Original Paper

Brain, Behavior and Evolution

Brain Behav Evol 1997;49:324–351

Molecular Insights into the Phylogeny of Mormyriform Fishes and the Evolution of Their Electric Organs

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Key Words
Mormyriformes
Molecular phylogeny
Electrocyte designs
Electric organ evolution
Electric fish
EOD

Abstract
In this report we generate a partial phylogeny of the mormyriform fishes using mitochondrial DNA sequences from twelve species of mormyriforms belonging to five genera. Electric organs and electric organ discharges are also examined. We have sequenced and aligned 373 bases from the mitochondrial 12S rRNA and 559 bases from the 16s rRNA from fourteen species of the superorder Osteoglossomorpha. Two non-mormyriform genera were used as outgroups. Three phylogenetic methods generated concordant partial phylogenies for these fish. Our analysis focuses on the genus *Brienomyrus,* which is a heterogeneous clade with at least eleven nominal species. Six morphs from Gabon had distinctive EODs but were morphologically 'cryptic' in that they all had the *brachyistius*-like body morphology. DNA analysis fully supports the EOD data that the six morphs represent distinct clades. The group from Gabon is monophyletic, while *B. brachyistius* from West Africa is a separate lineage. *B. niger,* a second distinct lineage, is a sister group to the six species from Gabon. *Petrocephalus* is the sister group of all the genera of the subfamily Mormyrinae so far analyzed, thereby confirming previous osteological results. *Gymnarchus niloticus* is the sister group of the family Mormyridae, also confirming an earlier phylogenetic hypothesis based on morphology. The molecular data adds polarity to electric organ characteristics. Stalkless electrocytes appear to be primitive. *Petrocephalus,* with non-penetrating stalked electrocytes innervated on the posterior side, represents an ancestral state for the Mormyridae, while *Marcusenius, Brienomyrus* and *Gnathonemus* with penetrating-stalked electrocytes, represent the apomorphic condition. Two species with doubly-penetrating electrocytes innervated on the posterior side may represent a transitional stage. At least two species of *Brienomyrus* appear to have reverted to non-penetrating stalked electrocytes, possibly through paedomorphosis.

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Introduction

With about 200 nominal species, the mormyriform electric fishes (order Mormyriformes, Osteoglossomorpha) comprise one of the most diverse clades of freshwater fishes from Africa and the largest group of electric fishes known. The mormyriforms have been the subject of widespread neurobiological interest ever since Lissmann [1951] discovered that they produce weak electric discharges. They have been used in behavioral, electrophysiological, anatomical, and developmental studies of the electric organs, electroreceptors, and the central nervous system, but little is known about their phylogenetic history. Most of the systematic work, based on traditional analyses of morphological characters, has not included phylogenetic or cladistic reasoning [Orts, 1967; Taverne, 1972; Daget et al., 1984]. According to Taverne [1972] and Gosse [1984] there are 199 nominal species of mormyriforms belonging to 18 genera. Taverne considers the order Mormyriformes to be composed of the families Gymnarchidae and Mormyridae. While the Gymnarchidae has a single species, *Gymnarchus niloticus,* the family Mormyridae is composed of two subfamilies: the Petrocephalinae (26 species) and the Mormyrinae (165 species). Since Taverne's work there have been several new species added to the group [Gosse, 1984], a number of revisions of genera based on regional data [Lévêque et al., 1990], and a number of studies of comparative biology of the group [Hopkins and Bass, 1981; Bass, 1986a, 1986b].

With the recent interest in the neurobiology and behavior of electric fish there is good reason to examine the phylogeny of this group using alternative data sets such as molecular information. In this paper we attempt a preliminary phylogenetic analysis of several subgroups of mormyriforms using mitochondrial DNA sequences.

Genus Brienomyrus

This paper will focus on the genus *Brienomyrus* (formerly *Marcusenius*), a morphologically heterogeneous clade with eleven known species. The genus *Brienomyrus* was established in 1971 by Taverne [1971a], but the literature on this genus has been particularly confusing, especially for fish from the forests of Central West Africa where one finds the greatest number of species. In previous publications on the mormyrids of Gabon, Hopkins and colleagues have shown that it is possible to identify live *Brienomyrus* on the basis of electric organ discharge (EOD) characteristics [Hopkins, 1981, Bass et al., 1986; Hopkins and Westby, 1986; Friedman and Hopkins, 1996]. Four morphologically similar species with distinguishable EODs, thought to be related to *Brienomyrus brachyistius* [Gill, 1862], were given temporary names such as *B. brachyistius* 'biphasic' (bp), *B. brachyistius* 'long biphasic' (lbp), *B. brachyistius* 'triphasic' (tp) and *B. brachyistius* 'monophasic' (mp) [Hopkins, 1980; Hopkins and Bass, 1981; Bass and Hopkins, 1983; Bass, 1986a; Bass et al., 1986]. Since the electric discharges in these mormyriforms are used for social communication and play a vital role in species recognition [Hopkins and Bass, 1981; Hopkins, 1986], these EOD types appear to define distinct clades which are reproductively isolated. Three of these species are undescribed, while one (identified as *B. brachyistius monophasic* by Hopkins [1980]) has been reidentified as *Brienomyrus batesii* [based on Boulenger, 1906]. Recent studies have revealed morphological, behavioral, and ecological differences between the EOD-types that confirm the species-level status of these groups; species descriptions are currently underway. In the past, without EODs, authors may have confused these species with other forms [see Trewavas, 1974; Bigorne 1990].

Taverne designated *Brienomyrus brachyistius* [Gill, 1862] as the type species for *Brienomyrus.* He also listed five other species for the genus: *B. niger* [Günther, 1866]; *B. sphecodes* [Sauvage, 1878]; *B. longianalis* [Boulenger, 1901]; *B. adustus* [Fowler, 1936], and *B. jacksoni* [Poll, 1967]. Taverne [1972] also defined two sub-genera, *Brienomyrus (Brienomyrus)* and *Brienomyrus (Brevimyrus),* placing *Brienomyrus (Brevimyrus) niger* into one sub-genus and the remainder into *Brienomyrus (Brienomyrus).* Since then, *B. jacksoni* was put into the genus *Paramormyrops,* and four new species were added to *Brienomyrus (Brienomyrus)*: *B. curvifrons* [Taverne, 1977]; *B. longicaudatus* [Taverne, 1977]: *B. hopkinsi* [Taverne and Thys van den Audenaerde, 1985], and *B. tavernei* [Poll, 1972]. Bigorne [1989] provided a revision of the *Brienomyrus* of West Africa and added *B. batesii* [Boulenger, 1906] (formerly *Hippopotamyrus*) to the genus. Mamonekene and Teugels [1993] added *B. kingsleyae* [Günther, 1896] (formerly *Pollimyrus*).

In addition to the difficulty of identifying some of the *Brienomyrus* in museum speciemens without the benefit of the EODs, confusion over *Brienomyrus* from Central Africa is in part due to confused synonomies for *Brienomyrus brachyistius.* The list includes *Mormyrus microcephalus* [Günther, 1867] with a type locality in the Ogooué River in Gabon; *Marcusenius longianalis* [Boulenger, 1901] from the Niger Delta, and *Marcusenius adustus* [Fowler, 1936] from Cameroon [Trewavas, 1974]. Molecular phylogenies, in conjunction with morphological and behavioral data can help us to understand the evolutionary relation-

ships among these confusing groups. This study sheds light on the relationships among morphologically similar *Brienomyrus* clades from Gabon using molecular methods.

Mitochondrial DNA (mtDNA)

In this study we use mtDNA sequences to estimate phylogenetic relationships. There is presently extensive information available about the mitochondrial genome of several vertebrate and invertebrate orders. In particular, ribosomal RNA genes have been shown to be suitable for phylogenetic studies addressing inter-specific as well as inter-familial realtionships. Among vertebrates, the 12S and the 16S rRNA genes have been utilized to study phylogenies among and between species and families of fish; [Meyer et al., 1993; Alves-Gomes et al., 1995], amphibians [Titus and Larson, 1995], reptiles [Knight and Mindell, 1993; Knight and Mindell, 1994], birds [Hedges and Sibley, 1994] and mammals, including rodents, bats, primates, and whales [Allard et al., 1992; Ammerman and Hillis, 1992; Milinkovitch et al., 1993; van der Kuyl et al., 1995].

We used 932 aligned sites of the 12S and 16S rRNAs to study the evolution of the mormyriforms. The resulting phylogeny permits an analysis of the evolution of electric organ characteristics.

Electric Organs and Electric Organ Discharges

Since Lissmann [1958], a number of biologists have reported on the electrical and anatomical characteristics of electric organs of mormyriforms [Bennett and Grundfest, 1961; Bennett, 1971; Schwartz et al., 1975; Hopkins, 1980, 1981; Hopkins and Bass, 1981; Bass, 1986a, 1986c; Bass et al., 1986]. It is widely recognized that the electric organ discharge (EOD) waveforms can be species-specific. The EODs vary widely between species – in duration, in number of peaks and inflection points, in polarity, and in frequency spectrum – and are used for purposes of species- and sex-recognition [Hopkins and Bass, 1981]. Yet, attempts to understand the evolution of electric organ design have been confusing, owing to the fact that there are competing selection pressures acting on their design characteristics [Bennett, 1971, Bass, 1986b].

The 'electrocytes' that make up the electric organ are flattened disk-shaped multi-nucleated cells with a diameter of up to several mm and a length of only 10 to 50 μm. One hundred or more disks lie packed together in four parallel columns in the elongate and cylindrical caudal peduncle of an electric fish. Each electrocyte has a complex stilt-root-like process emerging from one flat face or the other (usually posterior). These rootlets are smallest in diameter at the point where they fuse with the electrocyte face. They repeatedly fuse with others, increasing in diameter until they reach a large trunk where they receive synaptic input from electromotor nerve axons. This entire root system is called a 'stalk' [Bennett and Grundfest, 1961; Bennett, 1971] or a 'pédicule' [Szabo, 1960, 1961].

We recognize four principal types of electric organs among the mormyriforms we sampled (fig. 3). Our observations support and extend the observations of Ogneff [1898], Schlichter [1906], Gosse and Szabo [1960], Bennett and Grundfest [1961], Bennett [1971] and Bass [1968a], who recognized a number of other variations in morphology.

Materials and Methods

We conducted the original research herein in accordance with the guidelines for the humane and ethical treatment of animals set forth by the National Institutes of Health and supervised by the Cornell University Animal Care and Use Committee.

Specimens Used in This Study

Fourteen species were acquired through tropical fish importers in the United States, from field trips to Gabon in West Africa (by CDH) and from specimens sent to Dr. Axel Meyer from Benin in West Africa. The sources of material are summarized in table 1. Our specimen identifications were made after consulting the published accounts of Boulenger [Boulenger, 1909–1916] and Lévêque et al. [1990] and a number of regional publications. Five species from Gabon represent undescribed taxa. The Indian knife fish, *Notopterus chitala* [Hamilton-Buchanan, 1822] and the African butterfly fish, *Pantodon bucholzi* [Peters, 1877], both osteoglossomorphs, came from tropical fish importers and are included for outgroup comparison. *Gymnarchus niloticus* [Cuvier, 1829] and four mormyrids also came from tropical fish importers. One mormyrid, *Petrocephalus bovei* [Valenciennes, 1846] was fixed in the field in Benin and had no electrical recording.

We designate the five unidentified mormyrids from Gabon as *Brienomyrus sp. 1*, *Brienomyrus sp. 2*, *Brienomyrus sp. 3*, *Brienomyrus sp. 4*, and *Brienomyrus sp. 5*. (See table 1 for names of these unidentified species used in previous publications.) We also collected *Brienomyrus batesii*[1] [Boulenger, 1906] from Gabon. All six of the electrically distinct morphs collected in Gabon were previously putatively identified as being closest to *Brienomyrus brachyistius* based on morphological criteria, but all six can be reliably distinguished on the basis of electric organ discharge characteristics. Specimens from Gabon were collected in forest streams near Makokou (Ivindo River and Ogooué River drainage) or from forest-surrounded savanna streams near Franceville (Ogooué River drainage) (table 1).

EOD Recordings

To make an EOD recording we held a fish in a 10×30×15 cm Plexiglas aquarium with two silver/silver-chloride electrodes posi-

[1] In a previous publication (see Hopkins, 1981) we refer to two clades as *Hippopotamyrus batesii (rp)* and *Hippopotamyrus batesii (tp)*. We have recently determined that these two sibling species are close to the species description for *Brienomyrus sphecodes* (Sauvage). A redescription of *B. sphecodes* and descriptions of the new forms are currently underway.

Table 1. Specimens used in this study, listed according to species. Field numbers are used for field-collected specimens only. CU numbers are for voucher specimens in the Cornell University Vertebrate Collection. DNA sequence numbers refer to a single DNA sequence determination from a single PCR product

Species	Field #	CU#	DNA sequence	Locality
Brienomyrus sp. 1[1]	1002	CU75436	*M001‡	Gabon. Ivindo R.
	1005	CU75436	*M002‡	
Brienomyrus sp. 2[2]	1016	CU75439	M003	Gabon. Ivindo R.
	1018	CU75439	M004, M147	
	1019	CU75439	*M005, M148	
Brienomyrus sp. 3[3]	1020	CCU7544	*M006	Gabon. Ivindo R.
Brienomyrus sp. 4	1075	CU75456	*M007, M165	Gabon. Ogooué R.
Brienomyrus sp. 5	1097	CU75470	M008	Gabon. Ogooué R.
	1098	CU75470	*M009	
Brienomyrus batesii[4]	1352	CU75403	M175	Gabon. Ivindo R.
	1353	CU75403	*M176	
Brienomyrus niger	–	CU76349	M112	aquarium import
	–	–	M128, M145	
	–	–	*M143	
Marcusenius senegalensis	–	CU76347	M113	aquarium import
	–	–	*M144	
Gnathonemus petersii	–	CU76348	*M142	aquarium import
Brienomyrus brachyistius	–	CU76350	*M141, M164	aquarium import
	–	CU76153	M182	
	–	CU76153	M183	
Petrocephalus bovei	–	CU76360	*M050	Benin. Tobe.
Gymnarchus niloticus	–	–	*M053	aquarium import
Notopterus chitala	–	–	*M055	aquarium import
Pantodon bucholzi	–	–	*M054	aquarium import

‡ Samples M001 and M002, taken from two individuals thought to be the same species, diverged by more than 0.35% of their sequences and thus are both included in the phylogenetic analysis.
* Of the several sequences obtained for a given species, only one from each, indicated by the star (*), was used for the phylogenetic analyses. In the case of Brienomyrus sp. 1 (see note above) both sequences were used in the analysis.
[1] *Brienomyrus brachyistius* (bp) in Hopkins [1980]
[2] *Brienomyrus brachyistius* (tp) in Hopkins [1980]
[3] *Brienomyrus brachyistius* (1. bp.) in Hopkins [1980]
[4] *Brienomyrus brachyistius* (mp) in Hopkins [1980]

tioned at the ends, a ground lead in the center, and plastic tube in the center to give the fish shelter. The water, taken from the fish's home stream, had pH=6.5, conductivity=12 to 30 μS/cm, and temperature=25°C. We recorded electric organ discharge waveforms digitally using a Tektronix 222 field portable digital oscilloscope (8 bit accuracy, 512 points, 100 to 1,000 ksamples/s) or a custom-built pulse logger (Helpware; Psychology Department, University of Sheffield; 8 bit, 4,096 points, 1,000 ksamples/s). Digitized samples were stored on a portable computer (Dell 320N+) or on digital tape and analyzed later using custom-written software. We digitized the EOD at a normal gain to obtain the entire waveform undistorted, and at expanded gain, to record weaker prepulses and overshoots.

For *Petrocephalus bovei* and *Marcusenius senegalensis* we added field recordings made from specimens from the Niger River in Mali [C.D. Hopkins and P. Jacob, unpubl. observ.].

EOD Analysis

We subjected all EODs to quantitative analysis by measuring times, relative voltages, and slopes at a number of key points on the waveform (fig. 1A). We also generated power spectra of each EOD first by windowing the wave with a Hanning window and then taking its Fourier Transform (FFT) using Matlab (Mathworks, Inc., Natick, Massachusetts). From this we measured the peak frequency of the spectrum (fig. 1B). We measured the total duration of the wave as the time between the first and last points on the waveform that deviated from the baseline by more than 2.5% of the peak to peak height. All amplitude measurements were scaled to a percentage of the peak to peak height.

We designate the first head-positive peak of the EOD as P1, the following head-negative peak as P2. Some mormyrids with electrocytes having penetrating stalks also have an early head-negative peak which we designate as P0 [Bennett and Grundfest, 1961; Bennett, 1971]. The P0 can be small, so its presence is always verified at high vertical gain.

Sex Differences in EODs

We show EODs from representative specimens from mature males and females wherever we have field data for sexually mature individuals recorded during the breeding season. Otherwise, we present EOD

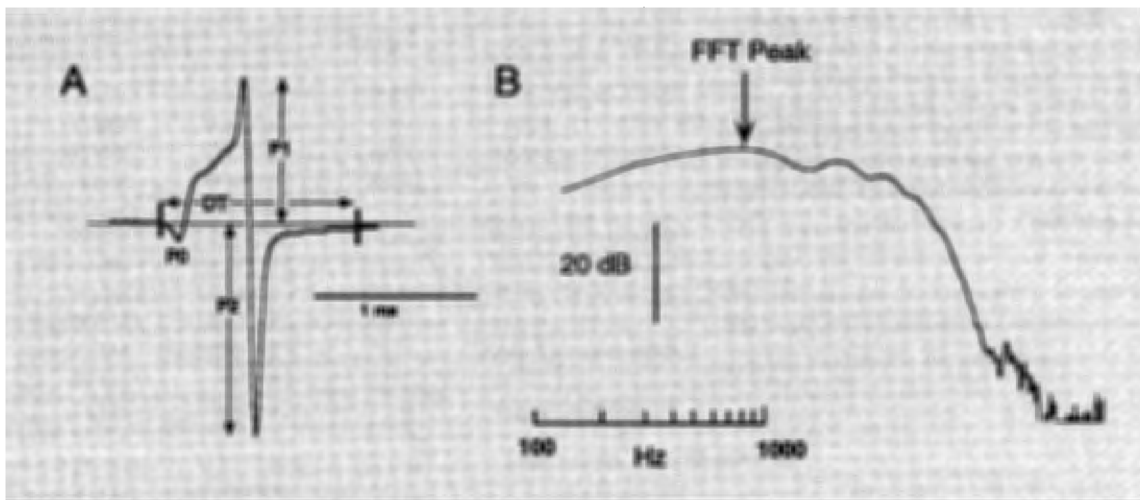

Fig. 1. A representative EOD and its Fourier Transform illustrate measures taken for table 2. (**A**) The EOD is from *Brienomyrus sp. 5* and is plotted with head-positivity upward. This EOD is typical for those species with electrocytes with penetrating stalks innervated on the anterior surface. The head-negative prepulse, P0, is thought to result from the action potential in the penetrating stalk system. Peak P1 occurs when the posterior faces of the electrocytes generate an action potential; peak P2 occurs when the anterior faces of the electrocytes generate an action potential. DT shows the total duration of the EOD. (**B**) The power spectrum of the EOD is shown to the right. The peak frequency of the power spectrum can be used to characterize the center of the energy spectrum for the EOD.

data only from the female or juvenile forms. Sex differences in EODs usually manifest themselves as differences in duration, but sex differences in *B. niger, B. brachyistius* and *G. petersii* (tables 2, 6) are known only from previous laboratory-based studies. A designation of 'no sex difference' implies that we have been unable to distinguish male and female EODs during the breeding season under field conditions.

Electric Organ Anatomy

Electric organs were fixed in phosphate-buffered 4% paraformaldehyde, with or without perfusion, in the field or laboratory, and the tails were removed. After removing scales we decalcified the tissue (12 hours in Calex II, Fisher Scientific), infiltrated it with methylacrylate, JB4 (Polysciences) overnight, catalyzed and embedded it in JB4, and sectioned it sagittally and transverse at 7 microns using a tungsten carbide knife. Sections were stained with Toludine blue and examined with a Leica DM microscope. The results were compared to the published anatomy of known species [see Bass, 1986a for review].

Tissue Samples for DNA

We extracted DNA and sequenced polymerase chain reaction (PCR) products of 29 tissue samples obtained from 24 specimens of fish representing 14 species in the superorder Osteoglossomorpha. Twenty-two specimens were mormyriforms; we also sampled one notopterid *(Notopterus)* and *Pantodon* (Pantodontidae) for outgroup comparison. Table 1 lists the specimens used in this study and the respective DNA sequences used in the phylogenetic analyses.

Tissue obtained in the field was preserved in 70% ethanol until the DNA extraction, whereas tissue from fish from dealers was used fresh. Voucher specimens were preserved and deposited in the fish collection at Cornell university (CU numbers in table 1).

DNA Extraction

Total genomic DNA was extracted from a piece of muscle or fin, following the protocol described by Kocher et al. [1989] with overnight incubation. The DNA was subsequently purified by two extractions with equilibrated phenol, one or two with phenol/chloroform/isoamyl alcohol (25:24:1), and one with chloroform/isoamyl alcohol (24:1). The DNA concentration in the extract was inferred by comparisons with DNA size markers of known concentration in standard electrophoresis in 0.8% agarose gels with 0.5 μg/ml ethidium bromide.

DNA Amplification and Sequencing

We used PCR (polymerase chain reaction) to amplify two segments of the mitochondrial genome of our samples. The first segment was approximately 400 bases long located in the 12S ribosomal RNA (rRNA) gene and the second about 600 bases long from the 16S rRNA.

Amplifications were done in a total volume of 25 μl, following the concentrations described in Kocher et al. [1989]. The 12S primers were modified from Kocher et al. [1989], and the sequences for the 16S primers were obtained from Palumbi et al. [1991]. The sequences are: 12S: L1,091: 5'-AAACTGGGATTAGATACCCCACTAT-3', and H1478: 5'-GAGGGTGACGGGCGGTGTGT-3'; and 16S: 16Sa-L: 5'-CGCCTGTTTATCAAAAACAT-3' and 16SB-H: 5'-CCGGTCT-GAACTCAGATCACGT-3'. The position of the 3'end of each primer in the L strand of the human mitochondrial genome are, respectively, 1,091, 1,478, 2,510, and 3,059 [Anderson et al., 1981].

Double stranded amplifications were carried out in 25 cycles with the following temperature profile: denaturation for 30 s at 93 °C; annealing for 30 s at temperatures varying between 50 and 60 °C, depending upon the optimal annealing temperature for the different genera; and extension for 30 s at 72 °C.

The PCR products of the four non-mormyrinae genera were sequenced by the dideoxynucleotide chain-terminator method (Sanger et al., 1977], following the standard protocol provided with the Sequanase 2.0 kit (United States Biochemical). All other PCR products were sequenced with a 370 a automated sequencer (Applied Biosystems, Inc.) following the procedures described in Alves-Gomes et al. [1995], with small changes. We initially checked the quality of our PCR products by running 5 μl of the amplified DNA in 0.8% agarose gels with 0.5 μg/ml ethidium bromide. The remaining 20 μl were cleaned by two filtrations using either Microcon-100 or Centricon-100 columns (Amicon, Inc.) following the recommendations of the manufacturer. The final filtered volume for the Microcon filters was normally less than 10 μl, and, in this case, ultrapure water was added to make a final volume of about 20 μl. The volume recovered from the Centricon filters was consistently around 45 μl. From the filtered DNA, 9.5 μl were used as a template for cycle sequencing (Applied-Biosystems, Inc.) each strand, using Taq polymerase and dye labeled terminators following the recommendations of the manufacturers, as described in Alves-Gomes et al. [1995]. All final sequences were obtained by reconciling sequences from both L and H strands.

Sequencing replicates were performed in several ways. For some samples we sequenced the same PCR product twice, for others we performed and sequenced a second PCR of the same DNA extraction, and in others, we sequenced PCR products from distinct DNA extraction of the same individual (see table 1).

Sequence Alignment

The DNA sequences were read and edited with the software SeqEd v. 1.0.3 (Applied Biosystems, Inc.). By comparing the sequences of this study with the secondary structures proposed for other teleosts for the same DNA segments [Alves-Gomes et al., 1995], we were able to identify regions corresponding to loops and stems and improve the alignment by prioritizing base-pair formation in the stems of the secondary structures. Regions corresponding to loops were aligned by eye, with the primary criterion that in the overall alignment transitions were considered less costly than transversions and the latter less costly than gaps.

After alignment of all 29 sequences (932 sites) we calculated the absolute number of sites at which two sequences differ, for every pair of taxa. Subsequently we computed a consensus sequence using the IUB single letter ambiguity codes for those sequences that differed in no more than three positions for all 932 aligned sites. We took a conservative approach, considering that divergences of up to 0.35% (calculated as in Mindell and Honeycutt [1990]) in any pairwise comparison could be due to some possible source of error in the final edited sequence [see Clark and Whittam, 1992]. Thus, specimens diverging by less than 0.35% were considered as belonging to the same genetic pool or population. Only one sequence was selected as a representative of each population for phylogenetic estimation. After this procedure, the new data matrix, now containing 15 sequences (see*'s in table 1), was subject to the phylogenetic analysis described below.

Phylogenetic Analysis

The 12S and 16S sequences were combined into a unique data set for all phylogenetic estimates. This was mainly due to the similarity of their nucleotide composition, proportion of conserved sites, and because we found no evidence suggesting that these two DNA segments are evolving under different evolutionary constraints.

For the phylogenetic inference we utilized three methods: (1) Maximum Parsimony (MP) trees, originated with the program PAUP v. 3.1.1 [Swofford, 1993] for the Macintosh; (2) distance-based estimates, obtained with the neighbor joining algorithm [Saitou and Nei, 1987], using various models for distance correction available in the program MEGA [Kumar et al., 1993]; and (3) maximum likelihood toplogies (ML), generated with the program FASTDNAML [Olsen et al., 1994] available in the DNA system package [Smith, 1988] at the University of California, San Diego. In all analyses, *Pantodon buchholzi* was designated as the outgroup following the well-accepted phylogenetic hypothesis [Lauder and Liem, 1983] for the superorder Osteoglossomorpha. Both gaps and ambiguities were treated as missing characters in almost every analysis. The only exception was MP, where gaps were considered as the fifth character state under one weighting scheme (see below).

Maximum Parsimony

Using PAUP [Swofford, 1993], we considered several cost relationships between transitions and transversions in order to compensate for possible saturation in transitional substitutions in our sequences and to assess the gain/loss of phylogenetic resolution when different weights were assigned to each class of substitution. Gaps were considered as a fifth character state only in one cost matrix, where every site to which a gap was assigned was given a cost of 1, i.e., a gap of length 5 was given a cost of 5. We used the following weighting schemes in MP: 'TS1TV1' meaning that the same cost of 1 step was assigned for each transition (TS) and each transversion (TV). In 'TS1TV1GP1', gaps (GP) were also considered as a character state and were given the same weight (1 step) as TS and TV. In 'TS1TV3' and TS1TV6', the respective costs assigned to each TV was three and six times that of each TS, and GP were omitted. In 'TVPARS', only TV were considered for phylogenetic reconstruction. In our last weighting matrix 'EOR' [Knight and Mindell, 1993; Collins et al., 1994a]. We determined the cost of each class of TS and TV by taking into consideration the base composition of each sequence and the expected/observed ratio (EOR) for the different types of base substitution for all pairwise comparisons, as described by Collins et al. [1994a]. When calculating EORs, we did not consider the direction of change, so A⇒T changes were not distinguished from T⇒A changes. After obtaining the EOR for each class of substitution, we normalized the values in relation to the lower EOR, and these values were rounded to the closest integer. The final cost matrix obtained by this approach and used in PAUP was: C-T=1; A-G=2; A-C=A-T=3; C-G=13 and G-T=15. In the EOR approach all sites with gaps and/or ambiguities were copletely excluded from the analysis [see Knight and Mindell, 1993; Collins et al., 1994a].

For each weighting scheme, with the exception of the EOR, branch and bound searches were performed with accelerated transformation (ACCTRAN) selected for character-state optimization. The upper bound for the searches was determined by the shortest tree found with one heuristic search with simple addition of taxa for the respective weighting scheme. Only minimal trees were kept, and zero-length branches were collapsed. For all searches where the characters were equally weighted (i.e., TS1TV1 and TS1TV1GP1 as well as TVPARS), the option for additional taxa chosen was 'furthest', whereas for the rest of the analyses, the option 'simple' was chosen [Swofford, 1993]. We opted to ignore invariant characters with PAUP. Using EOR, we performed 100 heuristic searches with random addition of taxa.

We tested the content of the phylogenetic information in our data set by performing a total of 1,000 bootstrap searches for each weighting scheme. In these analyses, 100 heuristic searches were executed

with 10 repetitions of random addition of taxa being performed at each replicate. The program retained groups with frequencies higher than 50%, and sampling of sites was done considering the non-ignored characters only. For each bootstrap replicate, only minimal trees were kept, zero length branches were collapsed, and starting trees were obtained by random stepwise addition. Branch swapping was performed according to the tree bisection reconnection algorithm (TBR). All minimal trees were saved.

Neighbor Joining

Neighbor Joining (NJ) trees were obtained with the program MEGA [Kumar et al., 1993]. Several models for distance correction were used. Assuming a constant rate of substitution among sites, we first estimated distances by computing the proportion of sites at which two sequences differ. Subsequently we used the following models to correct distance estimates for multiple hits: the Kumura (2 parameter) model, the Tamura model, and the Tajima-Nei model. In a second approach, we considered that the rates of substitution among sites fitted a gamma distribution, and we corrected distances for multiple hits using Kumura's and Tamura-Nei's models. In both cases, three values for the parameter 'a' of the gamma distribution were specified. This parameter is inversely proportional to the coefficient of variation of the substitution rate per site and has been estimated to be 0.47 for the mitochondrial control region and 2.0 for the amino acid sequences of cyctochrome-C [Kumar et al., 1993]. For Kimura correction, we used a=0.5, 1.0 and 1.5, and for the Tamura-Nei model, we used a=0.25, a=0.5, and a=1.0. All distances were calculated considering both TS and TV. Ambiguities were treated as missing characters, and sites with gaps were completely omitted from the entire matrix.

Maximum Likelihood

Using the program FASTDNAML [Olsen et al., 1994], we performed 10 maximum likelihood searches using a random number to 'jumble' the order of input of taxa. After each turn of taxa addition, global rearrangements in the tree were performed. In this procedure, each possible subtree is removed from the original tree and added back in all possible places. If a better tree is found, the process is repeated until each sub tree is tested without improvement of the tree. We also instructed the program to use the empirical base frequencies derived from the sequence data, towards the likelihood calculation.

Results

We describe first the species of fish we used, their electric organ discharges and their electric organ morphology. We then describe the DNA sequences and the phylogenetic analysis.

Description of EODs

The EOD waveforms of representative individuals of each species of mormyriform in our sample are shown in figure 2. All EODs are shown with head-positivity upward on the same time base. Quantitative data on the heights of P0 (if present) and P1/P2 ratios, peak frequency of the FFT of the EOD, and total durations are presented in table 2. In cases where we have made field recordings from adult males and females during the breeding season, we have included data on males and females separately. We define three types of EODs – I, II and III – produced by electric organs with four types of electrocytes – S, NP_p, P_a, and DP_p (see fig. 3 and table 2).

Gymnarchus niloticus [Cuvier, 1829]. The EOD of *Gymnarchus* is an approximately 300 Hz wave discharge composed of a head-positive monophasic pulses, approximately 1 ms in duration, superimposed on a head-negative baseline. We refer to monophasic EODs like this as type I (fig. 3). We have no information on sex differences in discharges, since animals smaller than about 100 cm length are sexually immature. Dahlgren [1914], Bennett [1971], Fessard [1958], and Schwartz et al. [1975] report, and we confirm, that the electrocytes in the electric organ (EO) have no stalks and are innervated on the posterior side. We refer to this type of electrocyte as type 'S' (i.e., 'stalkless') in figure 3.

Petrocephalus bovei [Valenciennes, 1846]. Our recordings of *Petrocephalus bovei* were made from the Niger River basin in Mali by C.D. Hopkins and Philippe Jacob (unpubl.). The EOD is simple and biphasic (i.e., type II), with no inflection points on the rising phase. The sexes are monomorphic in EOD and EO, thus the EODs for sexually mature males and females superimpose relatively precisely. The electric organ has electrocytes with non-penetrating stalks innervated on the posterior surface. We refer to this type of electrocyte as type 'NP_p' (Non-Penetrating, posterior innervation) in figure 3.

Marcusenius senegalensis [Steindachner, 1870]. Although the EOD appears to have only two phases, upon expanding the vertical gain it is clearly triphasic (type IIIa), with an early head-negative prepulse (P0) to every EOD. We make a distinction between EODs of type IIIa, where the height of P0 is less than 1% of the peak to peak height, and IIIb where P0 is greater than 1%. Aquarium imported specimens were all sexually immature, but specimens collected in the Niger River in Mali (by CDH and P. Jacob) showed a distinct sex difference in EODs (fig. 2). The P0 is larger in females than in males, and male EODs are twice as long in duration. Males have a lower FFT peak compared to females. Quantitative data are presented in table 2. The rising phase of the head positivity is smooth and shows little evidence of an inflection point. Electric organs in *M. senegalensis* have electrocytes with penetrating stalks, innervated on the anterior side of each cell (type 'P_a' in fig. 3).

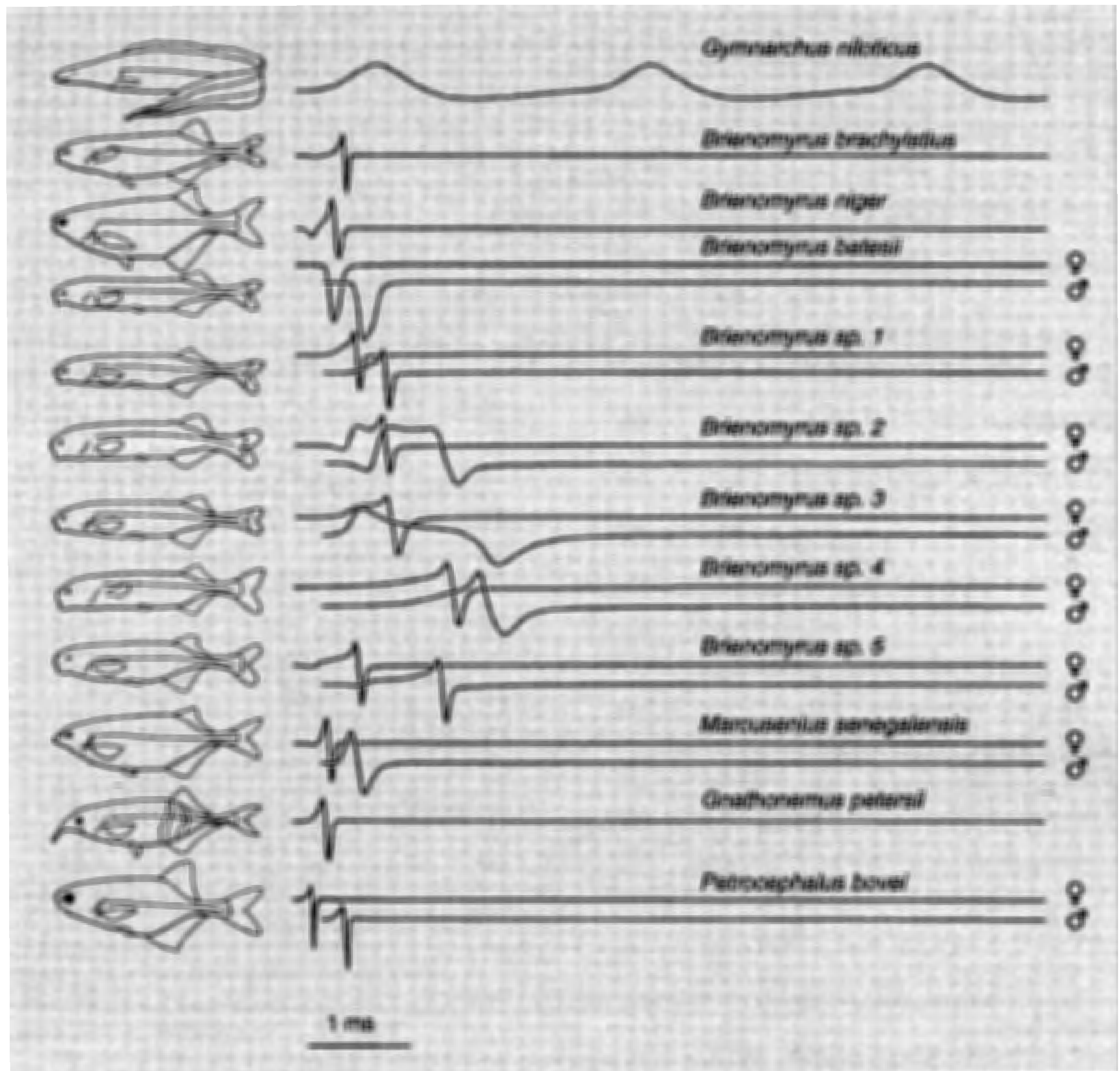

Fig. 2. The EODs of representative individuals of the 12 species of mormyriforms used in this study. All EODs are plotted on the same time base with head positivity upward. Representatives for each sex are illustrated only in those cases where the discharge has been recorded under field conditions from reproductively mature individuals. Although sex-differences occur in *B. niger, B. brachyistius,* and *G. petersii,* these differeces were compiled from laboratory studies involving animals treated with testosterone.

Gnathonemus petersii [Günther, 1862]. Like *M. senegalensis,* the discharge is triphasic with a small head-negative prepulse (P0), thus it is type IIIa. This species is known to have a sex difference in the discharge [see Landsman and Moller, 1988; Landsman et al., 1990], although we have no field data on the EOD. The electrocytes have penetrating stalks innervated on the anterior side of each cell [Bell et al., 1976] (type P_a in fig. 3).

Brienomyrus niger [Günther, 1866]. *B. niger* has a triphasic EOD (type IIIb). There are one or more distinct inflection points on the rising phase between P0 and P1. In some specimens, but not all, there is a small head-positive overshoot after P2. Males have a longer triphasic EOD than females, but this was not apparent on our imported specimens (P. Jacob, personal communication).

The electrocytes have penetrating stalks, but we noted consistently that the stalks are innervated on the posterior side, not the anterior side, and the innervated stalk immediately makes its first penetration through to the anterior side. The stalk travels across the anterior surface for a distance

Table 2. Characteristics of Electric Organs (EOs) and Electric Organ Discharges (EODs) of mormyriform fishes used in this study. EOD waveform types are I for monophasic, II for biphasic, and III for triphasic, with subtypes defined in the left column below. Electric organs fall into four categories: type S, for stalkless electrocytes with posterior innervation, type NP_p for electrocytes with non-penetrating stalks with posterior innervation, type P_a for those with penetrating stalks with anterior innervation, and type DP_p for those with doubly-penetrating stalks with posterior innervation. Some species have sex-differences in the waveform of the EOD and sexual dimorphism in the EO, noted either in the published literature or in this study, while others are known to be sexually monomorphic in EOD and EO. Quantitative measures of the EOD waveform are listed by sex: J refers to juvenile or sexually-immature individuals of unknown sex, F indicates adult females, and M indicates adult males. P0 is the height of peak 0, as percent of peak-to-peak height

Species	EOD	EO type	Sex difference in EOD	n	Sex	P0 (%)	EOD duration (ms)	FFT peak (Hz)	P1/P2 ratio
Brienomyrus sp. 1	IIIa	P_a	no	6	F	0.35	0.4977 ± 0.052	2,007	0.64
				8	M	0.56	0.544 ± 0.076	1,987	0.62
Brienomyrus sp. 2	IIIb	P_a	yes	16	F	6.09	1.213 ± 0.432	1,237	0.958
				13	M	10.66	4.04 ± 1.23	466	1.822
Brienomyrus sp. 3	IIIa	P_a	yes	13	F	0.25	1.18 ± 0.36	1,144	0.585
				1	M	0.69	3.09	390	0.866
Brienomyrus sp. 4	IIa	NP_p	yes	33	F	–	2.169 ± 0.89	651	0.708
				2	M	–	2.740 ± 0.68	488	1.267
Brienomyrus sp. 5	IIIb	P_a	yes	27	F	2.42	1.06 ± 0.168	938	0.583
				3	M	2.81	1.51 ± 0.124	1,008	0.643
Brienomyrus batesii	IIb	NP_p	yes (?)	1	F	–	0.390	291	0.003
				6	M	–	0.512 ± 0.072	189	0.0009
Brienomyrus niger	IIIb	DP_p	yes	5	J	10.20	0.365 ± 0.3	4,017	0.882
Marcusenius senegalensis	IIIa	P_a	yes	13	F	2.47	0.395 ± 0.44	5,566	0.719
				24	M	0.13	0.537 ± 0.154	2,515	1.028
Gnathonemus petersii	IIIa	P_a	yes	2	J	0.23	0.277	3,705	0.595
Brienomyrus brachyistius	IIIb	DP_p	yes	5	J	2.73	0.340 ± 0.110	5,128	0.587
Petrocephalus bovei	IIa	NP_p	no	5	F	–	0.198 ± 0.03	5,271	0.36
				6	M	–	0.211 ± 0.02	5,695	0.43
Gymnarchus niloticus	I	S	?	1	J	–	1.43	330	–

EODs

Type I: monophasic, head positive pulses emitted in a continuous wave at c. 300Hz.

Type II: biphasic head positive (+P1) then head negative (–P2) with no prepulse.

Type IIa: strictly biphasic (+P1, –P2).

Type IIb: primarily monophasic, P1 less than 1% of the peak-to-peak height.

Type III: triphasic, head negative (P0), head positive (P1), head negative (p2).

Type IIIa: apparently biphasic, weak P0 that is less than 1% of the peak-to-peak height (P1–P2).

Type IIIb: triphasic, P0 is greater than 1% of peak-to-peak height.

Electric Organs

Type S: stalkless, posterior innervated, cylindrical electrocyte.

Type NP_p: non-penetrating, stalk, posterior innervation.

Type P_a: penetrating stalk, anterior innervation.

Type DP_p doubly-penetrating stalk, posterior innervation.

before making a second penetration back through to the posterior side near the margin of the cell. We designate this type of electrocyte as DP_p, because of the Double Penetration with posterior innervation (fig. 3). It has not been previously reported and was at first mistaken for a typical P_a electrocyte. Only by careful inspection of the site of innervation do we see a difference between DP_p electrocytes and P_a. The DP_p electrocytes differ from the Doubly-Penetrating and Non-Penetrating stalk described by Bass [1986c] in that there appear not to be any non-penetrating stalks on the posterior side of the electrocyte.

Brienomyrus brachyistius [Gill, 1862]. Our specimens of *Brienomyrus brachyisius* came from tropical fish importers and are presumed to have originated in Nigeria. The EOD is triphasic (type IIIb). The fish apparently has a normal sex difference in its EOD, although this has not been reported

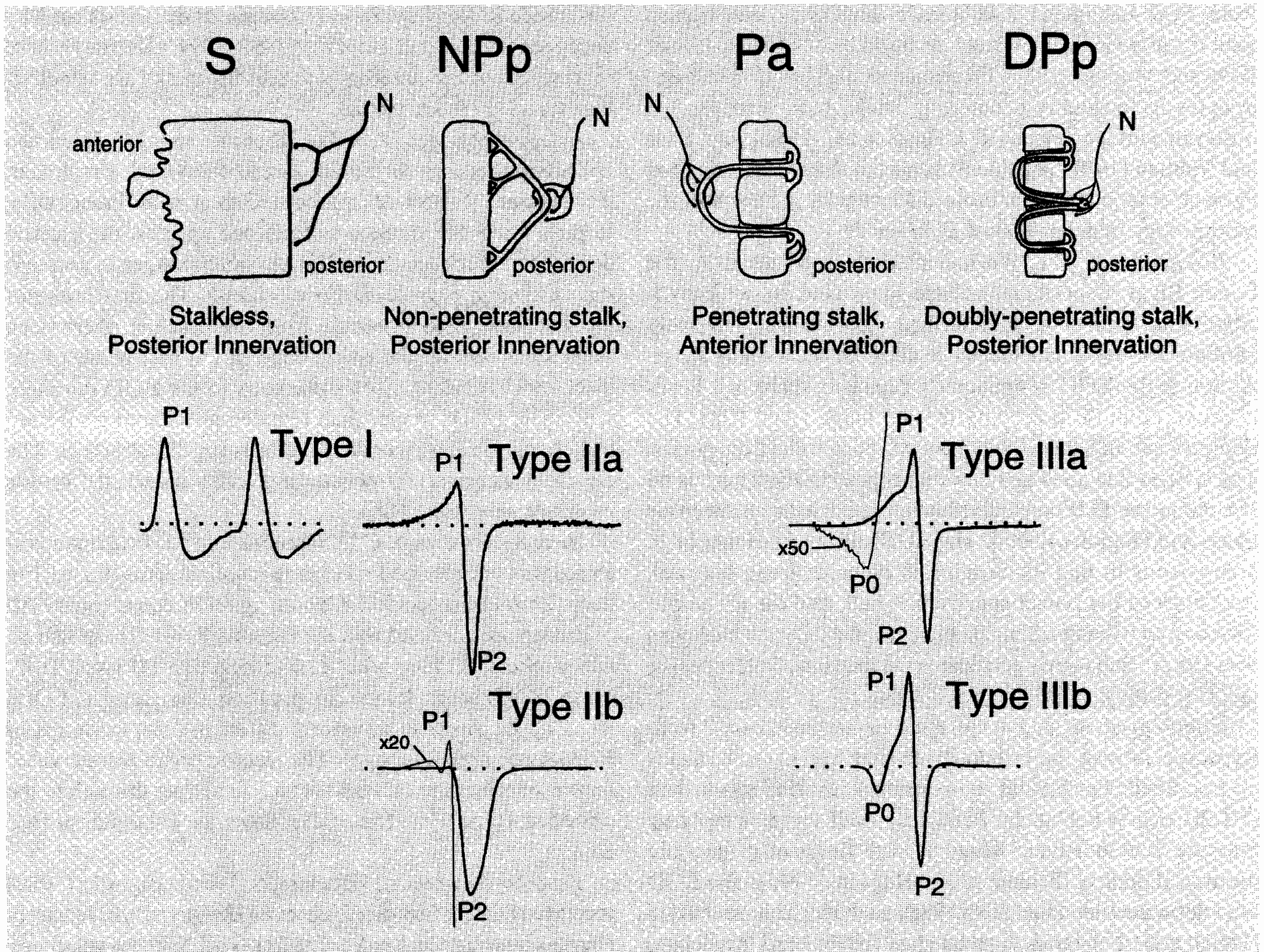

Fig. 3. Morphological diversity of electrocytes among the Mormyriforms, illustrated by four categories found among the species used in this study and the three corresponding types of electric organ discharges (EODs). Type S electrocytes, found in *Gymnarchus niloticus* have posterior innervation, and lack a stalk system. Type NP_p electrocytes have a well defined stalk system which is non-penetrating and innervated on the posterior side, represented by *Petrocephalus boveii, Brienomyrus batesii,* and *Brienomyrus sp. 4.* Type P_a electrocytes have a stalk system which penetrates once through the electrocyte and receives innervation on the anterior side. They are exemplified by *M. senegalensis, G. petersii, Brienomyrus sp. 1, Brienomyrus sp. 2, Brienomyrus sp. 3,* and *Brienomyrus sp. 5.* Type DP_p electrocytes have a stalk system which makes a double penetration through the electrocyte while the innervation is on the posterior side. They are exemplified by *Brienomyrus niger* and *B. brachyistius.* Type I EODs are monophasic, head positive discharges superimposed on a head-negative baseline. The discharge is wave-like. Type II discharges have two phases, P1 (positive) and P2 (negative). IIa discharges have a large positive phase, while IIb has a very small P1, so the discharge appears monophasic. Type III EODs are triphasic with peaks P0 (negative), P1 (positive), and P2 (negative). The P0 is less than 1% of the peak to peak height in IIIa, greater than 1% in IIIb. The thin lines (type IIb, type IIIa) show expanded traces.

from field specimens. Individuals that have been treated with testosterone have longer duration pulses [Bass, 1986a; Bass and Volman, 1987; Freedman et al., 1989]. The electrocytes have doubly-penetrating stalks innervated on the posterior surface (type DP_p). Bass et al. [1986] and Bass and Volman [1987] previously reported that this species has P_a electrocytes, but upon close inspection it is clear that the morphology matches that of *B. niger.*

Brienomyrus sp. 1. We collected this species from the Ivindo River basin of Gabon near Makokou. To our knowledge, this species is undescribed. The fish is relatively common in the small creeks leading to the Ivindo River. Although the EOD is apparently biphasic, there is a head-negative prepulse preceding the discharge (type IIIa). The rising phase of peak 1 has one or more inflection points. The species has a sexually monomorphic EOD. There is no change in the EOD of individuals treated with testosterone [Bass and Hopkins, 1985]. The EOD differs from that of *B. brachyistius* in that its duration is twice as long, the peak frequency of the power spectrum is half, and the P0 amplitude is much less than in *B. brachyistius.* The electrocytes also differ: they have penetrating stalks innervated on the anterior surface (type P_a) [Bass, 1986c].

Brienomyrus sp. 2. We collected *Brienomyrus sp. 2* from forest creeks near the Ivindo River near Makokou. It also is undescribed. Its habitat is restricted to the shallowest (10–20 cm) water at the heads of small creeks emptying into the Ivindo River, where it was frequently the only mormyrid present [Friedman and Hopkins,1996]. The EOD was described by Hopkins [1980] and Hopkins and Bass [1981], and its electric organ by Bass [1986c] and Bass et al., 1986]. This fish has a triphasic (type IIIb) discharge. Peak P1 has a strong inflection point on the rising phase that in some individuals, mainly very large females, can make a double-peak. There is a clear and pronounced sex difference in the EOD [Hopkins and Bass, 1981; Bass and Hopkins, 1983; Hopkins, 1983], with male EODs more than twice as long as female EODs. The waveform of the male is completely different from that of the female: it is not an isomorphically stretched female signal. The elctrocytes are innervated on the anterior side, and the stalks are penetrating (type P_a). The stalks are unusually large at the point where they penetrate through the electrocyte [Bass, 1986c].

Brienomyrus sp. 3. This species was common in the Ivindo River drainage near Makokou, Gabon, in forested streams with depths ranging from 0.3 m to 1.5 m. This represents another undescribed species, whose body morphology bears a superficial resemblance to the 'brachyistius-like' *Brienomyrus.* The EOD is type IIIa. The rising second phase has a marked inflection point. There is a clear sex difference in the waveform [Hopkins, 1980] modulated by androgens [Bass and Hopkins, 1983]. The electrocytes are innervated on the anterior side and the stalks are penetrating (type P_a) [Bass, 1986c].

Brienomyrus sp. 4. C.D. Hopkins and M. Friedman collected this fish in small forested-covered streams in the savanna district of southern Gabon near Franceville. Representing yet another undescribed species, the electric discharge is biphasic (type IIa) but much longer in duration than a typical *Petrocephalus* discharge. The first phase of the discharge rises smoothly from the baseline; there is an inflection point on the rising edge of the first phase of the discharge. There is a sex difference in the EOD duration, with male EODs longer than female but without a substantial change in the overall waveform (fig. 2, table 22). The electrocytes have non-penetrating stalks innervated on the posterior side (type NP_p).

Brienomyrus sp. 5. C.D. Hopkins and M. Friedman collected this undescribed species in small streams, 0.2 to 1 m deep, around Franceville, Gabon, again in dense forest. All of the streams drained into the Ogooué River. These fish all had triphasic EODs, with a head-negative P0 (type IIIb). Male EODs were longer in duration than female EODs (fig. 2). The triphasic waveform differs in form from that of *Brienomyrus sp. 2* (above). The electric organ is innervated on the anterior side on stalks which penetrate through to the posterior (type P_a). The stalks have an enlarged surface area.

Brienomyrus batesii [Boulenger, 1906]. Bigorne [1989] recently re-classified this species as *Brienomyrus* [formerly *Hippopotamyrus* in Taverne, 1972]. This fish was relatively uncommon in our collections, but we did collect it regularly from the Ivindo River, main channel, near Makokou, and in the medium-sized streams flowing into the river. Young individuals were found in the treeroots along the bank of the river. The EOD is highly distinctive because it appears almost entirely monophasic and head negative. Upon close inspection at high gain, one observes a characteristic head-positive prepulse (P1). We classify this biphasic discharge as type IIb (fig. 3) since the P1 phase is less than 1% of the peak to peak height. There may be a sex difference in the discharge, but our data are unconvincing. The electric organ is innervated on the posterior side, and the stalks are non-penetrating [Bass, 1986c].

DNA Sequences

All DNA sequences used in the phylogenetic analyses described in this study were deposited in GeneBank under accession numbers U33504–U33518 for the 12S rRNa and U33519–U33533 for the 16S rRNA. We were able to align

Table 3. Base composition (%) in the complete sequences versus base composition for the informative sites only. Underlined numbers depart noticeably from the averages for the group

Species	Complete				Informative only			
	A	T	C	G	A	T	C	G
Brienomyrus sp. 1	31.3	20.6	25.9	22.3	20.4	21.9	35.0	22.6
Brienomyrus sp. 1'	31.0	20.7	25.5	22.8	20.6	22.1	35.3	22.1
Brienomyrus sp. 2	31.3	20.6	25.8	22.4	20.6	22.1	33.8	23.5
Brienomyrus sp. 3	31.2	20.4	25.6	22.8	19.0	21.9	34.3	24.8
Brienomyrus sp. 4	31.2	20.8	25.6	22.4	21.2	23.4	32.8	22.6
Brienomyrus sp. 5	31.4	20.0	26.4	22.3	21.3	18.4	38.2	22.1
Brienomyrus batesii	31.2	20.2	26.1	22.5	19.9	19.9	36.8	23.5
Brienomyrus niger	31.1	20.8	25.5	22.6	20.6	24.3	32.4	22.8
Marcusenius senegalensis	31.5	20.6	25.9	22.0	22.5	21.0	36.2	20.3
Gnathonemus petersii	31.7	20.4	25.6	22.2	23.0	20.0	36.3	20.7
Brienomyrus brachyistius	31.9	20.3	25.8	22.0	24.8	18.8	35.3	21.1
Petrocephalus bovei	31.5	21.1	25.4	21.9	<u>28.5</u>	20.0	33.1	<u>18.5</u>
Gymnarchus niloticus	32.3	21.0	25.2	21.5	<u>33.9</u>	<u>24.2</u>	<u>27.4</u>	<u>14.5</u>
Notopterus chitala	33.2	20.6	25.1	21.2	<u>36.6</u>	19.8	<u>28.2</u>	<u>15.3</u>
Pantodon bucholzi	33.8	22.7	23.3	20.1	<u>43.6</u>	<u>30.8</u>	<u>17.1</u>	<u>8.5</u>

Table 4. Average of the observed number of each class of substitution in the L strand of the 12S + 16S rRNA for all pairwise comparisons, expressed as percent of total

	Transitions		Transversions				
	A↔G	T↔C	A↔T	A↔C	T↔G	C↔G	Total
Average number of substitutions	27.37	38.17	10.76	17.95	2.16	3.54	100

373 sites of the 12S rRNA and 559 of the 16S rRNA in our sequences (Appendix I). Seventy-three and sixty-eight percent of the sites were invariant in the 12S and 16S rRNA, respectively, totaling 658 invariant sites. From the variable sites, 50 were phylogenetically informative in the 12S rRNA and 88 in the 16S rRNA. Thus, we had a total of 138 sites in our sequences, for which at least two different kinds of nucleotides were present at each site, with each being represented by at least two of our sequences [Li and Graur, 1991]. Six sites became informative in addition to the original 138 sites when gaps were treated as a fifth character state under the TS1TV1GP1 cost matrix.

In the unaligned sequences, *Pantodon,* our designated outgroup, had the most conspicuous lengthwise variation, with gaps being necessarily asigned for 7 sites in the 12S rRNA and 37 sites in the 16S rRNA. *Gymnarnchus* had the next highest number of gaps, with 5 in the 12S rRNA and 14 in 16S rRNA. *Notopterus* had 16 gaps (6 in the 12S), and none of the other genera exceeded 5 gaps in the 12S and 10 gaps in the 16S rRNA. Every insertion/deletion event in our aligned sequences could be asssociated with unpaired sites in the secondary structure of both molecules, based on the model proposed by Alves-Gomes et al. [1995], with the exception of the gap in position 361 of the 12S rRNA, which has uncertain status because we have not sequenced the possible complementary sites. The great majority of insertion/deletion events were located in the two major hairpin loops of the 16S rRNA [see fig. 5a, b in Alves-Gomes et al., 1995].

The compositional bias computed for the 932 sites was nearly identical among all the osteoglossomorphs. The percentage of adenine in the sequences varied between 31% and 33.8%; cytosine between 23.3% and 25.9%; guanine 20.1–22.8%; and thymine 20–22.7% (table 3). However, when only those phylogenetically informative sites were computed, not only did the relative proportion of each base change, but the three non-mormyrid genera (*Gymnarchus, Notopterus* and *Pantodon*) had an unexpected behavior relative to the average composition of the remaining fish. *Pantodon* has the most extreme with the number of adenines being twice, and cytosines about one-third the average for the Mormyrinae (see table 3).

We also observed a strong substitution bias in our sequences, not only between TS and TV, but also among the six different types of TV. The average number of each class of substitution for all pairwise comparisons in shown in table 4. While the two types of TS (A⇔G and C⇔T) account for almost 65% of the total number of substitution, T⇔G transversions are the most rare type of substitution we encountered, representing only 2.1% of the total number of substitutions and being, in average, 17 times less frequent than C⇔T transitions and about 8 times less frequent than A⇔C transversions.

Phylogenetic Relationships

We found 272 equally parsimonious trees when only TV was used, as opposed to a maximum of 2 trees when TS and GP were included (table 5). The strict consensus of the 272 trees depicts the following clades as monophyletic assemblages: Mormyrifomes, Mormyridae, and Mormyrinae. However, the TV-based topology shows unresolved polytomy within the Mormyrinae. This suggests that TV might be effective in establishing relationships between more distantly related clades such as between the subfamilies Petrocephalinae and Mormyrinae, between the families Mormyridae and Gymnarchidae, and between mormyriforms and the other osteoglossomorphs. However, this class of substitution apparently is not abundant enough to elucidate relationships within more closely related fish, or within the Mormyrinae in our study. To resolve the phylogeny of those closely related taxa, TS is the main source of phylogenetic information in our data, since when it was considered, our results converged to a maximum of two possible topologies (fig. 4, 5). These two topologies differ only in the position of *Brienomyrus niger* and *Brienomyrus sp. 2*.

Table 5 summarizes the results of our different phylogenetic methods of analysis, and figures 4 and 5 depict the two resultant topologies recovered from our molecular data. All distance-based phylogenies produced a single topology which was identical to the phylogenetic hypothesis obtained with the Maximum Likelihood approach (fig. 5). In the case of Maximum Parsimony, with the exception of TV parsimony (when we obtained a polytomic consensus tree within the Mormyrinae), the phylogenetic position of 13 out of the 15 taxa included in our phylogenetic analysis remained unaltered, regardless of weighting scheme used (fig. 4, 5). The bootstrap values from Maximum Parsimony were always above 70% for the following clades: Mormyriformes (from 75% to 91%), Petrocephalinae (81–93%), Mormyrinae (90–100%), and *Brienomyrus sp.* from Gabon (85–99%).

Brienomyrus brachyistius consistently is placed outside the group represented by the *Brienomyrus* from Gabon and *Brienomyrus niger*. The close relationships among the *Brienomyrus* from Gabon suggests a monophyletic group. *Brienomyrus sp. 1* differs from *Brienomyrus sp. 1'* in only 7 out of 932 sites, pointing to a very close relationship and supporting the conclusion that it is a single species.

Table 5. Summary of phylogenetic methods and the results obtained for each cost matrix and correction model used

Phylogenetic method/ weighting scheme	Number of trees	Number of informative sites	Resultant topology
Maximum parsimony			
TS1TV1	2	138	Fig. 4
TS1TV1GP1	2	144	Fig. 4
TS1TV3	1	138	Fig. 4
TVPARS	272	53	*
EOR	2	105	Fig. 4
Neighbor joining			
(constant rate among sites)			
p-distance	1	105	Fig. 5
Jukes-Cantor	1	105	Fig. 5
Kimura	1	105	Fig. 5
Tamura	1	105	Fig. 5
Tamura-Nei	1	105	Fig. 5
Tajima-Nei	1	105	Fig. 5
(variable rate among sites – gamma distances)			
Kimura (a=0.5, 1.0 or 1.5)	1	105	Fig. 5
Tamura-Nei (a=0.25, 0.5, or 1.0)	1	105	Fig. 5
Maximum likelihood			
10 searches with random addition of taxa	1	138	Fig. 5

* The strict consensus topology obtained for TVPARS depicts an unresolved polytomic tree for the subfamily Mormyrinae. The exact topology is: *(Pantodon bucholzi, Notopterus chitala, (Gymnarchus niloticus, (Petrocephalus bovei, (Brienomyrus brachyistius, (Marcusenius senegalensis, Gnathonemus petersii, Brienomyrus niger, (Brienomyrus sp. 1, Brienomyrus sp. 1') Brienomyrus sp. 2, (Brienomyrus sp. 3, Brienomyrus batesii, Brienomyrus sp. 4, Brienomyrus sp. 5))))))*.

Discussion

Osteoglossiform 12S and 16S rRNA sequences show a characteristic substitution bias that has been documented in the mitochondrial and nuclear genomes of a wide spectrum of organisms [Brown, 1981; Brown et al., 1982; DeSalle et al., 1987; Thomas and Beckenbach, 1989; Marshall, 1992; Vawter and Brown, 1993]. Not only is TS more frequent

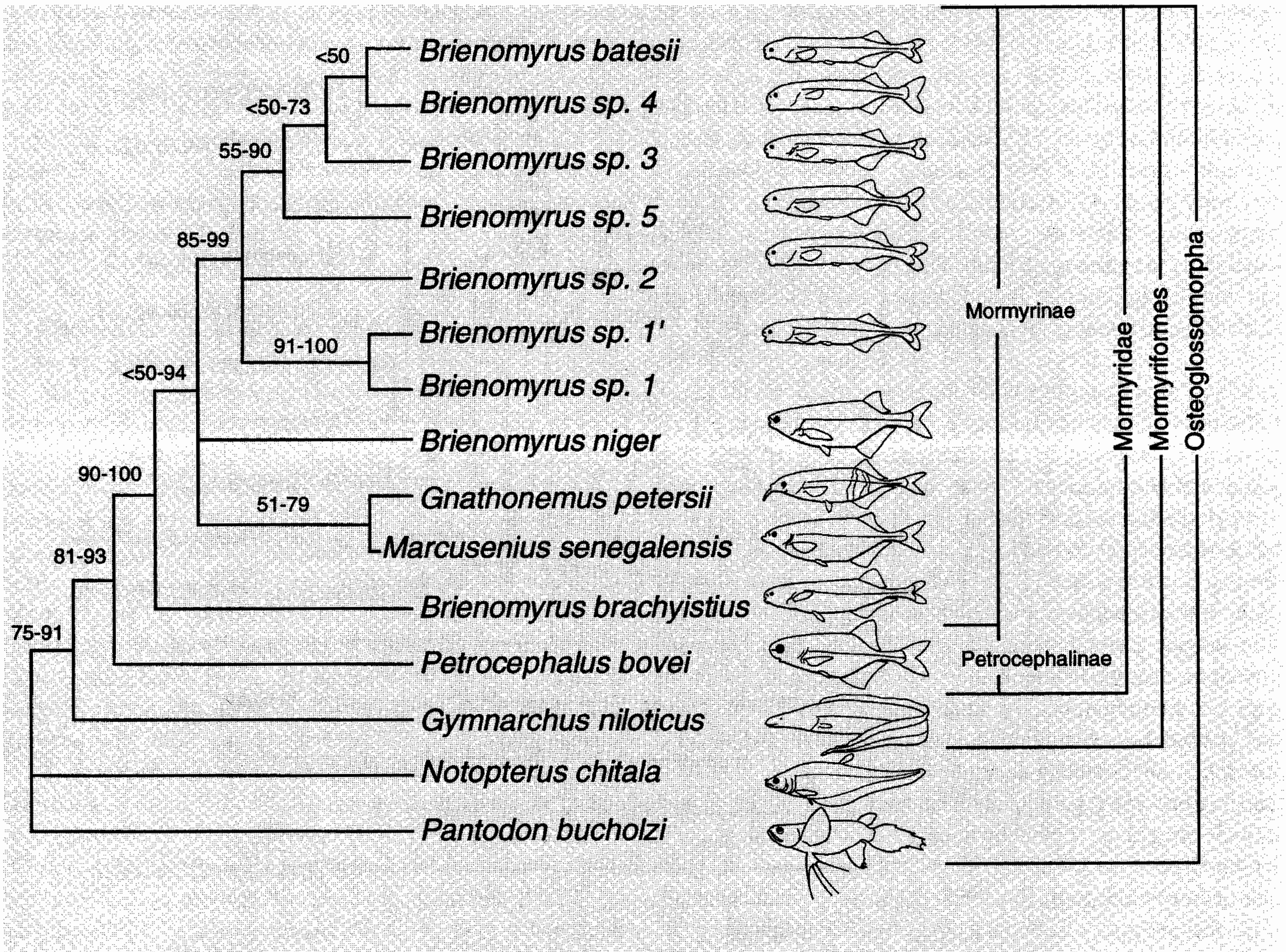

Fig. 4. Strict consensus phylogeny for the species used in this study derived from molecular data using the method of maximum parsimony (MP) with six distinct cost matrices for phylogenetic estimation (see table 5). The numbers over the branches represent the lowest and the highest bootstrap values (50% majority rule) for all cost matrices used without including TV parsimony. One thousand bootstrap replicates were performed for each cost matrix.

than TV, but the frequencies at which the different types of TS and TV occur are distinct. Transversions involving guanine for instance, are much less frequent than the other types of substitutions (table 3). Since TS tends to saturate faster than TV, authors have tended to assign smaller cost for TS in relation to TV in phylogenetic analyses, as divergence increase. However, it is less clear how to account for the potential problems caused by substitutional bias, considering each of the six possible types of substitutions (without differentiating the direction of change, i.e., considering A→T and T→A substitutions as a single class). The EOR approach, suggested by Knight and Mindell [1993] and Collins et al. [1994a] and applied in this study, attemps to partially compensate for compositional and substitutional bias by taking into consideration the expected and observed values for each type of substitution and the individual base frequencies. Several authors have pointed out potential problems of phylogenetic estimation based on DNA sequences with strong compositional and substitutional bias [Marshall, 1992; Collins et al., 1994], but their precise effect in phylogenetic estimates is still not clear. In our case, the cost matrix generated by the EOR approach produced a phylogenetic hypothesis for the Mormyriformes that is highly concordant with the topologies produced by the other matrices, which suggests a clean phylogenetic signal in our data.

eral cost matrices, methods of phylogenetic inference, and distance correction models as a more tangible way to recover the phylogenetic information in our data set. The robustness and congruence of the phylogenetic information contained in our data become clear if one considers that all alternative methods and costs used in our study converged to the same result (table 5). Nevertheless, our results are surprising in several aspects.

Phylogenetic Relationships of the Mormyridae Based on Mitochondrial DNA

After using the various methods, cost matrices, and correction models listed in table 5, we obtained only two resultant topologies for our data set. The only taxa that change their place in the two competing trees are *Brienomyrus niger* and *Brienomyrus sp. 2* under maximum parsimony. For the EOR cost matrix, *Brienomyrus niger* is depicted either as a sister group of *Gnathonemus* + *Marcusenius,* or sister group of the *Brienomyrus* complex from Gabon. In the second case for the two topologies obtained with TS1TV1 and TS1TV1GP1, *Brienomyrus sp. 2* is shown either in the same position as depicted in figure 5 or grouped with the clade formed by *B. batesii, Brienomyrus sp. 4, Brienomyrus sp. 3* and *Brienomyrus sp. 5.* The strict consensus topology for all maximum parsimony cost matrices is depicted in figure 4 and reflects the behavior of both *B. niger* and *Brienomyrus sp. 2* by showing these two taxa in a polytomic topology. Because all maximum likelihood searches converged to a unique topology that is the same topology recovered in all Neighbor Joining estimations, as well as under TS1TV3 and TS1TV6 with parsimony (table 5), we believe our figure 5 is the best estimate for the phylogenetic relationships among the taxa studied.

Starting at the deeper nodes, our hypothesis fully supports morphological studies which place the genus *Gymnarchus* as the sister group of the Mormyridae [Taverne, 1971a]. The number of genera of the family Mormyridae utilized in our study is reduced, nevertheless molecular and morphological data are also in agreement regarding the phylogenetic position of *Petrocephalus* within the family. According to mitochondrial sequences and morphological data, *Petrocephalus* is the sister group of all Mormyrinae utilized in our study (*Gnathonemus, Marcusenius,* and *Brienomyrus*). This is also in agreement with Taverne's results, which indicated that *Petrocephalus* belongs to a separate sub-family at the base of the Mormyridae.

Our results depart from previous studies based on morphology [Taverne, 1971b] when we consider the relationships within the subfamily Mormyrinae. Taverne has divided the subfamily Mormyrinae into two major clades, based upon the presence or absence of a single character, the lateral ethmoid bone [Taverne, 1972], Under Taverne's hypothesis, *Gnathonemus* and *Brienomyrus* are placed in the same clade because both lack the lateral ethmoid bone, whereas *Marcusenius* is placed into a different subgroup because it retains the bone. Contrary to Taverne's view, our results suggest that *Marcusenius* and *Gnathonemus* are more closely related to each other than either is to *Brienomyrus.* This implies that the lateral ethmoid alone may not be a reliable character to infer phylogenetic relationships among mormyrids.

At the moment, there is no other phylogenetic hypothesis for this group that can either corroborate or contradict our results. The great majority of previous work on mormyrid relationships are based on overall phenetic similarity [Taverne, 1972; Bigorne, 1990] and do not apply phylogenetic or cladistic reasoning. The only exception is the work of Agnèse and Bigorne [1992] which utilized genetic distances calculated on the basis of enzyme variability in 16 loci. Although Agnèse and Bigorne did not use an outgroup for comparison, their measures of genetic similarity resulted in a dendrogram depicting *Hippopotamyrus, Pollimyrus,* and *Mormyrops* as closely related to each other and relatively distant from *Petrocephalus.* Furthermore, according to their most parsimonious phylogenetic hypothesis, *Hippopotamyrus* and *Pollimyrus* (both having the lateral ethmoid) are more closely related to *Mormyrops* (without lateral ethmoid) than they are to *Marcusenius* (which also has the lateral ethmoid). These results support our suggestion that Taverne's conclusions regarding relationships between the genera, derived from the presence or absence of the lateral ethmoid, may have to be reexamined. The lateral ethmmoid may have been lost or gained independently within the Mormyrinae more than once.

The mitochondrial DNA results also contradict Taverne by suggesting that the genus *Birenomyrus* is not a monophyletic group. According to our findings, *Brienomyrus brachyistius* collected in West Africa represents a different lineage from the *Brienomyrus* from Gabon. Such a result was surprising, mainly because the external morphology of the West African and Gabon representatives is similar enough to confuse systematists and field researchers. However, DNA sequences suggest that these morphological similarities must be due to either parallel or convergent evolution or, even more probably, due to retention of ancestral characters in the Gabon species complex.

Recently, Bigorne [1989], in a revision of the *Brienomyrus,* moved *B. batesii* [Boulenger, 1906] from the genus *Hippopotamyrus* to the genus *Brienomyrus.* Given its close

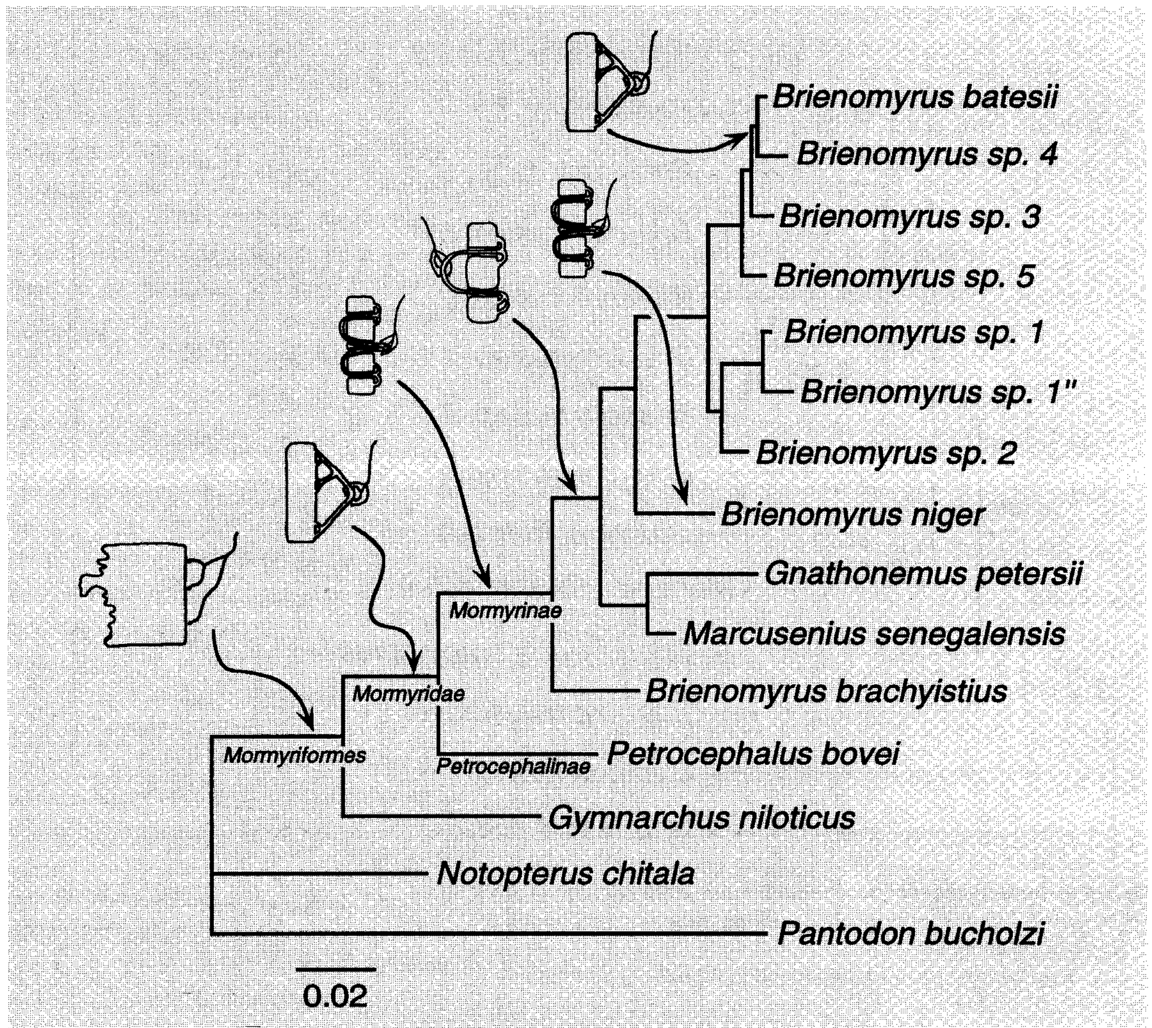

Fig. 5. Phylogenetic hypothesis for the species used in this study derived from molecular data using the maximum likelihood (ML) method. Ten searches with randomized addition of taxa produced this single topology. The exact same topology was obtained when we used twelve different methods for distance correction with the neighbor-joining approach and for MP under TS1TV1 and TS1TV6 (see table 5). The bars depict branch lengths for the ML tree. The morphologies of the electrocytes for each lineage are depicted as insets.

Another interesting aspect of mormyriform sequences, infrequently addressed in the molecular evolution literature, is the difference between species in base compositions expressed as a percentage for each nucleotide. This is especially so when considering the total number of aligned bases compared to the subset of phylogenetically informative sites. Considering just the informative sites, the outgroup *Pantodon* departs considerably from the average composition for the mormyrids by having an excess of adenine and thymine, and a depletion of cytosine and guanine. Also conspicuous are the variations in *Notopterus, Petrocephalus* and *Gymnarchus* (see underlined values in table 3). The fact that base compositions start to diverge as we go from within the subfamily Mormyrinae to Mormyriformes, and then to Osteoglossomorpha, indicates some as yet unclear tendency associated with variable/informative sites in the sequences. The causes of such variation still need further research. Additional studies specifically addressing this phenomena in different genes and organisms may help us to adjust our models for base substitutions in our phylogenetic algorithms.

Since there is no single best model that realistically accounts for all the substitutional and compositional bias found in the 12S and 16S ribosomal RNAs, we used sev-

relationship to the *Brienomyrus* in this study, we agree with this conclusion.

Our hypothesis, depicted in figure 4 and 5, also implies that the Gabon complex shares an immediate common ancestor with *Brienomyrus niger.* The clade formed by *Brienomyrus niger* plus the species complex of Gabon is a monophyletic clade that does not include the *Brienomyrus brachyistius* found in Nigeria. Taverne [1971b] placed *Brienomyrus niger* in the sub-genus *Brevimyrus.* Although we recognize the DNA affinity between *B. niger* and the six *Brienomyrus* from Gabon, in the absence of DNA data from the remainder of the named *Brienomyrus,* and in the absence of corroborating morphological data, we believe it premature to reclassify the Gabon specimens at this time. We propose to retain Taverne's nomenclature (i.e., *Brienomyrus*) until such time as a more thorough molecular and morphological study can be completed.

Implications for the Evolution of Electric Organs

In addition to the four classes of electric organs among those species we examined (table 2, fig. 3), two more are needed when considering other mormyrids. Type P_p organs, found in *Mormyrops,* have penetrating stalks with posterior innervation, and the EOD is inverted in polarity. Type DPNP organs have doubly-penetrating and non-penetrating stalks and are found in species of *Pollimyrus* and *Stomatorhinus* [Denizot et al., 1982; Bass, 1986a]. We use the phylogeny derived from molecular data to propose a model for the evolution of electric organs below (see fig. 5).

Type S. Only *Gymnarchus* has a type S electric organ, which is a flattened disk with no stalk system, innervated on the posterior side. Among mormyriforms, it is unique in its morphology and in its head-positive monophasic wave-like discharge. The anatomy is most similar to that of the non-apteronotid wave-discharging gymnotiforms of South America, which are very distantly releated. Bennet [1971] demonstrated that the monophasic EOD of *Gymnarchus* arises from the action potential in the posterior face of the electrocyte, which is a smooth surface. The anterior face, which is highly convoluted, is passive: it does not fire a spike, because its surface area is so greatly increased by surface invaginations that the current through this face is largely capacitative. Bennett estimates the capacitance of the non-innervated face to be 50 times that of the innervated face and speculates that the enormous increase in capacitance serves to reduce the D.C. component of the overall EOD in what otherwise would be a monophasic discharge. We suggest that type S electrocytes are plesiomorphic in relation to other electrocytes for mormyriforms.

Type NP_p. Three species in this study had Type NP_p electrocytes: *Brienomyrus batesii, Brienomyrus sp. 4,* and *Petrocephalus bovei.* The electrocytes are flattened disks with a well-developed stalk system emerging from the posterior face. The stalks start small but increase in diameter and end in a large trunk where the electromotor neurons make synaptic contact. All fish with type NP_p electric organs lacked head-negative prepulses in their EODs. We agree with Bennett and Grundfest [1961], who concluded that P0 prepulses are caused by inward currents in the stalk system being directed headward at the point where the stalk penetrates through the electrocyte. For those with NP_p electrocytes, P0 is absent, while it is present for all those with P_a or DP_p electrocytes.

Some fish with NP_p electric organs had a strong P1 phase (type IIa), while others had a weak one (type IIb). We have not found an anatomical correlate to explain this difference, P1 is usually caused by the firing of the posterior face of the electrocyte, while P2 is the firing of the anterior face. Accordingly, for *B. batesii* the magnitude of the action potential in the posterior face must be severely reduced but large enough to stimulate the anterior face to fire. We expect that there must be some molecular or ultrastructural feature of the electrocyte that reduces the amplitude of the discharge in the posterior face in type IIb EODs.

Petrocephalus bovei occupies a basal position in the phylogenetic trees derived from mtDNA (fig. 4, 5), as well as in trees derived from osteological studies [Taverne, 1972]. All *Petrocephalus* that have so far been examined have type NP_p electric organs [Bass, 1986c]. Because the electrocytes in *Petrocephalus* are relatively simple, without penetrations and without variations in duration for species or sex, we conclude that type NP_p electrocytes in *Petrocephalus* are plesiomorphic for the family Mormyridae.

We also conclude that type NP_p electrocytes in *Brienomyrus* differ from the type NP_p electrocytes in *Petrocephalus.* Those in *Brienomyrus* have sex differences, variations in duration, and inflections and plateaus in the waveform that are not found in the *Petrocephalus* EODs.

Type P_a. Six mormyrids in this study had type P_a electrocytes with type IIIa or IIIb EODs. All species with penetrating stalks produce discharges with a distinct head-negative prepulse, P0, which can be less than 1% of the peak to peak height of the discharge, so that the discharge looks biphasic, or greater than 1%, in which case it looks triphasic. Bennett and Grundfest [1961], showed a correlation between the surface area of the penetration and the magnitude of P0. We concur with this corrrelation in every case. Since the three main components to the EOD are present in both type IIIa and IIIb EODs, and since the electric organs have the same

basic morphology, with minor differences in the sizes and numbers of stalks, thickness of anterior versus posterior faces, and number of penetrations, we believe the critical feature of the electric organ is the penetrating stalk morphology. Most of the fish with type P_a organs have sex differences in EODs (tables 2, 6), and there are anatomical correlates of some of these sex differences [Bass et al., 1986].

Because of the complexity of the penetrating stalk morphology and the added complexity of the sex differences, we conclude that type P_a electrocytes are the more derived condition compared to type NP_p as seen in *Petrocephalus.*

Type DP_p. Two species, *B. niger* and *B. brachyistius* have type DP_p electrocytes with a doubly-penetrating stalk system innervated on the posterior side. The first penetration occurs immediately near the site of innervation while the second penetration is similar to that of the typical P_a electrocyte. We see no electrical correlate of the double penetration. The EODs from these two species have the same triphasic features as those species with P_a electrocytes. Inspection of figure 3 suggests that DP_p electrocytes are intermediate stages in electrocyte evolution, between NP_p and P_a.

At this time, we do not include type P_p and type DPNP electrocytes in this evolutionary analysis, since none of the species we studied had this type of electric organ. It is noteworthy that type P_p electrocytes, which are simply inverted versions of P_a electrocytes, are so far known only in the genus, *Mormyrops.* But the extent to which different *Mormyrops* have inverted electrocytes is unclear. Bass [1986c] reports their presence in *Mormyrops zanclirostris,* and Gose and Szabo [1960] show inverted organs in *M. deliciosus,* but three sets of authors have found polymorphisms within single species of *Mormyrops,* where the populations are divided between anterior innervated, (P_a) and posterior innervated (P_p) morphs [Gosse and T. Szabo, 1960; Moller and Brown, 1990; C.D. Hopkins and P. Jacob, unpubl. observ.]. Gosse and Szabo worked with *M. deliciosus;* Moller and Brown worked with *M. curviceps;* and Hopkins and Jakob worked with *M. anguilloides,* but Bigorne [1989], suggested that these three names are synonyms of *M. anguilloides,* so this may all be a single species phenomenon. None of these studies could relate the differences between P_a and P_p fish to sex or developmental condition. Type DPNP electrocytes appear in both *Pollimyrus* and *Stomatorhinus,* which are phylogenetically distantly related according to Taverne.

Evolutionary and Ontogenetic Considerations of Electrocyte Morphology

The phylogeny derived from DNA analysis suggests an hypothesis for how the four types of electrocytes evolved (fig.5). (1) Type S electrocytes, found in *Gymnarchus,* appear to represent the primitive condition. We conclude this because *Gymnarchus* occupies a basal position on the phylogenetic trees in figures 4 and 5, because the type S electrocytes bears some resemblance to the multi-nucleated muscle cells from which they are derived [Dahlgren, 1914; Srivastava and Szabo, 1972], and because these stalkless cells are the simplest in design. (2) Type NP_p electrocytes, as see in all of the Petrocephalinae, appear to be an early intermediate stage in evolution but plesiomorphic for the Mormyridae. These electrocytes have a simple stalk system which is unadorned, lacking in penetrations, and lacking any evidence for sex differences. (3) The penetrating stalks seen in both type P_a and DP_p electrocytes seems to be a more derived condition within this group. Each has a complex system of penetrating stalks, well-developed sex differences, and highly complex waveforms differing widely in duration. It is unclear which of these two morphologies represents the more primitive condition, but we suggest in figure 5 that DP_p electrocytes are an intermediate stage for *B. brachyistius* while DP_p electrocytes in *B. niger* represent a reversion. (4) The occurrrence of two closely related species of *Brienomyrus* with type NP_p electrocytes in our study suggests that type NP_p electrocytes may also evolve as a reversion from P_a to an even simpler design.

The ontogeny of electrocytes suggests a mechanism for reversion, although the only papers on the ontogeny of type P_a electrocytes were studies done by Szabo [1960] on larval material collected in the field by J. Daget in the late 1950's studies by Denizot et al. [1978, 1982] were on laboratory-bred *Pollimyrus isidori,* a species with doubly penetrating and non-penetrating stalks (type DPNP). Szabo [1960] examined larvae of *Mormyrops deliciosus* and *Hyperopisus occidentalis,* two species with penetrating stalks (both type P_a). He found that electrocytes in very young fish go through a three-stage development, starting with simple stalkless electrocytes, progressing to electrocytes with a well-developed non-penetrating stalks, and ending as the adult with electrocytes with penetrating, anterior innervated stalks. By contrast, *Mormyrus rume,* a species with type NP_p electrocytes, develops first stalkless and later non-penetrating stalks, but it never goes through a stage with penetrating stalks. We have observed the development of *Brienomyrus brachyistius* electrocytes going from non-penetrating electrocytes with posterior innervation to doubly-penetrating electrocytes with posterior innervation [C.D. Hopkins, unpubl. observ.].

We speculate that type NP_p electrocytes in *Brienomyrus* evolved through paedomorphosis, reverting to an earlier stage in ontogeny simply by arresting development at an

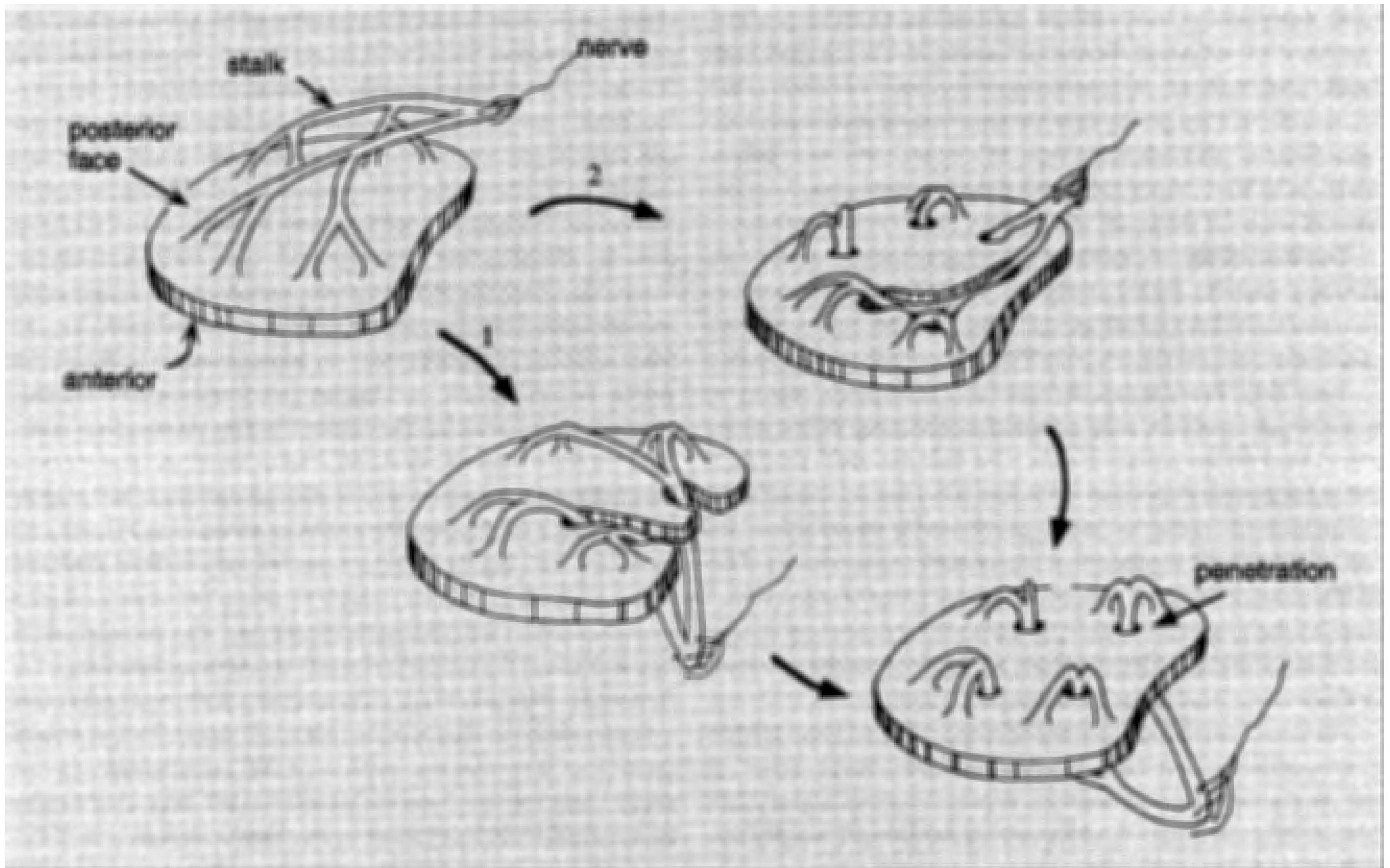

Fig. 6. An hypothesis for the ontogeny of P_a electrocytes among mormyriformes. The electrocyte first develops a non-penetrating stalk which is innervated on the posterior side of the electrocyte (above). It is proposed that the stalk system migrates, pulling the stalks through the edge of the electrocyte. Two possibilities are suggested: in (1) (middle) the proximal, thickened end of the stalk pulls through the edge of the electrocyte first, sealing around the stalk as it continues to migrate rostrally (below). In (2), the mid region of the stalk pushes through the electrocyte first, making a double penetration, and the proximal or thick end pulls through the edge later to make the P_a electrocyte shown below. Observations of larval electric organs in *Hyperopisus occidentalis* and *Mormyrops deliciosus,* both of which have P_a electrocytes in the adult electric organ but NP_p electrocytes in the same organ as juveniles [Szabo, 1960].

earlier stage. The actual mechanism for development of penetrating stalks is unknown, although it has fascinated microscopists ever since Fritsch [1891]. We suggest two possible mechanisms (fig. 6). In the first, the developing stalk, already innervated on the posterior side, migrates medially to the edge of the electrocyte, and then rostrally, so as to pull the smaller rootlets of the stalk through the edge of the cell, first as an invagination, and later as a discrete hole as the electrocyte body which closes around the stalk. In the second model, the thickened middle part of the developing stalk pushes through the face of the electrocyte from the posterior side to create a doubly-penetrating stalk system which is still innervated on the posterior side. Then the proximal end of the stalk migrates rostrally through the edge of the cell, pulling the innervated portion of the stalk entirely through to the anterior side.

According to the these models, arrested development may occur at two stages: failure to undergo any type of stalk migration at all causes a reversion to NP_p electrocytes, as seen in *Brienomyrus batesii* and *Brienomyrus sp. 4;* failure to undergo a complete migration of the large proximal ends of the stalks results in DP_p morphology as seen in *B. niger.* In neither case does this imply a loss of the molecular and physiological response to sex-steroids, which appears to be retained within the *Brienomyrus* in our samples, and also in several other *Brienomyrus* (see table 6) and would be quite independent of the mechanisms controlling the development of the penetrating stalk system.

Type NP_p Electrocytes in Other Genera

Analysis of electrocyte morphology from other mormyrids from the literature and from unpublished observations (table 6) supports the conclusion that type NP_p electric organs are an example of homoplasy, evolving several times (probably by paedomorphosis) in different mormyrid genera, although we do not have molecular data to independently assess the phylogeny. We find examples in both *Marcusenius,* and *Campylomormyrus.* For both of these genera, table 6 lists species with type NP_p and species with type P_a organs. We have not yet seen any other examples of DP_p electrocytes.

Some genera, such as *Mormyrus* and *Petrocephalus,* appear to have a conserved electric organ morphology (all NP_p), but much further study is needed to confirm this conclusion.

General Aspects of the Evolution of EOD Waveform Diversity

EOD waveforms are controlled by a number of critical features of the electric organ: the pattern of innervation, the geometry of the stalk system, the proliferation of the membrane surfaces, and the spatial distribution of ion channels in the electrocyte membranes. But EODs are also a type of behavior, and as such are under intense selection pressure for a variety of functions. At least two should be noted.

First, EODs are very probably adapted to the type of the micro habitat in which each fish lives, as a mechanism to maximize electrolocation capabilities and efficiency [Hopkins, 1974a; Hopkins and Heiligenberg, 1978; Heiligenberg and Bastian, 1980]. Vegetation, type of bottom, and depth are possible selective factors affecting the relative efficiency of a signal with, for example, a broader or narrower spectral frequency, or a lower or higher amplitude [Meyer, 1982; Hagedorn and Carr, 1985; Hopkins, 1988; von der Emde, 1990, 1992]. Dense vegetation, for instance, represents a more capacitative impedance than do rocks and mud, so fish living in areas with vegetation may opt for EODs with fast, high frequency characteristics which are preferable for sensing capacitative impedances. In order to avoid electrical jamming, mormyrids may opt for shorter duration pulses that avoid overlap compared to long duration pulses that overlap more frequently. Hopkins [1981] found an inverse correlation between inter-individual spacing and EOD duration among mormyrids in Gabon. All mormyrids have to cope with nonbiological background noise from lightning and other sources, and EOD waveforms may have evolved to differ from, or blend with the noise from lightning [Hopkins, 1981, 1986].

Second, EOD waveforms have probably evolved diversity as a mechanism of reproductive isolation allowing recognition of members of the same species and the distinction of males from females within a species [Crawford and Hopkins, 1989]. The diversity within the clade of *Brienomyrus* from Gabon is a good example. Not only do the EODs differ in waveform between species living in the same habitats, they also differ in the patterns of spikes evoked in electroreceptors, so they are coded differently by the nervous system [Hopkins, 1986], and they differ in the behavioral response they evoke in playback experiments [Hopkins and Bass, 1981; Hopkins, 1986]. Evidence accumulated from behavioral studies and from physiology of electrosensory responses indicates that EODs provide important cues for species and sex recognition among the mormyrids. Similar observations apply to the South American gymnotiforms [Hopkins, 1974b, 1974c, 1976; Hopkins and Heiligenberg, 1978; Hagedorn, 1988].

Phylogenetic Relationships among Brienomyrus

When we draw attention to the phylogenetic relationships among the *Brienomyrus* complex of Gabon, our mitochondrial DNA results are in agreement with independent data sets based on the electric organ discharges and electrocyte anatomy. The Gabon complex can be subdivided into several clades based on the type of EOD. These subgroups are fully supported by molecular data. Such general agreement provides a degree of reassurance in the phylogenetic signal of DNA sequences, as well as enhanced support for our hypothesis depicted in figures 4 and 5. The most surprising result from the molecular data is the finding that the genus *Brienomyrus* is not a monophyletic group. The type species for the genus, *Brienomyrus brachyistius,* appears to be a separate lineage from the *B. niger* + Gabon clades. Indeed, according to the molecular phylogeny, the clade of *Marcusenius* and *Gnathonemus* is closer to the Gabon *Brienomyrus* than either is to *B. brachyistius.* Ultimately this will demand a name change, but further analysis of all of the *Brienomyrus* will be necessary before splitting the genus into separate genera.

Concluding Summary. The use of molecular data supports data from morphological, ecological, behavioral, and physiological studies aimed at understanding phylogenetic relationships among the mormyriforms. The molecular data confirms the broad outline of mormyriform evolution, with *Gymnarchus* as the sister group to the family Mormyridae, and *Petrocephalus* as the sister group to the sub-family mormyrinae. Clearly, this study is only a beginning. Additional studies will be needed of critical genera such as *Mormyrus, Mormyrops, Hippopotamyrus, Marcusenius, Pol-*

Table 6. Data on electric organ types from mormyriforms, compiled from the literature, sorted by genus. Three genera (*Brienomyrus, Campylomormyrus,* and *Marcusenius*) have electric organs with both penetrating stalks (P_a– single border) and non-penetrating stalks (NP_p– double border), suggesting that paedomorphic reversion occurred more than once within the Mormyridae. The nomenclature based on Taverne's [1972] phylogeny

Genus	Species	EO type	EOD type	Sex diff.	Author(s)
Gymnarchus	niloticus	S	I	?	Dahlgren, 1914; Bennett, 1971
Petrocephalus	bovei	NP_p	IIa	no	Alves-Gomes & Hopkins
	sp. 1 (L.E.)	NP_p	IIa	no	*
	sp. 2 (S.E.)	NP_p	IIa	no	*
	sp. 3	NP_p	IIa	no	Bass, 1986c
Mormyrus	rume	NP_p	IIa	yes	Bennet & Grundfest, 1961; Crawford & Hopkins 1990; *
	subundulatus	NP_p	IIa	?	*
	kannume (=oxyrhynchus)	NP_p	?	?	Ogneff, 1898; Schlichter, 1906
	hasselquistii	NP_p	IIa	?	Fessard, 1958; *
Hyperopisus	bebe	P_a	IIIb	yes	*
	occidentalis	P_a	IIIb	?	Szabo, 1962
Hippopotamyrus	pictus	P_a	IIIb	yes	*
	psittacus	P_a	IIIb	?	*
Marccusenius	senegalensis	P_a	IIIa	yes	Alves-Gomes & Hopkins
	conicephalus	P_a	IIIb	?	Bass, 1986c
	furcidens	P_a	IIIa	?	*
	ussheri	P_a	IIIa	?	*
	cyprinoides	P_a	?	?	Ogneff, 1898
	paucisquamatus	NP_p	IIa	yes	Bass, 1986c
	moorii	NP_p	IIa	?	*
Pollimyrus	isidori	DPNP	IIa	yes	Bass et al., 1986
	petricolus	DPNP	IIIb	yes	*
	adspersus	DPNP	IIa	yes	*
	marchei	NP_p	IIa	?	*
Brienomyrus	brachyistius	DP_p	IIIb	yes	Alves-Gomes & Hopkins
	niger	DP_p	IIIb	yes	*
	sp. 1	P_a	IIIa	no	Bass, 1986c; Alves-Gomes & Hopkins
	sp. 2	P_a	IIIb	yes	Bass, 1986c; Alves-Gomes & Hopkins
	sp.3	P_a	IIIa	yes	Bass, 1986c; Alves-Gomes & Hopkins
	sp. 5	P_a	IIIb	yes	Bass, 1986c; Alves-Gomes & Hopkins
	sp. 4	NP_p	IIa	yes	Bass, 1986c; Alves-Gomes & Hopkins
	batesii	NP_p	IIb	yes?	Bass, 1986c; Alves-Gomes & Hopkins
	curvifrons	NP_p	IIa	yes	Bass (1986c); *
	longicaudatus	NP_p	IIa	yes	*
	kingsleyae	NP_p	IIa	?	Bass (1986c); *
Gnathonemus	petersii	P_a	IIIb	yes	Bell et al., 1976
Stomatorhinus	corneti	DPNP	IIIb	yes	Bass, 1986c
Boulengeromyrus	knoepffleri	NP_p	IIa	yes	Bass, 1986c
Campylomormyrus	tamandua	P_a	IIIb	?	Bennet, 1971
	compressirostris	NP_p	IIa	?	Bennett and Grundfest, 1961
Isichthys	henryii	NP_p	IIa	yes	Bass, 1986c;*
Paramormyrops	gabonensis	NP_p	IIa	yes	Bass, 1986c; *
Ivindomyrus	opdenboschi	NP_p	IIa	yes	Bass, 1986c; *
Mormyrops	zanclirostris	P_p	inverted IIIa	yes	Bass, 1986c; *
	deliciosus	P_a & P_p	IIIa and inverted IIIa	?	Gosse & Szabo, 1960
	curviceps	P_a & P_p	IIIa and inverted IIIa	?	Moller & Brown, 1990
	anguilloides	P_a & P_p	IIIa and inverted IIIa	?	*

* Unpublished observations of C.D. Hopkins, J.D. Crawford, and other colleagues.

limyrus, Campylomormyrus, Stomatorhinus, Paramormyrops, and *Boulengeromyrus,* before we can obtain a complete picture of mormyriform evolution.

The use of molecular data provides a framework for understanding the evolution of the electric organ. *Gymnarchus* appears to have the simplest and most plesiomorphic electric organ, with a drum-shaped electrocyte which lacks a stalk system. *Petrocephalus* appears to have the next most primitive electric organ, with electrocytes that have a simple stalk system which is non-penetrating and lacks sex differences. *Brienomyrus* and several other genera have electric organs with electrocytes that have advanced features, such as singly or doubly-penetrating stalks and a variety of sex differences. In *Brienomyrus* some species appear to have reverted to a simpler electrocyte with non-penetrating stalks, possibly through the mechanism of paedomorphosis.

The genus *Brienomyrus* stands as a remarkable example of speciation among mormyrids and as a reminder of the importance of electric organ discharges as a mechanism for species recognition and for reproductive isolation. Much remains to be learned about the mechanism of speciation in this diverse group of tropical rain forest electric fishes.

Acknowledgments

We dedicate this paper to the memory of Walter Heiligenberg, our friend, colleague, and mentor. We thank Margo Haygood for kindly providing laboratory space for DNA sequencing. The sequences of the four non-mormyrinae genera were generated by JA-G at Axel Meyer's (SUNY, Stony Brook) laboratory, to whom we express our appreciation. We thank Matthew Friedman for help with field work in Gabon, for computer programs for logging EODs, and helpful comments on the manuscript. We also thank Garry Harned and Mei-Hope Le for help with the histological preparation; Philippe Jacob, for help recording EODs in Mali; Masashi Kawasaki and Guillermo Ortí for sending tissue samples, Marshall Hopkins for artwork; and Rebecca Rundell, Garry Harned, and Wilhelm Harder who made helpful comments on the manuscript. This project was supported by NIMH grant MH37972 to CDH, by an NSF grant INB 91-06705 to Walter Heiligenberg, and by a Bazilian INPA-CNPQ doctoral scholarship to JA-G. CDH especially thanks Drs. Alain Georges of the CIRMF (Centre International de Recherches Médicales, Franceville) and Paul Posso of the Institut de Recherche en Écologie Tropicale in Makokou for the opportunity to work in Gabon.

Note Added in Proof

While this paper was in press, a new publication appeared [van der Bank and Kramer, 1996] in which results from an allozyme data set, recordings of electric organ discharges, habitats, and food preferences were used to infer a partial phylogeny of mormyrids. We include the reference here for the sake of completeness. Only three genera are common to their paper and ours, so it is difficult to compare the results of our two studies, however, the position of *Petrocephalus* in their proposed phylogeny (their fig. 4) is highly discordant from ours and also Taverne's [1972], which was derived from osteology. In our study we emphasize that EOD waveforms can be highly variable, even within a class of anatomically similar electric organ types. Further, we expect that competition between closely related clades can drive habitat and food preferences apart and may therefore not be a good source for synapomorphies. Finally, it may be arguable how efficient allozyme data can be as a genetic marker for distantly related fishes, given the variation observed at the level of populations. We therefore question the suitability of a number of the characters used by these authors and propose that the discordant position of *Petrocephalus* in their study may be related to the types of characters selected to estimate phylogeny.

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Appendix I

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Brienomyrus sp.1      GTAAACCTTGATGATAAATTACAAATATCATCCGCCAGGGGACTACAAGCGCATCGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Brienomyrus sp.1'     GTAAGCCTTGATGATAAATTACAAATATCATCCGCCAGGGGACTACAAGCGCATCGCTCGAAACCCAACGGACTTGGGGGTGCCCCACACCCACCTAGAG
Brienomyrus sp.2      GTAAACCTTGATGATAAACTACAAATATCATCCGCCAGGGGACTACAAGCGCATCGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Brienomyrus sp.3      GTAAACTTTGATGATAAGTTACAAATATCATCCGACAGGGGACTACAAGCGCATCGCTCGA?ACCCAAAGGACTTGGCGGTGCCCCACACCCACCTAGAG
Brienomyrus sp.4      GTAAACTTTGATGATAAGTTACAAATATCATCCGCCAGGGGACTACAAGCGCATCGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Brienomyrus sp.5      GTAAACTTTGATGATAAGTTACAAATATCATCCGCCAGGGAACTACAAGCGCATCGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Brienomyrus bat.      GTAAACTTTGATGATAAGTTACAAATATCATCCGCCAGGGGACTACAAGCGCATCGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Brienomyrus nig.      GTAAACCTTGATGATAAACTACAAATATCATCCGCCAGGGAACTACAAGCGTATCGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Marcusenius sen.      ATAAACCTTGATGATAAACTACAAATGTCATCCGCCAGGGAACTACAAGCGCATTGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Gnathonemus pet.      ATAAACTTTGATGATAGACTACAAATATCATCCGCCAGGGAACTACAAGCGTATCGCTCGAAACCCAACGGACTTGG?GGTGCCCCACACCCACCTAGAG
Brienomyrus bra.      ATAAACCTTGATGATAAATTACAAATATCATCCGCCAGGGAACTACAAGCGCATTGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Petrocephalus bov.    ATAAACTTTGATAATAAGATACCCATATTATCCGCCAGGGAACTACAAGCGCATTGCTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Gymnarchus nil.       ATAAAC?TTG?CAATAATGTACAAATATTATCCGCCAGGGAACTACAAGCGCATTGTTCGAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Notopterus chi.       ATAAACCTTGATAATAAAATACAAATATTATCCGCCAGGGAACTACAAGCGCATTGCTTAAAACCCAACGGACTTGGCGGTGCCCCACACCCACCTAGAG
Pantodon buc.         GTAAACTTTGATAGTAAAATACAATTACTACCCGCCAGAGTACTACAAGCGAATAGCTCGAAACCCAACGGACTTGGCGGTGCCCCAGACCCACCTAGAG

Brienomyrus sp.1      GAGCCTGTTCTATAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCCTCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CTCAAC
Brienomyrus sp.1'     GAGCCTGTTCTATAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCCTCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CTCAAC
Brienomyrus sp.2      GAGCCTGTTCTATAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCCTCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CTCAAC
Brienomyrus sp.3      GAGCCTGTTCTATAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCCTCTTCAG?CTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CTCAAC
Brienomyrus sp.4      GAGCCTGTTCTATAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCCTCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CTCAAC
Brienomyrus sp.5      GAGCCTGTTCTATAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCCTCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CTCAAC
Brienomyrus bat.      GAGCCTGTTCTATAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCCTCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CTCAAC
Brienomyrus nig.      GAGCCTGTTCTATAACTGACAATCCCCGTTCAACCTCACCACTCCTAGCCCCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGGATT-AAC
Marcusenius sen.      GAGCCTGTTCTATAACTGACAATCCCCGTTCAACCTCACCACCCCTAGCCCCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGGATC-AAC
Gnathonemus pet.      GAGCCTGTTCTATAACTGACAATCCCCGTTCAACCTCACCACCCCTAGCCCCTTCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGGATC-AAC
Brienomyrus bra.      GAGCCTGTTCTATAACTGACAATCCCCGTTCAACCTCACCACTCCTAGCCCCATCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGGACT-AAC
Petrocephalus bov.    GAGCCTGTTCTGTAACTGACAATCCCCGTTTAACCTCACCACCCCTAGCTTAATCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGG-CCCAAT
Gymnarchus nil.       GAGCCTGTTCTATAACTGACACTCCCCGTTTAACCTCACCATCCCTAGCCAAATCAGTCTATATACCACCGTCGCAAGCTCACCCTATGAAGGAC-CAAC
Notopterus chi.       GAGCCTGTTCTATAACTGACAATCCCCGTTAAACCTCACCACTTCTAGCCCTACCAGTCTATATACCACCGTCGTAAGCTCACCCTGTGAAGGAAACAAC
Pantodon buc.         GAGCCTGTTCTATAACTGACACCCCCCGTTAAACCTCACCACTCCTAGCCAACCCAGTGTATATACCACCGTCGCACGCTCACCCTATGAAGG-TCTAAT
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Appendix I (Continued)

```
Brienomyrus sp.1      AGTAAGCAAGATGGGCACACCCCAAAACGTCAGGTCGAGGTGTAGCATATGGAGTGGGAAGAAATGGGCTACATTTTCTGCCCA-CAGAACACTAACGGA
Brienomyrus sp.1'     AGTAAGCAAGATGGG?ACACCCCAAAACGTCAGGTCGAGGTGTAGCATATGGAGTGGGAAGAAATGGGCTACATTTTCTG?CCA-CAGAACACTAACGGA
Brienomyrus sp.2      AGTAAGCAAGATGGGCACAACCCAAAACGTCAGGTCGAGGTGTAGCATATGGAGTGGAAAGAAATGGGCTACATTTTCTGCCCA-CAGAACACTAACGGA
Brienomyrus sp.3      AGTAAGCAAGATGGGCAAAACCCAAAACGTCAGGTCGAGGTGTAGCATATGGAGTGGGAAGAAATGGGCTACATTTTCTGCCCA-CAGAACACTAACGGA
Brienomyrus sp.4      AGTAAGCAAGATGGGCAAAACCCAAAACGTCAGGTCGAGGTGTAGCATATGGAGTGGGAAGAAATGGGCTACATTTTCTGCCCA-CAGAACACT-ACGGA
Brienomyrus sp.5      AGTAAGCAAGATGGGCAAAACCCAAAACGTCAGGTCGAGGTGTAGCATATGGAGTGGGAAGAAATGGGCTACATTTTCTGCCCA-CAGAACACTAACGGA
Brienomyrus bat.      AGTAAGCAAGATGGGCAAAACCCAAAACGTCAGGTCGAGGTGTAGCACATGGAGTGGGAAGAAATGGGCTACATTTTCTGCCCA-CAGAACACTAACGGA
Brienomyrus nig.      AGTAAGCAAGATGGGCACAACCCAAAACGTCAGGTCGAGGTGTAGCATATGGAGTGGGAAGAAATGGGCTACATTTTCTATCTA-CAGAACATT-ACGGA
Marcusenius sen.      AGTAAGCAAGATGGGCACAACCCAAAACGTCAGGTCGAGGTGTAGCACATGGAGTGGAAGAAATGGGCTACATTTTCTGCCTA-CAGAACACC-ACGGA
Gnathonemus pet.      AGTAAGCAAGATGGGCACAACCCAAAACGTCAGGTCGAGGTGTAGCACATGGGGTGGGAAGAAATGGGCTACATTTTCTGCCTA-CAGAACACC-ACGAA
Brienomyrus bra.      AGTAAGCAAAATGGGCACAACCCAAAACGTCAGGTCGAGGTGTAGCACATGGAGTGGGAAGAAATGGGCTACATTTTCTGCCTA-CAGAATATY-ACGAA
Petrocephalus bov.    AGTAAGCAAGATGGGCACAACCCAACACGTCAGGTCGAGGTGTAGCACATGGGGCGGGAAGAAATGGGCTACATTTTCTG?CCAACAGAATACCAACGAA
Gymnarchus nil.       AGTAAGCAAAATGGGCACAACCCAGCACGTCAGGTCGAGGTGTAGCACATGGGATGGAAAGAAATGGGCTACATTCTCTGCCTAACAGAGTAC--ACGAA
Notopterus chi.       AGTAAGCAAGATGGGCACAGCCCAAAACGTCAGGTCGAGGTGTAGCACATGAAGTGGGAAGAAATGGGCTACATTTTCTGAA-A-CAGAATAT--ACGAA
Pantodon buc.         AGTATGCAAAATGATTAAAACTCAGCACGTCAGGTCGAGGTGTAGCGAATGGAGCGGGAAGAAATGGGCTACATTTTCTAAC-A-CAGAAAAC--ACGGA

Brienomyrus sp.1      CAACGTCATGAAACCTGACGTTTAAAGGAGGATTTAGCAGTAAAAAGAAAATAGAGAGTT-CTTTTGAAGCCGCCTGTCCGGTG-ACAATAAGTTTAACG
Brienomyrus sp.1'     CAACGTCATGAAACCTGACGTTTAAAGGAGGAT?TAGCAGTAAA?AGAAAATAGAGAGTT-CTTTTGAAGCCG?CTGTCCGGTG-ACAATAAGTTTAACG
Brienomyrus sp.2      CAACGTCATGAAACCTGACGTTTAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCCGCCTG?CCGGTG-ACAATAAGTTTAACG
Brienomyrus sp.3      CAACGTCATGAAATCTGGCGTTCAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCCGCCTGTCCGGTG-ACAATAAGTTTAACG
Brienomyrus sp.4      CAATGTCATGAAATCTGACGTTCAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCCGCCTGTCCGGTG-ACAATAAGTTTAACG
Brienomyrus sp.5      CAACGTCATGAAACCTGACGTTCAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCCGCCTG?CCAGTG-ACAATAAGTTTAACG
Brienomyrus bat.      CAACGTCATGAAATCTGACGTTCAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTTGCTTTTGAAGCCGCCTGCCCGGTG-ACAATAAGTTTAACG
Brienomyrus nig.      CGACGTCATGAAACCTGCCGTCCAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCCGCCTGCCCAGTG-ACAATAAGTTTAACG
Marcusenius sen.      CAACGCCATGAAACTTGCCGTTTAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCCGCCTGCCCAGTG-ACAATAAGTTTAACG
Gnathonemus pet.      CAACGCCATGAAATCTGTCGTTCAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCTGCCTG?CCAGTG-ACGATAAGTTCAACG
Brienomyrus bra.      CAACGTCATGAAA-CTGCCGTTTAAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGAGTT-CTTTTGAAGCCGCCTGCCCAGTG-ACGATAAGTTTAACG
Petrocephalus bov.    TAACGACATGAAA-CTGTCGTTTAAAGGAGGATTTAGCAGTAAAAAGAAAATAGAGAGTT-CTTTTGAAGCCGCCTGCCCAGTG-ACAATAAGTTTAACG
Gymnarchus nil.       CACTACCATGAAA-CTGGAAATTGAAGGAGGATTTAGCAGTAAAAAGAAAATAGAGAGTT-CTTTTGAAGCTGCCTGCCCAGTG-ACAATAAGTTAAACG
Notopterus chi.       TATCACCATGAAACCTGGTGCTCGAAGGAGGATTTAGCAGTAAAAAGAAAACAGAGCGTT-CTTTTGAAACCGCCTGCCCAGTG-ACCAAGAGTTAAACG
Pantodon buc.         TAATTCTATGAAA-CTAGAATTTCA?GGGGGATTTAGCAGTAAAAAGAAAATAGAGTGTT-CTATTGAAACCGCCTGTCCAGTGACATATAAGTTAAACG

Brienomyrus sp.1      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCCCCTGTCTCCTACTTCA
Brienomyrus sp.1'     GCCGCGGTATTTTAACCGTGGTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGTCCCCCTGTCTCCTACTTCA
Brienomyrus sp.2      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCCCCTGTCTCCTACTTCA
Brienomyrus sp.3      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCCCCTGTCTCCTACTTCA
Brienomyrus sp.4      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCTCCTGTCTCCTACTTCA
Brienomyrus sp.5      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCCCCTGTCTCCTACTTCA
Brienomyrus bat.      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCCCCTGTCTCCTACTTCA
Brienomyrus nig.      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGA?AGGTGTCACGAGGGCCCTCCTGTCTCCTACTTCA
Marcusenius sen.      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCTCCTGTCTCCTACTTCA
Gnathonemus pet.      GCCGCGGTATTTTAACCGTG?TAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCACCTGTCTCCTACTTCA
Brienomyrus bra.      GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCTCCTGTCTCCTACTTCA
Petrocephalus bov.    GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCTCCTGTCTCCTACTCCA
Gymnarchus nil.       GCCGCGGTATTTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGTGTCACGAGGGCCCTACTGTCTCCTCCTTCC
Notopterus chi.       GCCGCGGTATCTTAACCGTGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCTGTATGAAAGGCATTACGAGGGCCCAACTGTCTCCTACTTCA
Pantodon buc.         GCCGCAGTATTTTAACCGCGCTAAGGTAGCGTAATCACTTGTCTTTTAAATGAAGACCCGTATGAAAGGCACCACGAGGG?CCTACTGTCTCCTTCCCCA

Brienomyrus sp.1      AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAAGAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACTAATCAACCAATCGTGCCTAACAG
Brienomyrus sp.1'     AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAAGAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACTAATCAACCAATCGTGCCTAACAG
Brienomyrus sp.2      AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAAGAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACTAACCAACCAATCGTGCCTAACAG
Brienomyrus sp.3      AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAATAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACGAATCAACCAATCGTGCCTAACAG
Brienomyrus sp.4      AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAACAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACGAATCAACCAATCGTGCCTAACAG
Brienomyrus sp.5      AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAATAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACGAATCAACCAACCGTGCCTAACAG
Brienomyrus bat.      AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAACAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACGAATCAACCAATCGTGCCTAACAG
Brienomyrus nig.      AGTCTGTGAAATTGATCTGCCCGTGCAGAAGCGGGCATAATAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACCAATCAACCAATCGTGCCTAACAG
Marcusenius sen.      AGTCTGTGAAATTGATCTGCCCGTGCAGAA?CGGGCATAACAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACCAATCAATCAACCGTGCCTAACAG
Gnathonemus pet.      AGTCTGTGAAATTGATCTGCCCGTGCAGAAGCGGGCATAACAACATAAG?CGAGAAGACCCTGTGGAGCTTAAGACCAATCAATCAACCGTGCCTAACAG
Brienomyrus bra.      AGTCTGTGAAATTGATCTGCCCGTGCAGAAGCGGGCATAACAACATAAGACGAGAAGACCCTGTGGAGCTTAAGACTAATCAACCAACCGTGCCTAGAAG
Petrocephalus bov.    AGTCTATGAAATTGATCTGCCCGTGCAGAAGCGGGCATACCCCCATAAGACGAGAAGACCCTGTGGAGCTTAAGACTA-T-AACCAATCATGCCTAGTAA
Gymnarchus nil.       AGTCTGTGAAATTGATCTACCCGTGCAGAAGCGGGTATTCTAACATAAG?CGAGAAGACCCTGTGGAGCTTAAGATTA-TTAACCAATCACGCCTAGACA
Notopterus chi.       TGTCAGTGAAATTGATCTGTCCGTGCAGAAGCGGACATATTTACATAAGACGAGAAGACCCTGTGGAGCTTAAGAT-ACTAAATTAACCGCGCCTAGGAA
Pantodon buc.         AGTCTATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAATACATAAGACGAGAAGACCCTATGGAGCTTTAGAC-A-TAAGTCAATCATGCCTAAACA
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Appendix I (Continued)

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Brienomyrus sp.1      CCTATACACCCAATAGGAGATAAAAAGCTAAACAAGCATAACGACCCCTGATTG-AA-CTGTCTTCGGTTGGGGCGACCATGGGGGACAAAAAAGCCTCC
Brienomyrus sp.1'     CCTATACACCCAATAGGAGATAAAAAGCTAACCAAGCATAACGACCCCTGATTG-AA-CTGTCTTCGGTTGGGGCGACCATGGGGGACAAAAAAGCCTCC
Brienomyrus sp.2      CCTATCCCCCTAATAGGAAATAAAAAGCTAAACAAGCATAACGACCTGTGATTGTAA-CTGTCTTCGGTTGGGGCGACCGTGGGGGATAAAAAAGCCTCC
Brienomyrus sp.3      CCAATCCCCCTAACAGGAAATAAAAAGCTAAACAAGCATAACGACCCGTGATTG-AA-CTGTCTTCGGTTGGGGCGACCATGGGGGATAAAAAAGCCTCC
Brienomyrus sp.4      CTAATCCCCCTAACAGGAAATAAAAAGCTAAACAAGCATAACGACCCATGATTG-AA-CTGTCTTCGGTTGGGGCGACCATGGGGGATAAAAAAGCCTCC
Brienomyrus sp.5      CCCATCCCCCTAACAGGAAATAA-AAGCCAAACAAGCATAACGACCCATGATTG-AA-CTGTCTTCGGTTGGGGCGACCATGGGGGATAAAAAAGCCTCC
Brienomyrus bat.      CC-ATCCCCCTAACAGGAAATAAAAAGCTAAACAAGCATAACGACCCATGATTG-AA-CTGTCTTCGGTTGGGGCGACCATGGGGGATAAAAAAGCCTCC
Brienomyrus nig.      CCTATCAACCTAACAGGAAATAAGAAAC-AAGCAAGCATAACGACTTATGGTTG-AA-CTGTCTTCGGTTGGGGCGACCGTGGGGGATAAAAAAGCCTCC
Marcusenius sen.      CCAATCGCCCTAACAGGAAATAAAAAGCTAAACAAGCATAACGACTCATGATTG-AATCTGTCTTCGGTTGGGGCGACCATGGAGGACAAAAAAGCCTCC
Gnathonemus pet.      CCAACACCCC-AACAGGGAATAAAAAGCTAAGCAAGCATAACGAGTTATAATTA-AA-C-GTCTTCGGTTGGGGCGACCATGGAGGACAGAAAAGCCTCC
Brienomyrus bra.      CTCTCTGCCC-AACAGCAA-CAAAAAGCCAAACAAGCATAACGAACTATGGTTG-AA-CTGTCTTCGGTTGGGGCGACCATGGGGGACAAAAAAGCCCCC
Petrocephalus bov.    CCTACCCACC-AAACGGCA-CAAAAGGTCCAATAAGCTTAATGACA-ATGGTT-T-A-CTGTCTTCGGTTGGGGCGACCATGGGGGACAGAAAAGCCCCC
Gymnarchus nil.       G----CCATC-AAAC-----CAGAAAGC-AAACAAGCATA?CGACA-ATGGAT-TAATC-ATCTTCGGTTGGGGCGACCATGGAGGA?AAATAAGCCTCC
Notopterus chi.       CTAACCAGCCC-AAGGGCCCAAACACC--A?ACAAGCATAGCGACC-ATAATTA-AACTTATCTTCGGTTGGG?CGACCATGGAGGATAAAAAAGCCTCC
Pantodon buc.         CT---------ATA--AATAAATAAC--AAAGCATAATGAC-A---CTGACC-CA-CTTGTCTTCGGTTGGGGCGACCAAGGAGGAAAACACAGCCTCC

Brienomyrus sp.1      AAGAGGAAACAGGGGACCAGTTCAACCGATCCC-TAAGAGCCAAGAGCCACCGCTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGAC-ATTAGC
Brienomyrus sp.1'     AAGAGGA?ACAGGGGACCAGTTCAACCGATCCC-TAAGAACCAAGAGCCACCGCTCTAAGCAACAGAAAACTCTGGCCAATAATGATCCAGAC-ATTAGC
Brienomyrus sp.2      AAGAGGAAACAGGGGACCAGTTCAACCGATCCC-TAAGAGCCAAGAGCCACCGCTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGGC-ATTAGC
Brienomyrus sp.3      AAGAGGAAACAGGGGACCAGTTCAACCGATCCC-TAAGAGCCAAGAGCCACCGCTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGGC-ATTCGC
Brienomyrus sp.4      AAGAGGAAACAGGGGACCAGTTCAACCGATCCC-TAAGAGCCAAGAGCCACTACTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGGC-ACTCGC
Brienomyrus sp.5      AAGAGGAAACAGGGGACCAGTTCAACCGATCCC-TAAGAGCCAAGAGCCACCGCTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGGC-ATCCGC
Brienomyrus bat.      AAGAGGAAACAGGGGACCAGTTCAACCGATCCC-TAAGAGCCAAGAGCCACCGCTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGGC-ATTCGC
Brienomyrus nig.      AAGAGGAAGCAGGGGACCAGTTTAACCGATCCC-TAAGAGCCGAGAGCCACTACTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGGC-ACTAGC
Marcusenius sen.      AAGAGGAAGCAGGGGACCAGTTCAATTGATCCC-TAAGAGCTAAGAGCTACCACTCTAAGCAACAGAAAATTCTGACCAATAATGATCCAGGC-ACTAGC
Gnathonemus pet.      AAGAGGAAGCAGGGAACCAGTTTAATTGATTCC-TAAGAGCTAAGAGCCACCCCTCTAAGCAACAGAAAACTCTGACCAATAATGATCCAGAC-ACTAGC
Brienomyrus bra.      AAGAGGAAATAGGGGACCAGAT-AACTGATCCC-TAAGAGCCAAGAGCCACTACTCTAAGCAACAGAATACTCTGACCAATAATGATCCAGAC-ACAAGC
Petrocephalus bov.    AAGAAGAAGCAGGGGACCAATTACACCGATCCC-TAAGAGTTAAGAGCTACAACTCTAAACAACAGAAATATCTGACTAATAATGATCCAGG-AATAAAC
Gymnarchus nil.       AAGAGGAACCAGGGGGTCAATTACACAATACCCCTAAGAGCTAAGAGCCACATCTCTAAGCAACAGAGAAATCTGACCAATAATGACCCAGGTCCATGAC
Notopterus chi.       AAGAAGAAGCAGGGGATCAGTCACACAAT-CCC-TAAGAACCAAGAGCCACACCTCTAAGCAACAGAAAATTCTGACTAATAATGACCCAGGCCCA--GC
Pantodon buc.         TAGAAGAATTAA--GATTAATT-----AT-CTT-AAAGAACCGAGAGCTACTGCTCTAAGCAACAGAAAATTCTGACTACACA-GATCCGAAT---T-A-

Brienomyrus sp.1      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus sp.1'     CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus sp.2      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCAAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus sp.3      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus sp.4      CTGATCAGCGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus sp.5      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus bat.      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus nig.      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Marcusenius sen.      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCTCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Gnathonemus pet.      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCTCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Brienomyrus bra.      CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Petrocephalus bov.    CTGATCAACGGACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCCAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Gymnarchus nil.       CTGATCAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCTAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Notopterus chi.       CTGATTAACGAACCAAGTTACCCCAGGGATAACAGCGCAATCCTTTCCAAGAGCCCATATCGCCGAAAGGGTTTACGACCTCGATGTTGGATCAGGACAT
Pantodon buc.         TCGATTAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCTT?TTAAGAGCCCATATCGACAAAAGGGTTTACGACCTCGATGTTGG?TCAGGACAT

Brienomyrus sp.1      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Brienomyrus sp.1'     CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Brienomyrus sp.2      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Brienomyrus sp.3      CCTGGTGGCGAAAATTCTACCAAGGGTTCGGT
Brienomyrus sp.4      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Brienomyrus sp.5      CCTGGTGGCGAAAATTCTACCAAGGGT?CGTT
Brienomyrus bat.      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Brienomyrus nig.      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Marcusenius sen.      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Gnathonemus pet.      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Brienomyrus bra.      CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Petrocephalus bov.    CCTGGTGGCGAAAATTCTACCAAGGGTTCGTT
Gymnarchus nil.       CCTAGTGGCGAAAATTCTACCAAGGGTTCGTT
Notopterus chi.       CCTGGTGGCGAAAATTTTACCAAGGGTTCGTT
Pantodon buc.         CCTAGTGCCGAAAATTTTACTAAGGTTTCGTG
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