Biological Journal of the Linnean Society, 2003, **78**, 273–292. With 9 figures

Phylogenetic utility of the first two introns of the S7 ribosomal protein gene in African electric fishes (Mormyroidea: Teleostei) and congruence with other molecular markers

SÉBASTIEN LAVOUÉ[1,2], JOHN P. SULLIVAN[1] and CARL D. HOPKINS[1]*

[1]*Department of Neurobiology and Behavior, Cornell University, 263 Mudd Hall, Ithaca, NY, USA 14853*
[2]*Laboratoire d'Ichtyologie générale et appliquée, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75731 Paris cedex 05, France*

Received 20 May 2002; accepted for publication 6 November 2002

A series of recent molecular systematic studies of the African electric fishes (Mormyroidea) have challenged many aspects of their traditional taxonomy and precladistic hypotheses of their phylogeny. However, poor resolution of some interrelationships within the subfamily Mormyrinae in these studies highlights the need for additional data and analyses. Here we evaluate the phylogenetic information content of nucleotide sequences from the first two introns of the low-copy nuclear S7 ribosomal protein gene in 40 mormyroid species. Alignment of S7 sequences from 38 taxa within the subfamily Mormyrinae is non-problematic, but these are difficult to align with sequences of *Petrocephalus bovei* (Petrocephalinae) and *Gymnarchus niloticus* (Gymnarchidae), which we exclude from our analysis. There are no significant differences in base frequencies among these sequences and base compositional bias is low. Maximum parsimony (MP) analysis on the S7 dataset, designating *Myomyrus macrops* as the outgroup, generates a phylogenetic hypothesis for these taxa with a low level of homoplasy (RI = 0.87). We examine agreement between the S7 data with previously published mitochondrial (12S/16S, cytochrome *b*) and nuclear (rag 2) datasets for the same taxa by means of incongruence length difference tests and partitioned Bremer support (decay) analysis. While we find significant agreement between the S7 dataset and the others, MP analysis of the S7 data alone and in combination with the other datasets indicates two novel relationships within the Mormyrinae: (1) *Mormyrus* is the sister group to *Brienomyrus brachyistius* and *Isichthys henryi,* and (2) *Hippopotamyrus pictus* is the sister group of a clade, previously recovered, containing *Marcusenius senegalensis*. S7 data provide additional support for a number of clades recovered in the earlier molecular studies, some of which conflict with current mormyrid taxonomy. Inferred indels and a single inversion in the S7 fragment provide supplemental character support for many of these relationships. These phylogenetic results strengthen recent hypotheses concerning the evolution of electric organ structure in these fishes. The evolutionary characteristics of this nuclear marker and its phylogenetic utility in this group suggests that it could be widely useful for systematic studies at the subfamilial level in teleost fishes. *Biological Journal of the Linnean Society*, 2003, **78**, 273–292.

ADDITIONAL KEYWORDS: electric fishes – ILD – molecular evolution – PBS – systematics.

INTRODUCTION

The African families Mormyridae (18 genera, 180+ species) and Gymnarchidae (one monotypic genus) make up the Superfamily Mormyroidea, the largest group of freshwater electric fishes (Taverne, 1972). *Gymnarchus niloticus* occurs in the Niger and Nile basins; mormyrids are distributed throughout all African freshwaters except those in the northernmost Mahgreb and southernmost Cape provinces. Greatest mormyrid diversity and high levels of endemism occur in river and stream habitats within forested regions of the Congo, Lower Guinean and Upper Guinean ichthyofaunal provinces (Roberts, 1975). Recent discovery of many undescribed forms within the genus

*Corresponding author. E-mail: cdh8@cornell.edu

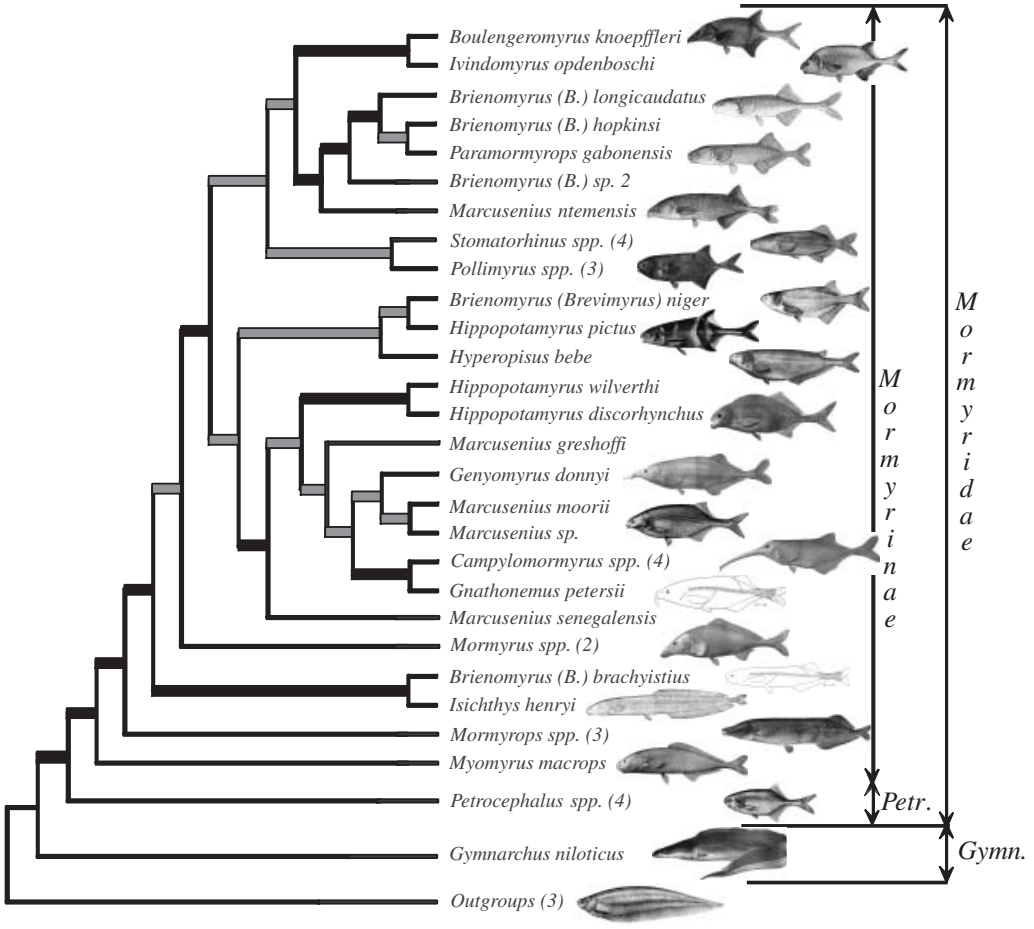

Figure 1. Proposed relationships of the mormyroid genera based on combined analysis of four DNA markers (partial sequences of mitochondrial 12S and 16S rRNA genes, complete sequences of mitochondrial cytochrome *b* gene and partial sequences of nuclear rag 2 gene) from Sullivan *et al*. (2000). Outgroups were notopterid fishes. Black thick branches correspond to well-supported relationships and grey thick branches correspond to weakly supported relationships according to these authors. Numbers in parentheses refer to the number of examined species for the corresponding monophyletic genera. Abbreviations: Petr. = Subfamily Petrocephalinae; Gymn. = Family Gymnarchidae. Most of the illustrations from Boulenger (1909–1916). Additional illustrations from Taverne & Géry (1968, 1975) and Taverne, Thys van den Audenaerde & Heymer (1976, 1977).

Brienomyrus in the Ogooué and Ntem rivers of Lower Guinea indicate that current taxonomy may often greatly underestimate real species-level diversity in this group (Hopkins, 1981; Sullivan, Lavoué & Hopkins, 2002). Mormyrids are largely nocturnal and possess complex electrosensory and electromotor systems used for electrolocation and communication (review in Moller, 1995).

In the late 1960s and 1970s, Louis Taverne revised the systematics of the mormyroids based on osteological studies (Taverne, 1968a,b, 1969, 1971b, 1972; Taverne & Géry, 1975; Taverne, Thys van den Audenaerde & Heymer, 1977) and proposed phylogenetic relationships for this group based on selected osteological characters, but without a cladistic analysis. Recent phylogenetic analyses of molecular data (Alves-Gomes & Hopkins, 1997; Lavoué *et al*., 2000; Sullivan, Lavoué & Hopkins, 2000) have supported the monophyly of the Momyroidea, the monophyly of the Mormyridae and the monophyly of Taverne's two mormyrid subfamilies, the Petrocephalinae and the Mormyrinae, but have rejected the monophyly of several mormyrin genera (*Brienomyrus*, *Marcusenius*, *Pollimyrus* and *Hippopotamyrus*) and have supported intergeneric relationships within the Mormyrinae different from those proposed by Taverne (1972). Basal relationships within the Mormyrinae are particularly well resolved in these studies: *Myomyrus* is found to be the sister group to all other taxa, within which *Mormyrops* is sister group to the others (Sullivan *et al*., 2000). The consensus hypothesis of mormyroid interrelationships from these molecular studies is presented in Figure 1. Despite

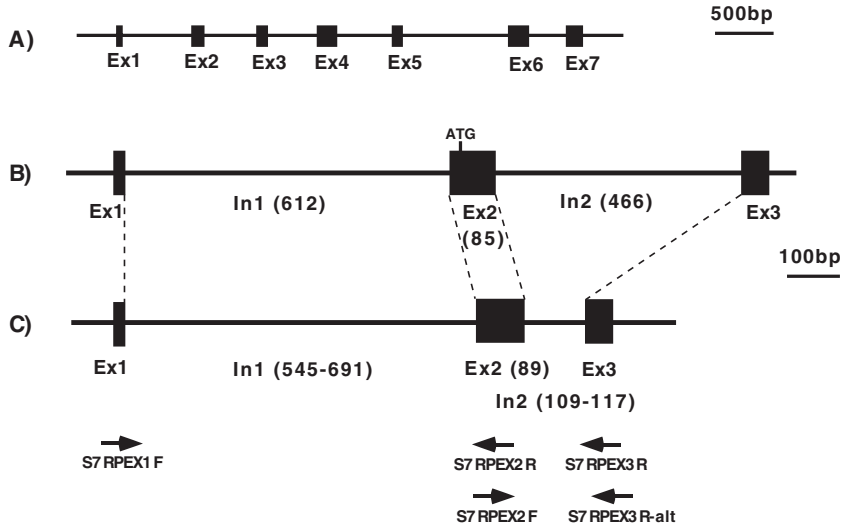

Figure 2. Diagram of the S7 gene structure and the primer positions. The gene diagram is drawn approximately to scale, except for the arrows designating the primer localization. Black boxes represent exons. (A) Complete S7 gene structure of puffer fish (Cecconi *et al*., 1996). (B) Partial S7 gene structure of the puffer fish corresponding to the region examined in this study (represents first two introns as well as second exon). (C) Amplified fragment for *Mormyrops nigricans*, with size range of introns in Mormyridae (in parentheses). Arrows indicate the locations and directions of the PCR and sequencing primers (see text for primers sequences).

these contributions, some intergeneric and interspecific relationships of this group remain poorly resolved (Alves-Gomes, 1999; Sullivan *et al*., 2000), especially within the subfamily Mormyrinae.

In this study we present new data from two introns and a single short exon within the nuclear gene coding for the S7 ribosomal protein (Chow & Hazama, 1998) for many of the same taxa included in Sullivan *et al*. (2000), representing all major mormyroid clades. The gene coding for the highly conserved ribosomal protein S7 consists of a single functional nuclear copy (Annilo, Stahl & Metspalu, 1995) (Fig. 2). The organization of this gene into seven exons separated by six introns is highly conserved in vertebrates, although the length of the introns varies considerably (Cecconi *et al*., 1996; Annilo *et al*., 1998). Interestingly, each of the six S7 introns in *Xenopus laevis* and *Fugu rubripes* contains coding sequences for the 'U17' small nucleolar RNA (snoRNA) (Cecconi *et al*., 1994, 1996). However, these sequences are degenerate in the first two introns of *F. rubripes* gene, and probably represent pseudogene copies.

We first describe the characteristics of the 5′ part of the S7 gene (including the first two introns as well as the second exon) in this group in order to evaluate its suitability for phylogenetic analysis. We then compare the results derived from phylogenetic analysis of this S7 region alone and in combination with cytochrome *b* (cyt *b*), 12S and 16S rRNA and rag 2 datasets used in previous systematic studies on mormyroids. We use the incongruence length difference test (Farris *et al*., 1994) and partitioned Bremer support (Bremer, 1994) to assess congruence among the datasets. Finally, we re-evaluate the recent hypotheses proposed by Sullivan *et al*. (2000) concerning the evolution of electric organs in the context of our revised hypothesis of mormyrid interrelationships.

MATERIAL AND METHODS

Representative taxa of the superfamily Mormyroidea were chosen to match those used in previous morphological and molecular systematic studies (Taverne, 1972; Sullivan *et al*., 2000). Species identifications, collection localities, voucher and museum accession numbers of specimens sequenced are listed in Table 1. We added only a single taxon not included in Sullivan *et al*. (2000) to the dataset, *Pollimyrus adspersus*. A total of 40 species were sampled representing both families Gymnarchidae and Mormyridae, the subfamily Petrocephalinae, and 16 of the 17 genera in the subfamily Mormyrinae. We preserved fresh tissue in the field in 90% alcohol or a DMSO/EDTA saturated salt solution (Seutin, White & Boag, 1991) and we extracted DNA from the tissue samples using the QIAamp Tissue Kit (QUIAGEN Inc., Valencia, CA, USA).

We amplified by polymerase chain reaction (PCR) an approximately 900-bp portion of the nuclear gene

Table 1. List of species examined in this study with collection origin and voucher number for each specimen (CU, Cornell University Museum of Vertebrates; MNHN, Museum National d'Histoire Naturelle; AMNH, American Museum of Natural History; CAR, Central African Republic)

Species	Origin	Voucher
Family: Mormyridae		
Subfamily: Mormyrinae		
Boulengeromyrus knoepffleri Taverne & Géry, 1968	Ivindo River, Gabon	CU 79692
Brienomyrus (Brienomyrus) brachyistius (Gill, 1862)	Aquarium import	CU 79741
Brienomyrus (Brienomyrus) hopkinsi Taverne *et al*., 1985	Ivindo River, Gabon	CU 78352
Brienomyrus (Brienomyrus) longicaudatus Taverne *et al*., 1977	Ivindo River, Gabon	CU 78355
Brienomyrus (Brienomyrus) sp.	Ivindo River, Gabon	CU 79740
Brienomyrus (Brevimyrus) niger (Günther, 1866)	Niger River, Mali	MNHN 1999–280
Campylomormyrus numenius (Günther, 1864)	Ubangui River, CAR	AMNH 228165
Campylomormyrus sp.*1*	Aquarium import	No voucher
Campylomormyrus tamandua (Günther, 1864)	Aquarium import	CU 79742
Campylomormyrus cf. *tamandua*	Sangha River, CAR	AMNH 228159
Genyomyrus donnyi Boulenger, 1898	Sangha River, CAR	AMNH 228154
Gnathonemus petersii (Günther, 1862)	Sangha River, CAR	AMNH 228157
Hippopotamyrus discorhynchus (Peter, 1868)	Lake Malawi, Malawi	CU 79743
Hippopotamyrus pictus (Marcusen, 1864)	Niger River, Mali	MNHN 1999–610
Hippopotamyrus wilverthi (Boulenger, 1898)	Ubangui River, CAR	AMNH 228164
Hyperopisus bebe (Lacépède, 1803)	Niger River, Mali	MNHN 1999–611
Isichthys henryi Gill (1862)	Ivindo River, Gabon	CU 79705
Ivindomyrus opdenboschi Taverne & Géry, 1975	Ivindo River, Gabon	CU 79698
Marcusenius ntemensis (Pellegrin, 1927)	Ivindo River, Gabon	CU 79706
Marcusenius greshoffi (Schilthuis, 1891)	Sangha River, CAR	AMNH 228160
Marcusenius moorii (Günther, 1867)	Ivindo River, Gabon	CU 79697
Marcusenius senegalensis (Steindachner, 1870)	Niger River, Mali	MNHN 1999–612
Marcusenius sp.	Sangha River, CAR	AMNH 228156
Mormyrops (Mormyrops) masiuanus Boulenger, 1898	Sangha River, CAR	AMNH 228163
Mormyrops (Mormyrops) nigricans Boulenger, 1899	Ogooué River, Gabon	AMNH 227337
Mormyrops (Oxymormyrus) zanclirostris (Günther, 1867)	Ivindo River, Gabon	CU 79707
Mormyrus rume Cuvier & Valenciennes, 1846	Niger River, Mali	MNHN 1999–613
Mormyrus ovis Boulenger, 1898	Sangha River, CAR	AMNH 228161
Myomyrus macrops Boulenger, 1914	Sangha River, CAR	AMNH 228166
Paramormyrops gabonensis Taverne *et al*., 1977	Ivindo River, Gabon	CU 79702
Pollimyrus isidori (Valenciennes, 1846)	Niger River, Mali	MNHN 1999–615
Pollimyrus adspersus (Günther, 1866)	Aquarium import	CU not registered
Pollimyrus marchei (Sauvage, 1878)	Ivindo River, Gabon	CU not registered
Pollimyrus Petricolus (Daget, 1954)	Niger River, Mali	MNHN 1999–616
Pollimyrus sp.	Sangha River, CAR	AMNH 228155
Stomatorhinus walkeri (Günther, 1867)	Louétsi River, Gabon	CU 79708
Stomatorhinus sp.*3*	Ivindo River, Gabon	CU 79703
Stomatorhinus sp.*2*	Sangha River, CAR	AMNH 228162
Subfamily: Petrocephalinae		
Petrocephalus bovei (Cuvier & Valenciennes, 1846)	Niger River, Mali	CU not registered
Family: Gymnarchidae		
Gymnarchus niloticus Cuvier, 1829	Aquarium import	CU 80334

coding for the S7 ribosomal protein, containing the complete first and second introns as well as the second exon using the two following PCR amplification primers, described by Chow & Hazama (1998): 5′-TGG-CCT-CTT-CCTTGG-CCG-TC-3′ (S7RPEX1F) and 5′-GCC-TTC-AGG-TCA-GAG-TTC-AT-3′ (S7RPEX3R). The forward primer S7RPEX1F and the reverse primer S7RPEX3R are located in the first exon and the third exon, respectively (Fig. 2). We designed an additional reverse primer later in this study for some problematic taxa: 5′-ACC-TTT-GCT-GCA-GTG-ATG-TT-3′ (S7EX3Ralt). We were unable to obtain a

PCR product with either of the reverse primers located in the third exon for *Petrocephalus bovei*. For this taxon we amplified only the first intron using the internal reverse primer S7RPEX2R, 5′-AAC-TCG-TCT-GGC-TTT-TCG-CC-3′ (Chow & Hazama, 1998). Double stranded amplifications were performed in 50-μL volumes containing 200 μM of each dNTP, 0.2 μM of each primer, 1.5 mM $MgCl_2$, 5 μL 10× buffer, 100 ng genomic DNA and 1 unit of Perkin Elmer AmpliTaq Gold. PCR runs were for 10 min at 95°C and 35 cycles of 1 min at 95°C, 1 min at 55°C and 1 min 30 s at 72°C; followed by a 7-min extension at 72°C. Amplifications were carried out on a Hybaid TouchDown thermocycler (Hybaid Limited, Teddington, Middlesex, UK). PCR products were purified using the Promega Wizard PCR Preps DNA purification kit (Promega, Madison, WI).

Sullivan *et al*. (2000) were unable to obtain complete cyt *b* sequences from *Hippopotamyrus pictus*, *Brienomyrus niger*, *Brienomyrus brachyistius*, *Stomatorhinus walkeri* and *S. sp1*, *Pollimyrus Petricolus* and *P. isidori*. For this study we were able to do so using the following primers: 5′-CTC-CGR-YCT-CCG-GAT-TAC-AAG-3′ (15930rev) and 5′-TGA-CTT-GAA-RAA-CCA-YCG-TTG-3′ (14724GLUDG) (Palumbi, 1996) using conditions described by these authors. However, we were unable to obtain the first 500 bp of the 5′ end of the cyt *b* sequence for *Stomatorhinus sp.2* which we coded as question marks in the aligned dataset. We amplified cyt *b*, 12S and 16S rRNA, and rag 2 sequences for *P. adspersus* with the primers and conditions described by Sullivan *et al*. (2000).

We sequenced the double-stranded PCR products in both directions with the same primers used for amplification by automated dye-terminator cycle chemistry on an Applied Biosystems 377 (Perkin-Elmer) automated sequencer. For *B. brachyistius* it was impossible to read the sequence generated by the primer S7PREX2F downstream of the position of a heterozygous indel in the second intron. We resequenced this specimen with an additional forward primer (S7PREX2F), complementary to the reverse primer S7PREX2R, to obtain a sequence. We edited the sequences with the Sequencher software package (Gene Codes Corp., Ann Arbor, MI, USA). Base composition, χ^2 tests for homogeneity of base composition across taxa and uncorrected pairwise distance values were calculated with PAUP* 4.0b8 (Swofford, 1999).

Sequences were deposited in GenBank under accession nos. AY124198–AY124317.

Alignment procedure, saturation analyses and phylogenetic reconstruction

We aligned the S7 gene sequences and re-aligned the 12S/16S sequence data using CLUSTAL X (Thompson *et al*., 1997) with three different parameter settings (gap opening/gap extension: 10/5, 7/5, 10/10). Sites which shifted relative position in the three alignments were excluded from phylogenetic analyses (Gatesy *et al*., 1993). Alignment of cyt *b* and rag 2 did not require gaps and was accomplished by eye.

To infer absolute substitutional saturation in S7 data, we plotted pairwise numbers of observed nucleotide differences ('adjusted distance' in PAUP*) against changes ('patristic distance' in PAUP*) inferred by maximum parsimony for each pair of taxa and for each type of substitution as described by Philippe *et al*. (1994) and Hassanin, Lecointre & Tillier (1998).

First, we conducted phylogenetic analyses on the S7 gene dataset. We compared the resulting topology with previously published results and results from phylogenetic analyses of the same taxa with three datasets (cyt *b*, 12S/16S and rag 2), each analysed separately. We then conducted incongruence length difference (ILD) tests to estimate any difference of phylogenetic signal among the four separate datasets after which we conducted a parsimony analysis on three combinations of the datasets (mitochondrial, nuclear and total). ILD test is not used here as a criterion to combine the individual datasets (Bull *et al*., 1993), but to explore phylogenetic signal in our data (Gatesy, O'Grady & Baker, 1999b). We performed the ILD tests as described by Farris *et al*. (1994), using PAUP* (Swofford, 1999), with 999 randomized replicates in addition to the original partition. For each replicate, we used heuristic searches with closest addition sequence option and tree bisection–reconnection (TBR) branch swapping. We removed all uninformative characters from each dataset before performing ILD tests (Cunningham, 1997b). All phylogenetic analyses employed maximum-parsimony (MP). The indels were coded using a test version of BARCOD (http://www.abi.snv.jussieu.fr/~billoud/Barcod/). This software implements the method described by Barriel (1994) and weights inferred indels equally, regardless of their size. We conducted heuristic searches with starting trees obtained via stepwise addition with 100 iterations of the random addition sequence and the TBR branch-swapping option using PAUP*. We calculated bootstrap proportions (1000 pseudoreplicates) and the Bremer support index (BSI) to assess the relative robustness of inferred relationships. In analyses of combined datasets, we calculated partitioned Bremer support (PBS) at each node (Baker & DeSalle, 1997). On an MP tree produced from a parsimony analysis of two or more combined data partitions, PBS analysis determines the additive contribution of each individual dataset (positive or negative) to the total BSI value at each node (Gatesy *et al*., 1999a). We used TreeRot (Sorenson, 1999) and PAUP* (Swofford, 1999) to calculate these indices.

Reconstruction of the electric organ evolution

We examined the evolution of two electric organ characters – the presence/absence of penetrating stalks, and the position of electrocyte innervation (posterior/anterior) – on the combined analysis MP tree by unweighted parsimony character reconstruction in the software application MacClade (Maddison & Maddison, 1997). Data on the structure of electric organs in the taxa used in this study are from Sullivan *et al*. (2000). Data on *P. adspersus* are from Alves-Gomes & Hopkins (1997).

RESULTS

Intron size

We obtained nucleotide sequences of the first and second introns as well as the second exon of the gene coding for the S7 ribosomal protein for 38 taxa representing 16 genera of the subfamily Mormyrinae and for *G. niloticus*. We were unable to obtain sequences for the second intron for *Petrocephalus bovei* (subfamily Petrocephalinae). The size of the first intron varies for all Mormyridae examined from 545 bp (*P. bovei*) to 630 bp (*Mormyrops zanclirostris*), but within the subfamily Mormyrinae, size variation is much smaller (610–630 bp). The second intron is shorter and ranged from 123 bp for *Campylomormyrus numenius* and *C. sp.1* to 132 bp for various *Mormyrops* species. The second exon is constant in length across Mormyroidea taxa (89 bp). The first and second intron sequences were compared to sequences in GenBank by a BLAST similarity search (Altschul *et al*., 1997). No significant similarity was found, with the exception of some short perfect matches of up to 18 bp to some human, fruit fly and *Arabidopsis thaliana* sequences. These matches appear to be spurious. Additionally, mormyrid intron sequences were compared by eye to functional sequences of the snoRNA U17 located in the third, fourth, fifth and sixth introns in the *Fugu rubripes* S7 gene (Cecconi *et al*., 1996). We found no obvious similarity between them, suggesting either that the first and second introns in mormyrids have never coded for snoRNA U17 or that sequence divergence associated with a loss of function has erased evidence of homology.

Within-individual sequence variation

Eighteen of the 40 specimens analysed in this study show intra-individual sequence variation. For most of these, two peaks of roughly equal intensity are seen at a single position on the chromatogram; all are located in intron sequences. In three specimens, an indel difference exists between distinct sequences of the same individual. The *Pollimyrus sp.1* and *B. brachyistius* specimens are polymorphic for two-base indels located in the first and second introns, respectively. For *Mormyrops masuianus*, a one-base indel is located in the first intron. The total number of base pair ambiguities within specimens ranges from zero (22 specimens) to 12 (in *Genyomyrus donnyi*). These genetic polymorphisms could be a consequence of different maternal and paternal alleles, or the presence of homeologous S7 copies. Contamination by a pseudogene sequence can be excluded since we detected no intra-individual variation in the exon sequences. Moreover, the primers used in this study have been shown to amplify only single copies of the S7 gene in other teleost groups (Chow *et al*., 2001). In the phylogenetic analyses, ambiguous sites are coded with an IUB ambiguity code, and sites exhibiting polymorphism for an intra-individual indel are excluded.

Base composition of the first two S7 introns

Statistically significant differences in base compositions across taxa as well as a strong base compositional bias can mislead the phylogenetic reconstruction (Swofford, 1999). The S7 sequences show a moderate bias towards adenine (25.2%) and thymine (34.3%). AT-rich intron sequences are common in fishes (Orti *et al*., 1996). A significant χ^2 value indicates that relative nucleotide composition varies across taxa. Examination of base frequencies by taxon indicates that those of *G. niloticus* and *P. bovei* differ significantly from the others. After exclusion of these sequences, a χ^2 test of base frequencies across the remaining taxa is not significant.

Alignment and indel coding

The sequence of *P. bovei* (the shortest of our dataset) has numerous deletions relative to sequences from other mormyrids and the sequence of *Gymnarchus niloticus* is highly divergent from the others. Because these sequences could not be aligned unambiguously to those from the Mormyrinae, both taxa were excluded from phylogenetic analyses. Sites within the mormyrin taxa that shifted alignment under the three different CLUSTAL X parameter settings were excluded from the phylogenetic analysis. These are positions 395, 396 and 671–685 in the first intron. The final alignment comprises 907 nucleotide positions. This alignment requires the insertion of 53 indels ranging in size from one to 28 base pairs: 40 in the first intron and 13 in the second intron. These are coded into a two-state (0/1) taxon by character matrix following Barriel (1994). In addition, a 22-bp segment of the first intron is inverted in some taxa relative to the others. The orientation of this segment is also coded as

one additional character (type 0/1) added to the indel character matrix. The inverted sequences were re-inverted to permit alignment with the other sequences and included in the parsimony analyses. The matrix consisting of 54 structural characters (53 indels, 1 inversion) was appended to the sequence data matrix. These characters were weighted equally to the nucleotide characters. Our final S7 data matrix contains 961 characters for 38 taxa. In all analyses reported here, *Myomyrus macrops* is chosen as outgroup to the remaining mormyrin taxa. Previous studies have supported the genus *Myomyrus* as the sister group of the rest of the subfamily Mormyrinae with high statistical confidence (Lavoué *et al*., 2000; Sullivan *et al*., 2000).

Genetic divergence

We examined sequence divergence among taxa for the aligned sequences of the two introns separately, treating gaps as missing data. For the first intron, the highest genetic divergence (about 13–15%) is observed, as expected, between *Myomyrus* and other Mormyrinae. Pairwise uncorrected distances range from 0 to 8% among the others. The sequences for *Pollimyrus marchei* and *Ivindomyrus opdenboschi* are identical, confirming the close relationship between these two taxa reported previously (Lavoué *et al*., 2000). For the second intron, the greatest divergence is observed between species of *Pollimyrus* (excluding *P. marchei*) and the other Mormyrinae (up to 21.6%). Out of a total of 961 positions for the whole S7 fragment (intron 1 = 719, exon 2 = 89, intron 2 = 153), 419 are variable (320, 12 and 87, respectively) and 230 are phylogenetically informative under parsimony (168, 6 and 56).

Distribution of variable sites along the S7 sequences and comparison with non-coding 12S/16S rRNA data

The distribution of variable sites along the S7 intron sequences among the mormyrin taxa is relatively uniform (Fig. 3A). There are proportionally fewer substitutions in the exon region, as expected. By comparison, 12S/16S rRNA sequences for the same taxa exhibit highly conserved regions that alternate with highly variable regions (Fig. 3B) corresponding to stems and loops, respectively (Orti *et al*., 1996; Miya & Nishida, 1998).

Saturation

Linear relationships are found between inferred and observed changes for all classes of substitution (slopes of the regression lines all above 0.98), indicating that these data are not saturated (Fig. 4). These observations suggest no reason for eliminating or down-weighting any class of substitution in the S7 data in the phylogenetic analysis. By comparison, the 12S/16S fragment shows strong evidence of substitutional saturation in pairwise comparisons of more distantly related taxa in this study (Fig. 3C).

Phylogenetic analysis of the S7 dataset

MP analysis based on equal weighting of each character yielded 66 trees of 619 steps, with a consistency index excluding uninformative characters (CI) of 0.749 and a retention index (RI) of 0.874. Figure 5 shows the strict consensus tree in which there are three polytomies. The strict consensus recovers the following well-supported relationships proposed by Sullivan *et al*. (2000). (1) *Mormyrops* is the sister group to all other Mormyrinae (excluding *Myomyrus macrops*); this result is supported by a high bootstrap proportion (BP = 97%), Bremer support index (BSI = 10) and three unique deletions. (2) *B. brachyistius* and *I. henryi* form a monophyletic group (BP = 100%, BSI = 17, two unique deletions). (3) Taxa of the genera *Marcusenius* (excluding *M. ntemensis*), *Hippopotamyrus* (excluding *H. pictus*), *Gnathonemus*, *Genyomyrus* and *Campylomormyrus* form a monophyletic group (BP = 100%, BSI = 7), which we refer to as the '*M. senegalensis* clade' in the rest of this study. (4) *Boulengeromyrus knoepffleri*, *I. opdenboschi* and *P. marchei* form a monophyletic group (BP = 99%, BSI = 4) that we call the '*B. knoepffleri* clade'. (5) *M. ntemensis*, *P. gabonensis* and the Lower Guinean species of *Brienomyrus* form a monophyletic group (BP = 99%, BSI = 7). An inversion of 22 bp in the first S7 intron of sequences of *Brienomyrus longicaudatus*, *B. sp*., *B. hopkinsi* and *P. gabonensis* relative to the sequences *M. ntemensis* and the rest of mormyrin taxa represents a synapomorphy for this clade. S7 data also support the paraphyly of the three species of *Brienomyrus* with respect to *P. gabonensis* (BP = 84%, BSI = 2, one unique insertion).

The novel sister group relationship between *H. pictus* and the *M. senegalensis* clade is well supported (BP = 100%, BSI = 9, two unique insertions, and one unique deletion) as is the sister group relationship of *Mormyrus* to *B. brachyistius* plus *I. henryi* (BP = 85%, BSI = 3). In addition, S7 data provide moderate support for a sister group relationship between genera *Pollimyrus* and *Stomatorhinus* (BP = 83%, BSI = 3) that was only weakly supported in the analysis of Sullivan *et al*. (2000).

Separate analyses of cytochrome *b*, rag 2 and 12/16S rRNA genes

To compare the phylogenetic results derived from the S7 dataset, we performed separate analyses on the fol-

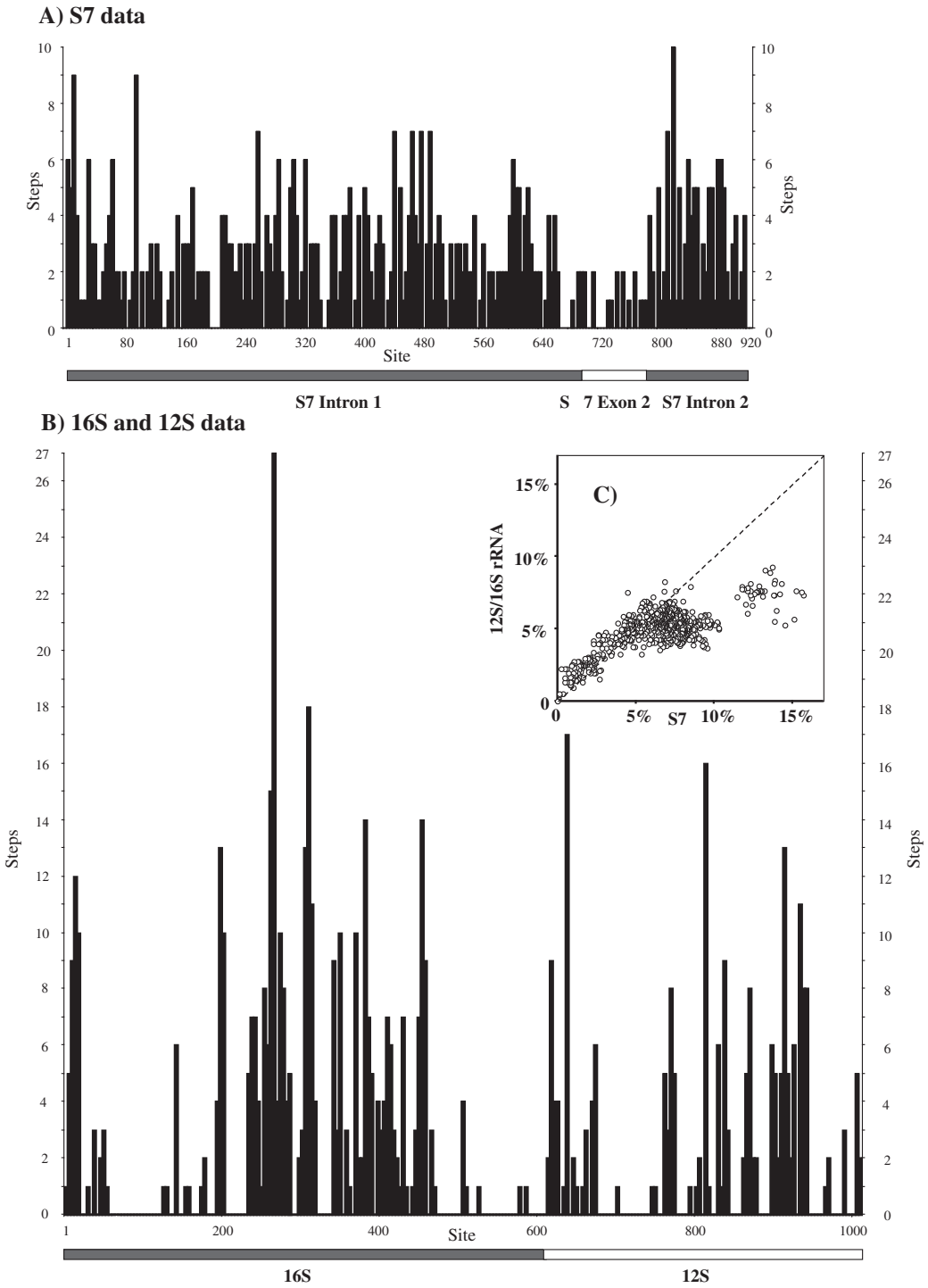

Figure 3. Relative nucleoditic variability for (A) S7 data and (B) 12S/16S rRNA data as estimated by the number of steps in one of the most parsimonious trees, using a 4-bp, non-overlapping window along the length of the molecules using MacClade (Maddison & Maddison, 1997). (C) Pairwise uncorrected genetic distance of the S7 gene are plotted against pairwise uncorrected genetic distance of the 12S/16S rRNA. The dashed line corresponds to $x = y$.

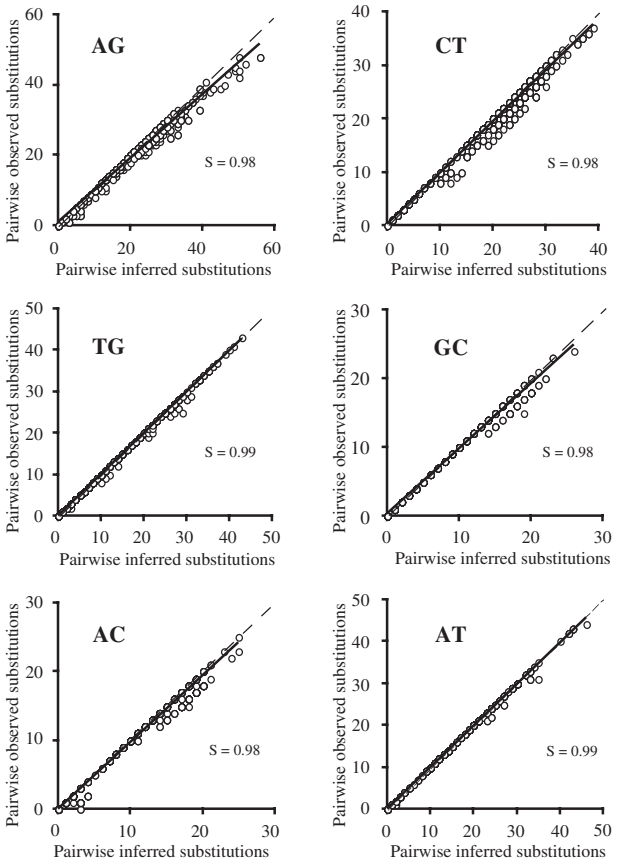

Figure 4. Saturation curves of the number of observed substitutions between two taxa for S7 data vs. the number of inferred substitutions in the most (or one of the most when more than one tree is recovered) parsimonious tree, for each of the six kinds of substitution (A-T, A-G, A-C, G-C, T-C, T-G). The dashed line corresponds to $x = y$, for which the number of inferred substitutions between two taxa is equal to the number of observed substitutions. The continuous line corresponds to the regression line. The slope of the regression line (S) is indicated.

lowing three datasets adapted from the study of Sullivan *et al*. (2000) for the same taxa: complete cyt *b* sequences (1140 characters), combined partial sequences of 12S & 16S rRNA (1021 characters), and partial sequences of rag 2 (1134 characters). Figure 6 presents the MP tree or the strict consensus of the MP trees derived from each of these analyses. Values for consistency indices (CIs) and retention indices (RIs) are lower for the mitochondrial datasets than for the nuclear datasets (Table 2), suggesting a higher level of saturation in the mitochondrial data. Downweighting types of substitution most subject to homoplasious change in the mitochondrial data (third position transitions for cyt *b* and transitions for 12S/16S) only slightly improves the mean CI and RI of the MP trees derived from these datasets (data not shown). However, these weighting schemes do not change any of the well-supported relationships in the unweighted separate analyses. Reflecting lower homoplasy in the nuclear datasets, both the S7 and the rag 2 data resolve more nodes above the 50% bootstrap level than do either the cyt *b* or 12S/16S data (Table 2).

Although numerous topological incongruities exist between the trees in Figure 6, high BP or BSI support few of the conflicting topologies. Only six intergeneric nodes are common to all datasets, indicated A to F in Figure 6. However, intergeneric relationships supported by BP above 50% are nearly identical in the two mitochondrial trees; the only significant difference concerns interrelationships among *B. hopkinsi*, *B. longicaudatus* and *P. gabonensis*. The rag 2 and S7 trees are similar, differing only in the position of genus *Mormyrus* and *H. pictus*. In addition, the nuclear datasets support one relationship not recovered by the mitochondrial datasets: the sister relationship between *B. knoepffleri* clade and *M. ntemensis* clade.

The positions of *H. bebe* and *B. niger* are highly unstable in all the individual datasets, and largely

Table 2. Summary information from MP analyses of individual and combined data sets

	No. of characters	No. of variable (informative) characters	No. of MP trees	Length	CI[2]	RI	Nodes with BP > 50%, total (intergeneric only)
S7[1]	961	419 (230)	66	619	0.749	0.874	26 (15)
Rag 2	1134	271 (151)	144	432	0.595	0.795	23 (12)
Nuclear (*nuc*)	2095	690 (381)	528	1064	0.671	0.831	27 (17)
Cytochrome *b*	1140	484 (395)	1	1885	0.343	0.496	21 (10)
12S/16S rRNA[1]	1021	238 (163)	15	646	0.395	0.651	21 (11)
Mitochondrial (*mt*)	2161	722 (558)	1	2556	0.352	0.535	24 (13)
Total (*nuc* + *mt*)	4256	1412 (939)	2	3643	0.423	0.618	28 (20)

[1]These data sets include the structural characters as coded by the software BARCOD (53 for S7 and 25 for 12S/16S rRNA).
[2]CI excluding uninformative characters.

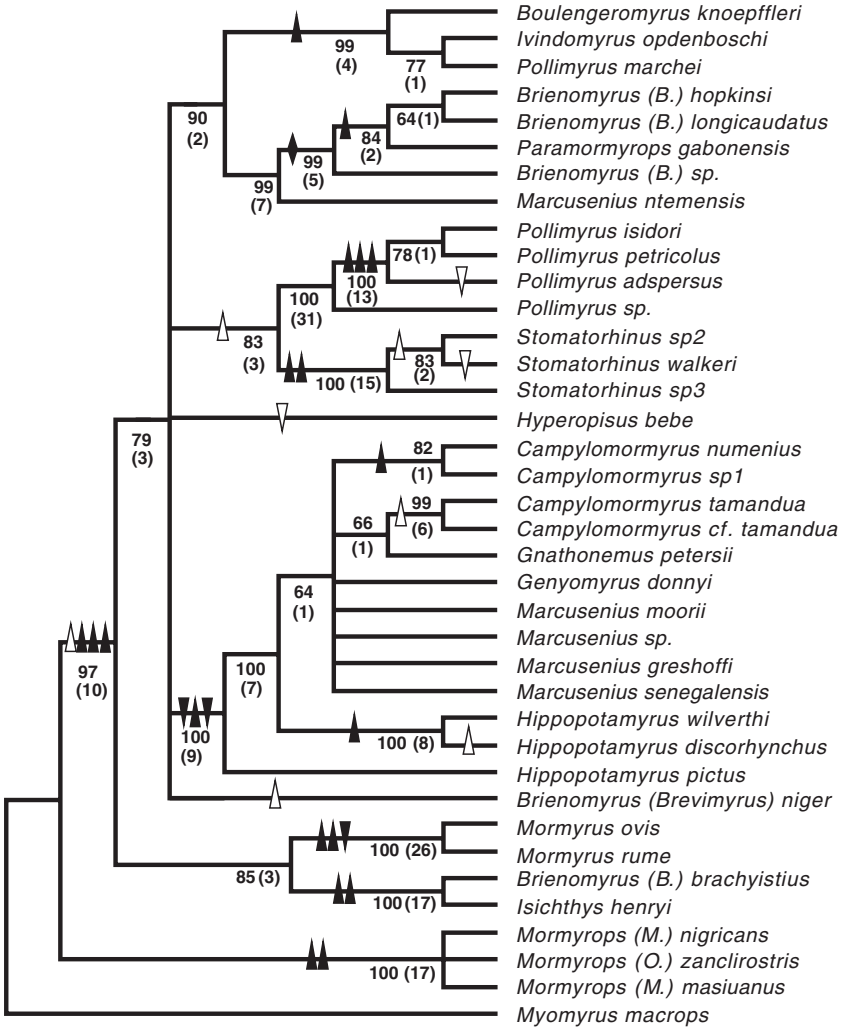

Figure 5. Strict consensus of 66 trees recovered in MP analysis of S7 sequences based on equal weighting of each character. The tree is rooted with *Myomyrus macrops*. Information for each tree is shown in Table 2. Numbers are bootstrap proportions (if >50%) and Bremer support index (in parentheses). Informative molecular structural changes as inferred under ACCTRAN optimization are shown by triangles (deletions), inverted triangles (insertions) and diamonds (inversion). Filled symbols refer to characters having CI = 1. Open symbols refer to characters having CI < 1.

unresolved in some of them (12S/16S rRNA and S7). The sister relationship between *Pollimyrus* and *Stomatorhinus* is recovered only by the S7 data, even with increased taxonomic sampling (Sullivan *et al*., 2000). Interestingly, the morphologically well-defined genus *Campylomormyrus* appears paraphyletic with respect to *Gnathonemus* in three of the four analyses (S7, cyt *b* and 12S/16S rRNA), although in all cases support for this topology is weak. In the tree derived from the rag 2 dataset, *Campylomormyrus* appears monophyletic with moderate support (BP = 64%, BSI = 1).

Character congruence between individual partitions according to the ILD test

The ILD test detects significant incongruence (at the $P < 0.05$ level) in three pairwise comparisons of these datasets (Table 3). The S7 dataset is incongruent with the rag 2 and cyt *b* datasets, and the rag 2 dataset is incongruent with the 12S/16Sr dataset. Bull *et al*. (1993) suggest incongruent data should not be combined for phylogenetic analyses. However, Gatesy *et al*. (1999a), who advocate a 'total evidence' method-

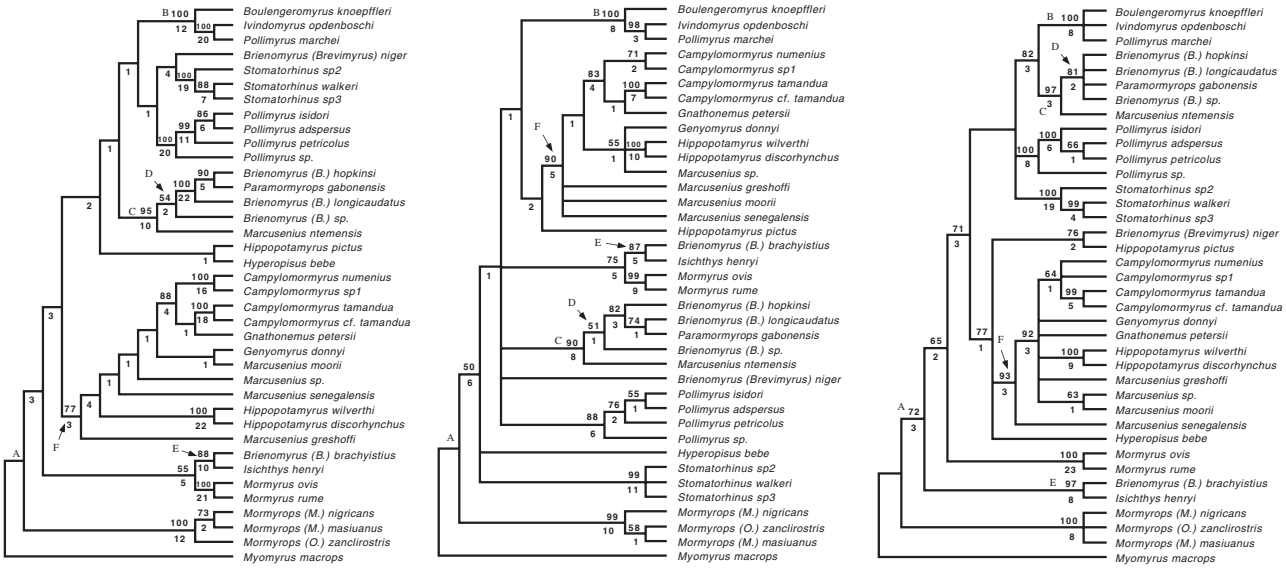

Figure 6. Maximum parsimony (MP) trees for individual genes. (A) Single MP tree recovered in analysis of cytochrome *b* sequences based on equal weighting of each nucleoditic substitution. (B) Strict consensus of 15 MP trees recovered in analysis of combined 12S and 16S sequences based on equal weighting of each character. (C) Strict consensus of 144 MP trees recovered in analysis of rag 2 sequences based on equal weighting of each nucleoditic substitution. Information for each tree is shown in Table 2. Each tree is rooted with *Myomyrus macrops*. Numbers above branches are bootstrap proportions (if >50%). Numbers below branches are Bremer support index. Letters indicate intergeneric clades common to all analyses of individual genes (including S7 data analysis, see Fig. 5).

Table 3. Results of the Incongruence Length Difference tests (Farris *et al*., 1994)

	Rag2	12S/16S	Cytochrome *b*	Nuclear	Total
S7	**0.001**	0.079	**0.010**	–	
Rag2	–	**0.035**	0.209	–	
12S/16S		–	0.266		
Mitochondrial				0.069	
Nuclear				–	
Total					**0.004**

Note: Numbers shown are *P* values. Numbers in bold indicate ILD test is significant at the $P < 0.05$ threshold.

ology, show that the combination of incongruent data can increase the resolution and the support within phylogenetic trees, revealing 'hidden signal' present in the separate datasets. Following this approach, we explore the results of combining datasets using PBS as an index of character congruence.

Combined analyses: mitochondrial vs. nuclear partitions

All characters were weighted equally in all combined analyses below. The individual datasets were first combined into two: nuclear (*nuc*) and mitochondrial (*mt*). Figure 7 presents the strict consensus of 528 MP trees derived from the *nuc* dataset (CI = 0.671, RI = 0.831) and the MP tree derived from the *mt* dataset (CI = 0.352, RI = 0.535). In the *nuc* tree, 27 nodes (17 intergeneric nodes) out of 34 have a BP above 50% compared to 24 nodes (13 intergeneric nodes) in the *mt* tree (Table 2). Comparing the trees, only one significant conflict is observed with respect to the interrelationships of *B. hopkinsi*, *B. longicaudatus* and *P. gabonensis*. Within the *mt* tree, the position of the genus *Mormyrus* is resolved and well supported

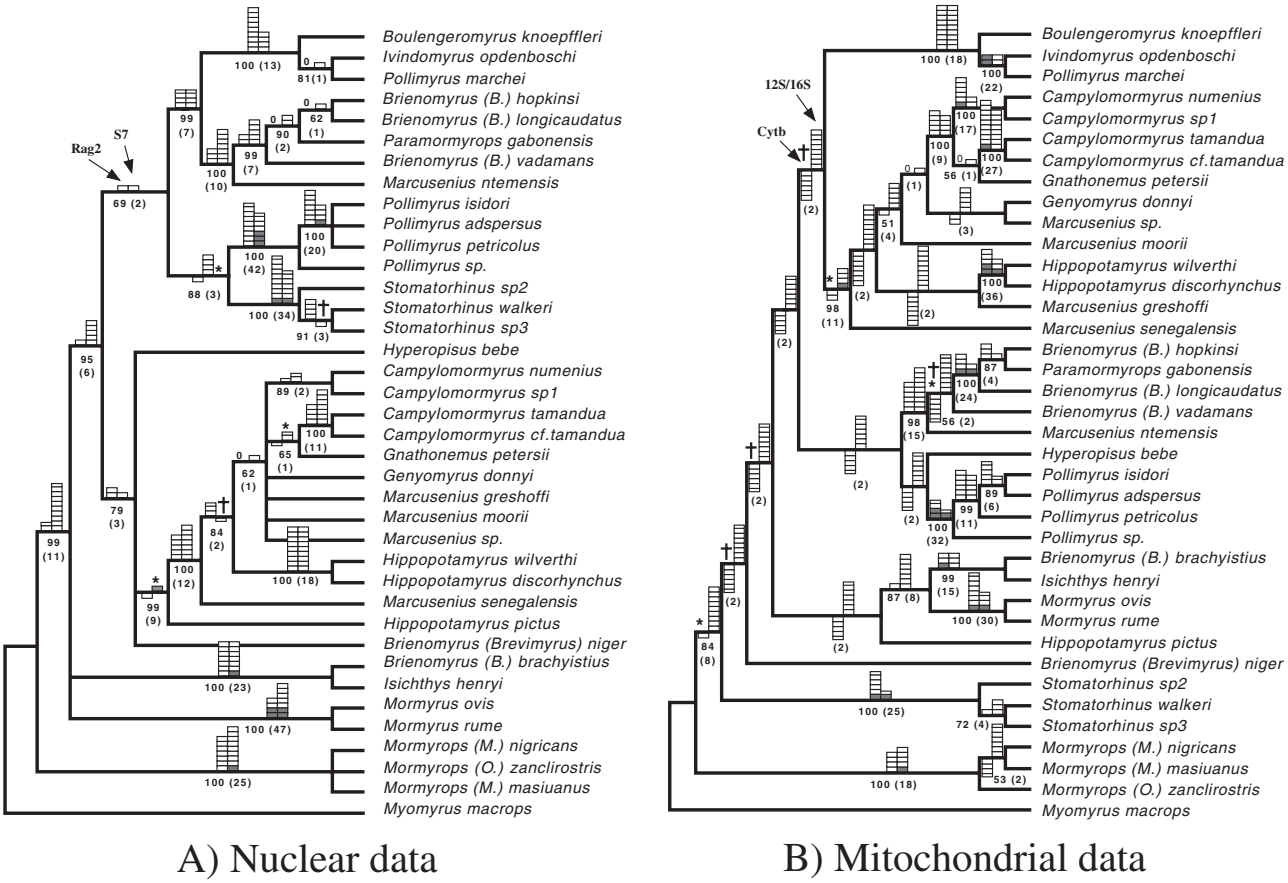

Figure 7. Maximum parsimony (MP) trees for combined mitochondrial and nuclear partitions. (A) Strict consensus of 528 MP trees recovered in analysis from the combined data set of nuclear markers (rag 2 and S7 data). (B) Single MP tree recovered in analysis from the combined data set of mitochondrial markers (12S/16S rRNA and cytochrome *b*). Information for each MP tree is shown in Table 2. Bootstrap proportions (>50%) and Bremer support index (in parentheses) are indicated below branches. Partitioned Bremer supports (PBS) for each individual data set are indicated with columns, above branches when positive and below branches when negative. Open bricks represent 1 'step'; shaded bricks represent 10 'steps'. The sum of negative and positive PBS for each individual data set and for a given node equal to BSI at this node is given. Asterisk and cross symbols refer to conflict between individual data sets as revealed by PBS.

(BS = 87%, BSI = 8) as well as the monophyly of (*Campylomormyrus*, *Gnathonemus*) clade (BS = 100%, BSI = 9), whereas the *nuc* dataset provides strong support for deeper nodes in the phylogeny.

Despite a significant ILD test result for the S7/rag 2 comparison, the examination of PBS in this analysis reveals that S7 and rag 2 data are in weak conflict at only five nodes (Fig. 7A), with negative values for a partition never greater than –1. In three of these nodes the topology is imposed by S7 (nodes marked by an asterisk, Fig. 7A) and in two by rag 2 (nodes marked by a cross, Fig. 7A). However, the position of the genus *Mormyrus*, which differs in the separate S7 and rag 2 analyses, is unresolved in the combined *nuc* analysis. Otherwise, the topology of this tree is globally consistent with the topologies of trees derived from the separate analyses of these two datasets.

PBS results in the *mt* analysis are unexpected (Fig. 7B). First, for 15 nodes, the 12S/16S rRNA and cyt *b* datasets are in conflict, sometimes strongly, despite the non-significant ILD test value between these datasets. For each of these nodes, 12S/16S PBS values are positive and cyt *b* values are negative. Secondly, negative PBS values for the cyt *b* dataset are observed at some nodes that are recovered in the separate analysis of the cyt *b* dataset (nodes marked by an asterisk, Fig. 7B). Thirdly, the BSI and PBS values are oddly identical at several nodes (for example, see nodes marked by a cross in Fig. 7B, at which BSI = 2, PBS (cyt *b*) = –4,5 and PBS (12S/16S) = +6,5). The topology of this tree is different from the topology of

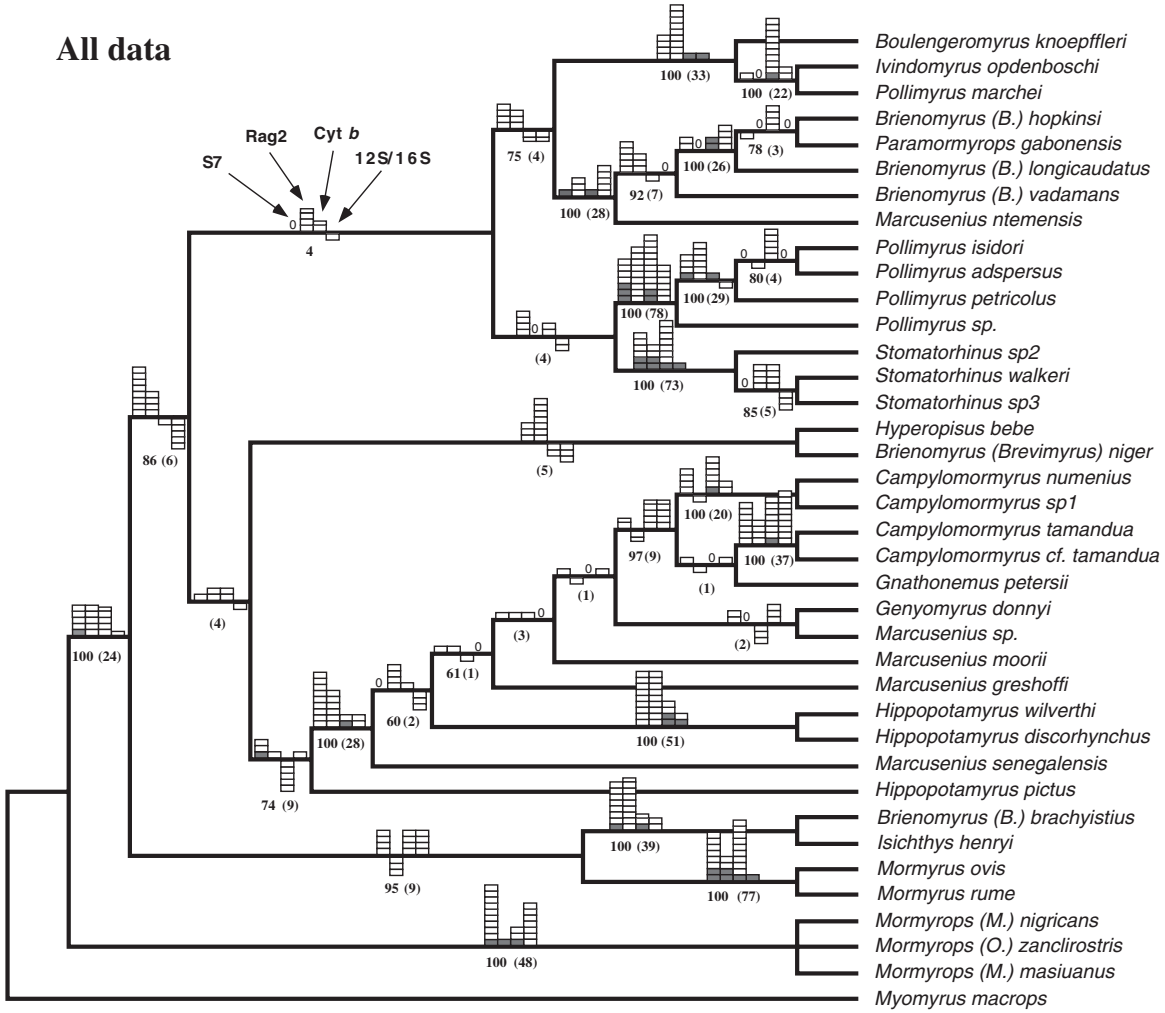

Figure 8. Strict consensus tree of two equally parsimonious trees from the combined separate data sets. Information for each tree is shown in Table 2. Bootstrap proportions (if >50%) and Bremer support index (in parentheses) are indicated below branches. Partitioned Bremer supports (PBS) for each individual data set are indicated with columns, above branches when positive and below branches when negative. Open bricks represent 1 'step'; shaded bricks represent 10 'steps'. The sum of negative and positive PBS for each individual data set and for a given node equal to BSI at this node is given. From left to right, columns represent PBS for S7, rag 2, cytochrome *b* and 12S/16S rRNA data sets.

trees derived from the separate analyses of the 12S/16S rRNA and cyt *b* datasets, most notably with respect to the position of three taxa: *H. bebe*, *B. niger* and *H. pictus*.

The ILD test value is not significant ($P = 0.069$) in a comparison between the *nuc* and *mt* datasets (Table 3). An ILD test comparing all four individual partitions simultaneously yields a significant result ($P = 0.004$).

Combined analyses of all data

MP analysis based on the complete dataset (4256 characters, 1412 variable and 939 potentially parsimony informative) results in two trees (CI = 0.423 and RI = 0.618). Figure 8 shows the strict consensus tree. The two shortest trees differ only in the relationships of *Mormyrops* species within the *Mormyrops* clade. Twenty-eight nodes (20 intergeneric nodes) out of 34 are supported by BP values above 50% (Table 2). The topology of this tree is very similar to that of the *nuc* combined data tree and the well-supported phylogenetic relationships previously identified are recovered with the exception of one terminal node joining *B. hopkinsi* and *P. gabonensis*. In addition, combination of *nuc* and *mt* data resolves the position of *Mormyrus* as the sister group of (*I. henryi*, *B. brachyistius*) clade with significant support (BP = 88% and BSI = 9).

PBS revealed limited conflicts between individual datasets. PBS values at nodes in this analysis fall into four classes. (1) Some nodes present positive PBS values for each dataset. These nodes define well-supported phylogenetic relationships that are not in conflict in the separate analyses. (2) At some nodes conflict is observed between the *nuc* and *mt* datasets; in each of these, values for the *nuc* partition are positive and values for the *mt* partition are negative. For example, the BSI at the (*B. knoepffleri* clade, *M. ntemensis* clade) node is the sum of the value of the *nuc* partition (+ 7) and the value of the *mt* partition (– 3). (3) Some nodes present strongly positive PBS values from a single partition, and relatively neutral values (weak positive, weak negative or PBS = 0) from the other partitions. For example, support for the *H. pictus*, *M. senegalensis* clade is concentrated within the S7 dataset. (4) At other nodes, the PBS value in one dataset is negative, in conflict with positive values in the other partitions. This is the case at the node supporting the monophyly of the *Mormyrus* and (*I. henryi*, *B. brachyistius*) clade.

DISCUSSION

The phylogenetic utility of the first two introns of the S7 gene

Perhaps because introns are 'generally believed to evolve too rapidly and too erratically to be of much use in phylogenetic reconstruction' (Kupfermann *et al*., 1999) relatively few systematic studies in teleost fishes have made use of them (Oakley & Phillips, 1999; Sides & Lydeard, 2000; Quattro, Jones & Oswald, 2001), although more studies in other vertebrate groups have done so (Kupfermann *et al*., 1999; Prychitko & Moore, 1997; Walton, Nedbal & Honeycutt, 2000; Mundy & Kelly, 2001; Weibel & Moore, 2002). Using RFLP, Chow & Hazama (1998) detected significant intraspecific variation in the first and second introns of S7 among yellowfin tuna (*Thunnus albacares*) populations and suggested that this fragment may be useful to investigate intraspecific genetic structure and phylogenetic relationships among closely related species. In contrast, our results show that very few nucleotide substitutions have taken place in these introns among seven closely related species of *Brienomyrus* from Gabon (Sullivan *et al*., 2002) (data not shown) and no variation exists between sequences of *P. marchei* and *I. opdenboschi*, also closely related species (Lavoué *et al*., 2000). However, our nucleotide sequence-based study provides evidence that the first two introns of the S7 gene possess a number of characteristics that make them useful molecular markers over much of the phylogenetic depth encompassed by our study group.

We observed no significant differences in base frequencies and a low base compositional bias among our sequences from the subfamily Mormyrinae. However, we found the base content in *P. bovei* and *G. niloticus* to be statistically different from the base content of taxa within the Mormyrinae, with a significant overrepresentation of guanine and cytosine. Although we observed few ambiguities in a multiple alignment of mormyrin sequences, alignment became difficult when gymnarchid and petrocephalin sequences were included. These characteristics effectively limit the phylogenetic depth at which this marker is useful.

The homoplasy content of this marker is very low, as shown by high consistency and retention indices on the most parsimonious trees, consistent with a low degree of observed substitutional saturation (Fig. 4). Comparison of sequences from the non-coding S7 introns with the 12S/16S rRNA fragments (which exhibit overall a similar level of uncorrected genetic divergence in our study group) calls attention to the relationship between global genetic variation (and its distribution along the sequence) and homoplasy in molecular markers. Substitution rate and functional constraints determine the level of homoplasy in sequence data. The mitochondrial 12S/16S rRNA genes exhibit a higher substitution rate than the S7 introns as observed between closely related taxa (Fig. 3C), but more sites are functionally constrained (and are invariant), resulting in a patchwork of conserved regions and hypervariable regions in which sites are prone to multiple hits (Fig. 3B). The first two S7 nuclear introns possess a comparatively slower substitution rate, but are structurally less constrained and variable sites are distributed more homogeneously along the sequence. Thus, the homoplasy content of this S7 fragment is considerably lower than for the 12S/16S rRNA region we examined.

Homoplasy is common with nucleotide substitution characters since only four character states are possible. Perhaps for this reason, base substitution synapomorphies for particular relationships are rarely identified in sequence-based phylogenetic studies. Instead, support for relationships is usually assessed by statistical indices such as bootstrap proportions, or by Bremer support. The high homoplasy content of nucleotide substitution characters can complicate phylogenetic analysis. Single structural molecular changes that are comparatively unconstrained by character state limitations such as insertions, deletions and inversions (i.e. introns, transposons or coding sequences) are characters less subject to homoplasy (Chow & Hazama, 1998; Hillis, 1999; Simmons, Ochoterena & Carr, 2001; van Dijk *et al*., 2001). Such characters can be useful in phylogenetic reconstruction and for diagnosing relationships for which morphoanatomical characters are lacking or in con-

flict. A number of recent studies have demonstrated the utility of such characters (Shimamura *et al*., 1997; Qiu *et al*., 1998, 1999; Gugerli *et al*., 2001; van Dijk *et al*., 2001; Venkatesh, Erdmann & Brenner, 2001). The first two introns of the S7 gene exhibit numerous structural modifications in our study group (54 indels for which 28 are informative) that are distributed essentially uniformly along the intron sequences. Coded as presence/absence characters in the program BARCOD and analysed separately from the sequence data, these data produce a strict consensus tree similar to that produced by the sequence data alone, with a mean CI of 0.85 (RI = 0.94). Twenty-three of 28 informative characters have a CI equal to 1. Some of these characters diagnose relationships for which no known morphological character support exists such as the sister relationships between *B. brachyistius* and *I. henryi*, the monophyly of the taxa belonging to a species flock of endemic Lower Guinean *Brienomyrus* (Sullivan *et al*., 2000), and the monophyly of all remaining mormyrin taxa when *Mormyrops* and *Myomyrus* are excluded.

Lastly, primers designed within the slowly evolving flanking exons permit amplification of the first two introns of the S7 gene in a large range of teleost taxa including Perciforms (Chow & Hazama, 1998), Salmoniforms (Chow & Hazama, 1998), Otophysi (He, pers. comm., pers. obs.) and Osteoglossiforms (this study).

Multiple genetic markers, congruence among them and the utility of PBS and ILD tests

In this study, we compared four different genetic datasets grouped by origin (*mt* vs. *nuc*) and by functional characteristics (coding vs. non-coding); three of these datasets contained sequences published previously, the *mt* coding gene cyt *b*, the *mt* non-coding 12S and 16S rRNA genes, and the *nuc* coding gene rag 2 (Sullivan *et al*., 2000). The 5′ part of the *nuc* S7 gene represents new data.

When multiple independent datasets are available for a phylogenetic study, the investigator can analyse each dataset separately and subsequently compare tree topologies obtained from each ('taxonomic congruence' (Bull *et al*., 1993; Miyamoto & Fitch, 1995; Huelsenbeck, Bull & Cunningham, 1996)) or combine all data prior to phylogenetic analysis ('total evidence' (Kluge, 1989)). Although advocates of these two approaches present them as philosophically opposed, all agree that use of multiple datasets improves phylogeny estimation. In this study we follow an empirical strategy, conducting both separate and simultaneous analyses (de Queiroz, 1993; Levasseur & Lapointe, 2001). Separate analyses allow us to examine and compare the phylogenetic signal content of each marker before simultaneous analyses of the combined datasets. We used the ILD (Farris *et al*., 1994) test as one method to assess congruence of phylogenetic signal among the datasets. Several studies point out that the results from congruence tests should not be used as criteria for combining individual datasets for two reasons. First, the reliability of the congruence tests, in particular the ILD test, has been questioned (Cunningham, 1997a; Yoder, Irwin & Payseur, 2001). Secondly, data sets which are in conflict over some parts of a tree may be congruent over other parts (Gatesy *et al*., 1999b).

In this study, the ILD test finds statistical incongruence in three pairwise comparisons of individual datasets: S7 vs. rag 2, S7 vs. cyt *b* and rag 2 vs. 12S/16S rRNA. In the first comparison between the two *nuc* genes, local topological incongruence between trees derived from the separate analyses is consistent with these results: S7 and rag 2 trees differ in their placement of *Mormyrus* species and *H. pictus* and in each tree these different topologies are well supported by character data. However, in the other two comparisons (cyt *b* vs. S7 and 12S/16S rRNA vs. rag 2), no strongly supported topological incongruence is observed between trees due to poor resolution of the *mt* trees and weak support for many of the resolved nodes within them. In these comparisons, the ILD tests could be returning significant, but largely meaningless, results as a consequence of the high homoplasy level in one of the datasets compared (Brower, DeSalle & Vogler, 1996; Graham *et al*., 1998), both mitochondrial datasets in this case.

Our simultaneous analyses produce better-supported trees than do the separate analyses. Our graphical representation of PBS (Baker & DeSalle, 1997) allows quick assessment of the relative support provided by each individual dataset in combined analyses (Figs 7 and 8). With the exception of the simultaneous analysis of *mt* datasets (see below), PBS values in combined analyses are generally consistent with the topology of trees recovered in the separate analyses. At certain nodes, positive PBS values reveal 'hidden signal' in some datasets, particularly for the noisy *mt* data when both *nuc* datasets are in conflict (e.g. see the position of *Mormyrus*).

However, PBS values are largely counterintuitive in the combined analysis of the *mt* datasets. At several nodes, the PBS values for each of the individual *mt* datasets are in conflict although no significant topological incongruence is observed on the trees derived from the separate analysis of these datasets and the ILD test detects no statistically significant incongruence between them. A possible explanation could be that the PBS values are influenced by the high homoplasy content of the *mt* data. Moreover, the PBS values for the cyt *b* partition are negative at some nodes of the combined data tree despite their presence

in the cyt *b*-only tree. It is possible that the different sets of MP trees recovered in combined vs. single dataset analyses results in differing optimizations of characters at these nodes.

Additional studies will be necessary to provide a better understanding of the complex relationship between the ILD test, PBS and homoplasy revealed in our results.

Phylogeny of Mormyrinae, overview

Alone and combined with the other datasets, the S7 data are in agreement with most of the recently proposed hypotheses of major group relationships within the Mormyrinae (Alves-Gomes & Hopkins, 1997; Lavoué *et al*., 2000; Sullivan *et al*., 2000). The position of the genus *Mormyrops* is particularly well established as the most basal taxon in the Mormyrinae after *Myomyrus*; S7 data provide three structural synapomorphies that strongly support the monophyly of the remaining Mormyrinae. The combination of the S7 sequences with the dataset of Sullivan *et al*. (2000) does not change the relative position of the following four clades: *Pollimyrus* plus *Stomatorhinus*; the *B. knoepffleri* clade; the *M. senegalensis* clade; and *H. bebe* plus *B. niger*. However, internodes between these clades receive poor support and no unique molecular synapomorphy diagnoses them. Perhaps more extensive morphological study will be able to provide synapomorphies for these four clades. Our data provide weak but consistent support (cyt *b*, 12S/16S and S7) for the paraphyly of the well-defined genus *Campylomormyrus*. This is an unexpected result in conflict with morphological data (Taverne, 1972). The sister group relationship between *H. bebe* and *B. niger* receives moderate support. Despite PBS analysis indicating that this relationship is supported only by nuclear data, this relationship has already been observed in an analysis of partial cyt *b* sequences (Lavoué *et al*., 2000). Paradoxically in this study, cyt *b* data seem incongruent with this relationship (see PBS, Fig. 8). More data are needed to ascertain the relationships between these two species. S7 data provide additional support for the monophyly of *Stomatorhinus* and *Pollimyrus*. Taverne (1972) proposed that *Stomatorhinus*, *Brienomyrus* and *Boulengeromyrus* form a monophyletic group based on 'osteological similarity' and did not assign a position for *Pollimyrus* within the Mormyrinae. More recently, a close relationship between *Stomatorhinus* and *Pollimyrus* was thought possible after the observation that they share the same complex electrocyte type, DPNp (doubly penetrating and non-penetrating stalk with posterior innervation), which is unique in the Mormyridae (Bass, 1986; Alves-Gomes & Hopkins, 1997). While finding that *Stomatorhinus* and *Pollimyrus* formed a monophyletic group (albeit with low support), Sullivan *et al*. (2000) re-examined the electrocyte structure for both genera and reclassified the electrocyte structure in *Stomatorhinus* as type Pa (possessing a single penetrating stalk system with anterior innervation). S7 data provide moderate to high support (BP, BSI) for the sister relationship between *Stomatorhinus* and *Pollimyrus*, although no one structural synapomorphy uniquely supports it.

The conclusions of the combined analyses presented here differ from those of Sullivan *et al*. (2000) in two main points, the position of the genus *Mormyrus* and the position of *H. pictus*.

Phylogeny of Mormyrinae, new findings

Taverne (1972) left the position of *Mormyrus* unresolved at the base of the tree of the Mormyrinae. Sullivan *et al*. (2000) proposed that the (*B. brachyistius*, *I. henryi*) clade diverged before the genus *Mormyrus*. Only moderate BP and BSI supported this topology and it was supported uniquely by rag 2 data. These authors did note that the position of *Mormyrus* reported here was recovered under particular character weighting scenarios. Our analysis of the S7 data alone and combined with the data adapted from Sullivan *et al*. (2000) indicate that *Mormyrus* is the sister group of *B. brachyistius* and *I. henryi* with high support. Other studies have not addressed the relative phylogenetic position of *Mormyrus* because of incomplete taxonomic sampling (Agnèse & Bigorne, 1992; van der Bank & Kramer, 1996; Lavoué *et al*., 2000).

Pappenheim (1906) described the genus *Hippopotamyrus* for *H. castor*. Myers (1960) described a new genus, *Cyphomyrus*, for some species in which the body was deep and the dorsal fin more elongated than the anal fin. However, Taverne (1971a) synonymised *Cyphomyrus* with *Hippopotamyrus*, forming a morphologically ill-defined and heterogeneous genus. In a study based primarily on allozyme data, Van der Bank & Kramer (1996) first suggested that the genus *Hippopotamyrus* (*sensu* Taverne) is polyphyletic, and proposed the reinstatement of *Cyphomyrus* as a valid genus. Sullivan *et al*. (2000) also showed *Hippopotamyrus* to be a polyphyletic genus, with two lineages. In our study, the diphyly of the genus *Hippopotamyrus* is strongly supported although with a different position for *H. pictus* than in Sullivan *et al*. (2000). We find the *M. senegalensis* clade and *H. pictus* to be sister groups, to the exclusion of *B. niger* and *Hyperopisus bebe*. *H. discorhynchus* and *H. wilverthi* form a monophyletic group, embedded in the *M. senegalensis* clade. Following the conclusion of van der Bank & Kramer (1996), the reinstatement of *Cyphomyrus* as a valid genus seems plausible (represented by *H. discorhynchus* and *H. wilverthi* in our study). However, additional sequences of representatives of the genus *Hippopota-*

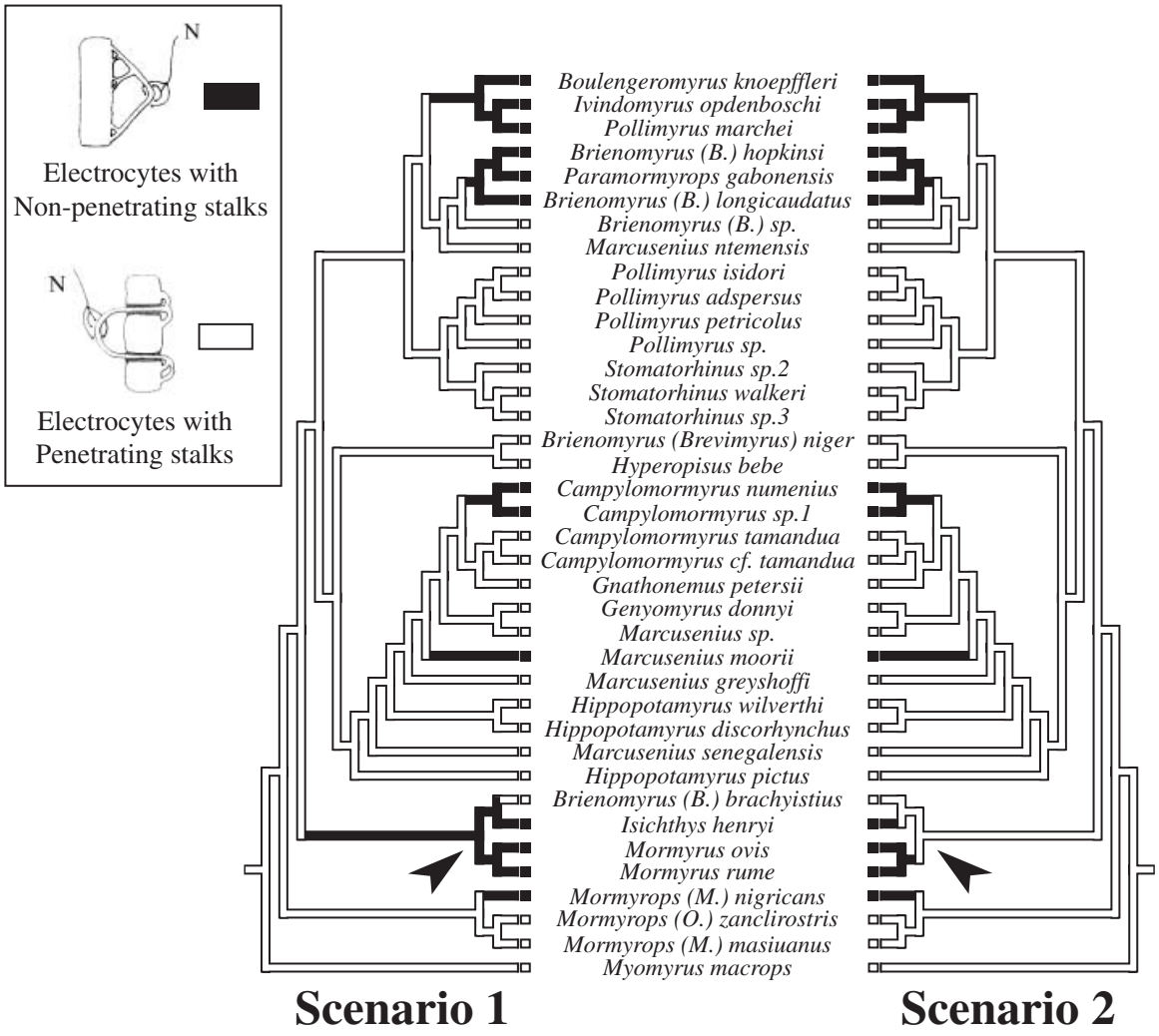

Figure 9. Reconstructions of the evolution of the presence/absence of the penetrating stalk electrocytes onto the two most parsimonious trees derived from the combined analysis using MacClade (Maddison & Maddison, 1997). Open branches refer to state 'presence of penetrating stalks'; closed branches refer to state 'non-penetrating stalks'. Arrowheads indicate differences between both scenarios. Abbreviation 'N' represents electromotor neuron. Diagrams of electrocytes come from Bass (1986).

myrus, especially from the type species *H. castor*, as well as osteological studies are needed to clarify the taxonomic status of all representatives of this polyphyletic genus.

Evolution of electric organs

The adult fishes from the family Mormyridae have electric organs derived from caudal muscle. These electric organs are composed of disc-shaped, multinucleated cells, called electrocytes. Two main types of electrocyte morphologies based on the presence or absence of penetrating stalks are currently recognized among Mormyridae (Bass, 1986; Alves-Gomes & Hopkins, 1997; Sullivan *et al*., 2000): non-penetrating stalk electrocytes (always innervated on the posterior face, type NPp) and penetrating stalk electrocytes. These authors distinguish four subtypes in this second group based on the position of the electrocyte innervation and other features of the stalk system: (1) penetrating stalk electrocytes innervated on the anterior face (type Pa); (2) penetrating electrocytes innervated on the posterior face (type Pp); this type is simply the inverted version of the Pa electrocyte; (3) doubly penetrating stalk electrocytes innervated on the posterior face (type DPp) and (4) doubly penetrating and non-penetrating stalk electrocytes innervated on the posterior face (type DPNP). The last three types possess penetrating stalks and therefore represent modified forms of type Pa.

First, we mapped the presence or absence of the penetrating stalks onto our two MP trees derived from the combined analysis using unweighted parsimony character reconstruction in MacClade (Maddison & Maddison, 1997). Two equiparsimonious reconstructions can be inferred for the evolution of the electrocyte stalk system in the subfamily Mormyrinae (Fig. 9). Both reconstructions require seven steps. Both reconstructions optimize the presence of the penetrating stalk as the ancestral state within Mormyrinae and the non-penetrating stalk electrocyte (type NPp) as a derived condition in agreement with the hypothesis of Sullivan *et al*. (2000). In the first, there are six independent reversals from penetrating stalks to non-penetrating stalks, and one transition from non-penetrating stalks to penetrating stalks. In the second scenario, there are seven independent reversals from penetrating stalks to non-penetrating stalks. Because both reconstructions require multiple independent paedomorphic transitions to electrocyte NPp, because our modified phylogeny generated a similar scenario for electrocyte evolution and finally because we believe that independent appearance of a complex character such as 'penetrating stalks' is less likely than paedomorphic reversals to a simpler condition, we favour this latter reconstruction. Thus, despite some differences between the combined data trees which include the S7 data and the earlier phylogenetic hypothesis proposed by Sullivan *et al*. (2000), conclusions about the evolution of the electric organs are unaltered.

Finally, we mapped the evolution of each particular electrocyte state (NPp, Pa, Pp, DPNP and DPp) onto one of the most parsimonious trees (the phylogenetic irresolution among *Mormyrops* species did not influence our perception of electrocyte evolution, Fig. 9). Pp and DPNP are shown to have originated once from Pa, while type DPp has evolved twice.

AKNOWLEDGEMENTS

We would like to thank J. H. Mve (IRAF, Gabon) for help with fieldwork; P. Posso (IRET) and J. D. Mbega (IRAF) for logistic assistance in Gabon; I. Lovette, J. Friel and K. Zamudio for comments on the manuscript; G. Harned for technical assistance in the laboratory. This work was funded by NSF (INT-9605176), NIMH (MH37972) and National Geographic Society grants (5801-96) to C.D.H.

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