, *Marcusenius* Boulenger 1905. Current status: valid as *Hippopotamyrus harringtoni* (Boulenger 1905)

macroterops, *Marcusenius* Boulenger 1920. Current status: valid as *Hippopotamyrus macroterops* (Boulenger 1920)

pappenheimi, *Marcusenius* Boulenger 1910. Current status: valid as *Hippopotamyrus pappenheimi* (Boulenger 1910)

paugyi, *Hippopotamyrus* Lévêque and Bigorne 1985. Current status: valid as *Hippopotamyrus paugyi* Lévêque and Bigorne 1985

pictus, *Petrocephalus* Marcusen 1864. Current status: valid as *Hippopotamyrus pictus* (Marcusen 1864)

retrodorsalis, *Marcusenius* Nichols and Griscom 1917. Current status: valid as *Hippopotamyrus retrodorsalis* (Nichols and Griscom 1917)

smithersi, *Marcusenius* Määr 1962. Current status: synonym of *Hippopotamyrus discorhynchus* (Peters 1852)

szaboi, *Hippopotamyrus* Kramer, van der Bank and Wink 2004. Current status: valid as *Hippopotamyrus szaboi* Kramer, van der Bank and Wink 2004

tanganicanus, *Marcusenius* Boulenger 1906. Current status: synonym of *Hippopotamyrus discorhynchus* (Peters 1852)

weeksii, Marcusenius Boulenger 1902. Current status: valid as *Hippopotamyrus weeksii* (Boulenger 1902)

Hippopotamyrus ansorgii (Boulenger, 1905)
(Figure 1E)

Marcusenius ansorgii Boulenger 1905
Hippopotamyrus ansorgii: Taverne 1971a: 104; Taverne 1971b: 136; Taverne 1972: 170.

Material examined
See Material and methods section.

Type specimens
Syntypes: BMNH 1905.5.29.62–63 (2).

Type locality
"Betweeen Benguella and Bihé". Benguella district includes the highlands of Bihé (Stieler 1910). The highlands are the origin of at least five major rivers flowing in all directions. The origin of the syntypes will therefore remain unclear until new evidence, possibly from historical notes, can be found giving more precise geographical information (an intensive search has so far been unsuccessful).

Diagnosis
nA, median 25 (range 24–26); nD, median 18.5 (range 18–19); SPc, median 16 (range 16–16); SLS, median 69.5 (range 67–72); LSo, mean 0.4365 (range 0.4259–0.4470) of HL; BD, mean 0.1966 (range 0.1891–0.240) of SL; HL, mean 0.2109 (range 0.2079–0.2139) of SL; PDL, mean 0.6444 (range 0.6347–0.6542) of SL; PAL, mean 0.6118 (range 0.5990–0.6245) of SL; pD, mean 0.3765 (0.3669–0.3861) of SL.

Hippopotamyrus ansorgii species complex specimens of uncertain status
Marcusenius ansorgii Boulenger, 1905. ZMH 1817. ZMH 1817, two specimens, Rio Cukimaala near north Lisboa (which has been renamed Huambo after 1975), a small tributary (headwater) of the Cunene in Angola, leg. Machado, 12 May 1961.

Marcusenius ansorgii Boulenger, 1905. ZMH 1790, two specimens, Rio Cuchi in Angola, a tributary of the Kubango (see map of Figure 2), no specific place given, leg. Ladiges and Voelker, 9 October 1961.

Hippopotamyrus ansorgii. SAIAB 67459, 67461–67464, 67467–67469, 17 specimens, and ZSM 29790, 29792–29807, 17 specimens from the Upper Zambezi River (Upper Zambezi morph, morph Ha1), East Caprivi, Namibia.

Hippopotamyrus ansorgii. SAIAB 67460, 67465, 67466, 18 specimens, ZSM 29773–29789, 29791, 18 specimens from the Kwando River (Kwando morph, morph Ha3), Caprivi Strip, Kongola bridge.

Hippopotamyrus szaboi Kramer, Van der Bank and Wink 2004
(Figure 1B)

Material examined

See Materials and methods section.

Type specimens

Holotype SAIAB 67143. Paratypes SAIAB 67144 (1), SAIAB 67145 (4), ZSM 29765–70 (6), SAIAB 67146 (4), SAIAB 67147 (1), ZSM 29771 (1), ZSM 29772 (1). (also referred to as 'morph Ha2' in present and earlier paper).

Type locality

Upper Zambezi River, Katima Mulilo, rocks in middle of river, 17°29′30″ S, 24°16′18″ E.

Diagnosis

CPL, mean 0.1838 (range 0.1671–0.2145) of SL; SLS, median 67 (range 61–73); PDL, mean 0.6624 (range 0.6337–0.6916) of SL; PAL, mean 0.6282 (range 0.5954–0.6596) of SL; pD, mean 0.3777 (range 0.3452–0.4006) of SL; HL, mean 0.258 (range 0.2349–0.2787) of SL; LSo, mean 0.4824 (range 0.4494–0.5203) of HL; BD, mean 0.2319 (range 0.2018–0.2651) of SL; nD, median 19 (range 18–21); nA, median 22 (range 21–25); head-positive EOD waveform (620–1800 s at 10% peak amplitude, 25°C). Mitochondrial cytochrome *b* DNA sequence, GenBank AY236983–8.

Hippopotamyrus longilateralis sp. nov.
(Figure 1A)

Material examined

See Materials and methods section.

Type specimens

Holotype SAIAB78793, Paratypes SAIAB 79502, 79503, 78778, 78779, 78793, 78783, 78787, ZSM 38561–ZSM 38567.

Type locality

Cunene River, just above Epupa Falls on the Namibian/Angolan border, 17°00′07″ S, 013°14′57″ E, altitude about 600 m.

Diagnosis

pD, mean 0.4013 (range 0.3791–0.4175) of SL; SLS, median 77 (range 74–80); PDL, mean 0.6273 (range 0.6123–0.6499) of SL; SPc, median 20 (range 19–22); nD, median 20 (range 18–21); nA, median 24 (range 22–25); PAL, mean 0.6020 (range 0.5906–0.6270) of SL; CPL, mean 0.2080 (range 0.1952–0.2202) of SL; HL, mean 0.2155 (range 0.1923–0.2289) of SL; LSo, mean 0.4943 (range 0.4578–0.5484) of HL; BD, mean 0.2256 (range (0.2037–0.2451) of SL; bipolar, pentaphasic waveform of EOD pulse (bipolar main pulse, about 230 μs). Mitochondrial cytochrome *b* and barcode sequences (GenBank, GU453680–GU453688 and HM065027–HM065034).

Description

Head broadly rounded with a terminally positioned mouth, lower jaw protruding less forward than upper; body long and slender, laterally compressed. Dorsal fin origin set far back and behind that of anal fin, situated about two-thirds of standard length from snout, obliquely orientated with anterior portion of the fin higher than the posterior portion, median number of rays 20 (18–21). Anal fin opposite dorsal fin but origin further anterior and also obliquely orientated, anteriorly lower and posteriorly higher, margin broadly rounded, median number of rays 24 (22–25). Scales cycloid with reticulate striae, except in centre, extending anteriorly to operculum and pectoral fins. SPc, median 20 (19–22). Caudal peduncle slender and subcylindrical over the entire length, usually slightly less than one-fifth in SL. Tail fin with broadly rounded lobes. EOD bipolar and pentaphasic, duration of the two main phases about 230 μs but whole pulse may last up to 5 milliseconds because of weak pre- and post-potentials (25°C). Dark brown with a vertical black band from dorsal origin that is only weak in most specimens.

Colour in preservation

Dark to medium brown, often with milky-grey hue, especially where opaque 'mormyrid skin' (electroreceptor organ area) present (and not rubbed off the underlying skin).

Ecology

The section of the Cunene River between Ruacana Falls and Epupa Falls is a permanently flowing river with a strong current. Water level is controlled by a hydroelectric power plant at Ruacana Falls (Namib Power) and a large dam (Calueque Dam) further upstream in Angola. The Ruacana Falls was completely dry during our survey, illustrating the extreme river regulation. A major daily difference in water level was

even detected at Epupa Falls (a straight line distance from Ruacana Falls of 115 km), where catches were good during lower water levels with no fish caught during high flow. Although the area around Epupa Falls is very dry, there are extensive stands of palm (Makalani, *Hyphaene petersiana*) and other trees, reeds and bushes that grow on the banks, including an occasional baobab. At Ruacana, which is wetter, the arboreal and other vegetation and bird life were more abundant and varied. Crocodiles were present at both Ruacana and Epupa waterfalls, with hippopotamus reported for the former, but not seen. Most of the *H. longilateralis* specimens were caught along well-vegetated and steep banks of the river. They therefore possibly prefer undercut banks with structure such as roots.

Distribution

Only known from the section of the Cunene River between below Ruacana Falls downstream to above Epupa Falls, a straight-line distance of 115 km. This section of the river forms part of the Namibian/Angolan border.

Relationships

Based on our phylogenetic analysis, *H. longilateralis* is the sister-group of all other members of the *H. ansorgii* species complex. Barcode (COI) sequence of paratype specimens were done for future reference.

Etymology

The specific name *longilateralis* is in reference to the long lateral line.

Remarks

Referred to as the lower Cunene morph in the present paper ('morph Ha4' in tables with limited space); *H. longilateralis* is distinguished by pD and SLS highest and PDL lowest among the species complex of seven; SPc and nD also high; distinctive bipolar, pentaphasic waveform of its EOD pulse, as opposed to monopolar DC pulses generated by the Upper Zambezi and Kwando morphs and *H. szaboi*. The species can also be distinguished on their phylogenetic relationships with mitochondrial cytochrome *b* and barcode sequences. We therefore recognize the lower Cunene morph as a new species, *H. longilateralis* sp. nov.

Discussion

The slender stonebasher, *H. ansorgii* (Boulenger, 1905), is described as a rare, current-loving species with a fragmented distribution in southern Africa (Van der Waal and Skelton 1984; Bell-Cross and Minshull 1988; Skelton 2001). On anatomical, electrical and in part genetic evidence, Kramer et al. (2004) have described six morphs of this species, one of which was recognized as the new species *H. szaboi*. We here present evidence for a seventh morph of the *H. ansorgii* species complex from the lower Cunene River, the new species *H. longilateralis*.

    Hippopotamyrus longilateralis is anatomically differentiated from all other known morphs, including the *H. ansorgii* types. Its EOD differs consistently from those of the

three other morphs that have been studied to date, and it genetically represents a monophyletic and divergent lineage compared with the Upper Zambezi and Kwando morphs, and a specimen from the Buzi River. *Hippopotamyrus longilateralis* appears to be specifically adapted to strong currents in its escarpment habitat. Their rear section is more strongly developed than in any other morph we have studied (pD and SLS are increased at the expense of PDL). In addition, their bipolar, multiphasic EOD is probably more advantageous than the monopolar DC pulses of its congeners from the Upper Zambezi and the Kwando: (1) reduced predation pressure from sharptooth and other catfish is likely because of less spectral energy in the low-frequency range (Hanika and Kramer 2000); (2) more possibilities in encoding individual differences (potentially of fitness relevance) are offered by a more complex EOD waveform with five phases rather than one (Markowski et al. 2008); (3) better electrolocation performance because a broadband test signal, such as that of *H. longilateralis*, will allow the detection of a broader range of capacitive impedances, such as prey organisms (Lissmann and Machin 1958; Meyer 1982; Von der Emde 1990).

Based on the electrophysiological work of Bennett (1971) and on the modelling approach of Westby (1984), Markowski et al. (2008) have shown that the strikingly different EOD waveforms found in a pair of parapatric sibling snoutfish species from southern Africa (*Pollimyrus castelnaui* and *Pollimyrus marianne*), can be mutually transformed to be identical, by making small changes in three physiological variables. EOD waveforms therefore seem to be much more plastic in evolutionary time and may become differentiated in rapidly evolving species more quickly than previously thought.

Genetic distances between *H. longilateralis* and the three morphs from the Upper Zambezi system were between 2.4 and 4% (Appendix 1, Table 4). A molecular clock of about 2% sequence divergence in 1 million years has been determined for mitochondrial genes (Wilson et al. 1987). This would correspond to a separation of the Cunene lineage from that of the Zambezi system morphs about 1.2–2 million years ago. Its basal position within this clade testifies to the hypothesis that Angolan rivers are key to understanding the phylogeography of snoutfish populations in southern Africa.

The number of fish common to two rivers divided by the total number of species yields a Similarity Index. A rather high Similarity Index of 0.55 for fish species inhabiting the Cunene and the Okavango suggests a former linkage between the two rivers (Skelton 1994). Such a linkage has been proposed via the ephemeral Colui, which is among the eastern headwaters of the Cunene (Moore and Larkin 2001, p. 66). However, a critical comparison of allopatric populations of *H. ansorgii* has not been made.

The evolution of the Cunene River is not well understood. It is assumed that it flowed into a large lake where the Etosha Pan is now, and was captured by a coastal river eroding backwards (Goudie 2005). The time of this event (which separated the Cunene from the Okavango–Zambezi systems) is not well constrained, and could be a consequence of the opening of the South Atlantic (suggested at about 110–130 million years ago, reviewed in Stankiewicz and de Wit 2006) some time in the Tertiary, or as recent as 35 000 years ago (reviewed in Goudie 2005).

The present cytochrome *b* data point to the lower Pleistocene, when a major rearrangement of the Zambezi River, possibly indirectly affecting the Cunene, occurred. First, inland extension of the headwaters of the short lower Zambezi captured the middle Zambezi via the Kariba gorge, and then the middle Zambezi beheaded the Upper Zambezi via the Batoka gorge, creating the present course of the main Zambezi. This latter event has been dated to 1.25–2 million years ago (reviewed in Moore and

Larkin 2001; Stankiewicz and de Wit 2006). It is tempting to speculate on the coincidence of the time estimates from the geological and hydrographic data with the molecular clock estimate for the origin of *H. longilateralis* presented in this paper as an example of vicariance affecting the phylogeny of fish.

Any improvement of our present state of knowledge regarding the linked river and fish evolution will only be possible with a better understanding of the Angolan fish fauna and river evolution.

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Appendix 1. Morphological differences and genetic distances between samples

Table 2. Variation in morphology within the *Hippopotamyrus ansorgii* species complex.

	PDL/SL	PAL/SL	LD/SL	LA/SL	LPF/HL	PPF/SL	pD/SL	CPL/SL	CPD/CPL	LSo/HL	OD/HL	HL/SL	HL/Na	BD/SL	nD	nA	SPc	SLS	SL (cm)
H. ansorgii – Types																			
Mean/Median*	0.6444	0.6118	0.1713	0.2176			0.3765	0.1882	0.3380	0.4365		0.2109	12.735	0.1966	18.5	25	16	69.5	10.27
SE/SIQ*	–	–	–	–			–	–	–	–		–	–	–					–
Min	0.6347	0.5990	0.1653	0.2168			0.3669	0.1724	0.2816	0.4259		0.2079	12.706	0.1891	18	24	16	67	10.1
Max	0.6542	0.6245	0.1772	0.2184			0.3861	0.2040	0.3944	0.4470		0.2139	12.765	0.2040	19	26	16	72	10.44
N	2	2	2	2			2	2	2	2		2	2	2	2	2	2	2	2
H. longilateralis sp. nov.																			
Mean/Median*	0.6273	0.6020	0.1788	0.2137	0.1631	0.1827	0.4013	0.2080	0.3564	0.4943	0.1828	0.2155	13.231	0.2256	20	24	20	77	14.44
SE/SIQ*	0.0018	0.0016	0.00143	0.0014	0.0023	0.0014	0.0019	0.0012	0.0034	0.0036	0.0041	0.0016	0.1418	0.0023	1	0.5	0.5	1.625	0.33
Min	0.6123	0.5906	0.1633	0.1997	0.1211	0.1684	0.3791	0.1952	0.3278	0.4578	0.1467	0.1923	11.813	0.2037	18	22	19	74	10.5
Max	0.6499	0.6270	0.1940	0.2321	0.1838	0.1956	0.4175	0.2202	0.4134	0.5484	0.2280	0.2289	14.646	0.2451	21	25	22	80	17.2
N	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
Upper Cunene Cukimaala morph																			
Mean/Median*	0.6484	0.6397	0.1696	0.2113			0.3666	0.1905	0.3637	0.4599		0.2258	11.0768	0.2323	15	20	20		10.86
Fish1	0.6378	0.626	0.1543	0.211			0.3567	0.1906	0.3554	0.4794		0.2102	10.68	0.2331	15	20	20		12.7
Fish2	0.6589	0.6534	0.1849	0.2115			0.3765	0.1905	0.3721	0.4404		0.2414	11.4737	0.2315	15	20	20		9.03
Rio Cuchi morph																			
Mean	0.6556	0.6181	0.1722	0.2092			0.3891	0.2086	0.327	0.5531		0.2012	10.0179	0.2238	18	22	16		8.46
Fish1	0.6494	0.6106	0.1673	0.2151			0.3835	0.2082	0.3206	0.4511		0.2341	11.75	0.2311	18	22	16		10.04
Fish2	0.6618	0.6255	0.1771	0.2032			0.3948	0.209	0.3333	0.6552		0.1684	8.2857	0.2366	18	22	16		6.89

H. szaboi																	
Mean/Median*	0.6624	0.6282	0.1799	0.2097	0.1819	0.3777	0.1838	0.3982	0.4824	0.258	13.68	0.2319	19	22	20	67	9.73
SE/SIQ	0.0037	0.0033	0.0023	0.0021	–	0.0032	0.0025	0.0104	0.0047	0.0027	0.3421	0.0039	1	0.5	0	2	0.704
Min	0.6337	0.5954	0.1614	0.1936	–	0.3452	0.1671	0.3052	0.4494	0.2349	11.07	0.2018	18	21	17	61	5.33
Max	0.6916	0.6596	0.1998	0.2222	–	0.4006	0.2145	0.4672	0.5203	0.2787	15.95	0.2651	21	25	22	73	14.6
N	19	19	19	19	1	19	19	19	19	19	18	19	19	19	19	12	19
Upper Zambezi morph																	
Mean/Median*	0.6459	0.6089	0.1795	0.2104	0.1916	0.3897	0.1989	0.3447	0.4927	0.2372	12.514	0.2208	19	22	16	66	7.53
SE/SIQ	0.002	0.0018	0.0019	0.0016	–	0.002	0.0018	0.0053	0.0043	0.0022	0.2648	0.0021	0.5	0.5	0.5	2.125	0.31
Min	0.6253	0.5804	0.1553	0.1912	–	0.3661	0.1728	0.2917	0.4583	0.1946	8.5	0.1942	15	20	16	58	3.71
Max	0.6762	0.6384	0.2008	0.2305	–	0.4104	0.223	0.4172	0.5565	0.2604	15.167	0.2462	21	24	18	71	10.77
N	34	34	34	34	1	34	34	34	34	34	34	34	34	34	34	21	34
Kwando morph																	
Mean/Median*	0.6583	0.6122	0.1583	0.1934	0.1709	0.3771	0.2071	0.3281	0.4688	0.2498	11.123	0.2143	18	22	16	67.5	5.51
SE/SIQ	0.0021	0.0018	0.0018	0.0021	–	0.0027	0.0018	0.0049	0.0038	0.003	0.1843	0.0023	0.5	0.125	0	2.25	0.275
Min	0.637	0.5886	0.1346	0.1683	–	0.3364	0.1803	0.266	0.4241	0.2176	9.222	0.1875	17	20	14	63	2.96
Max	0.6935	0.6409	0.1773	0.2163	–	0.4166	0.2242	0.4	0.5333	0.2972	13.214	0.2506	21	25	19	74	8.74
N	37	37	37	37	1	37	37	37	37	37	37	37	37	37	35	20	37

Notes: *Median and SIQ (semi-interquartiles) for count measures only (nD, nA, SPc, SLS).
Abbreviations of morphological characters are given in Materials and methods.

Table 3. Principal components analysis on correlations for morphological characters from four members of the *Hippopotamyrus ansorgii* species complex, that is, the Upper Zambezi morph, the Kwando morph, *Hippopotamyrus szaboi* and *Hippopotamyrus longilateralis* sp. n. (*N* = 119).

Eigen value	5.6575	2.8758	1.4264	0.9754	0.7531	0.5525	0.4077
Per cent	40.411	20.541	10.188	6.967	5.379	3.946	2.912
Cum Per cent	40.411	60.952	71.14	78.108	83.487	87.433	90.345
Eigenvectors							
PDL/SL	− 0.3174	0.289	0.0472	0.088	0.1088	0.1127	− 0.1825
PAL/SL	− 0.1476	0.4562	0.0602	0.2066	0.0805	0.2794	0.2774
LD/SL	0.3109	0.2048	− 0.3279	− 0.2694	0.1782	0.1383	0.055
LA/SL	0.3275	0.1409	− 0.3045	− 0.255	− 0.0927	− 0.0806	0.082
pD/SL	0.3107	− 0.2152	− 0.1886	− 0.1742	0.3014	− 0.0132	0.3485
CPL/SL	− 0.0173	− 0.5119	− 0.0067	0.2304	0.2945	0.258	− 0.0187
CPD/CPL	0.1941	0.4255	0.0583	0.2383	− 0.0135	− 0.4837	0.2249
LSo/HL	0.2153	0.0301	− 0.3018	0.5004	− 0.5425	0.4312	0.03
HL/SL	− 0.3251	0.2148	0.0624	− 0.114	0.3474	0.2454	0.1359
BD/SL	0.2455	0.2589	− 0.2603	0.1598	0.4203	0.1056	− 0.6563
nD	0.2809	0.0841	0.3683	− 0.3262	− 0.0721	0.5533	0.0671
nA	0.2491	0.1018	0.5051	− 0.2324	− 0.2344	− 0.0317	− 0.3521
SPc	0.3022	0.067	0.3426	0.3126	0.2812	0.0504	0.3285
SLS	0.3074	− 0.1455	0.2898	0.3532	0.181	− 0.1264	− 0.1456
Component loadings							
PDL/SL	− 0.755	0.4902	0.0564	0.0869	0.0945	0.0838	− 0.1165
PAL/SL	− 0.3511	0.7736	0.0719	0.2041	0.0699	0.2076	0.1771
LD/SL	0.7394	0.3474	− 0.3917	− 0.266	0.1546	0.1028	0.0351
LA/SL	0.779	0.2389	− 0.3637	− 0.2519	− 0.0804	− 0.0599	0.0523
pD/SL	0.739	− 0.365	− 0.2253	− 0.172	0.2616	− 0.0098	0.2225
CPL/SL	− 0.0412	− 0.8681	− 0.008	0.2275	0.2556	0.1918	− 0.0119
CPD/CPL	0.4616	0.7216	0.0696	0.2354	− 0.0117	− 0.3595	0.1436
LSo/HL	0.5121	0.051	− 0.3604	0.4942	− 0.4707	0.3205	0.0191
HL/SL	− 0.7733	0.3643	0.0746	− 0.1125	0.3015	0.1824	0.0867
BD/SL	0.5839	0.439	− 0.3109	0.1578	0.3648	0.0785	− 0.419
nD	0.668	0.1425	0.4398	− 0.3222	− 0.0625	0.4113	0.0428
nA	0.5925	0.1727	0.6033	− 0.2295	− 0.2034	− 0.0236	− 0.2248
SPc	0.7188	0.1137	0.4091	0.3088	0.244	0.0375	0.2098
SLS	0.7312	− 0.2467	0.3461	0.3488	0.1571	− 0.094	− 0.0929

Table 4. Genetic distances between *Hippopotamyrus longilateralis*, sp. nov. and other mormyrid species.

Species	Within	1	2	3	4	5	6
(1) *H. longilateralis*, sp. nov. (7)	0.1–0.4						
(2) *Hippopotamyrus* sp. (1)	–	2.4–2.6					
(3) *H. ansorgii* (10)	0.1–0.7	3.2–3.7	2.8–3.2				
(4) *H. szaboi* (6)	0.1–0.8	3.3–4.0	2.9–3.5	0.5–1.4			
(5) *Cyphomyrus* species (4)*	0.2–3.3	7.8–11.4	7.8–11.0	8.5–12.0	8.6–12.0		
(6) *Marcusenius altisambesi* (1)	–	9.5–9.6	9.6	11.2–12.0	11.3–12.0	8.9–9.1	
(7) *Pollimyrus castelnaui* (1)	–	11.9–12.1	12.5	12.4–13.0	12.3–13.3	12.4–16.4	13.0

Notes: The number of individuals that were assessed is shown in brackets.
*Summary of three species: *Cyphomyrus wilverthi* (2), *Cyphomyrus discorhynchus* (1) and *Cyphomyrus psittacus* (1).

Appendix 2. Electrical characters for male and female *Hippopotamyrus longilateralis*, sp. nov.
Table 5. Characters of the electric organ discharge (EOD) pulse waveform of *Hippopotamyrus longilateralis*, sp. nov.

	P0amp (V)	N0amp (V)	N1amp (V)	P2amp (V)	N0dur (ms)	P1dur (ms)	N1dur (ms)	P1N1sep (ms)	P1area (V × µs)	N1area (V × µs)
Females										
mean	0.0699	− 0.5933	− 1.7939	0.1206	0.1413	0.0793	0.1529	0.0742	0.0468	0.1445
S.E.	0.0072	0.0404	0.0639	0.0034	0.0057	0.0017	0.0033	0.0019	0.0008	0.0042
|minimum|	0.031	− 0.3671	− 1.4601	0.0999	0.1107	0.0675	0.1388	0.0667	0.0416	0.1228
|maximum|	0.1076	− 0.8521	− 2.1758	0.1407	0.1635	0.0868	0.1729	0.0892	0.0511	0.1613
N	11	11	11	11	11	11	11	11	11	11
Males										
mean	0.0737	− 0.5446	− 1.7765	0.1201	0.1379	0.078	0.147	0.0715	0.0451	0.138
S.E.	0.0046	0.0318	0.037	0.0036	0.0046	0.0028	0.0057	0.0023	0.0013	0.0048
|minimum|	0.0543	− 0.4078	− 1.522	0.099	0.1232	0.0644	0.1184	0.0638	0.0387	0.1149
|maximum|	0.0972	− 0.7831	− 1.9155	0.1307	0.1638	0.0935	0.178	0.087	0.0518	0.1637
N	11	11	11	11	11	11	11	11	11	11

Notes: Amplitudes re: P1amp = 1 V.
For abbreviations, see Materials and methods, and Figure 4.