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Phylogenetic Relationships of Mormyrid Electric Fishes (Mormyridae; Teleostei) Inferred from Cytochrome *b* Sequences

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The Mormyridae are African osteoglossomorph freshwater fishes of great interest because of their electric organs. They have become an important model in studies of electrophysiology and behavior but their phylogenetic relationships are poorly known. Phylogenetic relationships among mormyrids were determined by comparing cytochrome *b* sequences (588 bp) of 27 species belonging to 15 genera. Results showed that the *Petrocephalus* species (subfamily Petrocephalinae) are the sister-group of all other mormyrids (subfamily Mormyrinae). The monophyly of the Mormyrinae was supported, as well as three original intra-Mormyrinae clades. Three genera, *Marcusenius, Pollimyrus,* and *Brienomyrus,* were found to be polyphyletic with high support. Some of these polyphylies are tentatively explained. The results confirmed that the lateral ethmoid bone was lost several times within the Mormyrinae. These findings emphasize the necessity of systematic studies and taxonomic revision of the Mormyridae. The tree obtained from the mitochondrial data showed a single rise of each electrocyte type except for electrocyte with penetrating stalk ("Pa"). Constraining the single occurrence of electrocyte type Pa did not require an excessive number of extra steps (1.86%).

Key Words: **Mormyridae; electric fish; phylogeny; cytochrome *b;* electric organ.**

INTRODUCTION

The largest group of freshwater electrogenic fishes is the order Mormyriformes, with about 200 species (Gosse, 1984; Nelson, 1994; Boden *et al.,* 1997). New species are regularly described (Roberts, 1989; Bigorne and Paugy, 1990, 1991; Boden *et al.,* 1997). All these species are African endemics and are widely distributed in the freshwater (especially riverine) habitats of the continent except in the Cape and Maghrebian regions. The mormyrids reach their highest diversity in the Congolese (previously Zairian) ichthyofaunal province (Roberts, 1975) (Central Africa) with more than 100 species, where they account for 16.2% of the total fish species (Teugels and Guéguan, 1994). In some places, the mormyrids are the most abundant fishes, making up over 65% of the fish biomass (Petr, 1968). One of the most remarkable particularities in mormyrids is the presence of four electric organs (EOs), located in the caudal peduncle (Bennett, 1971), which enable them to emit weak electric discharges. Associated with electroreceptive structure, these fishes use electric discharge in object location (electrolocation) and social communication (Hopkins, 1986). These weak electric discharges are often species specific and may be useful as taxonomic characters (Hopkins, 1981; Crawford and Hopkins, 1989; Roberts, 1989; Bigorne and Paugy, 1991). Despite the great interest in mormyrid electrophysiology and behavior (reviews in Bullock and Heiligenberg, 1986; and Moller, 1995), the phylogenetic relationships within mormyrids is still poorly known.

The order Mormyriformes includes two families, the Mormyridae with 18 genera (Gosse, 1984) and the Gymnarchidae (a monospecific family), which are easily distinguishable by anatomical, in particular the lack of caudal and ventral fins for the Gymnarchidae (Taverne, 1972; Nelson, 1994), and electrophysiological characters (review in Kawasaki, 1993). The classification of Mormyridae has been successively studied by Boulenger (1898), Pappenheim (1906), and Myers (1960). The most recent classification of Mormyridae based on osteological characters (Taverne, 1968a,b, 1969, 1971a,b, 1972) recognizes two subfamilies: the Petrocephalinae (1 genus, *Petrocephalus*) and the Mormyrinae. Taverne (1972) made hypotheses on the relationships among the 16 genera of Mormyrinae known at that time but the osteological characters used present a high level of homoplasy, and most relationships are not well resolved or supported by synapomorphies. The difficulties in assessing phylogenetic relationships on the basis of osteological comparisons led us to address these questions using molecular data.

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Recently, Agnèse and Bigorne (1992) examined genetic variability at 16 protein-coding loci for eight species of West African mormyrids from five genera (*Hippopotamyrus, Marcusenius, Pollimyrus, Mormyrops,* and *Petrocephalus*). Because these authors didn't use any outgroup, phylogenetic implications are limited. However, their analysis revealed a large genetic difference between the *Petrocephalus* species and the other taxa. Van der Bank and Kramer (1996) analyzed allozyme data combined with morphological, behavioral, and ecological characters from five genera (*Hippopotamyrus, Marcusenius, Mormyrus, Petrocephalus,* and *Pollimyrus*) and used *Gymnarchus niloticus* Cuvier, 1829 as the outgroup. The resulting tree suggested that *Petrocephalus* and *Pollimyrus* are sister-groups and that *Hippopotamyrus* is paraphyletic. Alves-Gomes and Hopkins (1997) studied phylogenetic relationships between four mormyrid genera (*Marcusenius, Petrocephalus, Gnathonemus,* and *Brienomyrus*) and *Gymnarchus niloticus* with special attention to the genus *Brienomyrus* (with six species) using mitochondrial ribosomal DNA partial sequences (12S and 16S). *Gymnarchus niloticus* and mormyrids were found to be sister-groups. Within mormyrids, the genus *Petrocephalus* was the sister-group of the Mormyrinae. These authors also proposed the paraphyly of the genus *Brienomyrus* and the first hypothesis of evolution of electric organs based on a phylogeny.

There is currently no phylogenetic study of the mormyrids which includes all genera described. In this study we examined molecular phylogenetic relationships (using partial cytochrome *b* gene sequences) among 27 species of mormyrids representing all genera and subgenera recognized in Taverne (1972) and Gosse (1984), except *Isichthys, Stomatorhinus,* and *Heteromormyrus* (only one specimen is known for the latter genus (Taverne, 1972) and was lost during the last World War). The fossil record of osteoglossomorphs is well documented but there are few fossil mormyrids. Guo-Qing and Wilson (1996) estimated the emergence of the mormyriforms lineage at the early Oligocene (33 MA). The cytochrome *b* gene is an appropriate phylogenetic marker in fish at this maximum divergence time (Chen *et al.,* 1998). Using these phylogenetic relationships as a framework, we discuss the evolution of some osteological characters and electric organs.

MATERIAL AND METHODS

Twenty-nine specimens representing 27 species from the family Mormyridae and two outgroups, *Gymnarchus niloticus* (Gymnarchidae) and *Heterotis niloticus* (Cuvier, 1829) (Osteoglossidae), were studied (Table 1). Specimens of *Myomyrus pharao* Poll and Taverne, 1967 and *Genyomyrus donnyi* Boulenger, 1898 came from the ichthyological collections of the Royal Museum of Central Africa (MRAC) in Tervuren (Belgium), where they were preserved in alcohol after fixation in formaldehyde. *Campylomormyrus tamandua* (Günther, 1864) and *Gnathonemus petersii* (Günther, 1862) came from aquarium importers. All other specimens were collected in the field in Mali (1994) by R. Bigorne; in Ivory Coast (1996) by G. Teugels, G. Gourene, S. Lavoué, and S. Bariga; in Gabon (1997) by S. Lavoué; and in Ghana (1997) by Y. Fermon; and all specimens were preserved in alcohol. *Petrocephalus bovei* (Cuvier and Valenciennes, 1846) is a species complex rather than a valid species (Bigorne and Paugy, 1991; Bigorne *et al.,* in prep); for this reason, we studied two specimens from two origins. Because the taxonomy of the genus *Brienomyrus* is particularly uncertain, especially for the Gabon species, the identification of two specimens from Gabon was only to the level of genus, and they are referred as *B. sp1* and *B. sp2*. Most of the specimens used in this study are deposited at the Museum National d'Histoire Naturelle (MNHN) and the Royal Museum of Central Africa (MRAC) (Table 1).

Total DNA was extracted from tissues of muscles (preserved in 70% ethanol) using the procedure of Winnepenninckx *et al.* (1993). DNA was extracted from formaldehyde-fixed tissues (for *Myomyrus pharao* and *Genyomyrus donnyi*) following the protocol of Vachot and Monnerot (1996), modified by Lavoué and Agnèse (1998). DNA was amplified using the polymerase chain reaction (PCR) from the total genomic DNA extracts (300 to 1000 ng). The primers used were H15930 (CTT-CGA-TCT-TCG-RTT-TAC-AAG), L15047 (TAC-CTA-TAC-AAA-GAA-ACM-TGA-AA), L195 (GAA-ACC-GGM-TCA-AAC-AAC-CC), and cb3'L125 (TTC-TTY-GCC-TTC-CAC-TTC-TC). The temperature profile for 35 cycles of the amplification procedure was 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C followed by 4 min at 72°C.

Double-stranded PCR products were sequenced either after a cloning step or directly following availability. In the first case, the PCR products were ligated into a plasmid cloning vector and grown in *Escherichia coli* competent cells using the "pCR-Script SK(+) kit" (Stratagène). DNA was isolated from the cells by a miniprep protocol (Sambrook *et al.,* 1989). The double-strand insert was sequenced by the dideoxy chain termination method using the "T7 Sequencing Kit" (Pharmacia Biotech) and ^{35}S labeling. For each individual at least two independent clones were sequenced. In the second case direct sequencing was employed; 5 µl of 50 µl PCR products was used to carry out a sequencing reaction following the "Thermo Sequenase Cycle Sequencing Kit" (Amersham). Nonincorporated primers and nucleotides were initially digested enzymatically by 1 µl shrimp alkaline phosphatase and 1 µl Exonuclease I. Sequencing was performed with numbers of thermocycles containing denaturation, annealing, and extension steps at 95°C/30 s, 53°C/60 s, and 72°C/60 s for 30 cycles and 72°C for 10 min. Sequencing

TABLE 1

Specimens Analyzed in This Study with Their Geographic Origin (River and Country), Number Voucher, and Electric Organ (EO) Type

Species	Origin	Voucher	EO type
Family: Mormyridae			
Subfamily: Mormyrinae			
Marcusenius furcidens (Pellegrin, 1920)	[e]Bia River, Ivory Coast	MRAC 96-57-P-1	Pa[1]
Marcusenius senegalensis (Steindachner, 1870)	[b]Niger River, Mali	MNHN 1999-273	Pa[1]
Marcusenius ussheri (Günther, 1867)	[e]Bia River, Ivory Coast	MRAC 96-57-P-2	Pa[1]
Marcusenius conicephalus (Taverne *et al.*, 1976)	[a]Ivindo River, Gabon	Absent	Pa[2]
Marcusenius conicephalus (Taverne *et al.*, 1976)	[a]Ivindo River, Gabon	MNHN 1999-283	Pa[2]
Marcusenius moorii (Günther, 1867)	[a]Ivindo River, Gabon	MRAC 97-51-P-1	NPp[1]
Brienomyrus (Brevimyrus) niger (Günther, 1866)	[b]Niger River, Mali	MNHN 1999-280	DPp[1]
Brienomyrus (Brienomyrus) brachyistius (Gill, 1862)	[c]Agnébi River, Ivory Coast	MRAC not registered	?
Brienomyrus (Brienomyrus) sp. 1	[a]Ivindo River, Gabon	MNHN 1999-281	?
Brienomyrus (Brienomyrus) sp. 2	[a]Ivindo River, Gabon	MNHN 1999-282	?
Pollimyrus petricolus (Daget, 1954)	[b]Niger River, Mali	MNHN 1999-274	DPNP[1]
Pollimyrus isidori (Cuvier and Valenciennes, 1846)	[c]Agnébi River, Ivory Coast	MRAC not registered	DPNP[2]
Pollimyrus marchei (Sauvage, 1878)	[a]Ivindo River, Gabon	MRAC 97-51-P-4	NPp[1]
Campylomormyrus tamandua (Günther, 1864)	Aquarium import	Absent	Pa[2]
Gnathonemus petersii (Günther, 1862)	Aquarium import	Absent	Pa[2]
Mormyrops (Mormyrops) anguilloides (Linné, 1764)	[f]Bandama River, Ivory Coast	MRAC 96-57-P-4	Pa and Pp[1]
Mormyrops (Oxymormyrus) zanclirostris (Günther, 1867)	[a]Ivindo River, Gabon	Absent	Pp[2]
Mormyrus rume Cuvier and Valenciennes, 1846	[b]Niger River, Mali	MNHN 1999-275	NPp[2]
Mormyrus subundulatus Roberts, 1989	[f]Bandama River, Ivory Coast	MNHN not registered	NPp[1]
Hippopotamyrus psittacus (Boulenger, 1897)	[b]Niger River, Mali	MNHN 1999-276	Pa[1]
Boulengeromyrus knoepffleri Taverne and Géry, 1968	[a]Ivindo River, Gabon	Photo	NPp[2]
Paramormyrops gabonensis Taverne *et al.*, 1977	[a]Ivindo River, Gabon	MRAC 97-51-P-3	NPp[2]
Hyperopisus bebe (Lacépède, 1803)	[b]Niger River, Mali	MNHN 1999-277	Pa[1]
Ivindomyrus opdenboschi Taverne and Géry, 1975	[a]Ivindo River, Gabon	MRAC 97-51-P-9	NPp[2]
Myomyrus pharao Poll and Taverne, 1967	[d]Congo River, Congo	MRAC 82-25-P32-45	?
Genyomyrus donnyi Boulenger, 1898	[d]Congo River, Congo	MRAC 83-31-P39-40	?
Subfamily: Petrocephalinae			
Petrocephalus bovei (Cuvier and Valenciennes, 1846)	[f]Bia River, Ivory Coast	MRAC 96-57-P-5	NPp[1]
Petrocephalus bovei (Cuvier and Valenciennes, 1846)	[b]Niger River, Mali	MNHN 1999-278	NPp[1]
Petrocephalus soudanensis Bigorne and Paugy, 1990	[e]Volta Basin, Ghana	MNHN 1999-279	NPp[1]
Family: Gymnarchidae			
Gymnarchus niloticus Cuvier, 1829	[f]Niger River, Mali	Absent	S[1]
Family: Osteoglossidae			
Heterotis niloticus (Cuvier, 1829)	[f]Bandama River, Ivory Coast	MNHN not registered	Absent

Locality: [a]Mayibout, [b]Batamani, [c]Amebe, [d]Kisangani, [e]Wegbe (Dayi river), [f]indeterminate.
Literature source: [1]Alves Gomes and Hopkins (1997), [2]Bass (1986).

primers were initially radiolabeled with ^{33}P by kination.

DNA sequences have been deposited in GenBank under Accession Nos. AF095290 to AF095316 and AF095710 to AF095712. Sequence storage and alignment were performed using the program MUST (Philippe, 1993). Absolute mutational saturation was calculated for each codon position and for transitions and transversions separately by plotting the pairwise number of observed sequence differences (Y axis) against the corresponding pairwise number of inferred substitutions (X axis) in the most parsimonious tree from PAUP 3.1.1. (Swofford, 1993).

Phylogenetic relationships among Mormyridae were estimated by maximum-parsimony (MP) with PAUP 3.1.1. (Swofford, 1993). Neighbor-joining (NJ) (Saitou and Nei, 1987) using MUST (Philippe, 1993) and maximum-likelihood (ML) using PUZZLE 4.0 (Strimmer and von Haeseler, 1996) analyses were also conducted. Heuristic searches of the MP tree were performed with two weighting schemes: either all substitutions and positions were equally weighted or transitions at third position of codon were removed. Bootstrap proportions (BP) were calculated using PAUP through heuristic searches and 100 iterations. In parallel to this statistical evaluation of robustness of branches, an estimation of the Bremer support index (BSI) for each branch was performed (Bremer, 1994). A measure of the phylogenetic signal within these data was estimated by the skewness (g1) of tree-length distributions by generating 10,000 random trees (Hillis and Huelsenbeck, 1992) using PAUP 3.1.1. For NJ analyses, Kimura two-parameter distance correction (Kimura, 1980) was used. Statistical confidence of NJ evolutionary trees was assessed using bootstrapping (1000 iterations). For ML analyses, the Hasegawa–Kishino–Yano (1985) model, which incorporates observed bases frequencies and rates of transitions and transversions, was used. Data on the mormyrid electrocyte stalk complex was taken from the literature and not examined directly in this study. The evolution of the stalk complex of electrocytes was examined by mapping character states onto our phylogenetic hypothesis using MacClade (Maddison and Maddison, 1992). To evaluate the cost of a single rise of the electrocyte type Pa required for the molecular data, constrained weighted heuristic searches were used in PAUP 3.1.1.

RESULTS

Sequence Analysis

Over the 588-bp segment, 307 (52.2%) nucleotide positions were variable. Most variable sites (188, i.e., 61.2% of the total variable sites) were found at the third codon positions, 80 (26%) at the first codon positions, and 39 (12.8%) at the second codon positions. Only 495 bp were amplified and sequenced for *Myomyrus pharao, Genyomyrus donnyi,* and *Brienomyrus brachyistius* (Gill, 1866). Sequences obtained from two specimens of *Marcusenius conicephalus* (Taverne *et al.,* 1976) were found to be identical. Uncorrected sequence divergences (p distance) among different genera of Mormyridae ranged from 0.34 to 17.7%. The sequence divergence between the Petrocephalinae and the Mormyrinae ranged from 13.8 to 19.4%. As expected, the highest sequence divergence was observed between the outgroups (*Heterotis niloticus* and *Gymnarchus niloticus*) and the Mormyridae (20.0 to 26.9%).

The results of the saturation analysis are presented in Fig. 1. Plotting inferred transitions and transversions against pairwise observed transitions and transversions indicates a relatively linear relationship at the first and second positions of the codons. Transversions at the third positions are not saturated with superimposed substitutions in pairwise comparisons, except for *Heterotis niloticus* vs Mormyridae and *Gymnarchus niloticus* vs Mormyridae. Transitions at the third positions are strongly saturated with superimposed substitutions in all comparisons. The best trade-off between degree of saturation and loss of information is to use all types of substitutions at the first and second positions and only transversions at the third positions.

Phylogenetic Analysis

Over the 307 variable sites, 233 (39.6%) were informative for parsimony analysis (i.e., showing at least two kinds of nucleotides, each present at least twice). Of those phylogenetically informative sites, 168 (i.e., 72.1% of all informative sites) were at the third codon position, 51 (21.9%) were at the first codon position, and 14 (6%) were at the second codon position.

When the transitions at the third codon positions were excluded from the parsimony analysis, 8 equally parsimonious trees were found. Each tree was 528 steps long with a consistency index of 0.481 and a retention index of 0.590. A strict consensus of these 8 trees is presented in Fig. 2 and has three polytomies. The g1 value (−0.84) indicated the presence of a significant phylogenetic signal ($P = 0.01$). When all substitutions were considered, 39 equally parsimonious trees were found. Each tree was 1046 steps long with a consistency index of 0.460 and a retention index of 0.476. The strict consensus tree of these 39 trees also showed three polytomies (data not shown). The g1 value (−0.88) indicated the presence of significant phylogenetic signal ($P = 0.01$).

Results obtained by these two different analyses are similar and both support the monophyly of the family Mormyridae (BP ≥ 93% and BSI > 4). Within the Mormyridae, the genus *Petrocephalus* (Petrocephalinae) and the remaining genera of mormyrids (Mormyrinae) are sister-groups (BP ≥ 84% and BSI = 3). Three genera, *Pollimyrus, Marcusenius,* and *Brienomyrus,* are clearly polyphyletic. All analyses suggested the pres-

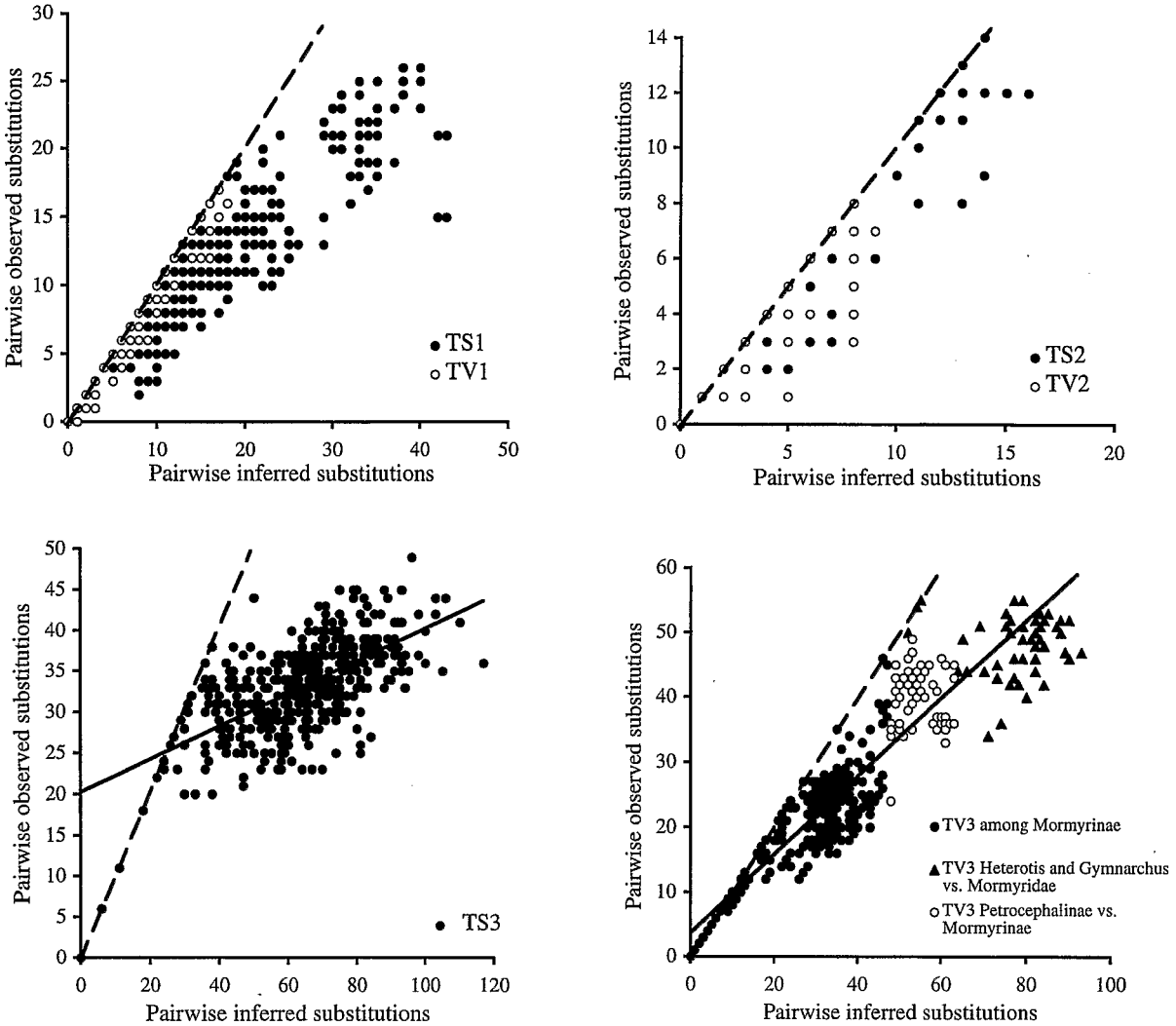

FIG. 1. Mutational saturation analysis of the sequence data set using COMP_MAT of MUST and PAUP. The dashed line represents the theoretical situation for which no saturation is observed (numbers of observed changes equal numbers of inferred changes). For TS3 and TV3, the continuous line represents the linear regression used to evaluate the level of saturation. TS, transitions; TV, transversions.

ence of three monophyletic groups within the Mormyrinae: (1) a clade grouping the species of the genera *Gnathonemus, Marcusenius* (without *M. conicephalus*), *Hippopotamyrus, Genyomyrus,* and *Campylomormyrus* (BP $\geq$ 71% and BSI $\geq$ 2); (2) *Ivindomyrus opdenboschi* (Taverne and Géry, 1975), *Boulengeromyrus knoepffleri* (Taverne and Géry, 1968), and *Pollimyrus marchei* (Sauvage, 1878) (BP = 100% and BSI > 4); and (3) a heterogeneous clade consisting of *Marcusenius conicephalus,* the two species of *Brienomyrus* from Gabon, and *Paramormyrops gabonensis* (Taverne *et al.,* 1977) (BP $\geq$ 66% and BSI = 2). Whatever the weighting scheme used, in Mormyrinae, *Myomyrus* represents the most basal lineage, although its position is never supported by high BP or BSI.

The tree (Fig. 2) presents a remarkable asymmetry in branch lengths. The basal branches are considerably shorter than those of the higher part of the tree. This is not due to a rooting artifact because Alves-Gomes and Hopkins (1997) found the same ingroup rooting point from another gene. The nodes discussed above were also found by the NJ and ML analyses with high statistical support (data not shown).

Saturation plots show a mutational saturation in transversions at the third position of the codon in *Gymnarchus niloticus* and *Heterotis niloticus.* To test the hypothesis of the effect of this saturation on intramormyrid phylogeny, *Gymnarchus niloticus* and *Heterotis niloticus* were eliminated from the analysis and the three species of *Petrocephalus* (Petrocephalinae) were chosen as the outgroups to root the Mormyrinae phylogeny. Removing these taxa apparently eliminates the observed saturation at the third position transversions (Fig. 1). Whatever the weighting scheme used (all substitutions or without third codon transitions), a topology similar to the one previously obtained was

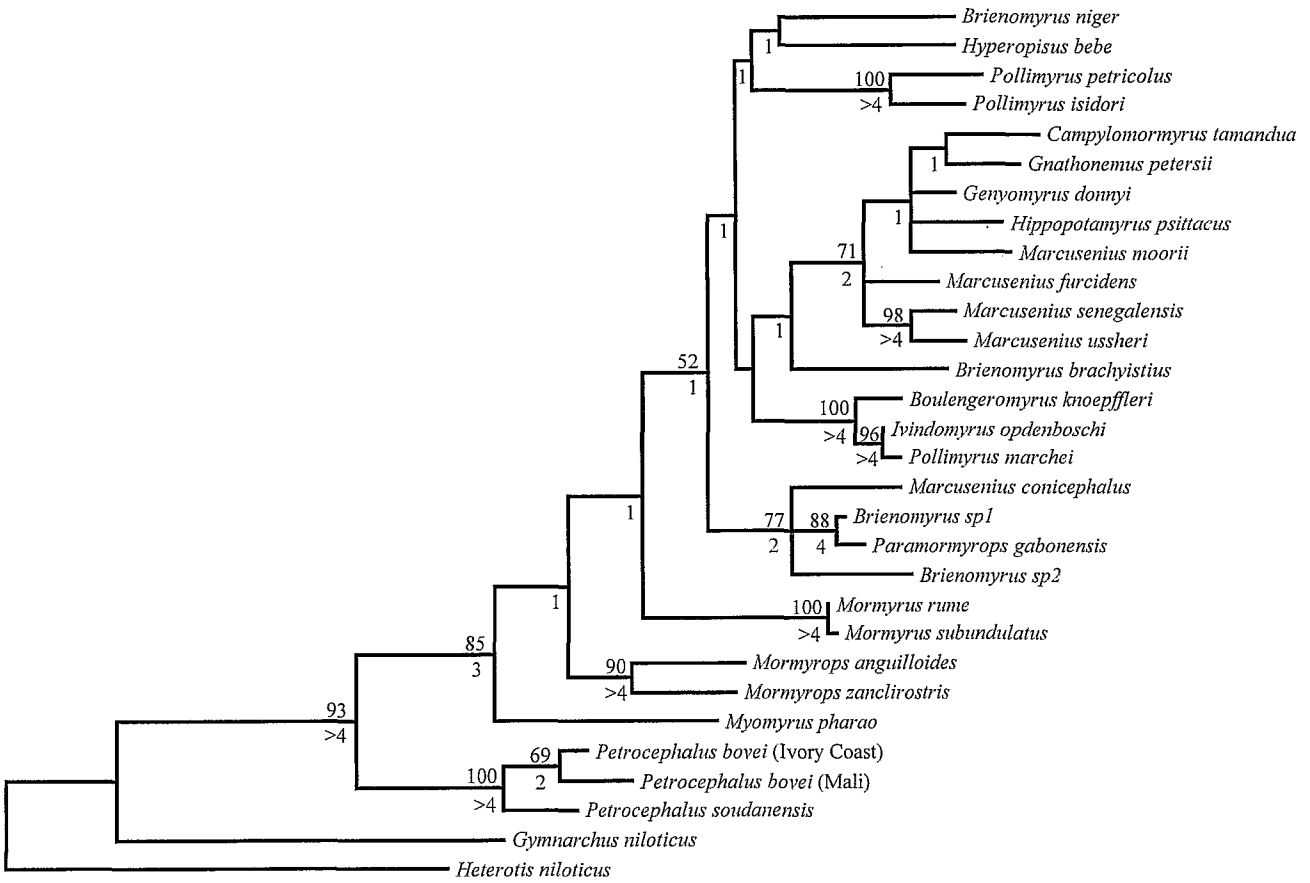

FIG. 2. Strict consensus tree of the eight equiparsimonious trees obtained from a heuristic search (PAUP 3.1.1.). Transitions at the third positions are excluded from the analysis (see Fig. 1). Length of each tree is 528 steps; C.I. = 0.481; R.I. = 0.59. The numbers above branches refer to the bootstrap proportions provided when above 50%. The numbers below branches refer to the Bremer support index. The length of this strict consensus with branch length (shown under ACCTRAN optimization) is 533 steps.

observed (Fig. 2). In this case, the same clades were supported by high BP or BSI.

DISCUSSION

Monophyly of Mormyridae and Sister-Group Relationships between Petrocephalinae and Mormyrinae

The monophyly of the Mormyridae without Gymnarchidae, contrary to some expressed doubts (Nelson, 1994), is clearly supported by our molecular data. Our results also showed that the family Mormyridae is composed of two sister-groups: species of the genus *Petrocephalus* and all other species. This is in agreement with the traditional nomenclature of Taverne (1969, 1972), who described two subfamilies (Petrocephalinae and Mormyrinae), and is also congruent with a partial phylogeny obtained by Alves-Gomes and Hopkins (1997).

However, Van der Bank and Kramer (1996) suggested that *Petrocephalus catostoma* (Günther, 1866) and *Pollimyrus castelnaui* (Boulenger, 1911) were sister-groups and provided characters supporting these relationships: (1) two allozyme products of identical mobility, (2) triphasic electric organ discharge (triphasic EOD) with head-negative main phase, (3) morphological similarity, and (4) food preferences for microcrustacea. We offer the following alternative interpretation of these characters. The relationships between *Pollimyrus* and *Petrocephalus* based on two uniquely shared isozymes may be the result of a convergent electrophoretic mobility gained twice, given the relationships supported by this study and by that of Agnèse and Bigorne (1992). The triphasic EOD with head-negative main phase seen in the two taxa is, first, atypical for the genus *Petrocephalus* and, second, known in several other mormyrid genera in addition to the two considered (Bass, 1986; Alves-Gomes and Hopkins, 1997). The external overall similarity between two taxa is not a proof of their close relationship. In the case of *Petrocephalus* and *Pollimyrus* the similarity is only superficial and the osteology of the two genera is very distinct (Taverne, 1969, 1971b). Last, many genera of Mormyridae share the same food habits and no attempt has

been made to establish the generality of these particular preferences within these two genera. Thus, the characters proposed by Van der Bank and Kramer (1996) to support a sister-group relationship between *Petrocephalus catostoma* and *Pollimyrus castelnaui* are problematic sources of phylogenetic information.

The Lateral Ethmoid Bone

Our results suggested relationships different from those implied in the classification of the subfamily Mormyrinae proposed by Taverne (1972). Under his hypothesis, all of the mormyrid genera without a lateral ethmoid bone are in a single clade (*Mormyrops, Myomyrus, Campylomormyrus, Genyomyrus, Gnathonemus, Boulengeromyrus,* and *Brienomyrus;* plus *Isichthys* and *Stomatorhinus,* which are not represented in this study). Alves-Gomes and Hopkins (1997) contradicted Taverne's view based on molecular evidence and proposed that the lateral ethmoid bone alone may not be a reliable character for inferring phylogenetic relationships among mormyrids. Our molecular data support this second hypothesis and indicate that the loss of the lateral ethmoid bone independently occurred several times in the Mormyrinae.

Polyphylies

As expected, the genus *Brienomyrus* is found to be polyphyletic (Alves-Gomes and Hopkins, 1997). More surprising are the polyphylies of *Pollimyrus* and *Marcusenius.* Such well-supported disagreements between our phylogenetic hypothesis and the classification at the genus level can have two origins: either the current taxonomical position of some species is wrong and requires reexamination or the phylogeny of mitochondrial DNA is different from the species tree (Maddison, 1997). This second hypothesis can have two causes: retention of ancestral polymorphism across past cladogenic events or introgression. If an ancestral species was polymorphic in its mtDNA, phylogenetic analysis of haplotypes of modern species might reveal the order in which the haplotypes originated within the ancestor and not the relationships of the species themselves (Agnèse *et al.,* 1997). The mtDNA of one species could also have been established in another by introgression of the mitochondrial genome without nuclear contamination. This phenomenon has already been observed in other fishes (Dowling *et al.,* 1989; Duvernell and Aspinwal, 1995; Mikai *et al.,* 1997; Gilles *et al.,* 1998).

Our results showed that *Marcusenius conicephalus* mtDNA from Gabon was closer to the mtDNA of the sympatric *Brienomyrus* and *Paramormyrops gabonensis* from Gabon than to the mtDNA observed in the four other *Marcusenius* species. To eliminate the hypothesis of DNA contamination, *Marcusenius conicephalus* sequences were obtained on two occasions separated by 6 months from different individuals. The genus *Marcusenius* was defined by Taverne (1971b) on the basis of its osteology and is characterized by a unique shape among mormyrids. We think it is possible that in this case the mitochondrial phylogeny differs from the species phylogeny (Doyle, 1992, 1997; Maddison, 1997). It seems more likely that the mtDNA haplotype of *M. conicephalus* is the result of introgressive hybridization than the result of ancestral polymorphism, since this haplotype is very different from those of other *Marcusenius* and is closer to the haplotypes of other species also endemic to the Ivindo River in Gabon and the N'tem River in Cameroon. Phylogenetic analysis of an unlinked nuclear locus would be useful in evaluating this hypothesis.

The genus *Pollimyrus* was also found to be polyphyletic. *Pollimyrus marchei* mtDNA was close to that of *Ivindomyrus opdenboshi* and *Boulengeromyrus knoepffleri* and very different from that of *P. petricolus* and *P. isidori.* Taverne (1971b) did not examine the osteology of *Pollimyrus marchei* and placed this species in the genus *Pollimyrus* without clear justification. The electrocyte structure in *P. marchei* is different from that found in other *Pollimyrus* species. *Pollimyrus marchei* has electrocytes with nonpenetrating stalk (NPp) (Bass, 1986), while *P. petricolus* and *P. isidori* have electric organs with electrocytes with double-penetrating and nonpenetrating stalks (type DPNP) (Bass, 1986; Alves-Gomes and Hopkins, 1997). This structure is complex and scarce in Mormyrinae (only *Stomatorhinus* shares this structure). The stalk structures of NPp and DPNP are very different. Although no osteological or morphological synapomorphy can be demonstrated, *P. marchei* is morphologically very close to *Ivindomyrus opdenboshi* and shares with it the same type of electrocyte (NPp). For these reasons, *P. marchei* probably does not belong in the genus *Pollimyrus.*

Brienomyrus is the third polyphyletic genus. The four species were clustered in three different groups: (1) *B. sp1* and *B. sp2* from Gabon group with *Paramormyrops gabonensis* and *Marcusenius conicephalus,* (2) *B. niger* represents a distinct lineage, and (3) *B. brachyistius* represents a second distinct lineage. These results confirm those of Alves-Gomes and Hopkins (1997). This genus is not morphologically well defined and it appears heterogeneous from the electrophysiological viewpoint (Bigorne, 1990; Alves-Gomes and Hopkins, 1997). These results emphasize the necessity of systematic study and taxonomic revision of *Brienomyrus.*

Original Clade

Our results clearly showed a close relationship among *Gnathonemus, Marcusenius, Campylomormyrus, Hippopotamyrus,* and *Genyomyrus.* This group cannot be characterized by any single uniquely derived character but can be characterized only by an original combination of three derived characters (each possibly found isolated elsewhere in the tree): (1) presence of a well-

developed submental swelling, (2) fusion between the antorbitar bone and the first infraorbitar bone into a unique lacrymal, and (3) electrocytes with penetrating stalks innervated on the anterior side of each cell (type Pa (Bass, 1986), except for *Marcusenius moorii* for which it is NPp, (i.e., electrocytes without penetrating stalk; see below). Type NPp electrocytes in *Marcusenius moorii* (as in *Brienomyrus*), could have arisen possibly through paedomorphosis from a Pa electrocyte (Alves-Gomes and Hopkins, 1997).

The Evolution of Mormyrid Electric Organs

The adult electric organs of the mormyrids have evolved from muscle tissue and are composed of electrocytes. The electrocytes are disk-shaped, multinucleated cells. Each cell has anterior and posterior faces. One face gives rise to a series of finger-like evaginations that fuse into a stalk system that is innervated in a restricted zone by spinal electromotor axons. The electrocyte structure and particularly the stalk system have been studied in great detail by several workers (Bennett and Grundfest, 1961; Szabo, 1961; Bass, 1986; review in Alves-Gomes and Hopkins, 1997). Bass (1986) and Alves-Gomes and Hopkins (1997) recognized five electric organ structures in mormyrids, based on the complexity of the stalk system: (1) nonpenetrating stalk electrocytes innervated on the posterior face (type NPp), (2) penetrating stalk electrocytes innervated on the anterior face (type Pa), (3) inverted penetrating stalk electrocytes innervated on the posterior face (type Pp) (this type is simply the inverted version of the Pa electrocyte; Pp electrocytes are found only in *Mormyrops*), (4) doubly penetrating stalk electrocytes innervated on the posterior face (type DPp), and (5) doubly penetrating and nonpenetrating stalk electrocytes innervated on the posterior face (type DPNP). In Gymnarchidae, the electric organs have the same muscular origin as in Mormyridae, though they present a number of anatomic differences. The electrocytes are stalkless and directly innervated by the spinal electromotor axons on the posterior face (type S). The anatomy of the stalk system has been described for 22 of the 27 species studied here (Table 1).

Based on their partial phylogeny and on electrocyte stalk system anatomy, Alves-Gomes and Hopkins (1997) suggested that primitive electrocyte structure in mormyriforms was stalkless (type S). Because there are only two families in mormyriforms and only *Gymnarchus niloticus* possesses the S organ, the outgroup criterion alone cannot establish with confidence whether the S type is ancestral relative to electrocytes with stalks. More data on the ontogenic development of each structure could be useful in resolving this issue. In Mormyridae, these authors proposed that the type NPp electrocyte is more ancestral than the type Pa electrocyte and suggested that reversions from Pa to NPp may have occurred. They proposed a paedomorphorphic mechanism to explain these reversions.

Tracing the evolution of electrocyte structure types within Mormyridae using MacClade (Maddison and Maddison, 1992) indicates that the ancestor of Mormyridae and Mormyrinae probably had electrocytes with nonpenetrating stalks (NPp). However, this conclusion is weakly supported by our data because the electrocyte type in *Myomyrus* is not known and no good resolution was found for the base of the tree in Mormyrinae. Thus, Pa, DPNP, and DPp organs could be derived from NPp as suggested by Alves-Gomes and Hopkins (1997). Our most parsimonious trees (discarding transitions at the third position) showed several occurrences of the electrocyte type Pa. In the less favorable optimization, Pa (and Pp) appears three times (the case of *Marcusenius conicephalus* being excluded because of possible introgression, see above). However, multiple occurrences of Pa are not supported by robust nodes. Constraining a single occurrence of the electrocyte type Pa does not require many extra steps (10 extra steps, i.e., 1.86%). Our mitochondrial data therefore do not strongly contradict the single rise of the Pa electrocyte type.

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