The Journal of Experimental Biology 203, 665–683 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2437

MOLECULAR SYSTEMATICS OF THE AFRICAN ELECTRIC FISHES (MORMYROIDEA: TELEOSTEI) AND A MODEL FOR THE EVOLUTION OF THEIR ELECTRIC ORGANS

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Accepted 25 November 1999; published on WWW 26 January 2000

Summary

We present a new molecular phylogeny for 41 species of African mormyroid electric fishes derived from the 12S, 16S and cytochrome *b* genes and the nuclear RAG2 gene. From this, we reconstruct the evolution of the complex electric organs of these fishes. Phylogenetic results are generally concordant with earlier preliminary molecular studies of a smaller group of species and with the osteology-based classification of Taverne, which divides the group into the Gymnarchidae and the Mormyridae, with the latter including the subfamilies Petrocephalinae (*Petrocephalus*) and Mormyrinae (all remaining taxa). However, we find that several genera previously recognized by Taverne are non-monophyletic. Within the Mormyrinae, the genus *Myomyrus* is the sister group to all the remaining taxa. Other well-supported clades within this group are recovered. A reconstruction of electrocyte evolution on the basis of our best-supported topology suggests that electrocytes with penetrating stalks evolved once early in the history of the mormyrids followed by multiple paedomorphic reversals to electrocytes with non-penetrating stalks.

Key words: electric fish, mormyrid, electric organ, phylogeny, systematics, 12S and 16S rDNA, cytochrome *b*, RAG2.

Introduction

The superfamily Mormyroidea (families Mormyridae + Gymnarchidae) *sensu* Nelson (1994) is a large group of freshwater fishes endemic to Africa, comprising more than 200 recognized species placed in 19 genera. They are distributed over most of the continent, with the exception of the Sahara, northernmost Mahgreb and southernmost Cape provinces (Lowe-McConnell, 1987; Roberts, 1975), and are most diverse in the river systems of Central and West Africa. Mormyroids are by far the most speciose extant lineage of the ancient teleost order Osteoglossiformes and represent a remarkable radiation within this otherwise relictual group. The monophyly of the mormyroids is supported by the derived presence of electric organs, matched electroreceptors and a greatly enlarged cerebellum in all taxa, amongst other characters (Taverne, 1972; Lauder and Liem, 1983).

While mormyroids were known to the ancient Egyptians, who accurately depicted them on the walls of their tombs (Brewer and Friedman, 1989), the discovery that these fishes produce and sense weak electric signals was made less than 50 years ago (Grundfest, 1957; Lissmann, 1951, 1958; Lissmann and Machin, 1958). Since then, mormyroids have become a model system for the study of electrogenesis, electroreception and electrocommunication (for recent reviews, see Bullock and Heiligenberg, 1986; Moller, 1995; Turner et al., 1999). Although advancements in our understanding of mormyroid systematics have lagged behind progress in these other areas, a modern phylogenetic hypothesis for these fishes is a prerequisite for placing the growing body of knowledge about them into an evolutionary context. In this paper, we present the most complete phylogenetic study of mormyroids to date. We have used maximum parsimony methods to analyze 3270 characters from the mitochondrial and nuclear genomes of 41 mormyroid species belonging to 18 genera. We use our phylogeny for the mormyroids to investigate the evolution of the electric organ in these fishes.

Materials and methods

Field and laboratory protocols

Table 1 lists the specimens used in this study from 41 mormyroid species belonging to 18 genera. The only recognized genus absent in our dataset is the questionable monotypic *Heteromormyrus* Steindachner 1866 from Angola. *Heteromormyrus pauciradiatus* is known only from a single specimen, which is now lost (Daget et al., 1984), and the validity of this genus is uncertain (Taverne, 1972). We use the

Table 1. *Specimens used in this study listed by genus and species*

Taxon	Original description	Field number	Catalog number	12S/16S	Cyt. *b*	RAG2	EO type	Number examined	Collection locality
Family Mormyridae:									
Genus *Boulengeromyrus*	Taverne and Géry, 1968								
1 *Boulengeromyrus knoepffleri***	Taverne and Géry, 1968	2248	CU 79692	•	•	•	NPp	1	Gabon, Ivindo R.
Genus *Brienomyrus*	Taverne, 1971								
2 *Brienomyrus brachyistius**	(Gill, 1863)	_	CU 79741	•	•[1]	•	DPp	5	Aquarium import
		_	MRAC no #		•				Ivory Coast, Agnébi R.
3 *Brienomyrus hopkinsi*	Taverne et al., 1985	2285	CU 78352	•	•	•	NPp	3	Gabon, Ivindo R.
4 *Brienomyrus longicaudatus*	Taverne et al., 1977	2289	CU 78355	•	•	•	NPp	1	Gabon, Ivindo R.
5 *Brienomyrus niger*	(Günther, 1866)	1123	MNHN 1999-280	•	•[1]	•	DPp	5	Mali, Niger R.
6 *Brienomyrus* sp.2	Teugels and Hopkins	2105	CU 79704	•	•		Pa	3	Gabon, Ivindo R.
.	(in preparation)	2425	CU 79740		•	•			Gabon, Ivindo R.
Genus *Campylomormyrus*	Bleeker, 1874								
7 *Campylomormyrus numenius*	(Günther, 1864)	2523	AMNH 228165	•	•	•	NPp	1	Central African Republic, Ubangi R.
8 *Campylomormyrus* sp.1		_		•	•	•	NPp	3	Aquarium import
9 *Campylomormyrus tamandua* (2)		2455	AMNH 228159	•	•	•	Pa	1	Central African Republic, Sangha R.
10 *Campylomormyrus tamandua* (1)	(Günther, 1864)	_	CU 79742	•	•	•	Pa	3	Aquarium import
Genus *Genyomyrus*	Boulenger, 1898								
11 *Genyomyrus donnyi***	Boulenger, 1898	2449	AMNH 228154	•	•	•	Pa	5	Central African Republic, Sangha R.
Genus *Gnathonemus*	Gill, 1862								
12 *Gnathonemus petersii**	(Günther, 1862)	2453	AMNH 228157	•	•	•	Pa	2	Central African Republic, Sangha R.
Genus *Hippopotamyrus*	Pappenheim, 1906								
13 *Hippopotamyrus discorhynchus*	(Peters, 1852)	_	CU 79743	•	•	•	Pa	1	Lake Malawi
14 *Hippopotamyrus pictus*	(Marcusen, 1864)	1273	MNHN 1999-610	•	•	•	Pa	3	Mali, Niger R.
15 *Hippopotamyrus wilverthi*	(Boulenger, 1898)	2519	AMNH 228164	•	•	•	Pa	1	Central African Republic, Ubangi R.
Genus *Hyperopisus*	Gill, 1862								
16 *Hyperopisus bebe***	(Lacépède, 1803)	1221	MNHN 1999-611	•	•	•	Pa	2	Mali, Niger R.
Genus *Isichthys*	Gill, 1863								
17 *Isichthys henryi***	Gill, 1863	2179	CU 79705	•	•	•	NPp	1	Gabon, Ivindo R.
Genus *Ivindomyrus*	Taverne and Géry, 1975								
18 *Ivindomyrus opdenboschi***	Taverne and Géry, 1975	2242	CU 79698	•	•	•	NPp	1	Gabon, Ivindo R.
Genus *Marcusenius*	Gill, 1862								
19 *Marcusenius conicephalus*	Taverne et al., 1976	2186	CU 79706	•	•	•	Pa	2	Gabon, Ivindo R.
20 *Marcusenius greshoffi*	(Schilthuis, 1891)	2482	AMNH 228160	•	•	•	Pa	1	Central African Republic, Sangha R.
21 *Marcusenius moorii*	(Günther, 1867)	2013	CU 79697	•	•	•	NPp	4	Gabon, Ivindo R.
22 *Marcusenius senegalensis*	(Steindachner, 1870)	1121	MNHN 1999-612	•	•	•	Pa	4	Mali, Niger R.
23 *Marcusenius* sp.		2450	AMNH 228156	•	•	•	Pa	1	Central African Republic, Sangha R.
Genus *Mormyrops*	Müller, 1843								
24 *Mormyrops masuianus*	Boulenger, 1898	2496	AMNH 228163	•	•	•	Pp	2	Central African Republic, Sangha R.
25 *Mormyrops nigricans*	Boulenger, 1899	_	CU 79745	•	•	•	NPp	1	Gabon, Ogooué R.
26 *Mormyrops zanclirostris*	(Günther, 1867)	2210	CU 79707	•	•	•	Pp	3	Gabon, Ivindo R.
Genus *Mormyrus*	Linné, 1758								
27 *Mormyrus ovis*	Boulenger, 1898	2476	AMNH 228161	•	•	•	NPp	1	Central African Republic, Sangha R.
28 *Mormyrus rume*	Valenciennes, 1846	1119	MNHN 1999-613	•	•	•	NPp	1	Mali, Niger R.
Genus *Myomyrus*	Boulenger, 1898								
29 *Myomyrus macrops*	Boulenger, 1914	2524	AMNH 228166	•	•	•	Pa	4	Central African Republic, Ubangi R.

Table 1. *Continued*

Taxon	Original description	Field number	Catalog number	12S/16S	Cyt. *b*	RAG2	EO type	Number examined	Collection locality
Genus *Paramormyrops*	Taverne et al., 1977								
30 *Paramormyrops gabonensis**	Taverne et al., 1977	2048	CU 79702	•	•	•	NPp	4	Gabon, Ivindo R.
Genus *Petrocephalus*	Marcusen, 1854								
31 *Petrocephalus bovei*	(Valenciennes, 1846)	1124	MNHN 1999-614	•	•	•	NPp	7	Mali, Niger R.
32 *Petrocephalus microphthalmus*	Pellegrin, 1908	2038	CU 79700	•	•	•	NPp	2	Gabon, Ivindo R.
33 *Petrocephalus simus*	Sauvage, 1879	2035	CU 79701	•	•	•	_	0	Gabon, Ivindo R.
34 *Petrocephalus soudanensis*	Bigorne and Paugy, 1990	_	MNHN 1999-279	•	•	•	_	0	Ghana, Volta R.
Genus *Pollimyrus*	Taverne, 1971								
35 *Pollimyrus isidori**	(Valenciennes, 1846)	1130	MNHN 1999-615	•	•	•	DPNP	5	Mali, Niger R.
		_	MRAC 97-51-P-4		•	•			Gabon, Ivindo R.
36 *Pollimyrus petricolus*	(Daget, 1954)	1145	MNHN 1999-616	•			DPNP	2	Mali, Niger R.
		_	MNHN 1999-274		•[1]	•			Mali, Niger R.
37 *Pollimyrus* sp.1		2445	AMNH 228155	•	•	•	DPNP	1	Central African Republic, Sangha R.
Genus *Stomatorhinus*	Boulenger, 1898								
38 *Stomatorhinus walkeri**	(Günther, 1867)	2550	CU 79708	•	•[1]	•	Pa	1	Gabon, Louétsi R.
39 *Stomatorhinus* sp. 1		2484	AMNH 228162	•	•[1]	•	Pa	0	Central African Republic, Sangha R.
40 *Stomatorhinus* sp. 2		2454	AMNH 228158	•	•[1]	•	_	0	Central African Republic, Sangha R.
41 *Stomatorhinus* sp. 3		2074	CU 79703	•	•[1]	•	Pa	3	Gabon, Ivindo R.
Family Gymnarchidae:									
42 *Gymnarchus niloticus***	Cuvier, 1829	_	CU 80334	•	•	•	S	1	Aquarium import
Family Notopteridae:									
43 *Chitala ornata*	(Gray, 1839)	_	CU 79744	•	•	•	_	0	Aquarium import
44 *Xenomystus nigri*	Günther, 1868	_	_	•	•	•	_	0	Aquarium import
Family Osteoglossidae									
45 *Pantodon buchholzi*	Peters, 1877	_	CU 80335	•	•	•	_	0	Aquarium import

Field numbers are indicated for field-collected specimens only.

Museum catalog numbers are abbreviated as follows: CU, Cornell University Fish Collection; MNHN, Museum National d'Histoire Naturelle (Paris); AMNH, American Museum of Natural History (New York); MRAC, Musée Royal de l'Afrique Centrale (Tervuren, Belgium).

All electric organ (EO) types (described in text and see Fig. 7) were determined histologically in the number of specimens indicated.

*Type species of genus by monotypy; **type species of genus by designation; _, not applicable or not examined.

Specimens identified here as *Campylomormyrus tamandua* (1) and (2) both key out to *Campylomormyrus tamandua* and resemble the type specimen, but differ from each other in gene sequences.

R, river.

[1]Only 800 bp sequenced; see text.

Dots indicate the gene(s) sequenced for each specimen.

notopterids *Chitala ornata* and *Xenomystus nigri* and the osteoglossid *Pantodon buchholzi* as outgroups for this study. According to Li and Wilson (1996) and Lauder and Liem (1983), the Notopteridae are the sister group to the mormyroids, while *Pantodon* represents a more distant outgroup. Most of these specimens were collected on field trips to Gabon (16 taxa), the Central African Republic (12 taxa), Mali (eight taxa), Ivory Coast (one taxon) and Ghana (one taxon). J. Snoeks provided *Hippopotamyrus discorhynchus* from Lake Malawi, and we obtained *Brienomyrus brachyistius*, *Campylomormyrus* sp.1, *Campylomormyrus tamandua* (1), *Gymnarchus niloticus*, *Chitala ornata*, *Xenomystus nigri* and *Pantodon buchholzi* from the aquarium trade. We identified specimens to species whenever possible by consulting original species descriptions, and we designated undescribed species or species of uncertain identification with numbers.

We killed the fishes using MS222 in accordance with NIH guidelines as supervised by the Cornell University Animal Care and Use Committee. Fresh tissue was preserved in the field in 90 % ethanol or a dimethylsulfoxide/EDTA saturated salt solution (Seutin et al., 1991). In most cases, DNA was extracted from the tissue samples using the QIAamp tissue kit (Quiagen Inc., Valencia, CA, USA).

This study directly builds upon that of Alves-Gomes and Hopkins (1997), who used partial 12S and 16S mitochondrial sequences to examine relationships among 12 mormyroid

species, and Lavoué et al. (1999), who used partial cytochrome *b* sequences in a phylogenetic analysis of 27 mormyroid taxa. We have again used the 12S, 16S and cytochrome *b* genes, but expanded taxonomic sampling and used longer sequences in the case of cytochrome *b*.

Primer sequences for the mitochondrial genes were taken from Palumbi (1996). These sequences are as follows: 12S forward, 5′-AAA CTG GGA TTA GAT ACC CCA CTA T-3′ (L1067); 12S reverse, 5′-GAG GGT GAC GGG CGG GCG GTG TGT-3′ (H1478); 16S forward, 5′-CGC CTG TTT ATC AAA AAC AT-3′ (L2510); 16S reverse, 5′-CCG GTC TGA ACT CAG ATC ACG T-3′ (H3080); cytochrome *b* forward, 5′-TGA TAT GAA AAA CCA TCG TTG-3′ (L14724); and cytochrome *b* reverse, 5′-CTC CAG TCT TCG rCTT TAC AAG-3′ (H15930). Certain taxa failed to amplify with primer L14724. For these, we used an alternative forward primer: 5′-TAC CTA TAC AAA GAA ACm TGA AA-3′ (L15047). Although some of the taxa sampled have been sequenced before for these same regions (Alves-Gomes and Hopkins, 1997; Lavoué et al., 1999), we obtained new sequences for all individuals included in this study with the exception of *Pollimyrus isidori*, for which we use the sequence of Lavoué et al. (1999).

To obtain an estimate of phylogeny from a locus independent of the mitochondrial genome, we also sequenced a portion of the nuclear recombination activating gene 2 (RAG2), which has recently been used in a study of beloniform fishes (Lovejoy, 1999). This gene and the closely linked RAG1 encode components of an enzyme involved in the recombination of immunoglobin and T-cell receptor genes. These genes appear as single copies in all vertebrates examined to date (Hansen and Kaattari, 1996; Willett et al., 1997).

Two forward and two reverse primers taken from Lovejoy (1999) were used in polymerase chain reaction (PCRs), certain combinations working best with different templates. The forward primers are 5′-TTT GGr CAr AAG GGC TGG CC-3′ (F1) and 5′-ArA CGC TCm TGT CCm ACT GG-3′ (F2). The reverse primers are 5′-GTr GAr TAG TAG GGC TCC CA-3′ (R4) and 5′-TGr TCC ArG CAG AAG TAC TTG-3′ (R6). These primers correspond to base pairs 90–110, 112–132, 1287–1307 and 1425–1446, respectively, in the zebrafish (*Danio rerio*) RAG2 gene (Willett et al., 1997).

Approximately 100–500 ng of total genomic DNA was used as template for 50 µl PCR reactions containing 1.25 units of Perkin Elmer AmpliTaq Gold and the following ingredients at the indicated final concentrations: Perkin Elmer GeneAmp PCR buffer II at 1×, each amplification primer at 0.2 µmol l^{-1}, each dNTP at 200 µmol l^{-1} and $MgCl_2$ at between 1.5 and 3 mmol l^{-1}.

Amplification conditions consisted of an initial 95 °C denaturation step for 10 min, 35 cycles of 94 °C for 1 min, annealing for 1 min (at 55–60 °C for the 12S and 16S fragments, at 42 °C for cytochrome *b* and at 53 °C for RAG2), extension at 72 °C for 1.5 min, followed by a final extension at 72 °C for 7 min. PCR products were purified using the Promega Wizard PCR Preps DNA purification kit (Promega, Madison, WI, USA).

We sequenced the double-stranded PCR products directly in both directions with the primers used for amplification on an Applied Biosystems 377 automated sequencer. We edited the sequences using the Sequencher software package (Gene Codes Corp., Ann Arbor, MI, USA). All sequences are available in GenBank (accession numbers AF201483–AF201660).

Alignment and phylogenetic inference

We aligned our 12S and 16S sequences using CLUSTAL W (Thompson et al., 1994) with three different parameter settings, which adjust the gap opening and gap extension costs relative to the base change cost. These were gap opening/gap extension: 10/5 (default), 7/5 and 10/10. The ‘transitions weighted’ option was used on all three alignments (identical bases receive a score of 3, transitional mismatches a score of 1, other mismatches a score of 0). Bases whose positions differed in the three alignments were excluded from the phylogenetic analyses to avoid errors in positional homology (Gatesy et al., 1993). We used the edited 12S and 16S datasets to create a matrix of gap characters using PAUPGap 1.0 (Cox, 1997). We preferred this method to coding gap positions as ‘fifth bases’ in PAUP so that shared gaps of any size receive the same weight in the analysis (i.e. a shared gap of five bases is treated as one character, not five). The matrix of two-state gap characters was appended to the NEXUS file consisting of the aligned sequences. These gap characters were given the same base weight as the nucleotide characters in those phylogenetic analyses in which they were included.

The NEXUS file containing the aligned sequences used for phylogenetic analysis is available at http://www.nbb.cornell.edu/neurobio/hopkins/hopkins.html or upon request from the authors.

We estimated the phylogenetic signal in the aligned sequence data using maximum parsimony (MP) analyses in PAUP* version 4.0b2 (Swofford, 1999). In all cases, the most parsimonious trees were obtained using 100 heuristic searches with random addition of the taxa to produce the starting tree. The default settings were used for all other parameters. We conducted separate MP analyses for the combined 12S and 16S data, the cytochrome *b* data and the RAG2 data. Relative support for internal nodes of these trees was estimated using bootstrap analysis (Felsenstein, 1985) consisting of 1000 pseudoreplicates in PAUP*. Bootstrap values are interpreted here as an estimate of relative support for nodes on a tree produced from a particular dataset, but are not equivalent to statistical probability values. We looked for evidence of mutational saturation for each class of nucleotide substitution and within each codon position of the cytochrome *b* and RAG2 genes by plotting a metric of the observed number of nucleotide differences in pairwise comparisons of taxa (the ‘adjusted character distance’ of PAUP*) against a metric of the corresponding number of substitutions inferred to have taken place on the branches joining each pair of species on a most-parsimonious tree (the patristic distance of PAUP*). This tree was produced from a preliminary unweighted MP analysis. Saturation is estimated by the extent

to which the slope of a linear regression departs from a value of 1 (Hassanin et al., 1998).

In cases of saturation of transitions, we examined the effect of down-weighting them relative to other character state changes through the use of step matrices and compensatory static character weights in PAUP*. For example, to down-weight certain transitions by half relative to all other character state changes, we used a step matrix that gave transversions a weight of 2 and transitions a weight of 1 at those sites. In addition, we placed a compensatory static weight of 2 on all characters not subject to the step matrix. In this way, transitions were down-weighted with respect to all other character state changes without otherwise altering weight relationships in the dataset.

We used the partition homogeneity or 'combinability' test of Farris et al. (1995), as implemented in PAUP*, to test for conflict of signal among the informative characters of the three datasets before pooling them into a combined dataset.

On the combined data we performed an unweighted MP analysis and, in addition, performed other MP analyses in which classes of mutation for which we found evidence of saturation were differentially down-weighted relative to other character state changes. There is no *a priori* objective method for selecting a single best weighting scheme for a parsimony analysis or of choosing a single best tree among those produced in the differently weighted analyses. For this reason, we adopted an approach similar to the successive approximations or '*a posteriori* reweighting' method of Farris (1969) in which a character's weight in a weighted parsimony search is set equal to the maximum rescaled consistency index (RCI) of that character on the set of trees obtained from an initial unweighted search. If necessary, this procedure is repeated until a single most parsimonious tree or set of trees is found. In our procedure, we expanded the set of candidate trees used to calculate each character's maximum RCI by including all shortest trees found in six separate MP analyses (five in which different *a priori* weighting schemes were applied and one unweighted). Our intention was to prevent a limited set of tree topologies from biasing the reweighted analysis.

To estimate support for nodes in this combined analysis, bootstrap values and decay indices were calculated with PAUP* and the software AutoDecay (Eriksson, 1997) respectively. In addition, we compared the nodes recovered in the *a posteriori* reweighted analysis with those present in the shortest trees produced in the original unweighted and five *a priori* weighted MP analyses.

Electric organ anatomy and character reconstruction

We examined electrocyte morphology using tissue fixed in 4% buffered formaldehyde embedded in JB-4 plastic (Polysciences), cut in sagittal plane at 6–7 μm and stained with Toluidine Blue, as described by Hopkins (1999a,b) and other sources (Bass, 1986b,c; Bass and Hopkins, 1983, 1985). Electrocytes were classified as to the presence or absence of penetrating stalks and the side of innervation using light microscopy. We explored the evolution of the mormyrid electrocyte on our preferred phylogenetic tree by searching for the most parsimonious reconstruction(s) of electrocyte character states using the software application MacClade 3.07 (Maddison and Maddison, 1997) combined with considerations of electrocyte ontogeny.

Results

12S and 16S sequences

For the analysis of the 12S and 16S sequences, we used *Chitala ornata* and *Xenomystus nigri* as the sole outgroups since alignment was appreciably improved when the more distant *Pantodon buchholzi* was excluded from the dataset. After the removal of *Pantodon buchholzi*, the alignment of all 395 bases of the 12S gene fragment was identical under the three different parameter settings in CLUSTAL W. Of these sites, 100 (25%) are variable, with 75 (19%) being parsimony-informative. In the 16S fragment, 45 characters changed position among the three CLUSTAL W alignments and were excluded from later analyses, leaving 571 aligned bases. Of these sites, 151 (26.5%) are variable in the dataset, with 113 (20%) being parsimony-informative. From the aligned and edited 12S and 16S datasets, 29 gap characters were identified and coded using PAUPGap 1.0. Of these, 20 are parsimony-informative.

Nucleotide composition (Table 2) was very similar between the 12S and 16S fragments. Considering only informative sites, *Gymnarchus niloticus* and the notopterid outgroup taxa are relatively lower in G and higher in A content than the others. This pattern is the same as that reported by Alves-Gomes and Hopkins (1997). Nevertheless, a χ^2-test to detect non-homogeneity of base frequencies across taxa did not approach significance on the informative-characters-only dataset.

In Fig. 1, the 12S, 16S and cytochrome *b* uncorrected genetic distances (p-distances) calculated in all possible pairwise comparisons of the taxa used in this study are plotted against the corresponding values for the RAG2 gene. Similar p-distances in corresponding pairwise comparisons for the 12S and 16S fragments indicate similar evolutionary characteristics. For this reason and because of similar base composition and a non-significant *P*-value of a partition homogeneity test in PAUP, the 12S and 16S datasets were pooled for subsequent analysis. Parsimony analysis of this pooled dataset with and without the gap characters revealed that their inclusion allows the resolution of several additional nodes. When the 29 gap characters were included and given the same weight as the nucleotide data, a heuristic search yielded 10 most parsimonious trees of 834 steps with a Consistency Index (CI) of 0.38 and a Retention Index (RI) of 0.62. Fig. 2 shows a consensus of the results for the combined 12S, 16S and gap data when three different weighting schemes are employed. All nodes recovered above the 50% bootstrap level in the three analyses were compatible.

The results of the 12S and 16S parsimony analysis support the monophyly of the ingroup (mormyroid) taxa, with *Gymnarchus niloticus* (Gymnarchidae) forming the sister group to the all the mormyrid taxa. Within the mormyrids, all four included *Petrocephalus* species form a clade that is the sister

Table 2. *Nucleotide composition of the gene fragments used in this study and results of χ^2-tests of homogeneity of base composition across taxa*

Gene	Nucleotide				*N*	χ^2
	A	C	G	T		
12S						
All	30.9 (29.9–32.9)	27.4 (25.6–28.5)	22.4 (21.6–23.0)	19.4 (17.9–20.7)	395	NS
Informative	24.1 (20.0–36.9)	37.0 (27.0–44.0)	17.8 (13.5–22.7)	21.1 (13.3–28.4)	75	NS
16S						
All	31.0 (29.4–32.1)	25.7 (24.2–26.9)	22.4 (21.2–23.3)	20.9 (19.8–21.7)	616	NS
Informative	30.1 (24.1–37.9)	29.8 (23.4–34.8)	19.0 (14.7–24.3)	21.1 (15.0–28.8)	113	NS
Cytochrome *b*						
Position 1, all	25.3 (22.9–29.5)	26.2 (23.6–29.1)	25.9 (21.6–28.6)	24.5 (19.9–24.5)	380	NS
Position 1, informative	20.1 (15.6–35.6)	42.7 (34.4–48.9)	23.1 (14.4–29.3)	14.0 (9.7–20.0)	90	NS
Position 2, all	19.6 (17.8–20.5)	25.5 (24.2–26.5)	13.0 (11.3–13.7)	41.9 (40.8–44.7)	380	NS
Position 2, informative	8.7 (0–16.0)	41.4 (24.0–52.0)	14.7 (8.0–21.1)	35.2 (28.0–56.0)	25	NS
Position 3, all	41.1 (36.4–45.7)	44.8 (32.0–51.5)	3.6 (1.8–7.9)	10.5 (5.8–21.5)	381	***
Position 3, informative	37.5 (32.8–42.5)	47.0 (32.4–54.3)	3.8 (1.8–8.3)	11.7 (6.3–23.5)	336	***
RAG2						
Position 1, all	20.2 (16.8–21.4)	25.3 (24.3–26.2)	35.4 (34.4–38.4)	19.1 (17.2–20.1)	378	NS
Position 1, informative	35.7 (16.4–40.8)	23.6 (19.7–37.0)	20.1 (15.8–34.2)	19.9 (10.5–22.4)	76	NS
Position 2, all	30.5 (28.7–31.0)	20.0 (19.6–21.6)	26.5 (25.9–27.0)	23.1 (21.9–23.8)	378	NS
Position 2, informative	23.7 (11.9–30.2)	26.5 (23.3–37.2)	32.8 (20.9–39.5)	17.0 (11.6–21.4)	43	NS
Position 3, all	14.4 (10.0–18.8)	37.9 (33.6–40.5)	29.6 (25.9–38.1)	18.1 (11.7–20.9)	378	NS
Position 3, informative	18.9 (10.9–24.5)	33.7 (27.9–37.6)	23.4 (20.3–37.0)	23.9 (13.9–27.4)	237	NS

***Statistically significant ($P<0.001$); NS, not significant.

group to the remaining taxa. Within this latter group, *Myomyrus macrops* forms the sister group to the other mormyrids. Most other higher-level relationships remain unresolved. However, the genera *Stomatorhinus*, *Pollimyrus*, *Mormyrops* and *Mormyrus*, which are represented by more than one taxon, all appear monophyletic with strong support. A clade of four species of *Campylomormyrus* plus *Gnathonemus petersii* is strongly supported. This clade is nested within a larger clade including *Genyomyrus donnyi* and species of *Marcusenius* and *Hippopotamyrus*. *Paramormyrops gabonensis* is nested within a clade of *Brienomyrus* species from Gabon. Included in this well-supported clade is *Marcusenius conicephalus*, which failed to group with other *Marcusenius* species. *Brienomyrus brachyistius*, the type species of the genus, does not form a clade with the *Brienomyrus* species from Gabon, but instead is the sister taxon to *Isichthys henryi*. *Brienomyrus niger* clusters neither with *B. brachyistius* nor with the Gabon *Brienomyrus* species. *Ivindomyrus opdenboschi* and *Boulengeromyrus knoepffleri*, two monotypic species both endemic to the Ivindo/Ntem system of Gabon and Cameroon, form a strongly supported clade.

Cytochrome b

With the exception of *Hippopotamyrus pictus*, for which cytochrome *b* primers failed to amplify a fragment, cytochrome *b* sequences were obtained for all taxa used in the 12S and 16S dataset. We obtained 1141 nucleotides of this gene for 37 taxa and 800 nucleotides for seven taxa (indicated in Table 1) which failed to amplify with the L14724 cytochrome *b* primer. For these seven taxa, we used the L15047 forward primer. Finally, for *Pollimyrus isidori*, which failed to amplify with any of our primers, we used a 500 base sequence from Lavoué et al. (1999). Alignment of the cytochrome *b* sequences was straightforward: no gaps/insertions were observed. Codon position was easily determined, and no stop codons were observed within any of the sequences.

In our plot of pairwise-adjusted character distances *versus* corresponding pairwise patristic distances inferred from a preliminary MP tree, we observed significant evidence of saturation in third-position transitions at greater genetic distances. Saturation of third-position transversions was only evident in pairwise comparisons between *Pantodon buchholzi* (the most distant outgroup taxon sequenced) and other taxa. For this reason, we opted to exclude *Pantodon buchholzi* from the cytochrome *b* dataset in further analyses. We rejected the alternative of retaining *Pantodon buchholzi* but excluding third-position sites, since these account for approximately 75% of the informative characters in cytochrome *b*. Saturation of third positions was less severe for the remaining outgroups *Xenomystus nigri* and *Chitala ornata*.

Over the 1141 base fragment, 529 (46%) of nucleotide positions are variable and, of these, 451 (39.5%) are parsimony-informative (363 variable/336 informative in the

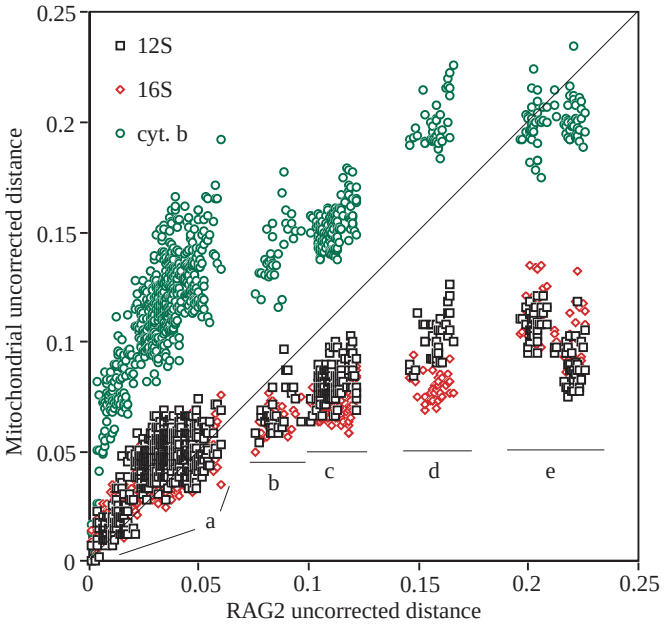


Fig. 1. Comparative evolutionary rates of the four gene fragments used in this study. The 12S, 16S and cytochrome *b* (Cyt. *b*) uncorrected genetic distances (p-distances) calculated in all possible pairwise comparisons of the taxa used in this study are plotted against the corresponding values for the RAG2 gene. The evolutionary rates of the three mitochondrial genes appear to slow at higher RAG2 distances, probably as a result of increasing saturation of variable sites in these genes relative to RAG2. Note the clustering of the data along the RAG2 axis into five distinct groups. Cluster a (RAG2 p-distance 0–0.06) contains all pairwise comparisons made within subfamily Mormyrinae, with the exclusion of the genus *Myomyrus*; cluster b (RAG2 p-distance 0.08–0.09) contains all pairwise comparisons of *Myomyrus* with other Mormyrinae; cluster c (RAG2 p-distance 0.10–0.12) contains all pairwise comparisons of species of *Petrocephalus* (Petrocephalinae) with species of Mormyrinae; cluster d (RAG2 p-distance 0.15–0.17) contains all pairwise comparisons of *Gymnarchus* (Gymnarchidae) with species of Mormyridae (Mormyrinae + Petrocephalinae); cluster e (RAG2 p-distance 0.20–0.22) contains all pairwise comparisons of the outgroup taxa *Xenomystus nigri* and *Chitala ornata* (Notopteroidea) with species of Mormyroidea (mormyrids + *Gymnarchus*).

third position, 126 variable/90 informative in the first position and 40 variable/25 informative in the second position). These ratios are similar to those reported by Lavoué et al. (1999).

We found nucleotide composition in the third-position sites (Table 2) to be strongly biased towards C and A while poor in T and G content. In addition, base composition varied greatly across taxa. A χ^2-test of base frequencies across taxa was highly significant (P<0.0001), indicating a lack of homogeneity. An excess of T and a lack of C compared with most other taxa is apparent in *Gymnarchus niloticus* (21.5 % T, 32 % C) and in the notopterid outgroup taxa (15–17.5 % T, 38–41 % C). A base composition that differs significantly across taxa, as we found here, can impact on maximum parsimony and other phylogenetic reconstruction methods which may incorrectly group taxa with convergently or primitively similar base compositional biases (Swofford, 1999). Strong bias in nucleotide composition is also evident in the informative sites of the first two positions, although χ^2-tests for heterogeneity across taxa did not approach significance (Table 2).

Considered as a unit, cytochrome *b* appears to evolve at approximately three times the rate found in 12S and 16S at lower genetic distances and closer to twice the rate of these genes at higher genetic distances in the taxa we studied (Fig. 1). The apparent relative slowing of cytochrome *b* compared with 12S and 16S at increasing genetic distance could be due to mutational saturation of third-position transitions (noted above) obscuring true genetic distance. Third-position saturation is undoubtedly in part the result of the overabundance of A and T (together comprising 86 % of all bases), which greatly increases the likelihood of homoplasious change.

A heuristic search on the complete unweighted cytochrome *b* dataset produced two most parsimonious trees (2453 steps, CI excluding uninformative characters 0.32, RI 0.47). Because evidence of saturation in the cytochrome *b* fragment was present only for the third position sites (see above), only third-position transitions were down-weighted relative to other changes in the weighted analyses. Single most parsimonious trees were obtained when third-position transitions were given weights of 0.5× and 0.25× relative to other characters, although several nodes differed in these two trees. Notably, in none of these trees does *Gymnarchus niloticus* form a clade with the other ingroup taxa, but instead appears as the sister taxon to *Xenomystus nigri*. Only when third-position transitions are down-weighted to one-tenth (or less) of the weight of all other character state changes does *Gymnarchus niloticus* cluster with the ingroup taxa. However, this extreme weighting also affects relationships in more terminal portions of the tree. We interpret the failure of the ingroup taxa to appear as a clade in analyses in which third positions are not heavily down-weighted as the effects of mutational saturation and the similar base compositional biases in the third positions of the *Gymnarchus* and notopterid cytochrome *b* sequences, noted above.

Fig. 3 shows the results of a combinable component consensus of three MP bootstrap trees obtained from the cytochrome *b* data under three different weighting regimes. Excepting the failure of *Gymnarchus niloticus* to group with the mormyrid taxa, every supra-generic clade recovered above the 50 % bootstrap level in the combined 12S and 16S bootstrap analysis is also recovered in the cytochrome *b* bootstrap analysis (allowing for the absence of *Hippopotamyrus pictus* from the cytochrome *b* dataset). Several additional clades are recovered as well. The first of these is a clade of the four *Campylomormyrus* species. These species plus *Gnathonemus petersii* form a clade with a *Marcusenius moorii* plus *Marcusenius* sp. 1 sister pair and *Genyomyrus donnyi*. In addition, *Marcusenius conicephalus* forms the sister taxon to the *Brienomyrus*/*Paramormyrops* clade. Some intrageneric relationships in the genera *Petrocephalus*, *Mormyrops*, *Pollimyrus* and *Stomatorhinus* and within the Gabon *Brienomyrus*/*Paramormyrops* clade

differ between the MP bootstrap analyses of this dataset and the 12S/16S dataset. Despite these differences, a partition homogeneity test between the two datasets did not approach significance.

RAG2

We obtained 1143 bases of the RAG2 gene for all 45 taxa in Table 1. Insertions/deletions (indels) were observed only between the *Pantodon buchholzi* sequence and the others. These are four indels varying from one to three codons in length which were easily aligned by eye by maintaining codon structure and identifying flanking codons for amino acid residues identical to those in the other sequences. Of the 1143 bases, 578 (50.6 %) are variable and 356 (31.1 %) of these are parsimony-informative (335 variable/237 informative in the third position, 148 variable/76 informative, in the first position and 95 variable/43 informative in the second position).

Base composition for each position of the RAG2 fragment is shown in Table 2. χ^2-tests for non-homogeneity of bases across taxa did not approach significance for any codon position in RAG2.

We observed no evidence of saturation in third-position

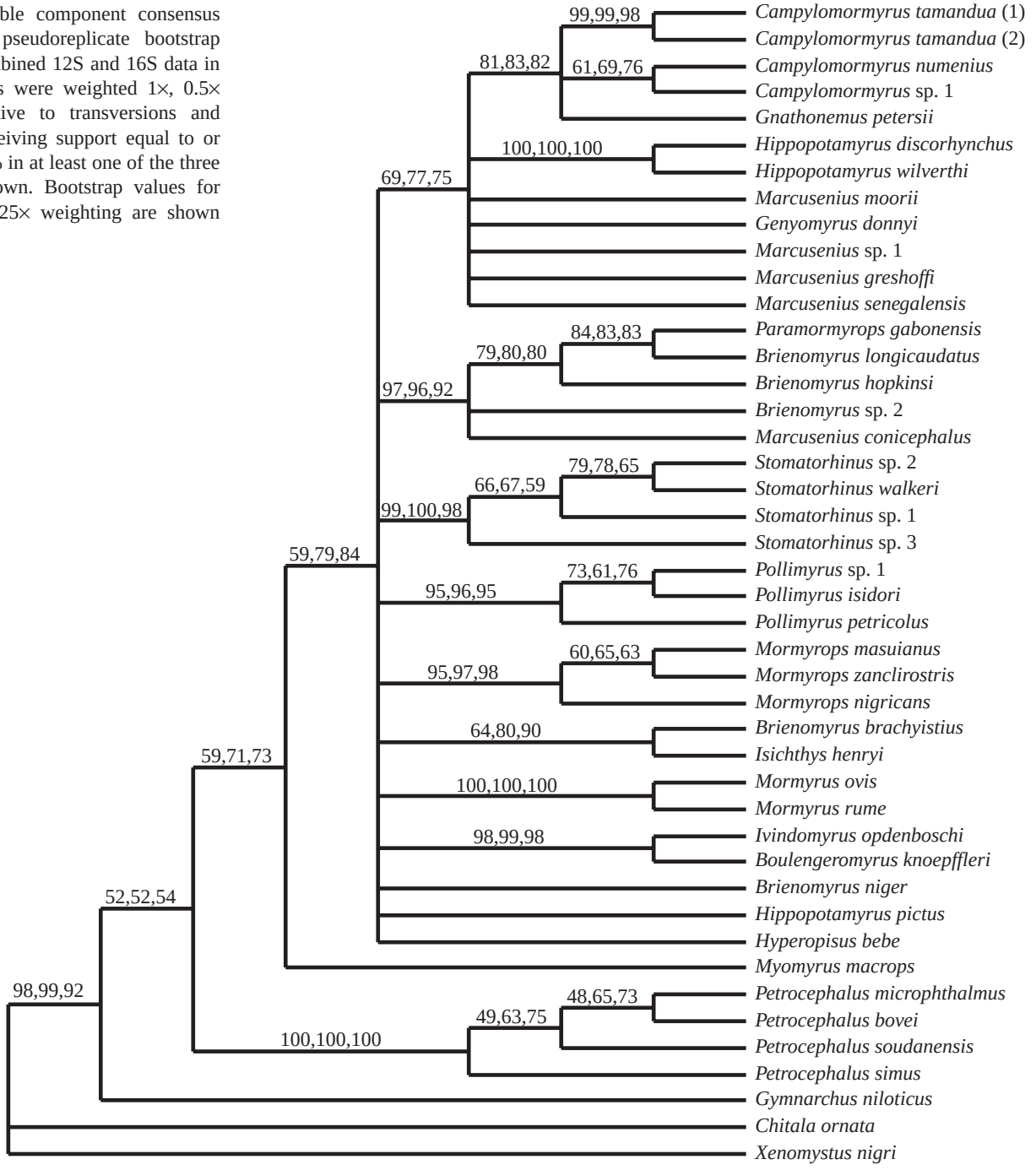


Fig. 2. Combinable component consensus of three 1000 pseudoreplicate bootstrap trees for the combined 12S and 16S data in which transitions were weighted 1×, 0.5× and 0.25× relative to transversions and gaps. Nodes receiving support equal to or greater than 50 % in at least one of the three analyses are shown. Bootstrap values for 1×, 0.5× and 0.25× weighting are shown from left to right.

transitions when we plotted PAUP's pairwise-adjusted character distances against the patristic distances calculated from a tree produced by a preliminary maximum parsimony analysis. RAG2 uncorrected p-distance in pairwise comparisons of closely related taxa is approximately half that of the 12S and 16S p-distance and less than 20 % of that of cytochrome *b* p-distance in the same comparisons (Fig. 1). The apparent evolutionary rate of RAG2 increases relative to that of the mitochondrial genes in pairwise comparisons of more distantly related taxa such that, at intermediate overall genetic distance, RAG2 p-distances are roughly equal to those of the 12S and 16S fragments. In pairwise comparisons between outgroup and ingroup taxa involving greater genetic distance, RAG2 p-distances surpass those of the 12S and 16S fragments and are roughly equal to those of the cytochrome *b* gene. We interpret this pattern to be the result of increasing mutational saturation of the mitochondrial genes at greater genetic distances relative to RAG2, which shows no evidence of saturation. Also notable is the large genetic distance in the RAG2 data separating *Myomyrus macrops* from other species within Taverne's subfamily Mormyrinae (Fig. 1).

A heuristic MP search on the entire RAG2 dataset produced

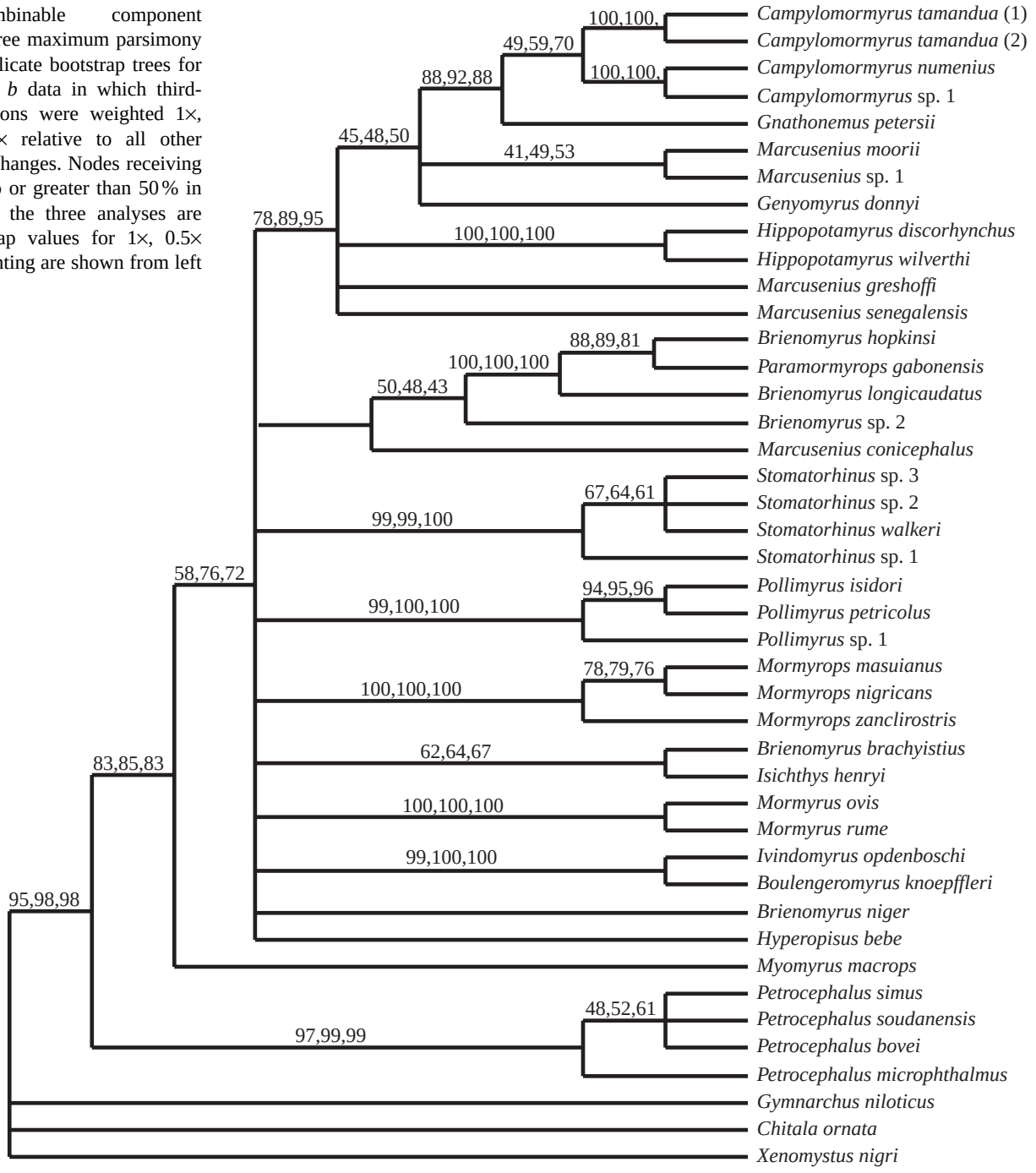


Fig. 3. Combinable component consensus of three maximum parsimony 1000 pseudoreplicate bootstrap trees for the cytochrome *b* data in which third-position transitions were weighted 1×, 0.5× and 0.25× relative to all other character state changes. Nodes receiving support equal to or greater than 50 % in at least one of the three analyses are shown. Bootstrap values for 1×, 0.5× and 0.25× weighting are shown from left to right.

144 equally parsimonious trees, each 1226 steps long (CI excluding uninformative characters 0.56, RI 0.72). A strict consensus of these trees is shown in Fig. 4. Bootstrap support values are indicated to the left of each node. This tree is better resolved than either of the mitochondrial bootstrap consensus trees and is consistent with the suprageneric relationships recovered in them.

In the RAG2 tree, *Brienomyrus niger* and *Hippopotamyrus pictus* are resolved as sister taxa that, together with *Hyperopisus bebe*, are placed in an unresolved polytomy at the base of the *Campylomormyrus*/*Gnathonemus*/*Genyomyrus*/*Marcusenius*/*Hippopotamyrus* clade. This large clade is sister to another that is resolved into a polytomy of three subclades. Two of these consist of the species of *Pollimyrus* and *Stomatorhinus*, respectively. The third contains the clade consisting of several *Brienomyrus* species from Gabon plus *Paramormyrops gabonensis*. Again, as in the mitochondrial trees, *Marcusenius conicephalus* forms the sister taxon to this

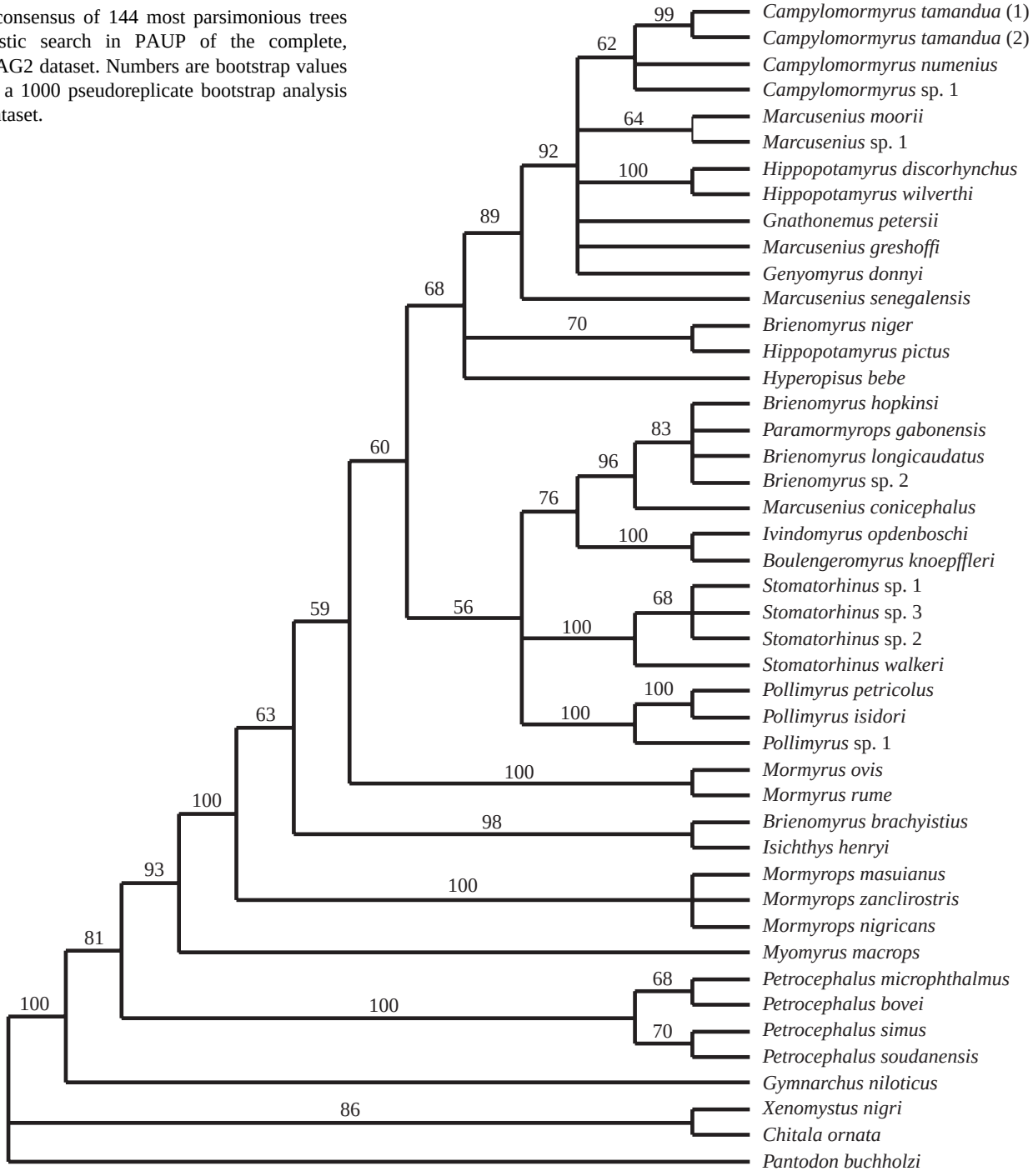
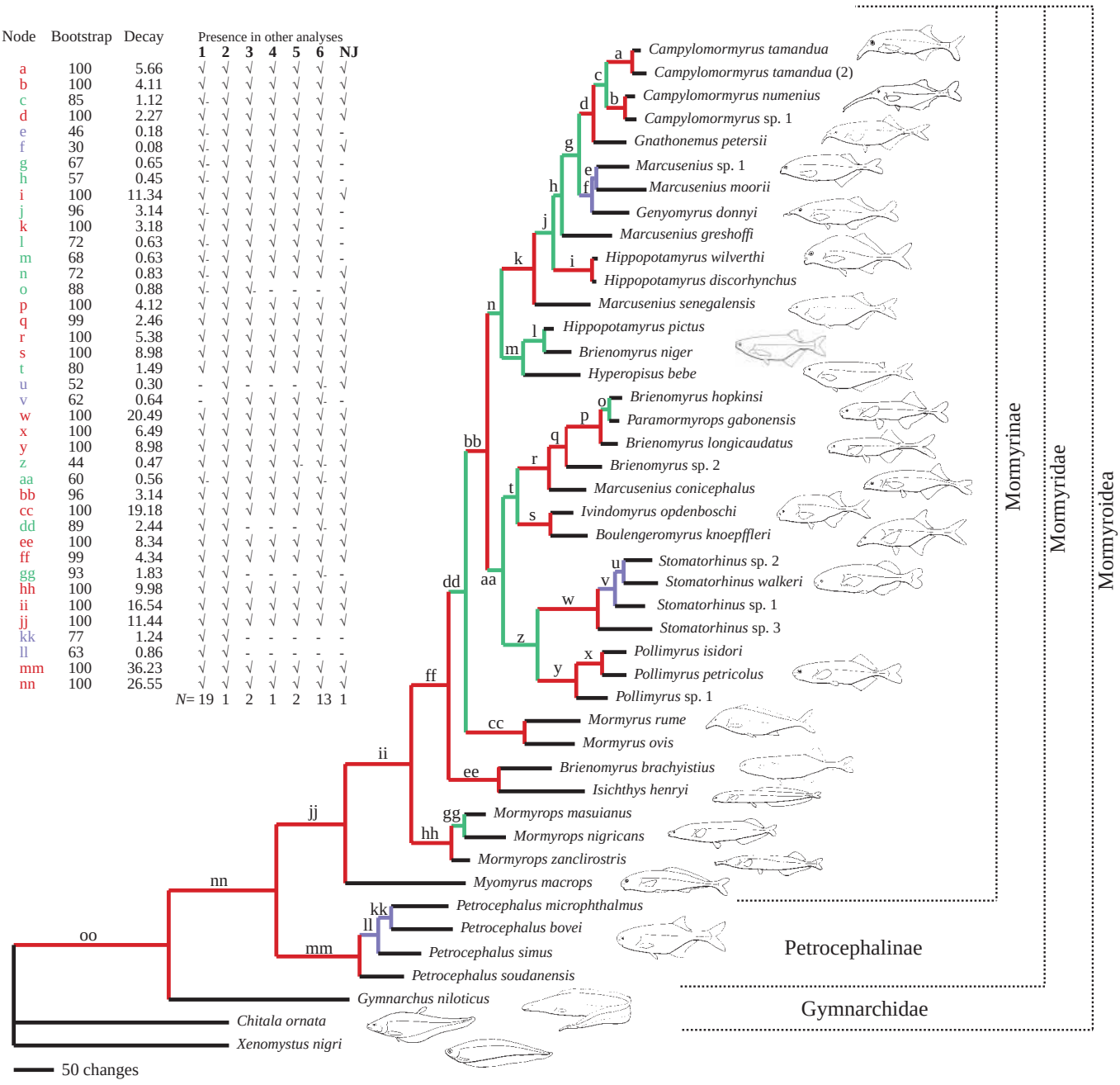


Fig. 4. Strict consensus of 144 most parsimonious trees from a heuristic search in PAUP of the complete, unweighted RAG2 dataset. Numbers are bootstrap values obtained from a 1000 pseudoreplicate bootstrap analysis on the same dataset.



Node	Bootstrap	Decay	Presence in other analyses 1	2	3	4	5	6	NJ
a	100	5.66	√	√	√	√	√	√	√
b	100	4.11	√	√	√	√	√	√	√
c	85	1.12	√-	√	√	√	√	√	√
d	100	2.27	√	√	√	√	√	√	√
e	46	0.18	√-	√	√	√	√	√	-
f	30	0.08	√-	√	√	√	√	√	√
g	67	0.65	√-	√	√	√	√	√	-
h	57	0.45	√-	√	√	√	√	√	-
i	100	11.34	√	√	√	√	√	√	√
j	96	3.14	√-	√	√	√	√	√	-
k	100	3.18	√	√	√	√	√	√	-
l	72	0.63	√-	√	√	√	√	√	-
m	68	0.63	√-	√	√	√	√	√	-
n	72	0.83	√-	√	√	√	√	√	√
o	88	0.88	√-	√	√-	-	-	-	√
p	100	4.12	√	√	√	√	√	√	√
q	99	2.46	√	√	√	√	√	√	√
r	100	5.38	√	√	√	√	√	√	√
s	100	8.98	√	√	√	√	√	√	√
t	80	1.49	√	√	√	√	√	√	√
u	52	0.30	-	√	-	-	-	√-	√
v	62	0.64	-	√	√	√	√	√-	-
w	100	20.49	√	√	√	√	√	√	√
x	100	6.49	√	√	√	√	√	√	√
y	100	8.98	√	√	√	√	√	√	√
z	44	0.47	√	√	√	√	√-	√-	√
aa	60	0.56	√-	√	√	√	√	√-	√
bb	96	3.14	√	√	√	√	√	√	√
cc	100	19.18	√	√	√	√	√	√	√
dd	89	2.44	√	√	-	-	-	√-	√
ee	100	8.34	√	√	√	√	√	√	√
ff	99	4.34	√	√	√	√	√	√	√
gg	93	1.83	√	√	-	-	-	√-	-
hh	100	9.98	√	√	√	√	√	√	√
ii	100	16.54	√	√	√	√	√	√	√
jj	100	11.44	√	√	√	√	√	√	√
kk	77	1.24	√	√	-	-	-	-	-
ll	63	0.86	√	√	-	-	-	-	-
mm	100	36.23	√	√	√	√	√	√	√
nn	100	26.55	√	√	√	√	√	√	√
		N=	19	1	2	1	2	13	1

Fig. 5. Single most parsimonious tree obtained from the combined, *a posteriori* reweighted, 12S, 16S, cytochrome *b* and RAG2 characters. In this analysis, each character was assigned a weight equal to its maximum RCI on one or more of the most parsimonious trees produced by six prior MP searches. Five of these employed step matrices to downweight transitions relative to transversions. In the sixth, transitions and transversions were weighted equally. Branch lengths are proportional to number of unweighted character state changes calculated under ACCTRAN. 1000 pseudoreplicate bootstrap values and decay indices are shown for each node in the table above. The presence or absence of each node in each of the six MP analyses is indicated. √, present in shortest tree or all shortest trees; √–, present in at least one of the shortest trees; –, absent in shortest tree or all shortest trees. Weightings used in these analyses are (1) 1:1/1:1, (2) 2:1/2:1, (3) 2:1/4:1, (4) 4:1/10:1, (5) 4:1/1:0 and (6) 1:0/1:0, where the first ratio indicates the relative weight placed on transversions *versus* transitions over the entire 12S+16S dataset, and the second ratio indicates the relative weight placed on transversions *versus* transitions in the third positions of cytochrome *b*. Weights were applied in a stepmatrix in PAUP along with compensatory static weights such that transitions at these selected sites were down-weighted with respect to all other character state changes while weight ratios between transversions at these sites and other characters were unchanged (see Materials and methods). The presence or absence of each node in a neighbor-joining tree was calculated from the Kimura three-parameter distances indicated in the column marked NJ. The number of shortest trees obtained by each analysis is indicated in the bottom row of the table. The authors' subjective confidence in nodes is indicated by color: red, high confidence; green, moderate confidence; blue, low confidence.

clade. The sister group to this larger clade is *Ivindomyrus opdenboschi* plus *Boulengeromyrus knoepffleri*. Several additional clades not obtained in the mitochondrial analyses are resolved in the RAG2 tree as successive outgroups to the taxa discussed above. These are (in descending order down the tree) the two species of *Mormyrus*, the sister pair of *Brienomyrus brachyistius* and *Isichthys henryi*, and the clade comprising the three *Mormyrops* species. As in the mitochondrial trees, *Myomyrus macrops* appears as the sister taxon to all other Mormyrinae, the species of *Petrocephalus* are the sister taxon to all the Mormyrinae, and *Gymnarchus niloticus* is the sister taxon to all Mormyridae. With *Pantodon buchholzi* included as the designated outgroup, the two notopterid taxa appear as sister taxa to each other and as the sister group to the mormyroids.

Combined analysis

We pooled the three datasets after partition homogeneity tests among them failed to detect incompatibility. The notopterids *Xenomystus nigri* and *Chitala ornata* were included as the only outgroups. We retained *Hippopotamyrus pictus* in this analysis despite the absence of a cytochrome *b* sequence for this taxon (all cytochrome *b* characters were coded as 'missing'). This dataset contains 3270 characters, 969 of which are parsimony-informative. A heuristic search on the unweighted dataset produced 19 equally parsimonious trees (4308 steps, CI excluding uninformative characters 0.38, RI 0.57). Suprageneric relationships in the strict consensus of these 19 trees are consistent with the well-supported relationships in the analyses of the individual datasets. However, several nodes inside the sister group to the *Mormyrus* clade, which had been resolved in the MP analysis of the RAG2 data alone, are lost in this combined analysis.

To establish character weights for an *a posteriori* reweighted MP analysis of the combined data, we calculated the rescaled consistency index (RCI) of each of the 969 informative characters on the above 19 trees plus 13 other shortest trees produced from five weighted parsimony searches. Each of these employed different *a priori* down-weighting of transitions in the 12S/16S datasets and in the third-position sites of the cytochrome *b* dataset (see legend to Fig. 5). Each character was then assigned a weight equal to the highest RCI found for that character on one or more of these 32 trees: 184 of these characters received a weight of 1, 212 characters received a weight of 0 and 785 characters received a fractional weight between 0 and 1. The mean weight of all informative characters was 0.321, and the median weight was 0.20. A parsimony analysis on this *a posteriori* reweighted dataset yielded a single most parsimonious tree of 1179.61 steps (Fig. 5). We assigned nodes recovered in the *a posteriori* reweighted tree to three subjective confidence classes: high, moderate and low (indicated by red, green and blue, respectively, in Fig. 5) on the basis of their performance in bootstrap and decay analyses and their presence or absence in the five *a priori* weighted MP analyses and the equally weighted MP analysis. Specifically, a node was given a rating of high confidence if it had a bootstrap value on the *a posteriori* reweighted tree of 90 % or higher, a decay index of 2 or above, and if it was present in all most parsimonious trees recovered in the one unweighted and five *a priori* weighted parsimony analyses. Nodes with lesser performance were rated moderate or low confidence. We rank only six of the 40 nodes on this tree as low-confidence nodes. Only one of these weak nodes, node f, affects a hypothesis of a suprageneric relationship. We regard the relationships between *Genyomyrus donnyi*, *Marcusenius moori* and *Marcusenius* sp.1 to be uncertain with respect to the well-supported *Campylomormyrus* + *Gnathonemus* clade.

An alternative topology to one clade we classify as 'moderate confidence' based on a high bootstrap value and decay index appears in three of five *a priori* weighted MP analyses (analyses 3, 4 and 5 in Fig. 5) and deserves mention. In these analyses, node dd is absent, and the two species of *Mormyrus* form a clade with the *Brienomyrus brachyistius* and *Isichthys henryi* sister pair.

All suprageneric relationships depicted in the *a posteriori* reweighted tree of the combined data are compatible with those recovered above the 50 % bootstrap level in the MP analyses of the individual datasets.

Evolution of the mormyroid electric organ

Because of the remarkable diversity of electric organ discharge waveforms among mormyroids (Hopkins, 1980, 1981, 1986, 1999b; Hopkins and Bass, 1981) and the corresponding diversity of electric organs, we examined the evolution of the electric organ in mormyroids using a simplified version of our tree (see Fig. 7). We incorporated into this tree all high or moderate confidence suprageneric nodes, collapsing the low-confidence nodes e and f in Fig. 5. In addition, for the sake of simplicity, we collapsed clades of single genera that were found to be monomorphic with respect to electrocyte anatomy into single terminals (e.g. the clades of *Petrocephalus*, *Mormyrus*, *Stomatorhinus* and *Pollimyrus* species). Furthermore, we removed *Mormyrops masuianus* from this tree since its position within the *Mormyrops* clade was not strongly resolved and its absence would have no effect on the reconstruction.

We observed six types of electrocyte among the species included in this study. These data are summarized in Table 1. Type S electrocytes found in *Gymnarchus niloticus* are stalkless. There are two major categories of stalked electrocytes. In the first, stalks arising from the posterior face of the electrocyte are non-penetrating and receive innervation on the posterior side (non-penetrating stalk with posterior innervation, type NPp, shown in Fig. 6E for *Petrocephalus bovei*). All fish with electrocytes of this type produce simple biphasic electric organ discharge (EOD) waveforms. In the second category, the stalk penetrates through the electrocyte. Several different forms within this category are observed. In the simplest of these, the stalk penetrates through the electrocyte to the opposite side, where it receives innervation. Electrocytes with stalks arising from the posterior face that penetrate through to receive innervation are called type Pa (penetrating stalk with anterior innervation, shown in Fig. 6I

for *Myomyrus macrops*). Those with stalks arising from the anterior face that penetrate through to receive innervation from the posterior face are called type Pp (penetrating stalks with posterior innervation). In *Brienomyrus brachyistius* and *Brienomyrus niger*, the stalk penetrates through the electrocyte a second time to receive innervation on the face from which it originated (doubly penetrating stalk with posterior innervation, type DPp). Species of only one genus, *Pollimyrus*, have both doubly penetrating and non-penetrating stalks (type DPNP). In contrast to Bass (1986a,c) and Alves-Gomes and Hopkins (1997), we find all species of *Stomatorhinus* have type Pa electrocytes. The EOD waveform produced by each electric organ morphotype is a function of the direction of current flow through the stalk and the relative order in which the anterior and posterior electrocyte faces depolarize (see Bass, 1986a,c).

At the base of the mormyroid tree, we use the parsimony criterion in conjunction with available developmental data to choose a most likely scenario for electric organ evolution. The sister group to the mormyrids, *Gymnarchus niloticus*, has stalkless (S-type) electroctyes and an electric organ fundamentally different in structure and organization from the mormyrid adult electric organ (Dahlgren, 1914; Denizot et al.,

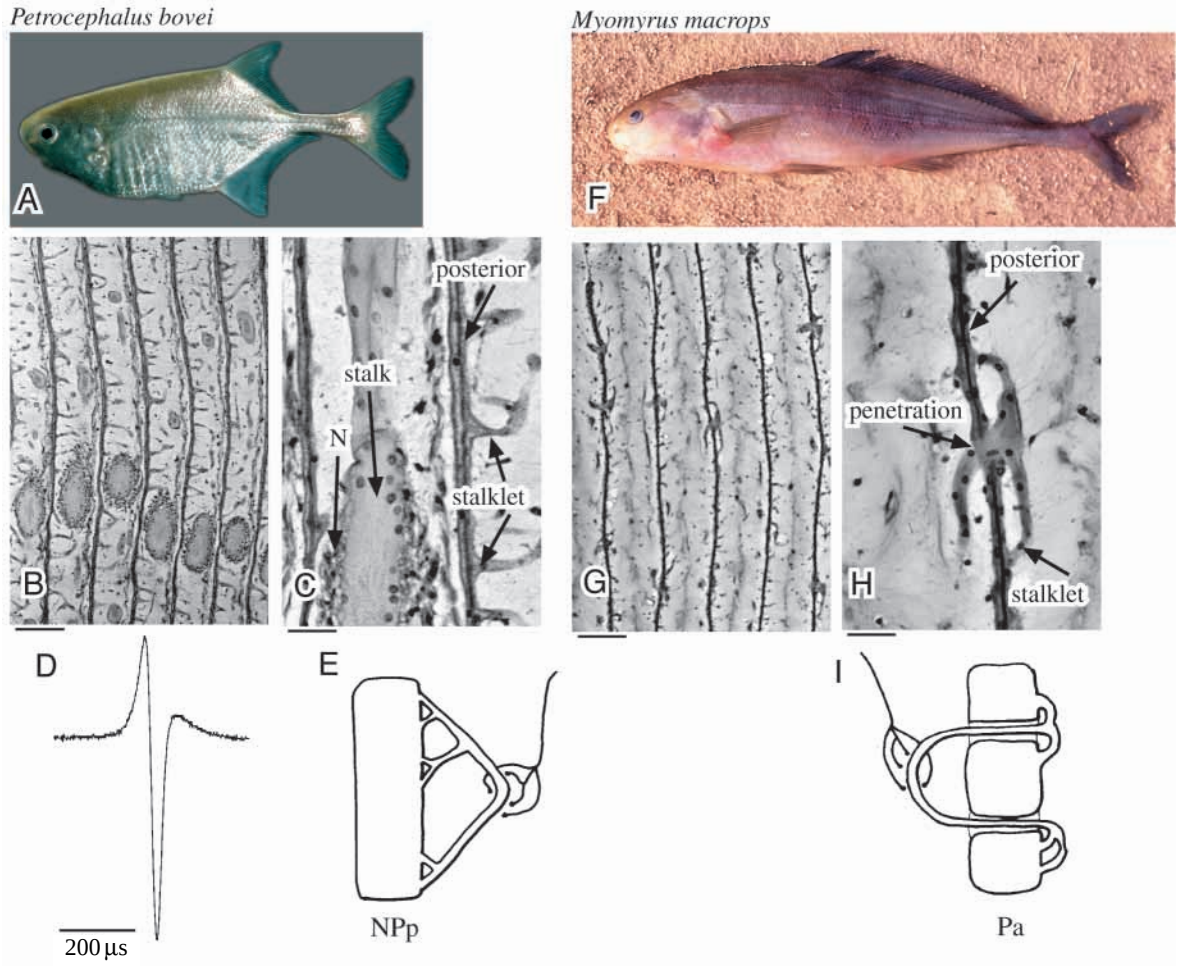
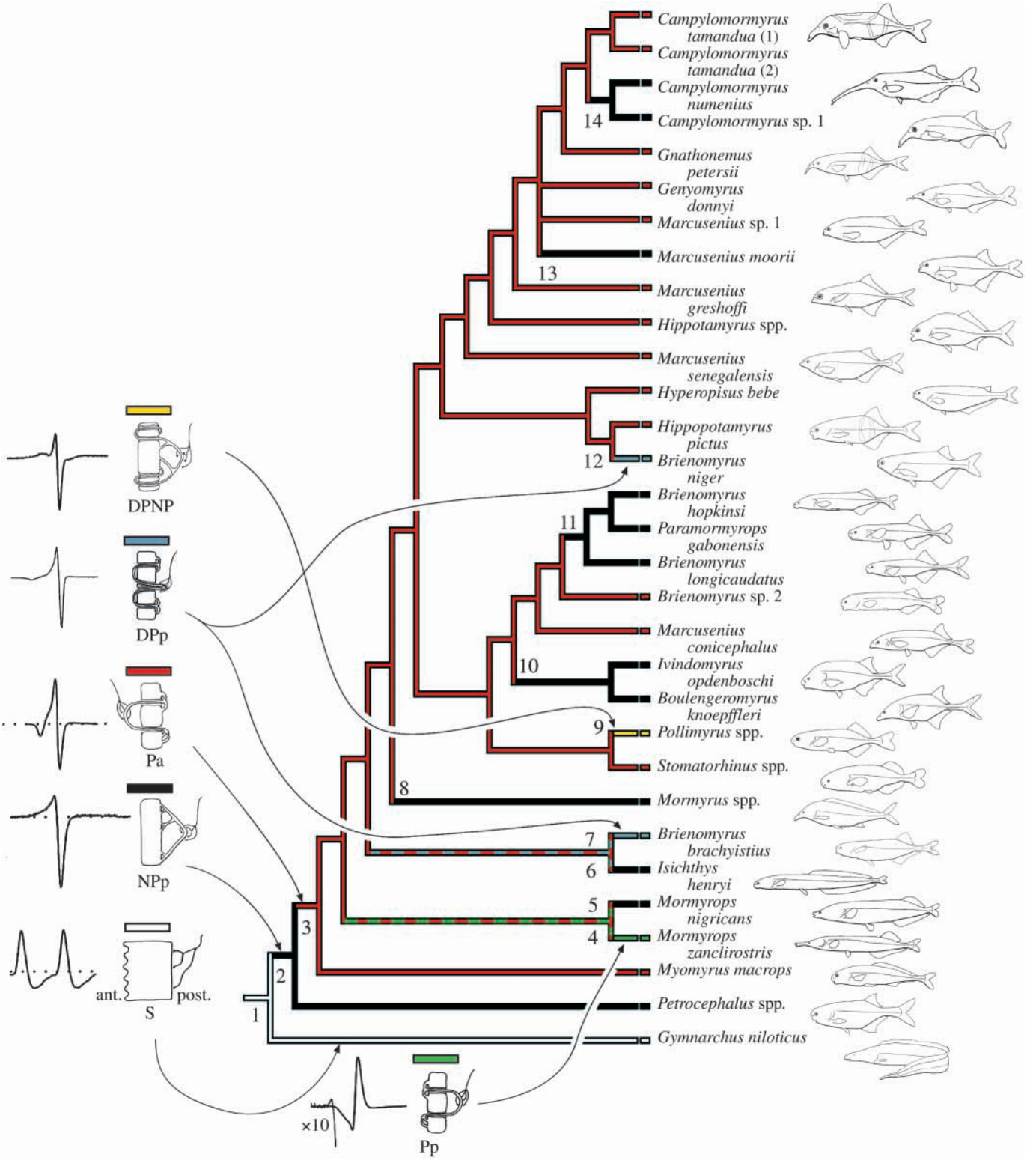


Fig. 6. Two basal genera of mormyroids differing in the structure of their electric organs. All species of *Petrocephalus* so far examined, including *P. bovei* shown here (A–D), have electrocytes with non-penetrating stalks innervated on the posterior side (type NPp). (A) Photograph of a 94 mm (standard length) female *Petrocephalus bovei* from the Niger River in Mali. (B,C) Sagittal section through the electric organ of *Petrocephalus bovei* showing multiple electrocytes with stalklets emerging from the posterior surface of the flattened cell. Stalklets join to form progressively larger stalks. Nerve fibers (N) synapse on the enlarged stalk, which is also on the posterior side of the electrocyte. (D) The electric organ discharge (EOD) of *Petrocephalus bovei* is illustrated with head-positivity upwards. The initially head-positive peak is caused by the firing of the posterior face of the electrocyte; the second head-negative phase is caused by the anterior face firing a spike. The final head-positive overshoot may be caused by capacitative coupling or by a long-lasting potential from the posterior face or stalk. Not all species of *Petrocephalus* have this final head-positive overshoot in their EOD. (E) A schematic drawing of the electrocyte morphology with its neural innervation. (F–I) All species of *Myomyrus* so far examined, including this juvenile *Myomyrus macrops* (standard length 165 mm) from the Sangha River, Central African Republic (F), have electrocytes with penetrating stalks. (G,H) Sagittal sections of the electric organ showing stalklets emerging from the posterior face, penetrating through to the anterior (type Pa). The EOD waveform is unknown for this species. (I) A schematic drawing of a type Pa electrocyte in sagittal view, with the innervation of the stalk on the anterior side of the electrocyte. Scale bars: 100 µm in B and G, 25 µm in C and H.

1978, 1982; Kirschbaum, 1987). While the *Gymnarchus niloticus* organ and the mormyrid adult organ are clearly homologous at some level, we believe the most immediate homologue to the electric organ in *Gymnarchus niloticus* is the larval electric organ in mormyrids, which develops soon after hatching and degenerates as the adult organ develops (Denizot et al., 1978, 1982; Kirschbaum, 1987, 1995). Both are present in the medial part of the deep lateral muscle rostral to the caudal peduncle. In both, the electrocytes are arranged myotomically, with myofibrils present, and in both only the caudal electrocyte face is electrically active, so the EOD waveforms are monophasic. This is in contrast to the adult electric organ of mormyrids which is restricted to the caudal peduncle, in which the myotomic arrangement has been lost, myofibrils are largely



absent, both electrocyte faces are electrically excitable and EOD waveforms have both positive and negative phases.

Given this, there exist two possible reconstructions for the electric organ at the base of the mormyroid tree (node 1, Fig. 7). First, the common ancestor of all mormyroids may have had an electric organ and electrocytes much like that of *Gymnarchus niloticus*. In this case, the developmentally separate adult electric organ present in living mormyrids would have evolved in the immediate common ancestor of mormyrids (node 2, Fig. 7). Alternatively, the common ancestor of all mormyroids could have possessed separate larval and adult electric organs, as do extant mormyrid taxa, and a subsequent paedomorphic loss of the adult organ took place in the lineage leading to *Gymnarchus*.

We favor the first scenario for the evolution of electric organs. If the origins of the larval and adult electric organs are considered as two separate evolutionary steps, the first hypothesis represents the most parsimonious reconstruction.

In the subfamily Mormyrinae, one form of non-penetrating stalked electrocyte (NPp) and four forms of penetrating stalk electrocyte (Pa, Pp, DPp and DPNP) occur in extant taxa. All electrocytes examined from *Petrocephalus* species in the sister subfamily Petrocephalinae are of type NPp. In addition, ontogenetic study of the electrocytes in *Brienomyrus brachyistius*, which have penetrating stalk electrocytes, has demonstrated that the developing, but functional, electrocyte first passes through a stage identical to the NPp condition before penetrations develop (C. D. Hopkins, unpublished observations). Similar observations in *Hyperopisus bebe* and *Mormyrops deliciosus* were made by Szabo (1960). For this reason and because penetrating stalk electrocytes are absent in the basal-most lineage of mormyrids, we hypothesize that NPp is the primitive condition for the mormyrid electrocyte (node 2, Fig. 7).

Above this node, MacClade generates two equally parsimonious reconstructions when the electrocyte is coded as a binary character with states corresponding to non-penetrating and penetrating electrocyte stalks (all varieties of penetrating stalk are treated as a single character state). Both reconstructions require eight steps. In the first of these, penetrating stalks originate four times from ancestors with non-penetrating stalks, and there are four reversals to the non-penetrating condition. In the second reconstruction (shown in Fig. 7 and equivalent to a reconstruction using the ACCTRAN algorithm), the penetrating stalks originate once in the common ancestor of the Mormyrinae, with seven reversals to non-penetrating stalks at the nodes numbered 5, 6, 8, 10, 11, 13 and 14. We favor this latter reconstruction since we regard multiple independent origins of a particular modification to an ancestral and ontogenetically antecedent morphology as less likely than a single origin followed by multiple paedomorphic reversals.

Superimposing the four forms of penetrating stalk electrocyte (Pa, Pp, DPp and DPNP) onto this reconstruction, additional patterns emerge. Electrocyte type DPNP is shown to have originated once in the genus *Pollimyrus*, while type DPp has evolved twice, in the distantly related Pa-type ancestors of *Brienomyrus niger* and in *Brienomyrus brachyistius*.

The remaining form of penetrating stalk electrocyte, type Pp, is known only from several species of *Mormyrops* (e.g. *M. zanclirostris* and *M. masuianus* in this study). This electrocyte is simply reversed in anterior/posterior polarity from type Pa. Within the genus *Mormyrops*, other species possess Pa-type electrocytes, including *Mormyrops deliciosus* (Gosse and Szabo, 1960) and *Mormyrops curviceps* (Moller and Brown, 1990), while still others have type NPp (e.g. *M. nigricans* in this study).

Fig. 7. A hypothesized evolutionary history of electrogenesis among mormyroid fishes reconstructed on the most parsimonious tree for the group derived from mitochondrial and nuclear DNA sequence data (outgroups omitted). Type S electrocytes found in *Gymnarchus niloticus* are 'stalkless.' ant., anterior; post., posterior. Type NPp electrocytes have non-penetrating stalks with posterior innervation; the remaining electrocytes all have penetrating stalks, including type Pa electrocytes with penetrating stalks with anterior innervation, type Pp electrocytes with penetrating stalks with posterior innervation, type DPp electrocytes with doubly penetrating stalks with posterior innervation and type DPNP electrocytes with doubly penetrating and non-penetrating stalks with posterior innervation. A diagram represents each type of electrocyte; arrows point to its hypothesized origin on the tree. Representative electric organ discharge waveforms accompany each type of electrocyte. On the basis of character optimization in MacClade and developmental data (see text), we hypothesize a single origin of the penetrating-stalk-type electrocyte early in the history of the group. Alternating color bars to the left of a clade indicate that the ancestral type of penetrating stalk electrocyte is equivocal. Numbers refer to hypothesized character state changes and are discussed in the text.

Discussion

Phylogenetic conclusions and agreement with previous studies

The only available higher-level hypothesis of mormyroid interrelationships has been that of Taverne, who organized the modern taxonomy of the group in the course of his osteological studies (Taverne, 1967, 1968, 1969, 1971b, 1972; Taverne and Géry, 1968). Taverne's classification divided the group into two families, the Gymnarchidae, consisting of the monotypic genus *Gymnarchus*, and the Mormyridae, comprising the remaining genera. Within the Mormyridae, he recognized two subfamilies: the Petrocephalinae (genus *Petrocephalus*) and the Mormyrinae (the remaining genera). Taverne diagnosed the Mormyrinae by the loss of the basisphenoid bone. The results of our phylogenetic analysis of molecular data support these taxonomic divisions. Within the Mormyrinae, Taverne proposed a large subdivision diagnosed by the loss of the lateral ethmoid bone, although he did not assign this group any formal taxonomic category. These taxa are not recovered as a monophyletic group in our study, and we infer that the lateral ethmoid has in fact been lost several times within the Mormyrinae. The phylogenetic tree of Taverne (1972) depicts additional relationships within the Mormyrinae that are not explicitly supported by character data and many of which differ substantially from relationships recovered in our data analysis.

Only recently has mormyroid systematics begun to benefit from cladistic studies. Agnèse and Bigorne (1992) studied enzyme variability at 16 presumptive loci among 22 populations of 11 species belonging to five mormyrid genera. In one analysis, the presence and absence of alleles were coded as characters in a Wagner parsimony analysis. Phylogenetic conclusions from the unrooted network produced from these data were limited. However, despite the authors' claims that their analysis 'genetically characterizes' the three species of the genus *Marcusenius* used, their network instead stipulates their paraphyly, regardless of the position of the root. Different taxonomic sampling in that study and the present study makes comparisons difficult, although our data also show the genus *Marcusenius* to be an artificial group.

Van der Bank and Kramer (1996) examined the relationships between eight mormyrid species using cladistic methods (among others) to analyze a dataset consisting of allozyme, morphological, behavioral and ecological characters and reported a sister-group relationship between genera *Petrocephalus* and *Pollimyrus*. Some of the character analysis used to create their dataset has since been criticized (Alves-Gomes, 1999; Alves-Gomes and Hopkins, 1997; Lavoué et al., 1999). In our analysis, species of *Petrocephalus* form the sister group to all other mormyrid species, and species of *Pollimyrus* are nested far within this latter group.

Alves-Gomes and Hopkins (1997) produced a phylogeny of seven mormyroid taxa plus two outgroups from maximum parsimony and maximum likelihood analysis of 932 bases from the mitochondrial 12S and 16S genes and presented a model for the evolution of the electric organ in these taxa. This study found *Gymnarchus niloticus* to be the sister taxon to the other included mormyroids, and one species of *Petrocephalus* to be the sister group to the other included mormyrids. Furthermore, the genus *Brienomyrus* was found to be non-monophyletic. These results are consistent with those from our data analysis.

In the most inclusive study to date, Lavoué et al. (1999) sequenced a 588-base-pair portion of the cytochrome *b* gene to infer relationships among 27 mormyroid taxa, including representatives of all nominal genera with the exception of *Isichthys*, *Stomatorhinus* and *Heteromyrus*. Their results supported the monophyly of the Mormyridae (exclusive of the Gymnarchidae) and a sister-group relationship between species of *Petrocephalus* and all remaining mormyrids, consistent with Taverne's classification and the findings of Alves-Gomes and Hopkins (1997). In addition, three clades within the Mormyridae were supported: (1) a clade including species of the genera *Gnathonemus*, *Marcusenius* (minus *Marcusenius conicephalus*), *Hippopotamyrus*, *Genyomyrus* and *Campylomormyrus*; (2) a sister-group relationship between the two monotypic genera *Ivindomyrus* and *Boulengeromyrus*; and (3) a clade consisting of two species of *Brienomyrus* from Gabon and *Paramormyrops gabonensis* to which *Marcusenius conicephalus* formed the sister group. In addition, these authors tentatively placed the genus *Myomyrus* as the sister group to the remaining Mormyrinae. These results are consistent with those we report here.

Our analysis of the data presented here provides particularly strong resolution of basal nodes in the mormyroid tree: the position of *Gymnarchus niloticus* as the sister taxon to all other mormyroids, the position of *Petrocephalus* species as the sister group to all other mormyrids and the position of *Myomyrus* as the sister taxon to the remaining Mormyrinae. This position for *Myomyrus*, weakly supported by data presented by Lavoué et al. (1999), is supported with high confidence in our analysis, although it has never been suggested from morphological evidence. Above *Myomyrus*, there is very strong support for a sister-group relationship between the species of *Mormyrops* and the remaining Mormyrinae. Genera represented by more than a single species and that appear as monophyletic groups in our analysis are *Petrocephalus*, *Mormyrops*, *Mormyrus*, *Pollimyrus*, *Stomatorhinus* and *Campylomormyrus*. Nominal genera that do not appear as monophyletic groups in our analysis are *Marcusenius*, *Hippopotamyrus* and *Brienomyrus*.

Lavoué et al. (1999) found the genus *Marcusenius* to be polyphyletic. In our larger study, both the genera *Marcusenius* and *Hippopotamyrus* are rendered polyphyletic. We think it is likely that these two genera have been defined with characters plesiomorphic for the larger clade, which includes *Genyomyrus*, *Gnathonemus* and *Campylomormyrus*. *Marcusenius conicephalus*, however, is found not belong to this clade, but instead is strongly supported as the sister taxon to a clade consisting of *Brienomyrus* species from Gabon and *Paramormyrops gabonensis*, also from Gabon. This result was also reported in Lavoué et al. (1999). To explain it, Lavoué et al. (1999) suggested that a disjunction between the mitochondrial phylogeny and the true species phylogeny could have resulted from hybridization and introgression of a foreign mitochondrial genome into an ancestor of *M. conicephalus*. However, additional support for this relationship from the independent nuclear RAG2 locus allows us to reject this hypothesis. Closer scrutiny of morphological characters in *Marcusenius conicephalus* is needed.

Another strong, but non-intuitive, result of this study, not reported elsewhere, is the sister-group relationship between *Brienomyrus brachyistius* and *Isichthys henryi*. While dissimilar in appearance, these taxa share nearly identical distributions throughout the coastal drainages of the Upper and Lower Guinea ichthyofaunal provinces of West and Central Africa (type 7 distribution; see Lévêque, 1997). A third species, unavailable for this study, but morphologically similar to *Brienomyrus brachyistius* and sharing this distribution, is *Brienomyrus longianalis*. This species probably represents a third member of this clade.

Lavoué et al. (1999) reported a sister-group relationship between *Ivindomyrus opdenboschi* and *Pollimyrus marchei*, both species from the Ivindo River of Gabon. We recovered identical sequences for all gene fragments from our specimen of *Ivindomyrus opdenboschi* and specimens tentatively identified as *Pollimyrus marchei*. Because of uncertainty in specimen identification, we decided to exclude all specimens of *Pollimyrus marchei* from this study.

Taverne (1971b) erected the genus *Brienomyrus* and designated *Marcusenius brachyistius* Gill 1863 the type

species of the genus. Nominal species of this genus do not form a clade on our tree. *Brienomyrus niger*, which Taverne (1971a) placed into the separate subgenus *Brevimyrus*, appears at the base of the *Marcusenius*/*Hippopotamyrus*/*Gnathonemus*/*Campylomormyrus* clade along with *Hippopotamyrus pictus* and *Hyperopisus bebe*. A third clade of nominal *Brienomyrus* species appears as the sister group to *Marcusenius conicephalus* in our study, as noted above. *Paramormyrops gabonensis* is nested within these species of *Brienomyrus*. Relationships among these and additional taxa belonging to this clade are the subject of additional study by the authors. All available data point to the Lower Guinea ichthyofaunal province and, in particular, the Ogooué River Basin as the center of diversity for this group. (Although not included here, other putative members of this clade occur in the Congo River Basin.) Two successive outgroups to this clade, *Marcusenius conicephalus* and the *Boulengeromyrus knoepffleri*/*Ivindomyrus opdenboschi* sister pair, are endemic to the Ivindo River, an Ogooué tributary (and to the Ntem River, a separate coastal drainage whose headwaters co-mingle with those of the Ivindo). We take this combination of phylogenetic and distributional data to be evidence that this region is, in addition, the center of origin for the *Brienomyrus*/*Paramormyrops* clade.

The results of our phylogenetic analysis of molecular data demonstrate a need for taxonomic revisionary work and renewed study of morphological characters within mormyroids with the aim of establishing genera that reflect natural groups. In addition, if the novel relationships within these fishes suggested here and by Lavoué et al. (1999) are accurate, these should be supported by morphological, as well as molecular, synapomorphies.

Electric organ evolution

Our tree-based hypothesis (Fig. 7) suggests that penetrating stalked electrocytes evolved once early in the history of modern mormyrids and that the occurrence of electric organs with non-penetrating electrocytes within the Mormyrinae is the result of multiple independent reversals to the ancestral condition. To confirm that penetrating stalks have only evolved once in these fishes would require a more detailed analysis of phylogenetic relationships within genera that contain both species with penetrating and species with non-penetrating stalked electrocytes, such as *Brienomyrus*, *Marcusenius*, *Mormyrops* and *Campylomormyrus*. To date, our analysis has sampled too few species from each of these large clades to do so.

Our phylogenetic tree does allow us to formulate a likely scenario for the process of electric organ evolution in mormyrids, although we may only speculate on the adaptive significance of penetrating stalks or the reasons for the multiple independent reversals to non-penetrating stalked electrocytes.

If we assume that the first mormyrids used their electric organ discharges both for communication and for electrolocation, we can imagine three important advantages for penetrating stalks over non-penetrating stalks. First, fish with penetrating stalks might be very effective in reducing the direct current (DC) components of their electric organ discharges. Bennett (1971) and Bennett and Grundfest (1961) and others have noted that fish with penetrating stalks all produce an initial head-negative phase in their discharges. This pre-pulse might facilitate balancing the positive and negative phases of the rest of the electric discharge, thereby eliminating undesirable DC components to the EOD waveform. By so doing, a fish would be able to generate an electrolocation signal that would not jam its own DC-sensitive ampullary receptors, which are used for passive sensing of weak low-frequency signals from external sources such as prey or predators. Reducing the DC component of the EOD would also reduce its electrical conspicuousness to catfish predators, which also carry DC-sensitive ampullary electroreceptors and might use this component of the discharge as a homing beacon for prey.

Second, fish with penetrating stalks might be able to produce EOD waveforms with higher spectral frequencies than fish without them. The penetrating stalk should extend the bandwidth of the spectrum of the EOD waveform to higher frequencies by adding an extra modulation into the waveform without significantly increasing the duration of the overall pulse. When combined with a tuned electroreceptor, such as the Knollenorgan, acting as a matched filter (see Bass and Hopkins, 1984; Hopkins, 1983), these fish might make use of the more private unused high-frequency electrical bandwidth for intraspecific communication, especially when several species of mormyrids are already in sympatry. This would be an advantage for extending the distance of electrical communication in the presence of jamming EODs from heterospecifics, because it would reduce the noise in the signal channel.

Third, fish with penetrating stalks might be able to produce complex EOD waveforms that could serve in species recognition and reproductive isolation. Complex EOD waveforms might be especially useful when several similarly discharging species occur in sympatry.

There must also have been reasons why selection favored reversal to non-penetrating stalked electrocytes in certain clades. We have noticed that a majority of the fish with EODs of very long duration (over 2 ms in duration) tend to have non-penetrating stalked electrocytes. Aside from the ancestral condition seen in *Petrocephalus*, in which all the known EODs are short in duration and rather simple in waveform, the longest-duration EODs reported for the more derived mormyroids are from *Paramormyrops gabonensis* (>10 ms), males of *Isichthys henryi* (>4 ms), males of *Mormyrus rume* (>20 ms) and *Campylomormyrus numenius* (>10 ms), all species with non-penetrating electrocytes. In clades of mormyrids in which penetrating stalked electrocytes are ubiquitous, a reversal to non-penetrating stalks may have allowed the evolution of long-duration EODs that might have been impossible with penetrating stalks.

Indeed, long-duration EODs may arise by the forces of sexual selection under the influence of female choice, as has been argued by Hopkins et al. (1990) for South American pulse gymnotiform fishes (Hopkins, 1999b). Since long-duration EODs may be more costly to produce compared with shorter EODs of the same amplitude, females may select mates that are capable of generating these costly discharges because they are reliable and ‘honest’ indicators of the overall health and quality

of a potential mate. Thus, while penetrating stalks might have pushed the signal bandwidth to higher frequencies, non-penetrating stalks may allow the signal bandwidth to relax to lower frequencies, especially if the fish had already evolved the molecular machinery necessary both to slow the duration of the action potentials and to modulate the duration of the EOD seasonally under the influence of steroid hormones, as have many of the genera of mormyrids apart from *Petrocephalus* (for a review, see Bass, 1986a). The forces of sexual selection, operating on overall pulse duration, and the requirements for unique EOD waveforms for species recognition may have been factors in the reversal of the electric organ from penetrating stalked to non-penetrating stalked electrocytes in several of the more speciose clades of mormyroids.

This research was supported in part by grants to C.D.H. from the National Geographic Society (5801-96), the NSF (INT-9605176) and the NIMH (Grant MH37972). We thank Garry Harned for help with histology, Melanie Stiassny for helping fund J. Sullivan's collecting trip to the Central African Republic. Paul Posso, IRET, Jean-Daniel Mbega and Jean Hervé, Mve IRAF, provided logistic help in Gabon. Emanuel Vreven, African Museum, Belgium, helped with field work. S.L thanks the Museum National d'Histoire Naturelle for support for field work and a travel grant to Ithaca, New York. J. Snoeks provided mormyrid specimens from Lake Malawi. We gratefully acknowledge the Cornell University Evolutionary Genetics Core Facility for use of facilities and we thank K. Zamudio and G. Harned for comments on our manuscript.

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