ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/13087014

Systematic biology of gymnotiform and mormyriform electric fishes: Phylogenetic relationships, molecular clocks and rates of evolution in the mitochondrial rRNA genes

Article *in* Journal of Experimental Biology · June 1999

Source: PubMed

CITATIONS
73

READS
130

1 author:

José A Alves-Gomes
Instituto Nacional de Pesquisas da Amazônia
42 PUBLICATIONS **1,063** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Biomonitoring View project

All content following this page was uploaded by José A Alves-Gomes on 27 November 2015.

The user has requested enhancement of the downloaded file.

The Journal of Experimental Biology 202, 1167–1183 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB2077

SYSTEMATIC BIOLOGY OF GYMNOTIFORM AND MORMYRIFORM ELECTRIC FISHES: PHYLOGENETIC RELATIONSHIPS, MOLECULAR CLOCKS AND RATES OF EVOLUTION IN THE MITOCHONDRIAL rRNA GENES

JOSÉ A. ALVES-GOMES*

Instituto Nacional de Pesquisas da Amazônia, Coordenação de Pesquisas em Biologia Aquática, Manaus, AM 69083-000, Brazil

*e-mail: puraque@inpa.gov.br

Accepted 25 January; published on WWW 21 April 1999

Summary

The phylogenetic relationships of both African and South American electric fish orders are reviewed at their intra-ordinal level taking into consideration recent studies in which cladistic principles have been employed. Several concordant topologies emerge from the different data sets, but some unsettled issues still remain. From the studies available, a consensus topology has been suggested for the Mormyriformes and for the Gymnotiformes. Subsequently, the evolutionary relationships of these two electric fish clades are considered within each respective superorder, i.e. in relation to the other osteoglossomorph and ostariophysan orders. The inter-ordinal phylogenies are used as a framework to test the molecular clock hypothesis with two gene fragments of the mitochondrial genome. Gymnotiformes, Siluriformes and Characiformes are accumulating mutations at the same pace in relation to their respective outgroups, but for all the other combinations of sister clades tested the molecular clock can be statistically rejected. Fossil records are then surveyed and used to calibrate absolute rates of genetic differentiation for each main lineage (orders) of both osteoglossomorphs and ostariophysans. The most conserved regions (stems) of the 12S and 16S gene fragments used are evolving at an average rate of $0.123\,\%\,10^6$ years^{-1} for the osteoglossomorphs and $0.137\,\%\,10^6$ years^{-1} for the ostariophysans, with no significant difference between these two values. The rate of mutation in the loops, the faster-evolving segments, estimated for closely related electric fish taxa is $0.82\,\%\,10^6$ years^{-1} for four *Brienomyrus* species and $1.01\,\%\,10^6$ years^{-1} for the four eigenmanniid genera. When the entire molecule (loops + stems) is considered, the rate of mutation in both mormyriforms and gymnotiforms converges to a rounded value of $0.23\,\%\,10^6$ years^{-1}.

Key words: electric fish, systematics, molecular clock, Gymnotiformes, Mormyriformes, Osteoglossomorpha, Ostariophysi, molecular evolution, phylogeny.

Introduction

Since the first studies by Lissmann and Machin around the middle of this century (Lissmann, 1951; Lissmann and Machin, 1958; Machin and Lissman, 1960), neurobiologists have learned a great deal about ‘how’ and ‘why’ the electrogenic and electrosensory systems (EESs[1]) of electric fish perform in one particular way or another (for reviews, see Bullock and Heiligenberg, 1986; Moller, 1995). However, studies combining physiological, anatomical and behavioral findings only infrequently consider the phylogenetic history of the fish, despite the broad recognition of the importance of such an evolutionary approach. Phylogenetic hypotheses deliver an indispensable perspective to comparative studies, not only by permitting the study of character evolution within a proper evolutionary framework of sister and outgroup relationships but also by bringing the concept of relative time (sister groups have the same age) into the study of how such changes occur.

The present article focuses on the evolutionary biology of the orders Mormyriformes (superorder Osteoglossomorpha) and Gymnotiformes (superorder Ostariophysi), two teleosts clades widely separated within the evolutionary history of fishes (Fig. 1), but which have independently developed a very elaborate and similar EES. In both gymnotiforms and mormyriforms, the field generated during each electric organ discharge (EOD) is monitored by an array of several types of electroreceptors distributed over the fish’s skin. Disturbances in the voltage gradient across the excitable membrane of the

[1]In this paper, the abbreviation EESs will be employed to refer to both the electrogenic and the electrosensory systems as a functional unit. The use of this abbreviation is intended to emphasize the conjunct performance of the two systems, which have not always appeared simultaneously in teleost evolution. There is at least one electrogenic teleost that is not electroreceptive (*Astroscopus*), and there are several electroreceptive fish lineages that do not produce electric organ discharges (Bullock et al., 1983).

electroreceptors, caused by an interfering source, are encoded at the level of electroreceptors and sequentially sent to higher centers of the nervous system where the information is processed. The EES is used by the fish for electrolocation and communication, and plays a crucial role in how the fish senses and deals with the external world.

Despite the series of outstanding similarities between the EESs of gymnotiforms and mormyriforms at several levels of their sensory and motor anatomy and physiology (Finger et al., 1986; Zakon, 1986; Kawasaki, 1993), it is well established that these two groups 'invented' their EESs completely independent of one another. The two ancestral teleost lineages that later gave rise to the living mormyriforms and gymnotiforms had been separated for at least 140 million years, as inferred from the oldest fossil assigned to the osteoglossomorphs (see below), and there is no evidence that either electric organs or electroreceptors were present in any of the most basal ostariophysans (in the case of gymnotiforms) or osteoglossomorphs (in the case of mormyriforms).

In this article, a consensus phylogenetic hypothesis for Gymnotiformes and Mormyriformes is first proposed, according to evidence gathered from several studies which employed cladistic principles to analyze morphological, behavioral and molecular data sets. Phylogenetic relationships were considered at both the intra- and inter-ordinal level. By adopting a relatively well-corroborated phylogeny for both electric fish orders within each respective superorder, and having DNA sequences available for all relevant lineages considered, the relative rate method was used to determine whether the DNA sequences used in this study are evolving in a clock-like manner in both osteoglossomorphs and ostariophysans. After the molecular clock hypothesis had been tested in the molecular data set, the fossil records of each main lineage were used to calibrate absolute rates of genetic differentiation between the different fish orders.

The temporal framework provided by the molecular clock and fossil records, despite being subject to some methodological and sampling inaccuracy, can be used to estimate roughly the time of divergence between any of the fish lineages used in this study. The results shown here represent an initial and tentative step in the sense of bringing evolutionary time into the study of EES evolution. As an immediate consequence, a more likely estimate of the time of appearance of particular features inherent to each fish lineage, such as the different structures, connections and behaviors associated with the EES, can be proposed.

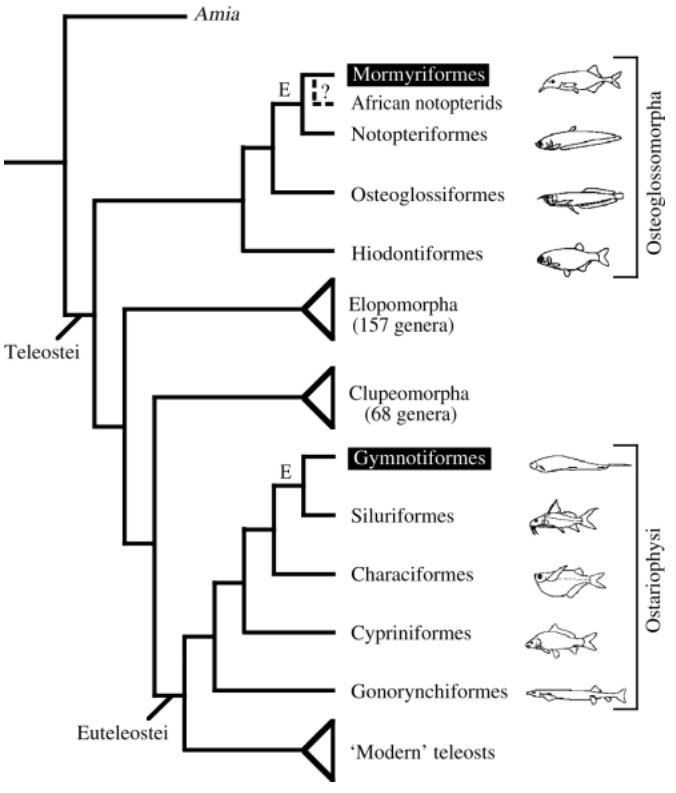

Fig. 1. Partial teleost phylogeny indicating the phylogenetic position of the two electric fish orders Gymnotiformes and Mormyriformes. E, electroreceptive capability. Current evidence suggests that the order Notopteriformes may not be monophyletic and that African notopteriforms, which are electroreceptive, may be in fact the sister group of Mormyriformes.

Methodological rationale

The nomenclature adopted in this article for the osteoglossomorph orders was adapted from Taverne (1979), with the main difference being that Taverne's Notopteroidei is treated here as an order (Notopteriformes). The ostariophysan and the gymnotiform clades follow Fink and Fink (1996) and Alves-Gomes et al. (1995), respectively.

The present study was conducted using a mitochondrial DNA data matrix formed primarily from published sequences available in GenBank. The complete data matrix comprised 39 taxa, 17 of the superorder Osteoglossomorpha and 22 of the superorder Ostariophysi. At least one representative of each main lineage within each of the superorders was included in the analyses. Two segments of the mitochondrial genome were used, the 12S and the 16S rRNA genes, which together make a few more than 800 sites. Table 1 provides further information about the taxa used and the accession numbers of the sequences in GenBank. Only the sequence for the siluriform genus *Pangasius* has not been published previously. All the methodological procedures used to obtain the nucleotide sequences for both 12S and 16S rRNA fragments in *Pangasius* followed the protocols described by Alves-Gomes and Hopkins (1997).

This study focuses on the South American and African electric fishes, and therefore a larger number of representatives of these groups (13) was included. Efforts were made to give equivalent representation to the gymnotiform and mormyriform sequences. Unfortunately, sequences were available for only five genera of the mormyriform subfamily Mormyrinae. Considering that this subfamily contains 17 recognized genera and that their phylogenetic relationships are still not well

Table 1. *List of taxa used in the present study*

List of taxa	GenBank accession number
Gonorhynchiformes	
Kneria sp.	GB: U33990, U34028
Parakneria sp.	GB: U33991, U34029
Cypriniformes	
Cyprinus carpio	GB: X61010
Crossostoma lacustre	GB: M91245
Characiformes	
Hepsetus odoe	GB: U33852, U33992
Tetragonopterus sp.	GB: U33973, U34010
Siluriformes	
Helogenes sp.	GB: AF072136, AF072150
Malapterurus sp.	GB: U15261, U15237
Pangasius sp.	GB: AF072750, AF072751
Gymnotiformes	
Gymnotidae	
Gymnotus sp.	GB: AF072137, AF072151
Sternopygidae	
Sternopygus macrurus	GB: U15252, U15228
Rhamphichthyoidea	
Steatogenys elegans	GB: U15252, U15228
Apteronotidae	
Apteronotus albifrons	GB: U15275, U15226
Eigenmanniidae	
Eigenmannia cf virescens 1	GB: U15269, U15245
Eigenmannia cf virescens 3	GB: AF072144, AF072158
Eigenmannia cf virescens 4	GB: AF072146, AF072160
Eigenmannia cf virescens 5	GB: AF072145, AF072159
Eigenmannia humboldtii	GB: AF072147, AF072161
Eigenmannia sp.	GB: AF072148, AF072162
Archolaemus blax	GB: AF072149, AF072163
Distocyclus conirostris	GB: U15279, U15246
Rhabdolichops eastwardi	GB: AF072141, AF072155
Mormyriformes	
Gymnarchidae	
Gymnarchus niloticus	GB: U33514, U33529
Petrocephalinae	
Petrocephalus bovei	GB: U33518, U33533
Petrocephalus sp.	GB: X99173, X99174
Mormyrinae	
Brienomyrus brachyistius	GB: U33505, U33520
Gnathonemus petersii	GB: U33513, U33528
Marcusenius senegalensis	GB: U33515, U33530
Brienomyrus niger	GB: U33506, U33521
Brienomyrus batesii	GB: U33504, U33519
Brienomyrus sp. 1	GB: U33507, U33523
Brienomyrus sp. 2	GB: U33509, U33524
Brienomyrus sp. 3	GB: U33510, U33525
Brienomyrus sp. 4	GB: U33511, U33526
Brienomyrus sp. 5	GB: U33512, U33527
Notopteriformes	
Notopterus chitala	GB: U33516, U33531
Osteoglossiformes	
Pantodon bucholzi	GB: U33517, U33532
Osteoglossum ferrerai	GB: X99172, X99171
Hiodontiformes	
Hiodon olosoides	GB: X99170, X99169

established, it is assumed that only a fraction of the total genetic divergence within mormyrines was sampled. In the case of the gymnotiforms, enough representatives of each main lineage were included to ensure that most of the potential genetic variability in the order was taken into consideration. In both groups of electric fish, one genus was over-represented as a means of comparing the intra-generic genetic divergence between the South American and African electric fishes. Hence, the average genetic divergence found among representatives of the gymnotiform genus *Eigenmannia* was computed and compared with the divergence found among representatives of the genus *Brienomyrus* of the mormyriforms.

The sequences of the 39 taxa were aligned taking into consideration the secondary structure of each molecule, as described in detail by Alves-Gomes et al. (1995) and Alves-Gomes and Hopkins (1997). Briefly, as the rRNA molecules fold to form their secondary structure, individual nucleotides can form pairs with complementary bases (A–T and C–G). The paired nucleotides define the stems in the secondary structure, whereas the unpaired sites form either loops or bulges. For a great variety of vertebrates, it has been shown that the unpaired sites are normally more variable than the stems (Gutell and Fox, 1988; Wheeler and Honeycutt, 1988; Gutell et al., 1992; Dixon and Hillis, 1993; Gutell, 1993). Therefore, it may become difficult to obtain an unambiguous alignment for these highly variable sites as the evolutionary distance between the clades increases. In this study, all the sites for which alignment was dubious when the 39 taxa were considered were excluded from the analysis. In other words, the analyses were performed upon the most conservative sites of both molecules for which the alignment was unequivocal throughout the entire data matrix. The aligned sequences can be obtained by direct request from the author.

Genetic distances (d), or the number of substitutions per site, were calculated without being corrected for multiple hits (P distance) for each possible pair of taxa in the data matrix, where d is the absolute number of mismatches per total number of sites compared. Sites with missing characters and gaps were ignored in the affected pairwise comparisons. The average distances between each one of the main clades within each superorder were calculated subsequently.

To test whether the substitution rates within each lineage were accumulating mutations in a steady manner in both osteoglossomorphs and ostariophysans (molecular clock hypothesis), the distances between each lineage within a pair of sister taxa and their immediate common outgroup were computed and compared (relative rate test), as described by Easteal (1992) and Li (1997). For instance, if we consider three clades A, B and C, where A and B are sister groups and C is the sister group of (A+B), the molecular clock hypothesis can be accepted if the distance from the outgroup C to each one of the ingroups A and B is not significantly different, i.e. $d_{C\text{-}A}=d_{C\text{-}B}$. If the number of taxa present in each clade exceeds one, then $d_{C\text{-}A}$ and $d_{C\text{-}B}$ are computed as the average distance for all pair-wise comparisons between the representatives of A and C and of B and C, respectively.

Once the molecular clock hypothesis had been statistically tested, the relevant fossil records for each main lineage were surveyed, and the oldest fossil of each clade was brought into the phylogenetic context as a benchmark reference to calibrate absolute rates of genetic differentiation.

Results and discussion

The aligned data set had a total of 810 sites, 354 in the 12S rRNA and 456 in the 16S rRNA. The phylogenetic hypotheses generated by the cladistic analysis of these data sets have been published for both the gymnotiforms (Alves-Gomes et al., 1995; Alves-Gomes, 1998) and the mormyriforms (Alves-Gomes and Hopkins, 1997). Details of the base composition, functional constraints and substitution bias are found in these papers and conform with the results obtained for the same genes for other teleosts (Bargelloni et al., 1994; Ortí et al., 1996; Montoya-Burgos et al., 1997; Simons and Mayden, 1998).

The present paper does not emphasize the phylogenetic hypotheses generated by the molecular data set alone, but instead attempts to compile a consensus phylogenetic hypothesis molded by several sources of data, as discussed below. The main purpose of the molecular data set is to compute the average genetic divergence between the clades and to test the molecular clock hypothesis. Table 2 presents information about the mean genetic divergence calculated between the main clades of this study.

The subsequent discussion takes the following form: (1) a consensus topology for gymnotiforms and mormyriforms is compiled from several published papers; (2) the results of relative rate tests are discussed, and it is shown that the molecular clock hypothesis holds only for some of the ostariophysan lineages included in this study; (3) the oldest fossil records of each ostariophysan and osteoglossomorph order are surveyed and used to calibrate absolute rates of genetic divergence, and (4) the absolute geological time of split between the different orders is estimated.

Systematics of Gymnotiformes

According to the most recent revision of Mago-Leccia (1994), the order Gymnotiformes encompasses 27 genera and 97 species. In the last 5 years, however, with the increasing interest of systematists in the group and with the addition of new collecting techniques in the Amazon, such as bottom-trawling in the main rivers and the more intensive use of 'electric fish detectors' in previously unprobed habitats, several new taxa have been discovered. Recently, two new apteronotid

Table 2. *Average distance for all pairwise comparisons between the representatives of the main clades of the Ostariophysi and Osteoglossomorpha used in this study*

	Gon	Cyp	Cha	Sil	Gym	Hio	Ost	Not
Gonorhynchiformes	–							
Cypriniformes	15.23±0.21							
	22.90±0.77	–						
Characiformes	15.64±0.36	11.91±0.74						
	24.80±0.34	16.37±1.33	–					
Siluriformes	16.75±1.89	12.15±2.89	11.30±2.80					
	27.26±5.51	17.02±5.70	16.09±5.90	–				
Gymnotiformes	16.48±0.52	11.60±0.83	11.55±1.03	12.90±2.50				
	26.47±1.49	15.94±1.49	16.07±1.88	19.31±5.48	–			
Hiodontiformes	19.11±0.32	15.11±0.90	15.05±0.00*	16.85±2.61	15.06±1.16			
	33.09±0.49	22.44±1.76	23.21±0.45	28.30±6.64	23.43±2.70	–		
Osteoglossiformes	22.60±0.78	18.90±0.19	19.05±1.18	19.53±0.34	20.70±1.10	18.30±0.74		
	44.20±2.83	33.39±0.42	34.63±4.30	35.25±1.81	39.38±3.57	29.51±2.50	–	
Notopteriformes	18.46±0.52	14.19±0.67	15.56±0.61	16.49±1.35	17.62±0.74	14.84‡	12.45±1.12	
	30.56±1.29	21.35±1.81	25.33±0.83	27.40±3.23	30.50±2.03	22.12	17.88±1.39	–
Mormyriformes	20.26±0.75	16.55 ±0.82	16.25±0.87	18.31±0.97	18.04±0.85	15.01±0.48	14.06±0.69	08.69±0.74
	36.58±2.24	26.62±2.08	26.03±2.40	31.48±2.84	30.81±2.51	21.69±0.92	20.64±1.84	11.03±1.21

Values are means ± S.D. (*N* varies (2–169) for each pairwise comparison, according to Table 1).

*The two distances were the same.

‡Only one representative of each clade was available in the data matrix.

The upper values in each pairwise comparison correspond to the P (uncorrected) distances, or the average number of substitutions per 100 sites, whereas the lower numbers represent the distances corrected for multiple hits using the Tamura–Nei model.

Gon, Gonorhynchiformes; Cyp, Cypriniformes; Cha, Characiformes; Sil, Siluriformes; Gym, Gymnotiformes; Hio, Hiodontiformes; Ost, Osteoglossiformes; Not, Notopteriformes.

(*Magosternarchus* and *Tembeassu*) and one rhamphichthyoid (*Iracema*) genera, plus several species from different families have been described, currently totaling approximately 110 gymnotiform species (Costa and Campos-da-Paz, 1991; Campos-da-Paz and Paepke, 1994; Mago-Leccia, 1994; Albert and Miller, 1995; Albert and Fink, 1996; Campos-da-Paz, 1996; Lundberg et al., 1996; Triques, 1996a,b, 1998). Additional new taxa, including new genera and species, are currently in the process of being described by several authors (J. P. Sullivan, unpublished results; J. G. Lundberg, unpublished results; R. Campos-da-Paz, unpublished results; J. A. Alves-Gomes, unpublished results). If this tendency holds, it is possible that the total number of nominal species of gymnotiforms may exceed 150 in the next 5–10 years.

As a group, the monophyly of gymnotiforms has never been disputed, but for some time the gymnotiforms were considered to be closely related to the characins (Rosen and Greenwood, 1970; Mago-Leccia and Zaret, 1978). This idea was radically modified after Fink and Fink (1981) presented a list of synapomorphies, including electroreceptive capability, pointing to a monophyletic gymnotiform–siluriform clade. Since then, Fink and Fink's hypothesis has been strengthened by additional evidence (Alves-Gomes, 1995; Dimmick and Larson, 1995; Fink and Fink, 1996). At present, it is broadly accepted that South American electric fishes and the catfishes are indeed sister groups, i.e. they share an immediate common ancestor that is not shared with any other teleost lineage.

Concerning the intra-ordinal relationships of the gymnotiforms, the taxonomic scheme derived from the studies of Mago-Leccia (1976, 1978) was the most frequently adopted version of a systematic classification of gymnotiforms by the early 1990s, albeit that Mago-Leccia's studies were not explicitly cladistic. In the last 5 years, however, several authors have addressed the gymnotiform phylogeny, including morphological, behavioral, molecular and fossilized data (Triques, 1993; Gayet et al., 1994; Alves-Gomes et al., 1995; Albert, 1999; Albert and Campos-da-Paz, 1998; Alves-Gomes, 1998; for a review, see Campos-da-Paz and Albert, 1998). The authors have reached moderately different conclusions depending upon the data set used, but several concordant relationships have emerged. Most of the groups proposed by Mago-Leccia (1978) as monophyletic clades on the basis of morpho-osteological features (predominantly his 'morphotypes') have been supported by independent data sets in more recent studies. However, there are three particularly problematic clades.

First, by taking into consideration the existing morphological evidence at the time *versus* the results obtained using molecular data (12S and 16S rRNA sequences) plus additional data associated with the EES, Alves-Gomes et al. (1995) argued that Mago-Leccia's family Sternopygidae was not a monophyletic clade. *Sternopygus* was suggested to represent a different lineage from the remaining genera of the family (*Rhabdolichops*, *Archolaemus*, *Distocyclus* and *Eigenmannia*), and a new family Eigenmanniidae, excluding *Sternopygus*, was proposed. More recently, however, additional morphological characters supporting the sister group relationships between *Sternopygus* and the eigenmanniids have been suggested (Albert and Fink, 1996; Albert, 1999; Albert and Campos-da-Paz, 1998). Alves-Gomes (1998) has addressed these new proposed synapomorphies and re-evaluated the mitochondrial phylogeny of the group, including *Sternopygus*, all eigenmanniid genera and several species not previously used, plus representatives of every other main gymnotiform lineage. The new analyses confirmed *Sternopygus* as a separate lineage within gymnotiforms, despite the new eigenmanniid taxa added. In addition, the new proposed synapomorphies placing the genus together with the eigenmannids were shown to be problematic in several aspects; therefore, from the evidence compiled, *Sternopygus* was still considered by Alves-Gomes (1998) to represent a different lineage from the eigenmanniids. Clearly, the discussion has not yet terminated, and additional studies are required to settle this matter definitively. Besides the unsettled phylogenetic position of the genus *Sternopygus*, there is also a debate about the phylogenetic relationships among the eigenmanniids (*sensu* Alves-Gomes, 1995), since the morphological data have generated four different hypotheses about their intergeneric relationships (Mago-Leccia, 1978; Fink and Fink, 1981; Triques, 1993; Albert and Fink, 1996; Albert and Campos-da-Paz, 1998). The mitochondrial phylogeny obtained by Alves-Gomes (1998) fully supports the alternative suggested by Mago-Leccia (1978) about eigenmanniid phylogeny, in which *Eigenmannia* and *Distocyclus* are sister groups, *Archolaemus* is the sister group of (*Eigenmannia* + *Distocyclus*) and *Rhabdolichops* is the sister group of them all.

The second gymnotiform group that is also contentious is the family Hypopomidae (*sensu* Mago-Leccia, 1976). Mago-Leccia (1976, 1978) initially defined a rhamphichthyoid morphotype including all the representatives of the families Hypopomidae and Rhamphichthyidae. Further, this author placed all the short-snouted genera (*Hypopomus*, *Racenisia*, *Brachyhypopomus*, *Microsternarchus*, *Steatogenys* and *Hypopygus*) in the family Hypopomidae, whereas he kept in the separate family Rhamphichthyidae the genera with long snouts and/or tubular mouth (*Rhamphichthys*, *Gymnorhamphichthys* and now *Iracema*). This hypothesis remained undisputed for almost two decades, but recent studies based both on molecular and morphological data (Alves-Gomes et al., 1995; Sullivan, 1997; J. P. Sullivan, personal communication) suggest that *Steatogenys* and *Hypopygus*, formerly hypopomids, are likely to be more closely related to *Rhamphichthys* and *Gymnorhamphichthys*. Therefore, although rhamphichthyoids are clearly monophyletic, Hypopomidae, as defined by Mago-Leccia (1976), appears to be an unnatural assemblage.

The third gymnotiform clade for which phylogenetic relationships are still unsettled is the specious family Apteronotidae. Although there is a general consensus among authors that Apteronotidae is a monophyletic group, the relationship between the genera still needs extensive additional studies. Albert (1999) and Albert and Campos-da-Paz (1998)

have originated phylogenetic hypotheses for the genera in the family based mainly upon anatomical characters, but unpublished molecular results point to quite different topologies (J. A. Alves-Gomes, unpublished results). Among the few agreements concerning the apteronotids are the probable paraphyletic status of the genus *Apteronotus* and the close relationship between *Magosternarchus* and *Sternarchella* (Lundberg et al., 1996; J. A. Alves-Gomes, unpublished results). Uncovering the phylogeny among the apteronotids may be a challenging issue, but the evolutionary relationships nested within the most diversified gymnotiform clade may reveal interesting questions about their biology and evolutionary history.

In summary, the current evidence suggests that within the order Gymnotiformes there are at least the following main monophyletic lineages: Sternopygidae, (Electrophoridae + Gymnotidae), (Rhamphichthyidae + Hypopomidae) and (Eigenmanniidae + Apteronotidae).

The interrelationships among these seven main lineages are also somewhat controversial, and several hypotheses have been proposed (for a comprehensive review, see Campos-da-Paz and Albert, 1998). For instance, there is no agreement about which of the extant gymnotiform represents the oldest South American electric fish lineage. It has been suggested that apteronotids are the extant representatives of the most plesiomorphic gymnotiforms, primarily because they possess a caudal fin, as do the other ostariophysans outgroups (Triques, 1993; Gayet et al., 1994). However, several other studies using molecular and morphological data suggest that the apteronotids are in fact a more derived group within the order (Mago-Leccia, 1994; Alves-Gomes et al., 1995; Albert, 1999; Albert and Campos-da-Paz, 1998), and the presence of a caudal fin in this group should be considered a more recent and autapomorphic reversal. Other authors have suggested that gymnotiforms should be divided into two major monophyletic groups: one containing all the wave-type species [(*Sternopygus* + eigenmanniids) apteronotids], and the second all the pulse-type genera (Mago-Leccia, 1994; Albert, 1999). Alves-Gomes et al. (1995) argued in favor of *Sternopygus* as the most plesiomorphic gymnotiform on the basis of their DNA sequence analyses and the relatively simple architecture and physiology associated with the pacemaker circuitry in this genus (Rose et al., 1987; Keller et al., 1991). However, according to mitochondrial rRNA data alone, neither the position of *Sternopygus* nor that of the (*Gymnotus* + *Electrophorus*) clade could be definitely established within the order, and it would therefore still be a bit premature to advocate a monophyletic assemblage for the pulse-type fish. Fig. 2 depicts a scheme with a proposed consensus about gymnotiform phylogeny compiled from the studies mentioned above.

Systematics of Mormyriformes

Despite the rich material that mormyriforms have been providing for anatomical and physiological studies for decades, the phylogenetic relationships among the great majority of the genera are still poorly understood. With few exceptions, the studies addressing taxonomic aspects of mormyriform biology are essentially of a descriptive nature and do not use any explicit method of phylogenetic estimation. The series of papers by Taverne (1969, 1970, 1971a,b, 1972) probably includes the most detailed anatomical description of the order, but unfortunately it lacked any supplementary analyses based upon cladistic reasoning and, therefore, its immediate use as a source of phylogenetic hypotheses is not possible.

The group is considered to be monophyletic and composed of approximately 196 species distributed into 18 genera (Boden et al., 1997). Curiously, the basal mormyriform lineages (Gymnarchidae and Petrocephalinae, see below) are monogeneric clades, and the great majority of the species are included in the subfamily Mormyrinae, which has 16 genera, more than 170 species and a great number of undescribed taxa (Bass, 1986; Hopkins, 1986; Alves-Gomes and Hopkins, 1997).

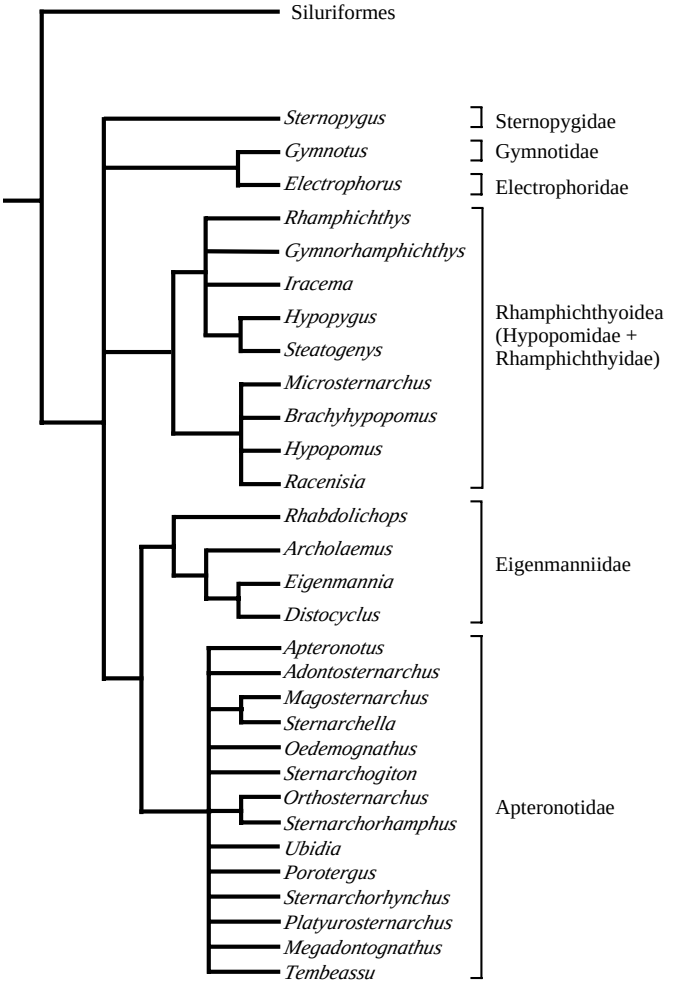

Fig. 2. Consensus phylogeny for the order Gymnotiformes derived from studies based upon cladistic analyses of morphological, physiological and molecular data. There is no current agreement among authors about the phylogenetic position of *Sternopygus* or of the *Gymnotus* + *Electrophorus* clade. Much remains to be done in relation to the internal phylogeny of Apteronotidae.

As in the case of the gymnotiforms, only recently have more explicit methodological principles of phylogenetic analysis been used in studies addressing mormyriform relationships. Agnèse and Bigorne (1992) and Van Der Bank and Kramer (1996) have used genetic distances derived from protein electrophoresis to examine the phylogenetic relationships among five and six mormyriform genera, respectively. Van Der Bank and Kramer (1996) also considered behavioral and ecological characters in association with the genetic distances to obtain their final hypothesis. Benveniste (1995) has produced an important work based on maximum parsimony analysis of morphological characters for the basal mormyriform clades, and Alves-Gomes and Hopkins (1997) used mitochondrial DNA sequences to assess the phylogenetic relationships among five mormyriform genera. Unfortunately, none of these studies had a good representation of the Mormyrinae in their analyses, and the inter-generic relationships within this subfamily are therefore still in need of much work.

By examining the results of the studies mentioned above, the only conspicuous discrepancy between the authors is the awkward phylogenetic position of *Petrocephalus* according to the final results of Van Der Bank and Kramer (1996), who departed radically from the proposals of the remaining authors (see below). Otherwise, it is possible to infer a phylogenetic hypothesis that is reasonably well supported by morphological and molecular characters (Fig. 3), at least for the basal clades of the mormyriforms.

According to many sources of evidence, including morphological and molecular studies, the sister group of Mormyriformes is the order Notopteriformes (Greenwood, 1971, 1973; Taverne, 1979; Lauder and Liem, 1983; Benveniste, 1995; Li and Wilson, 1996; Alves-Gomes and Hopkins, 1997). However, the fact that the African notopteriform genera *Xenomystus* and *Papyrocranus* are electroreceptive, whereas the Asian genus *Notopterus* is not, has given rise to the possibility that the order Notopteriformes is not monophyletic and that the electroreceptive xenomystines are in fact the sister group of the Mormyriformes (Braford, 1982, 1986). The phylogenetic study of Benveniste (1995) based upon morphological characters also shows some indication, although slight, that Notopteriformes may indeed be paraphyletic and that the electroreceptive genera may in fact be more closely related to mormyriforms. This hypothesis is in a great need of being tested using alternative data sets.

Within the Mormyriformes, there are two main clades: a monotypic clade containing only *Gymnarchus niloticus* (family Gymnarchidae), and the family Mormyridae encompassing all the remaining mormyriform genera. The recognition of *Gymnarchus* as a mormyriform, despite its conspicuous dissimilarity from a 'typical' mormyrid external morphology, goes back to Erdl (1847; cited in Benveniste, 1995), but Günther (1880) and Boulenger (1898, 1907) were probably the most incisive authors to consolidate *Gymnarchus* in the clade with the remaining mormyriforms. Boulenger (1907) divided the Mormyridae into two subfamilies: Gymnarchinae and Mormyrinae. Although the taxonomic rank of these two clades has bounced back and forth as being two subfamilies within Mormyridae, or two families of the order Mormyriformes, there is no current dispute that *Gymnarchus* stands as the most basal lineage of the order Mormyriformes and is therefore the sister group of all the remaining genera (family Mormyridae in this study). This is corroborated by an impressive amount of evidence, including morphology, DNA sequences and isoenzyme data (Greenwood, 1971; Taverne, 1979; Benveniste, 1995; Van Der Bank and Kramer, 1996; Alves-Gomes and Hopkins, 1997).

The family Mormyridae is subdivided into two clades (here considered subfamilies): Petrocephalinae, with the single genus *Petrocephalus* and approximately 26 species; and Mormyrinae, with approximately 16 genera and more than 170 species (Hopkins, 1986). Petrocephalinae is believed to be the sister group of Mormyrinae (Taverne, 1972; Benveniste, 1995; Alves-Gomes and Hopkins, 1997), but this hypothesis was disputed by Van Der Bank and Kramer (1996). When only the enzymatic data generated by these authors is used, *Petrocephalus* is depicted as a sister group of the mormyrines in four out of six resultant topologies. However, because the authors include behavioral and other morphological characters in their final topology, *Petrocephalus* jumps into the middle of the mormyrines. Alves-Gomes and Hopkins (1997) have commented about possible reasons for such discrepant positioning of *Petrocephalus*. It is quite probable that food habits, habitat preference and at least some of the isoenzymes used are evolving too fast to be used in establishing evolutionary relationships that have occurred so far in the past, as is the case for the basal mormyriforms (Avise, 1994). The present study adopts the fairly well corroborated view that Mormyrinae is monophyletic and that Petrocephalinae is its sister group.

The relationships among the mormyrines are still very poorly understood. The clade includes complex assemblages of morphologically similar genera for which systematic studies are lacking. Taverne (1979) divided the genera into two major groups according to the presence or absence of the lateral ethmoid bone (see Fig. 2 in Hopkins, 1986), but studies using molecular data suggest that this bone may not be a good character to sort out the relationships among the mormyrines, once molecular studies portray genera with and without lateral ethmoids as sister groups (Agnèse and Bigorne, 1992; Alves-Gomes and Hopkins, 1997).

The few references explicitly addressing phylogenetic relationships among the mormyrines (Agnèse and Bigorne, 1992; Van Der Bank and Kramer, 1996; Alves-Gomes and Hopkins, 1997) are not very informative since, individually, these studies include only a small fraction of the 16 extant genera for the subfamily. Only *Marcusenius* was common to the three studies mentioned, and the results are therefore not comparable. Nevertheless, mitochondrial DNA studies suggest that the genus *Brienomyrus* is not monophyletic (Alves-Gomes and Hopkins, 1997), whereas neither *Hippopotamyrus* nor *Marcusenius* is monophyletic according to Van Der Bank and

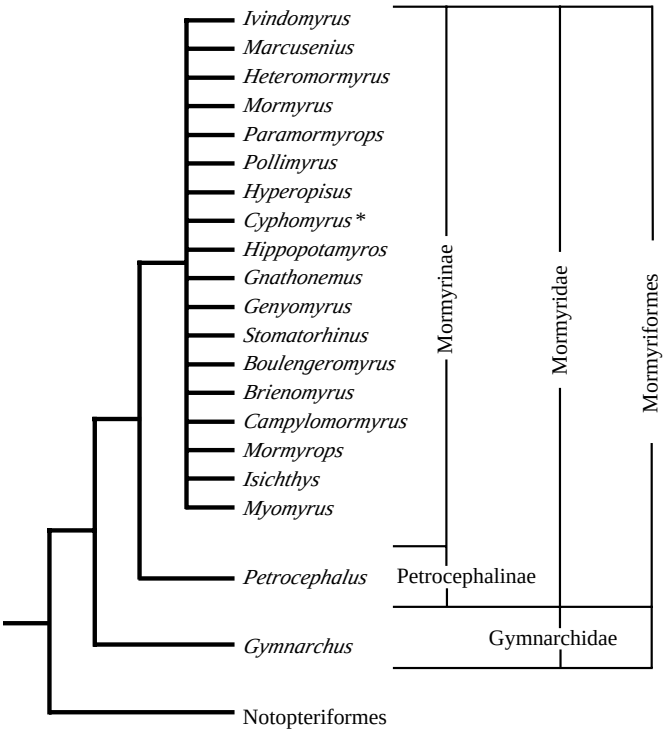

Fig. 3. Consensus phylogeny for the order Mormyriformes derived from studies based upon morphological, behavioral and molecular characters. Despite the relatively good agreement about the phylogenetic position of the most basal clades of the order, there is no current phylogenetic hypothesis available for the diverse subfamily Mormyrinae. *It was recently proposed that the genus *Cyphomyrus* Myers should be reinstated (Van Der Bank and Kramer, 1996).

Kramer (1996) and Agnèse and Bigorne (1992), respectively. Much remains to be discovered about the higher level phylogeny of the mormyrines. Fig. 3 depicts a proposed consensus topology for the mormyriform phylogeny based upon the studies mentioned above.

Testing the molecular clock: are the 12S and 16S rRNA genes evolving at the same pace in the Osteoglossomorpha and in the Ostariophysi?

The idea of molecular clocks originated with Zuckerkandl and Pauling (1962, 1965), who first suggested that mutations accumulate at a roughly constant rate over time and, therefore, that genetic divergence could be used to estimate the time of a split between lineages. Several subsequent studies have demonstrated that the divergence rates are highly variable among genes, among lineages and among different domains within a gene (Britten, 1986; Martin and Palumbi, 1993; Vawter and Brown, 1993; Ortí et al., 1996; Alves-Gomes and Hopkins, 1997; Caccone et al., 1997). Therefore, the idea of an 'universal clock' ticking at a constant rate had to be re-evaluated and updated to a more constrained one when the idea of 'local' clocks was invoked. Local comparisons, broadly speaking, essentially imply comparisons of homologous segments of DNA between closely related lineages or organisms with similar life styles. A uniform rate of mutation (the molecular clock) should not be expected if organisms with different thermal habits, efficiency of DNA repair mechanisms and nucleotide generation times (body size + metabolic rate + generation time) are compared (Martin and Palumbi, 1993; Rand, 1994; Hillis, 1996). In the present study, comparisons are made between tropical and subtropical freshwater fish lineages that do not have, in principle, any major apparent discrepancies in their physiological and bio-ecological characteristics. Thus, to test whether osteoglossomorphs and ostariophysans are accumulating mutations in their 12S and 16S gene segments in a clock-like fashion, the relative rate method was employed (Easteal, 1992; Li, 1997). In this test, the molecular clock can be accepted if the relative rates of divergence between two lineages with reference to an external outgroup are statistically the same. The results of this test for the clades used in the present study are shown in Table 3.

Table 3 shows that the rate at which mutations are accumulating in the gene fragments used in this study are statistically the same between gymnotiforms, siluriforms and characiforms, whereas cypriniforms are apparently accumulating mutations at a slightly slower rate than the former groups in relation to the gonorhynchiform outgroups. Although highly speculative, the fact that, in general, the cypriniforms are more abundant in higher (colder) latitudes, whereas the Characiphysi orders (Characiformes + Siluriformes + Gymnotiformes) are mainly associated with tropical regions may have influenced the results obtained here. The slower nucleotide generation time detected in

Table 3. *Relative rate test applied to the different ostariophysan and osteoglossomorph sister clades and their immediate outgroup to test the molecular clock hypothesis*

Pair of clades Outgroup×ingroup	Average P distance (%) Mean	S.D.	Reject molecular clock? *t*-test, *P*	d.f.
Ostariophysi				
Cha×Gym	11.55	1.03		
Cha×Sil	11.30	2.80	No (P=0.690)	30
Cyp×Cha	11.91	0.74		
Cyp×(Gym+Sil)	11.72	1.32	No (P=0.770)	34
Gon×Cyp	15.23	0.21		
Gon×(Gym+Sil+Cha)	16.31	0.93	Yes (P=0.012)	38
Osteoglossomorpha				
Ost×Mor	13.60	0.30		
Ost×Not	14.06	0.69	Yes (P=0.005)	28
Hio×Ost	18.30	0.74		
Hio×(Not+Mor)	15.38	1.19	Yes (P<0.0001)	15

Gon, Gonorhynchiformes; Cyp, Cypriniformes; Cha, Characiformes; Sil, Siluriformes; Gym, Gymnotiformes; Hio, Hiodontiformes; Ost, Osteoglossiformes; Not, Notopteriformes; Mor, Mormyriformes.

cypriniforms in relation to characiphysans may be a result of ancient evolutionary events associated with the historical biogeography of the group that may have confined the cypriniforms to colder environments, including lower metabolic rates.

Among the osteoglossomorphs, the differences in the rates were significant for all comparisons made. Mormyriformes and Notopteriformes are not evolving at the same rate in relation to Osteoglossiformes, and the genetic distance between the osteoglossiforms and the hiodontiforms is larger than the distance between the hiodontiforms and the notopteriform + mormyriform clade. According to the molecular data of the present study, the mormyriforms are accumulating mutations faster than the notopteriforms, and the osteoglossiforms are evolving, in relation to the hiodontiforms, at a faster rate than the remaining osteoglossomorph clades. Having a single representative for the Notopteriformes and for the Hiodontiformes could be a source of bias. However, the facts that the order Hiodontiformes consist of only two living species and that the Notopteriformes have only four genera and eight extant species suggest that increasing the number of representatives would not affect the results in the case of the hiodontiforms. In the case of the Notopteriformes, it would be quite interesting and prudent to include at least one African genus and to perform further analyses, not only to verify the apparent differential rates but also to test the possible paraphyly of Notopteriformes mentioned above.

Fossil records

As the paleontological records are inserted into phylogenetic hypotheses taking into consideration cladistic principles, they can provide a powerful framework for evolutionary inference because they may be used to obtain a time reference to estimate absolute rates of divergence between clades.

For the purpose of subsequent considerations, probably the most relevant cladistic postulate to be reviewed is that sister groups, by definition and independently of the taxonomic rank, have the same age. If we apply this simple concept to well-supported phylogenies for which fossil records are also available, we have an absolute time frame to be used as an estimate of the earliest times of appearance for the lineages considered (Lundberg, 1993, 1998). For instance, consider a hypothetical fossil A, dating from 10^7 years ago, and which can be unambiguously classified as the sister-group of an extant clade B on the basis of a series of shared derived characters (synapomorphies). It sounds almost too obvious to state that, 10^7 years ago, clade A had enough characters inherent to his own lineage (autapomorphies) that it could be identified as a separate biological entity from clade B. Nevertheless, because A and B are sister groups, the fossil record allows us to infer that 10^7 years ago is the minimal age to be considered as the divergence time between A and B. Clearly, these minimal ages are underestimates of real splitting times since, at the time of the fossilization, the two sister lineages already existed as separated biological units and, in fact, both lineages may be much older than the fossil. In other words, we have no direct means of knowing how long a lineage may have existed before the first fossil was preserved. However, when accurately identified and geologically dated, fossils constitute the best estimates for the minimal age of a clade's existence (Lundberg, 1998).

Gymnotiformes and Mormyriformes represent two clades of teleost fish that are widely separated in terms of their phylogeny (see Fig. 1) and for which the fossil record is not very helpful in establishing minimal times of divergence. There is a single fossil record for a gymnotiform fish, the eigenmanniid †*Ellisella* (Fink et al., 1999), described from the Late Miocene (approximately 7.5×10^6 years ago) of Bolivia by Gayet and Meunier (1991), whereas no mormyriform fossil is known up to the present. Under these circumstances, the fossil records of relevant outgroups can be used to estimate approximate absolute rates of evolution for both ostariophysans and osteoglossomorphs. From the estimated rates, the time of differentiation of individual pairs of taxa can be estimated.

Ostariophysan fossils

There is abundant material about ostariophysan fossils and, as expected, still unsettled issues about the taxonomic and phylogenetic status of several of these records (Lundberg, 1993, 1998; Fink and Fink, 1996; Grande, 1997; Gayet and Meunier, 1998; and references herein). These circumstances turn the full interpretation of the data available into a rather complex matter that exceeds the scope of the present paper. Here, the objective is to obtain the ages for the oldest fossils unmistakably associated with each main lineage, since they represent the closest estimate of divergence time between lineages whose sequences were used in this study.

Gymnotiformes

The only existing gymnotiform fossil, the eigenmanniid †*Ellisella*, determines a minimal age for the eigenmanniids of approximately 7.5×10^6 years (Gayet and Meunier, 1991; Fink et al., 1999), but provides no information about the ages of the other clades.

Siluriformes

Several fossils from Late Cretaceous (66.4×10^6 to 97.5×10^6 years ago) deposits in South and North America are thought to belong to various siluriform lineages (Frizzell, 1965; Cione et al., 1985; Cione, 1987; Gayet, 1988a,b; Gayet and Meunier, 1998; Lundberg, 1998). Such evidence suggests that siluriforms were well differentiated and reasonably diversified by the Maastrichtian (66.4×10^6 to 74.5×10^6 years ago). Therefore, the South American electric fish lineage, being the sister group of catfishes, also had to be in existence by the Late Cretaceous.

Characiformes

Until recently, a series of fossils from Maastrichtian deposits in Bolivia (66.4×10^6 to 74.5×10^6 years ago) were considered as the oldest fossils known for the group (Gayet, 1982, 1995;

Arratia and Cione, 1996). However, Werner (1994, cited in Gayet and Meunier, 1998) reports a characiform fossil from the Cenomanian (91×10^6 to 97.5×10^6 years ago) from Sudan, which becomes the oldest otophysan (Cypriniformes + Characiformes + Siluriformes + Gymnotiformes) fossil reported so far.

Cypriniformes

Considering that the phylogeny depicted in Fig. 1 is largely accepted as the correct hypothesis about Ostariophysi phylogeny, and therefore that the cypriniform lineage must be the oldest lineage among the Otophysi, there are no indisputable cypriniform fossils older than the Paleocene (57.8×10^6 to 66.4×10^6 years ago) presently known (Gayet, 1995). Cypriniform fossils have been described from the Miocene of Africa (Novacek and Marshall, 1976), from the basal Eocene of Eurasia (Patterson, 1975) and from the middle Eocene of North America (Grande et al., 1982; Grande, 1984). Nevertheless, none of them can be used to set the minimal age for the cypriniform lineage, since older characiform and siluriform fossils have been recorded (see above).

Gonorhynchiformes

The oldest Anotophysi (Ostariophysi with the exclusion of the Otophysi) fossil is a chanid from the Early Cretaceous (Late Hauterivian–Early Barremian) from Spain, 119×10^6 to 131×10^6 years old (Poyato-Ariza, 1994; Lundberg, 1998).

Ostariophysi

The oldest fossil putatively identified as an ostariophysan, †*Tichlingenichthy*, was reported from Late Jurassic (144×10^6 to 163×10^6 years ago) deposits (Arratia, 1997).

Osteoglossomorpha fossils

As in the case of the ostariophysans, the phylogeny of the osteoglossomorphs taking into consideration all the fossil evidence is complex, particularly because of the number of fossils described and the lack of consensus about the phylogenetic and taxonomic status of several of them. For instance, Li and Wilson (1996) have synthesized a phylogenetic hypothesis for the Osteoglossomorpha in which 23 clades are considered and only 11 are extant. Several other osteoglossomorph fossils were not included in their study, such as some of those listed by Taverne (1979) and Gayet (1991). Here again, the focus is on the oldest fossils from each lineage in order to establish the minimal ages of differentiation of the clades involved.

Mormyriformes + Notopteriformes

There are no fossils currently described that can be identified as either a notopteriform or a mormyriform. However, if the fossil †*Ostariostoma* from the Late Cretaceous–Early Paleocene of North America is indeed the sister group of the clade Notopteriformes + Mormyriformes, as suggested by Li and Wilson (1996), the ancestral lineage that later differentiated into Notopteriformes + Mormyriformes was in existence in the Early Paleocene (62.3×10^6 to 66.4×10^6 years ago). From their current distribution, and if the fossil is not too far off from the real time of divergence, it is probable that both African electric fish and the notopteriforms evolved after the separation of Africa and South America, which happened between 106×10^6 and 84×10^6 years ago according to Pitman et al. (1993) or approximately 112×10^6 years ago according to ammonite and foraminifera data (Bengtson and Koutsoukos, 1991).

Osteoglossiformes

The Osteoglossiformes, as considered here, includes the Arapaimidae + Osteoglossidae + Pantodontidae. The last two families are considered to be sister groups (Lauder and Liem, 1983; Li and Wilson, 1996), and for the present study DNA was obtained for *Pantodon* (family Pantodontidae) and *Osteoglossum* (family Osteoglossidae). Determining the minimal ages for Pantodontidae + Osteoglossidae may become a complicated exercise because of an ongoing debate about the phylogenetic position of the phareodontines, a group including several fossils found in deposits ranging from the Late Cretaceous to the Tertiary of several continents (Taverne, 1979; Lundberg, 1993; Li and Wilson, 1996; Gayet and Meunier, 1998), and †*Singida*, a fossil genus from the Middle Miocene (Li and Wilson, 1996). Several alternative hypotheses have been suggested for the phylogenetic position of both phareodontines and †*Singida* (Taverne, 1979; Li, 1996; Li and Wilson, 1996; Li et al., 1997a,b; Gayet and Meunier, 1998). However, for our purpose, the important fossil to be considered is the oldest fossil that could be unambiguously assigned to one of these three lineages. †*Laeliichthys* (Silva Santos, 1985) from the Aptian (113×10^6 to 119×10^6 years ago) of Brazil, is the oldest fossil putatively placed in the same lineage as the two extant arapaimid genera (the neotropical *Arapaima* and the African *Heterotis*). †*Laeliichthys* implies that the minimal age for the common ancestor of *Arapaima* and *Heterotis* is the Aptian (113×10^6 to 119×10^6 years ago) or at about the final moments of contact between Africa and South America. The fossil genus †*Tanolepis* (=*Tanichthys*) from Early Cretaceous (97.5×10^6 to 144×10^6 years ago) deposits in East Asia (Li and Wilson, 1996; Jin, 1994, cited in Li et al., 1997b) has been considered the sister group of (Osteoglossiformes + Mormyriformes + Notopteriformes) by Li and Wilson (1996). However, more recently Li et al. (1997b) placed this clade within a lineage that is the sister group of the Osteoglossiformes alone. In either case, a reasonable estimate of the minimal age of the common ancestor of the Osteoglossiformes is around the Jurassic–Cretaceous boundary.

Hiodontiformes

The order Hiodontiformes is monogeneric, and the fossil species (†*Hiodon consteniorum*) from the Eocene (36.6×10^6 to 57.8×10^6 years ago) of North America is assumed to be the sister group of the extant genus *Hiodon* (Li and Wilson, 1996; Li et al., 1997b). Furthermore, the putative sister group of *Hiodon*, the fossil genus †*Eohiodon*, is known from Early to Late Eocene (Li et al., 1997b). Therefore, the minimal age for

the *Hiodon* + †*Eohiodon* lineage is Eocene. The oldest hiodontiform according to Li and Wilson (1996) is †*Yanbiania* from the Early Cretaceous (97.5×10^6 to 144×10^6 years ago) in Asia. This dating corroborates the inference obtained from †*Tanolepis* and †*Laeliichthys* that Hiodontiformes and the clade (Osteoglossiformes + Notopteriformes + Mormyriformes) must have been separated since the end of the Jurassic or for more than 140×10^6 years.

Osteoglossomorpha

The oldest fossil record assigned for the lineage of the osteoglossomorphs is the fossil genus †*Lycoptera* from the Late Jurassic–Early Cretaceous (Greenwood, 1970; Lundberg, 1993; Li and Wilson, 1996) or between 144×10^6 and 163×10^6 years ago. †*Lycoptera* is considered to be the sister group of all osteoglossomorphs, and therefore it sets the minimal time for the separation of the Osteoglossomorpha lineage from the other teleost lineages or the minimal age for when they diverged from an ostariophysan ancestral.

Calculating absolute rates of divergence

The information about minimal ages retrieved from the fossil records can be used to estimate the absolute rates at which the different lineages are accumulating mutations in their mitochondrial genes. Since the fossil records establish the minimal age of a clade's existence, the values obtained from these computations always represent maximal rates of genetic differentiation. To establish these absolute rates, the oldest fossil in each monophyletic group was used in conjunction with the average distance between the sister lineages. For example, the sister group of the Characiformes (Cha) is the Siluriphysi, i.e. a clade comprising both Siluriformes (Sil) + Gymnotiformes (Gym) (Fink and Fink, 1981, 1996). Therefore, the average distance of all pairwise comparisons between the characiforms *versus* the gymnotiforms plus the siluriforms was divided by the age of the oldest fossil record for these three clades to determine the absolute rate of mutation for Characiformes and the Siluriphysi. The sister group lineages considered in these computations were derived from the phylogenetic hypothesis shown in Fig. 1 and are as follows for the ostariophysans: Sil × Gym, Cha × (Sil + Gym), Cyp × (Cha + Sil + Gym) and Gon × (Cyp + Cha + Sil + Gym). For the osteoglossomorphs, they are Not × Mor, Ost × (Not + Mor) and Hio × (Ost + Not + Mor). Abbreviations are shown in Table 2. Because the fossil records are associated with geological strata with time boundaries, both a lower and an upper value can be obtained for the maximal rate of mutation. These values were calculated as follows: LDR=(d–S.D.)/upper time boundary of geological time and UDR=(d+S.D.)/lower time boundary of geological time, where LDR is the lower limit for a maximal absolute divergence rate, d is the average distance for all the possible pair of taxa between the sister clades considered, S.D. is the standard deviation, and UDR is the upper limit for the maximal absolute divergence rate. Table 4 compiles the calculations for all pairs of sister lineages.

The absolute rates calibrated using fossil records shown in Table 4 represent upper and lower estimated limits of the real evolutionary rate. It is expected that the true values lie somewhere between the values obtained for all pairs of sister lineages. Consequently, an average rate was calculated for osteoglossomorphs and for ostariophysans taking into consideration the upper and lower values obtained from the fossil records within each superorder. These averages obtained for each superorder are not significantly different (t-test, P=0.505), and the values from both clades can therefore be used to compute a single averaged value for all the clades. The average divergence rate for all pairs of taxa considered in Table 3, including both ostariophysan and osteoglossomorph rates, is 1.31×10^{-9} substitutions site^{-1} year^{-1}, or 0.13 % divergence 10^6 years^{-1}. To interpret this rate better, two important aspects must be considered: first, the distances used for calculations were P distances. These values are not corrected for multiple hits in the molecules and therefore tend to be an underestimation of the real value. If, for instance, we consider the Tamura–Nei model of substitution (as implemented in PAUP* Version 4.0.0d64, Swoford) to calculate the values in Table 4, the total average rate obtained is 0.19 % divergence 10^6 years^{-1}. Second, the rate of 0.13 % 10^6 years^{-1} is the average mutation rate obtained from the most conserved sites of both genes, since all sites with ambiguous alignment across all the taxa considered were excluded from the analyses. Therefore, to obtain the rates for the loops and for the entire molecule, two extra data sets were considered: one contained only one species from each eigenmanniid genus (*Rhabdolichops*, *Archolaemus*, *Distocyclus* and *Eigenmannia*) for the gymnotiforms, and the other contained four species of the genus *Brienomyrus* (*B. batesii*, *B. niger*, *B. brachyistius* and *B.* sp.) for the Mormyriformes. In these data sets, the sequences are similar enough to allow a complete alignment of the genes, including the most variable regions corresponding to the loops.

By using the average value of 0.13 % divergence 10^6 years^{-1}, taken from Table 4, with the average genetic distance calculated for the conserved regions only between eigenmannids (3.85 %) and the *Brienomyrus* species (2.72 %), we obtain a value of $3.85/0.13=29.61\times10^6$ years and $2.71/0.13=20.84\times10^6$ years for the minimal divergence time for the four eigenmannid genera and the four *Brienomyrus* species, respectively. These dates were, in turn, used to estimate the mutational rates of the loops alone and of the entire molecule (stems + loops). The average divergence among the eigenmannids in the isolated loops is 29.96 %, a 7.78-fold increase in relation to stems. These values produce a mutational rate of $29.96/29.61=1.01$ % 10^6 years^{-1} for the loops alone. When loops and stems are considered together, the average divergence between the four eigenmanniid genera is 6.74 %, which implies an average rate of 0.23 % 10^6 years^{-1} for the entire fragment (loops + stems).

The four species of the genus *Brienomyrus* have an average divergence of 17.18 % for the loops and of 4.71 % when the entire fragments of the two molecules are considered. If the same calculations made for the eigenmannids are performed

Table 4. *Absolute rates of genetic differentiation for the sister lineages of ostariophysans and osteoglossomorphs estimated from fossil records*

	Average P distance (%)		Fossil calibration (million years)		Maximal divergence rates (% per 10^6 years)	
Pair of taxa	Mean	S.D.	Lower	Upper	Lower, LDR	Upper, UDR
Ostariophysi						
Gym×Sil	12.90	2.50	66	97.5	0.107	0.2
Cha×(Gym+Sil)	11.52	1.46	91	97.5	0.103	0.143
Cyp×(Gym+Sil+Cha)	11.72	1.32	91	97.7	0.106	0.143
Gon×(Gym+Sil+Cha+Cyp)	16.31	0.93	119	131	0.117	0.144
				Ostariophysan average	0.137±0.042	
Osteoglossomorpha						
Mor×Not	8.69	0.74	62.3	66.4	0.120	0.151
Ost×(Not+Mor)	13.95	0.81	113	144	0.091	0.131
Hio×(Ost+Not+Mor)	15.38	1.19	113	144	0.099	0.147
				Osteoglossomorph average	0.123±0.024	
				Overall average*	0.131	

*There is no significant difference (*t*-test, P=0.505) between the absolute average rate of ostariophysans and osteoglossomorphs.

LDR, lower limit for maximal divergence rate; UDR, upper limit of maximal divergence rate.

Gon, Gonorhynchiformes; Cyp, Cypriniformes; Cha, Characiformes; Sil, Siluriformes; Gym, Gymnotiformes; Hio, Hiodontiformes; Ost, Osteoglossiformes; Not, Notopteriformes; Mor, Mormyriformes.

for the *Brienomyrus* species, we find that the loops are evolving at a rate of $0.82\,\%\,10^6$ years^{-1} and the entire molecules at an average rate of $0.23\,\%\,10^6$ years^{-1}.

The values obtained above indicate that, in both African and South American electric fish, the two ribosomal RNA genes of the mitochondria are evolving at similar pace of approximately $0.23\,\%\,10^6$ years^{-1}.

There are only a few papers currently available that may be used for a direct comparison with the results obtained in the present study. Several authors have estimated the evolutionary rate of mitochondrial genes, but the majority have looked either at different genes or at organisms other than fish. Three main DNA fragments are used as molecular markers in the mitochondrial genome that are sources of evolutionary rate estimates: the D-loop, cytochrome *b* (cyt *b*) and the rRNAs genes. For vertebrates, the D-loop has been estimated to evolve at rates ranging from 1–1.5 $\%\,10^6$ years^{-1} in salamanders (Shaffer and McKnight, 1996) to 7.0 % in hominoids (Horai et al., 1995). The cyt *b* gene has been shown to have a mutational rate that varies according to the codon position (Martin et al., 1992; Cantatore et al., 1994; Caccone et al., 1997), but average values considering all positions vary from $0.40\,\%\,10^6$ years^{-1} in turtles (Bowen et al., 1993) to $0.77\,\%\,10^6$ years^{-1} in salamanders (Caccone et al., 1997). Briolay et al. (1998) estimated a value of $0.371\,\%\,10^6$ years^{-1} for the first and second positions on the cyt *b* gene in cypriniform fishes. For the rRNA genes, Caccone et al. (1997) estimated $0.38\,\%\,10^6$ years^{-1} for 12S and 16S rRNA for the European salamanders, whereas the same authors calculated $0.47\,\%\,10^6$ years^{-1} for the North American clades. Lundberg (1998) estimated the divergence rates for *Colossoma* (Characiformes) and its sister group (either *Piractus* or *Mylossoma*, depending upon the author) on the basis of solid *Colossoma* fossil evidence, and calculated a value of $0.21–0.26\,\%\,10^6$ years^{-1}. These values were based upon genetic divergences obtained with the entire molecule, including the loops (Ortí, 1997), and are in perfect agreement with the values obtained here. Lundberg (1998) also determined the mutation rate for callichthyids catfishes (*Corydoras* and *Callichthys*) using the oldest callichthyid fossil known, found in the Argentinean Paleocene (58.5×10^6 years ago), and found rates of $0.05–0.08\,\%\,10^6$ years^{-1}. In this case, only the most conserved regions of the same 12S and 16S gene fragments used here were considered. The values suggest a slightly slower rate for callichthyids in relation to the other fish studied, but if we consider the possible uncertainty associated with the ages of the fossils, we may find that the values found for callichthyids are much closer to those shown in Table 4 than to the values originally suggested for terrestrial vertebrates.

In summary, the rates of $0.23\,\%\,10^6$ years^{-1} found in this study for the entire molecule and of $0.13\,\%\,10^6$ years^{-1} for the most conserved regions of the rRNA genes appear to be in accord with the values of Lundberg (1998) for characiform and siluriform fish and the values of Caccone et al. (1997) for the rRNA genes in salamanders, particularly if we take into consideration the hypothesis that fish may have a slower rate of mutation than terrestrial vertebrates (Martin et al., 1992; Martin and Palumbi, 1993).

Absolute rates and minimal times of differentiation

The average absolute mutation rate of $0.13\,\%\,10^6$ years^{-1} found for the most conserved stretches (stems) of the 12S and 16S rRNA can be used to estimate the minimal time for the

split between the main sister lineages in both ostariophysans and osteoglossomorphs. Table 5 depicts these calculations. The values obtained are in good agreement with the known paleobiology of the groups and, apparently, the estimate of $0.13\,\%\,10^6$ years^{-1} for the stems represents a reasonable 'preliminary approach' to the correct value. However, the fact that this value was obtained using the P distance, which does not take into consideration possible multiple hits in the DNA sequences, must be kept in mind. Therefore, the value of $0.13\,\%\,10^6$ years^{-1} must be considered a conservative value, probably representing a slight underestimate of the real value.

A comprehensive analysis of Table 5 and its implications for the evolutionary history of osteoglossomorphs and ostariophysans will be presented elsewhere. However, there are a few interesting aspects involving the electric fish clades that I would like to point out. It seems that the differentiation of characiforms, siluriforms and gymnotiforms has occurred within a relatively short period, since the average genetic distance between each pair of orders is very close and, consequently, the estimated times of split derived from these distances plus fossil records (Table 5) are also quite similar. These close values also seem to account for at least part of the relative lack of resolution between these clades obtained using phylogenetic studies of mitochondrial genes (Ortí and Meyer, 1997; Alves-Gomes, 1995). Furthermore, the values of splitting time shown (Table 5) for these orders constitute a good match with the final period of separation of South America and Africa (84×10^6 to 112×10^6 years ago; Pitman et al., 1993; Bengtson and Koutsoukos, 1991). The complex dynamics of geological events and landmass contact, including South America, Africa and Europe, during the final stages of Pangea/Gondwana fragmentation, have probably influenced dramatically the differentiation processes of these fish clades. Alves-Gomes (1995) suggested a biogeographical hypothesis for these otophysan groups which also implies a relatively fast radiation of the four orders in approximately the same time frame obtained in Table 5.

The genetic distances among the Mormyriformes and between the mormyriforms and the notopteriforms, in comparison with the distances between the gymnotiforms and their outgroups, suggest that the African electric fish clade is younger than the Gymnotiformes. Further, they imply that the Mormyriformes differentiated well after the complete separation between Africa and South America.

These temporal considerations associated with the evolution of African and South American electric fish, and consequently their EESs, represent an initial and preliminary step in establishing a temporal backbone to which the current comparative and evolutionary studies of their nervous systems can be related. Further and more focused studies of genetic distances within each electric fish order may reveal more refined time frames in which physiological, anatomical and behavioral characters have evolved in electric fish. For instance, the genetic divergence for the entire molecule (stems + loops) among all taxa of the genus *Eigenmannia* studied is approximately 3.85 % (not to be confused with the value of 3.85 % divergence found for the most conserved regions of 12S and 16S rRNA among the four eigenmannid genera employed previously). Considering that the rate of mutation of $0.23\,\%\,10^6$ years^{-1}, it is possible to estimate that the common ancestor of all the *Eigenmannia* species sampled for this study is approximately 16.7×10^6 years old. For all the specimens classified as *Brienomyrus* in this study, a genetic divergence of 4.03 % implies a common ancestor with an age of approximately 17.5×10^6 years.

Concluding remarks

Electric organ discharges and electroreceptive capabilities

Table 5. *Estimated minimal time of split between the main sister lineages of ostariophysans and osteoglossomorphs using the overall average of 0.131 % per million years divergence rate found in Table 4*

	Average P distance (%)		Minimal time of split (million years)	
Pair of taxa	Mean	S.D.	Lower	Upper
Ostariophysi				
Gym×Sil	12.90	2.5	79.39	117.56
Cha×(Gym+Sil)	11.52	1.46	76.79	99.08
Cyp×(Gym+Sil+Cha)	11.72	1.32	79.39	99.54
Gon×(Gym+Sil+Cha+Cyp)	16.31	0.93	117.40	131.60
Osteoglossomorpha				
Mor×Not	8.69	0.74	60.69	71.98
Ost×(Not+Mor)	13.95	0.81	100.31	112.67
Hio×(Ost+Not+Mor)	15.38	1.19	108.32	126.49
Osteo × Ostario	18.05	1.66	125.11	150.46

Gon, Gonorhynchiformes; Cyp, Cypriniformes; Cha, Characiformes; Sil, Siluriformes; Gym, Gymnotiformes; Hio, Hiodontiformes; Ost, Osteoglossiformes; Not, Notopteriformes; Mor, Mormyriformes.

are found in the South American gymnotiform and in the African mormyriform fishes. According to fossil records, these two lineages of fish separated more than 140×10^6 years ago, and current evidence suggests that their common ancestor was neither electroreceptive nor electrogenic. It is quite remarkable, therefore, that after this prolonged period of independent evolution, these two fish clades have evolved a number of very elaborate similarities associated with their sensory and motor biology, completely independently of one another. The electrogenic and electrosensory systems (EESs) of mormyriforms and gymnotiforms are used for electrolocation and communication, and the design, physiology and *modus operandi* of the two systems are extraordinarily similar, if not identical, in several ways. Despite the great scientific interest in these two fish groups, much remains to be studied about their evolutionary biology and phylogenetic relationships. In this article, the current studies of mormyriform and gymnotiform phylogeny that have used cladistic principles have been reviewed, and a consensus phylogeny for both groups has been proposed. The genetic distances between mormyriforms and gymnotiforms in relation to the other clades of their respective superorders were estimated using the 12S and 16S rRNA genes, and the molecular clock hypothesis was tested in both Osteoglossomorpha and Ostariophysi. Characiformes, Siluriformes and Gymnotiformes are accumulating mutations at similar rates, but the molecular clock was rejected for the remaining osteoglossomorph and ostariophysi lineages. In a subsequent analysis, the oldest fossil records for each main lineage within each superorder were surveyed and used in conjunction with genetic distances to calibrate absolute rates of genetic differentiation. The stems of the 12S and 16S rRNA are evolving at an overall average rate of $0.13\,\%\,10^6$ years^{-1} for both osteoglossomorphs and ostariophysans whereas, in the loops alone, mutations are accumulating at the rate of $0.82\,\%\,10^6$ years^{-1} in *Brienomyrus* and of $1.01\,\%\,10^6$ years^{-1} among eigenmanniids. The average rate for the whole molecule is approximately $0.23\,\%\,10^6$ years^{-1} in both clades. The present study represents an initial and preliminary attempt to bring a temporal perspective into the study of the evolution of the EES of gymnotiforms and mormyriforms.

A considerable portion of this paper was conceived during a visit to Ted Bullock's laboratory at Scripps Istitution of Oceanography in La Jolla. Without Ted's support and always inspiring attitude towards science, it would have been much more difficult to start and complete this enterprise. This article has benefited tremendously from several stimulating discussions with John Lundberg. My thanks to Ray Turner and Len Maler for reviewing and for several suggestions for improving the manuscript. I also thank David Swofford for allowing me to use an experimental version of PAUP*. Maria João do Amaral, João Bacelo, Cristina Compaan, Kyara Formiga and Jacqueline Batista helped on several fronts during the elaboration of this manuscript. This reserach was partially financed by PPI 3260-INPA.

References

Agnèse, J.-F. and Bigorne, R. (1992). Premières données sur les relations génétiques entre onze espèces ouest-africaines de Mormyridae (Teleostei, Osteichthyes). *Rev. Hydrobiol. Trop.* **25**, 253–261.

Albert, J. S. (1999). *Phylogenetic Systematics of the American Knifefishes (Teleostei: Gymnotiformes)*. Miscellaneous Publications of the Museum of Zoology of the University of Michigan (in press).

Albert, J. S. and Campos-da-Paz, R. (1998). Phylogenetic systematics of Gymnotiformes with diagnoses of 60 clades: a review of available data. In *Phylogeny and Classification of Neotropical Fishes* (ed. L. R. Malabarba, R. E. Reis, R. P. Vari, C. A. S. Lucena and Z. M. S. Lucena), pp. 419–446. Porto Alegre: Museu de Ciencias e Tecnologia – PUC/RS. (in press).

Albert, J. S. and Fink, W. L. (1996). *Sternopygus xingu*, a new species of electric fish from Brazil (Teleostei: Gymnotoidei), with comments on the phylogenetic position of *Sternopygus*. *Copeia* **1996**, 85–102.

Albert, J. S. and Miller, R. R. (1995). *Gymnotus maculosus*, a new species of electric fish (Chordata: Teleostei: Gymnotoidei) from Middle America, with a key to species of Gymnotus. *Proc. Biol. Soc. Washington* **108**, 662–678.

Alves-Gomes, J. A. (1995). The phylogeny and evolutionary history of the South American electric fishes (order Gymnotiformes). PhD thesis, University of California, San Diego.

Alves-Gomes, J. A. (1998). The phylogenetic position of the South American electric fish genera *Sternopygus* and *Archolaeumus* (Ostariophysi: Gymnotiformes) according to 12S and 16S mitochondrial DNA sequences. In *Phylogeny and Classification of Neotropical Fishes* (ed. L. R. Malabarba, R. E. Reis, R. P. Vari, C. A. S. Lucena and Z. M. S. Lucena), pp. 447–460. Porto Alegre: Museu de Ciencias e Tecnologia – PUC/RS. (in press).

Alves-Gomes, J. A. and Hopkins, C. D. (1997). Molecular insights into the phylogeny of mormyriform fishes and the evolution of their electric organ. *Brain Behav. Evol.* **49**, 324–350.

Alves-Gomes, J. A., Ortí, G., Haygood, M., Heiligenberg, W. and Meyer, A. (1995). Phylogenetic analysis of the South American electric fishes (order Gymnotiformes) and the evolution of their electrogenic system: a synthesis based on morphology, electrophysiology and mitochondrial sequence data. *Mol. Biol. Evol.* **12**, 298–318.

Arratia, G. (1997). *Basal Teleosts and Teleostean Phylogeny*. Munich: F. Pfeil.

Arratia, G. and Cione, A. (1996). The record of fossil fishes of southern South America. In *Contributions of Southern South America to Vertebrate Paleontology* (ed. G. Arratia), pp. 9–72. Munich: F. Pfeil.

Avise, J. C. (1994). *Molecular Markers, Natural History and Evolution*. New York: Chapman & Hall.

Bargelloni, L., Ritchie, P. A., Patarnello, T., Battaglia, B., Lambert, D. M. and Meyer, A. (1994). Molecular evolution at subzero temperatures: mitochondrial and nuclear phylogenies of fishes from antarctica (suborder notothenioidei), and the evolution of antifreeze glycopeptides. *Mol. Biol. Evol.* **11**, 854–863.

Bass, A. H. (1986). Species differences in electric organs of mormyrids: Substrates for species-typical electric organ discharge waveforms. *J. Comp. Neurol.* **244**, 313–330.

Bengtson, P. and Koutsoukos, E. A. M. (1991). Ammonite and forminiferal dating of the first marine connection between the central and south Atlantic. 1er Colloque Stratigraphie et

Paleogeographie des bassins sedimentaires ouest-Africans, 11e Coll. Africain de Micropaleont. Abstracts.

Benveniste, L. (1995). Phylogenetic systematic of *Gymnarchus* (Notopteroidei) with notes on *Petrocephalus* (Mormyridae) of the Osteoglossomorpha. MSc thesis, The City College of New York.

Boden, G., Teugels, G. G. and Hopkins, C. D. (1997). A systematic revision of the large-scaled *Marcusenius* with description of a new species from Cameroon (Teleostei; Osteoglossomorpha; Mormyridae). *J. Nat. Hist.* **31**, 1645–1682.

Boulenger, G. A. (1898). A revision of the genera and species of fishes of the family Mormyridae. *Proc. Zool. Soc. Lond.* **1898**, 775–821.

Boulenger, G. A. (1907). *Zoology of Egypt. The Fishes of the Nile.* London: Hugh Rees Ltd.

Bowen, B. W., Nelson, W. S. and Avise, J. C. (1993). A molecular phylogeny for marine turtles: trait mapping, rate assessment and conservation relevance. *Proc. Natl. Acad. Sci. USA* **90**, 5574–5577.

Braford, M. R., Jr (1982). African, but not Asian notopterid fishes are electroreceptive: Evidence from brain characters. *Neurosci. Lett.* **32**, 35–39.

Braford, M. R., Jr (1986). African knifefishes – The xenomystines. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 453–464. New York: John Wiley & Sons.

Briolay, J., Galtier, N., Brito, R. M. and Bouvet, Y. (1998). Molecular phylogeny of Cyprinidae inferred from cytochrome b DNA sequences. *Mol. Phyl. Evol.* **9**, 100–108.

Britten, R. J. (1986). Rates of DNA sequence evolution differ between taxonomic groups. *Science* **231**, 1393–1398.

Bullock, T. H., Bodznick, D. A. and Northcutt, R. G. (1983). The phylogenetic distribution of electroreception: Evidence for convergent evolution of a primitive vertebrate sense modality. *Brain Res. Rev.* **6**, 25–46.

Bullock, T. H. and Heiligenberg, W. (1986). *Electroreception.* New York: John Wiley & Sons.

Caccone, A., Milinkovitch, M. C., Sbordoni, V. and Powell, J. (1997). Mitochondrial DNA rates and biogeography in European newts (genus *Euproctus*). *Syst. Biol.* **46**, 126–144.

Campos-da-Paz, R. (1996). Redescription of the Central American electric fish *Gymnotus cylindricus* (Ostariophysi: Gymnotiformes: Gymnotidae), with comments on character ambiguity within the ostariophysan clade. *J. Zool. Lond.* **240**, 371–382.

Campos-da-Paz, R. and Albert, J. S. (1998). The gymnotiform 'eels' of tropical America: A history of classification and phylogeny of the South American knifefishes (Teleostei: Ostariophysi: Siluriphysi). In *Phylogeny and Classification of Neotropical Fishes* (ed. L. R. Malabarba, R. E. Reis, R. P. Vari, C. A. S. Lucena and Z. M. S. Lucena), pp. 401–418. Porto Alegre: Museu de Ciencias e Tecnologia – PUC/RS. (in press).

Campos-da-Paz, R. and Paepke, H.-J. (1994). On *Sternarchorhamphus hahni*, a member of the rhamphicthyid genus *Rhamphichthys* (Ostariophysi: Gtmnotiformes). *Ichthyol. Explor. Freshwaters* **5**, 155–159.

Cantatore, P., Roberti, M., Pesole, G., Ludovico, A., Milella, F., Gadaleta, M. N. and Saccone, C. (1994). Evolutionary analysis of cytochrome b sequences in some perciformes: evidence for a slower rate of evolution than in mammals. *J. Mol. Evol.* **39**, 589–597.

Cione, A. (1987). The late Cretaceous fauna of Los Alamitos, Patagonia, Argentina. II. The fishes. *Rev. Mus. Argentino Cienc. Nat.* **3**, 111–120.

Cione, A. L., Pereira, S. M., Alonso, R. and Arias, J. (1985). Los bagres (Osteichthyes, Siluriformes) de la formación Yacoraite (Cretácico tardío) del Noroeste Argentino. Consideraciones biogeográficas y bioestratigraficas. *Ameghiniana* **21**, 294–304.

Costa, W. J. E. M. and Campos-da-Paz, R. (1991). Description d'une nouvelle espèce de Poisson électrique du genre néotropical *Hypopomus* (Siluriformes: Gymnotoidei: Hypopomidae) du Sud-Est du Brésil. *Rev. Fr. Aquariol.* **18**, 117–120.

Dimmick, W. W. and Larson, A. (1996). A molecular and morphological perspective on the phylogenetic relationships of the otophysan fishes. *Mol. Phyl. Evol.* **6**, 120–133.

Dixon, M. T. and Hillis, D. M. (1993). Ribosomal RNA secondary structure: compensatory mutations and implications for phylogenetic analysis. *Mol. Biol. Evol.* **10**, 256–267.

Easteal, S. (1992). A mammalian molecular clock? *BioEssays* **14**, 415–419.

Erdl, M. P. (1847). Beschreibung des Skeletes von *Gymnarchus niloticus*, nebst Vergleichung mit Skeleten formverwandter Fishes. *Abh. K. bayer Akad. Wiss., Math.-Phys. K1* **5**, 209–252.

Finger, T. E., Bell, C. C. and Carr, C. E. (1986). Comparisons among electroreceptive teleosts. Why are electrosensory systems so similar? In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 465–481. New York: John Wiley & Sons.

Fink, S. V., Albert, J. S. and Fink, W. L. (1999). Phylogenetic relationships of the fossil gymnotoid *Ellisella kirschbaumi* (Ostariophysi: Gymnotoidei). *J. Vert. Paleont.* (in press).

Fink, S. V. and Fink, W. L. (1981). Interrelationships of the ostariophysan fishes (teleostei). *Zool. J. Linn. Soc.* **72**, 297–353.

Fink, S. V. and Fink, W. L. (1996). Interrelationships of Ostariophysan Fishes (Teleostei). In *Interrelationships of Fishes* (ed. M. L. J. Stiassny, L. R. Parenti and G. D. Johnson), pp. 209–249. San Diego: Academic Press.

Frizzell, D. L. (1965). Otoliths of new fish (*Vorhisia vulpes*, n. gen., n. sp., Siluroidei?) from Upper Cretaceous of South Dakota. *Copeia* **1965**, 178–181.

Gayet, M. (1982). Découverte dans le Crétacé supérieur de Bolivie des plus anciens Characiformes connus. *C.R. Acad. Sci. Paris* **294**, 1037–1040.

Gayet, M. (1988a). Le plus ancien crâne de Siluriforme: *Andinichthys bolivianensis* nov. gen., nov. sp. (Andinichthyidae nov. fam.) du Maastrichtien de Tiupampa (Bolivie). *C.R. Acad. Sci. Paris* **307**, 833–836.

Gayet, M. (1988b). Nouveaux Siluriformes du Maastrichtien de Tiupampa (Bolivie). *C.R. Acad. Sci. Paris* **390**, 867–872.

Gayet, M. (1991). 'Holostean' and Teleostean fishes of Bolivia. In *Fosiles y Facies de Bolivia*, vol. 12 (ed. R. Suarez), pp. 453–494. Revista Tecnica de Yacimientos Petroliferos Fiscales Bolivianos.

Gayet, M. (1995). Fossil relatives of Ostariophysi (Pisces, Teleosteans). *Bull. Soc. Zool. Fr.- Evol. Zool.* **120**, 347–360.

Gayet, M. and Meunier, F. J. (1991). Première découverte de Gymnotiformes fossiles (Pisces, Ostariophysi) dans le Miocène supérieur de Bolivie. *C.R. Acad. Sci. Paris* **313**, 471–476.

Gayet, M. and Meunier, F. J. (1998). Maastrichtian to early late Paleocene Ostechtyes of Bolivia: additions and comments. In *Phylogeny and Classification of Neotropical Fishes* (ed. L. R. Malabarba, R. E. Reis, R. P. Vari, C. A. S. Lucena and Z. M. S. Lucena), pp. 85–110. Porto Alegre: Museu de Ciencias e Tecnologia – PUC/RS. (in press).

Gayet, M., Meunier, F. J. and Kirschbaum, F. (1994). *Ellisella kirschbaumi* Gayet and Meunier, 1991, gymnotiforme fossile de Bolivie et ses relations phylogénétiques au sein des formes actuelles. *Cybium* **18**, 273–306.

Grande, L. (1984). Paleontology of the Green River formation with a review of the fish fauna. *Bull. Geol. Surv. Wyoming* **63**, 1–333.

Grande, L., Eastman, J. T. and Cavender, T. (1982). *Amyzon gosiutensis*, a new catostomid fish from the Green River Formation. *Copeia* **1982**, 523–532.

Grande, T. (1997). Interrelationships and historical biogeography of ostariophysan fishes with emphasis on the gonorynchiformes (Actinopterygii: Teleostei). *J. Vert. Paleont*. **17**, 50A–51A.

Greenwood, P. H. (1970). On the genus *Lycoptera* and its relationship with the family Hiodontidae (Pisces, Osteoglossomorpha). *Bull. Brit. Mus. Nat. Hist. (Zool.)* **19**, 257–285.

Greenwood, P. H. (1971). Hyoid and ventral gill arch musculature in osteoglossomorph fishes. *Bull. Br. Mus. Nat. Hist. (Zool.)* **22**, 1–55.

Greenwood, P. H. (1973). Interrelationships of osteoglossomorphs. In *Interrelationships of Fishes* (ed. P. H. Greenwood, R. S. Miles and C. Patterson), pp. 307–332. London: Academic Press.

Günther, A. (1880). *An Introduction to the Study of Fishes* (reprinted 1993). New Delhi: Discovery Publishing House.

Gutell, R. R. (1993). Collection of small subunit (16S- and 16S-like) ribosomal RNA structures. *Nucleic Acids Res*. **21**, 3051–3054.

Gutell, R. R. and Fox, G. E. (1988). A compilation of large subunit RNA sequences presented in a structural format. *Nucleic Acids Res.* (Suppl.) **16**, r175–r269.

Gutell, R. R., Schnare, M. N. and Gray, M. W. (1992). A compilation of large subunit (23S- and 23S-like) ribosomal RNA structures. *Nucleic Acids Res.* (Suppl.) **20**, 2095–2109.

Hillis, D. (1996). Applications of molecular systematics: The state of the field and a look to the future. In *Molecular Systematics* (ed. D. Hillis, C. Moritz and B. K. Mable), pp. 515–543. Sunderland, MA: Sinauer Associates, Inc.

Hopkins, C. (1986). Behavior of Mormyridae. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 527–576. New York: John Wiley & Sons.

Horai, S., Hayasaka, K., Kondo, R., Tsugane, K. and Takahata, N. (1995). Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. *Proc. Natl. Acad. Sci. USA* **92**, 532–536.

Jin, F. (1991). A new genus and species of Hiodontidae from Xintai, Shandong. *Vert. PalAsiatica.* **29**, 46–54.

Kawasaki, M. (1993). Independently evolved jamming avoidance responses employ identical computational algorithms: a behavioral study of the African electric fish, *Gymnarchus niloticus*. *J. Comp. Physiol.* A **173**, 9–22.

Keller, C. H., Kawasaki, M. and Heiligenberg, W. (1991). The control of pacemaker modulations for social communication in the weakly electric fish *Sternopygus*. *J. Comp. Physiol.* A **169**, 441–450.

Lauder, G. V. and Liem, K. F. (1983). The evolution and interrelationships of the actinopterygian fishes. *Bull. Mus. Comp. Zool.* **150**, 95–197.

Li, G.-Q. (1996). A new species of Late Cretaceous osteoglossid (Teleostei) from the Oldman Formation of Alberta, Canada and its phylogenetic relationships. In *Mesozoic Fishes – Systematic and Paleoecology* (ed. G. Arratia and G. Viohl), pp. 285–298. Munich: F. Pfeil.

Li, G.-Q., Grande, L. and Wilson, M. V. H. (1997a). The species of *Phareodus* (Teleostei: Osteoglossidae) from the Eocene of North America and their phylogenetic relationships. *J. Vert. Paleont*. **17**, 487–505.

Li, G.-Q. and Wilson, M. V. H. (1996). Phylogeny of Osteoglossomorpha. In *Interrelationships of Fishes* (ed. M. L. J. Stiassny, L. R. Parenti and G. D. Johnson), pp. 163–174. San Diego: Academic Press, Inc.

Li, G.-Q., Wilson, M. V. H. and Grande, L. (1997b). Review of *Eohiodon* (Teleostei: Osteoglossomorpha) from Western North America, with phylogenetic reassessment of Hiodontidae. *J. Paleont.* **71**, 1109–1124.

Li, W.-H. (1997). *Molecular Evolution*. Sunderland, MA: Sinauer Associates, Inc.

Lissmann, H. W. (1951). Continuous electrical signals from the tail of a fish, *Gymnarchus niloticus* Cuv. *Nature* **167**, 201–202.

Lissmann, H. W. and Machin, K. E. (1958). The mechanisms of object location in *Gymnarchus niloticus* and similar fish. *J. Exp. Biol*. **35**, 451–486.

Lundberg, J. G. (1993). African–South American freshwater fish clades and continental drift: Problems with a paradigm. In *Biological Relationships between Africa and South America* (ed. P. Goldblatt), pp. 157–199. New Haven: Yale University Press.

Lundberg, J. G. (1998). The temporal context for diversification of neotropical fishes. In *Phylogeny and Classification of Neotropical Fishes* (ed. L. R. Malabarba, R. E. Reis, R. P. Vari, C. A. S. Lucena and Z. M. S. Lucena), pp. 49–68. Porto Alegre: Museu de Ciencias e Tecnologia – PUC/RS. (in press).

Lundberg, J. G., Cox Fernendes, C., Albert, J. S. and Garcia, M. (1996). *Magosternarchus*, a new genus with two new species of electric fishes (Gymnotiformes: Apteronotidae) from the Amazon River Basin, South America. *Copeia* **1996**, 657–670.

Machin, K. E. and Lissman, H. W. (1960). The mode of operation of the electric receptors in *Gymnarchus niloticus*. *J. Exp. Biol.* **37**, 801–811.

Mago-Leccia, F. (1976). Venezuelan gymnotiform fishes: a preliminary study for a revision of the group in South America. PhD thesis, Universidad Central de Venezuela.

Mago-Leccia, F. (1978). Los peces de la familia Sternopygidae de Venezuela. *Acta Cient. Venez.* **29**, 1–89.

Mago-Leccia, F. (1994). *Electric Fishes of the Continental Waters of America*. Caracas: Clemente Editores, C.A.

Mago-Leccia, F. and Zaret, T. M. (1978). The taxonomic status of *Rhabdolichops troscheli* (Kaup, 1856) and speculations on gymnotiform efolution. *Env. Biol. Fish.* **3**, 379–384.

Martin, A. P., Naylor, G. J. and Palumbi, S. R. (1992). Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature* **357**, 153–155.

Martin, A. P. and Palumbi, S. R. (1993). Body size, metabolic rate, generation time and the molecular clock. *Proc. Natl. Acad. Sci. USA* **90**, 4087–4091.

Moller, P. (1995). *Electric Fishes – History and Behavior*. London: Chapman & Hall.

Montoya-Burgos, J.-I., Muller, S., Weber, C. and Pawlowski, J. (1997). Phylogenetic relationships between Hypostominae and Ancistrinae (Siluroidei: Loricariidae): first results from mitochondrial 12S and 16S rRNA gene sequences. *Rev. Suisse Zool.* **104**, 185–198.

Novacek, M. J. and Marshall, L. G. (1976). Early biogeographic history of ostariophysan fishes. *Copeia* **1976**, 1–12.

Ortí, G. (1997). Radiation of characiform fishes: Evidence from mitochondrial and nuclear DNA sequences. In *Molecular Systematics of Fishes* (ed. T. Kocher and C. Stepien), pp. 219–243. San Diego: Academic Press.

Ortí, G. and Meyer, A. (1997). The radiation of characiform fishes

and the limits of resolution of mitochondrial ribosomal DNA sequences. *Syst. Biol.* **46**, 75–100.

Ortí, G., Petry, P., Porto, J. I. R., Jégu, M. and Meyer, A. (1996). Patterns of nucleotide change in mitochondrial ribosomal RNA genes and the phylogeny of piranhas. *J. Mol. Evol.* **42**, 169–182.

Patterson, C. (1975). The distribution of Mesozoic freshwater fishes. *Mem. Mus. Nat. D'hist. Nat., Paris, Serie A, Zool*. **88**, 155–174.

Pitman, III, W. C., Cande, S., LaBrecque, J. and Pindell, J. (1993). Fragmentation of Gondwana: the separation of Africa from South America. In *Biological Relationships between Africa and South America* (ed. P. Goldblatt), pp. 15–34. New Haven: Yale University Press.

Poyato-Ariza, F. J. (1994). A new Early Cretaceous gonorhynchiform fish (Teleostei: Ostariophysi) from Las Hoyas (Cuenca, Spain). *Occ. Pap. Mus. of Nat. Hist. Univ. Kansas* **164**, 1–37.

Rand, D. M. (1994). Thermal habit, metabolic rate and the evolution of mitochondrial DNA. *Trends Ecol. Evol.* **9**, 125–131.

Rose, G., Keller, C. and Heiligenberg, W. (1987). 'Ancestral' neural mechanisms of electrolocation suggest a substrate for the evolution of the jamming avoidance response. *J. Comp. Physiol.* A **160**, 491–500.

Rosen, D. E. and Greenwood, P. H. (1970). Origin of the Weberian apparatus and the relationships of the ostariophysan and gonorhynchiform fishes. *Am. Mus. Novitates* **2428**, 1–25.

Shaffer, H. B. and McKnight, M. L. (1996). The polytipic species revisited: genetic differentiation and molecular phylogenetics of the tiger salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. *Evolution* **50**, 417–433.

Silva-Santos, R. (1985). *Laeliichthys ancestralis*, novo gênero e espécies de Osteoglossiformes do Aptiano da Formação Areado, estado de Minas Gerais, Brasil. *VIII Congresso Bras. de Paleotol. Ser. Geol. 27, Paleontol./Estrat.* **2**, 151–167.

Simons, A. M. and Mayden, R. L. (1998). Phylogenetic relationships of the Western North American phoxinins (Actinopterygii: Ciprinidae) as inferred from mitochondrial 12S and 16S ribosomal RNA sequences. *Mol. Phyl. Evol.* **9**, 308–329.

Sullivan, J. P. (1997). A phylogenetic study of the neotropical hypopomid electric fishes (Gymnotiformes: Rhamphichthyoidea). PhD thesis, Duke University.

Taverne, L. (1969). Etude ostéologique des genres *Boulengeromyrus* Taverne et Géry, *Genyomyrus* Boulenger, *Petrocephalus* Marcusen (Pisces, Mormyriformes). *Mus. Roy. Afr. Centr. Ann. Soc. R. Zool. Belg.* **174**, 1–85.

Taverne, L. (1970). Note sur l'ostéologies du genre *Gymnarchus* Cuvier (Pisces, Mormyriformes). *Bull. Acad. R. Belg. Cl. Sci.* **56**, 63–78.

Taverne, L. (1971a). Note sur la systématique des poissons Mormyriformes. Le probléme des genres *Gnathonemus* Gill, *Marcusenius* Gill, *Hippopotamyrus* Pappenhein, *Cyphomyrus* Myers, et les nouveaux genres *Pollimyrus* et *Brienomyrus*. *Rev. Zool. Bot. Afr.* **84**, 99–110.

Taverne, L. (1971b). Ostéologie des genres *Marcusenius* Gill, *Hippopotamyrus* Pappenhein, *Cyphomyrus* Myers, *Pollimyrus* Taverne et *Brienomyrus* Taverne (Pisces Mormyriformes). *Mus. Roy. Afr. Centr. Ann. Soc. R. Zool. Belg.* **188**, 1–144.

Taverne, L. (1972). Ostéologie des genres *Mormyrus* Linné, *Mormyrops* Müller, *Hyperopisus* Gill, *Isichthys* Gill, *Mormyrus* Boulenger, *Stomayorhinus* Boulenger et *Gymnarchus* Cuvier. Considérations générales sur la systématique des poisson du l'ordre des Mormyriformes. *Mus. Roy. Afr. Centr. Ann. Soc. R. Zool. Belg.* **200**, 1–194.

Taverne, L. (1979). Ostéologie, phylogenèse et systematique des Téléostéens fossiles et actuels du super-ordre des Ostéoglossomorphes. Troisième partie. Évolution des structures ostéologiques et conclusions générales relatives à la phylogenèse et à la systematique du super-ordre. *Bull. Acad. R. Belg. Cl. Sci. Addendum* **43, F. 3**, 1–168.

Triques, M. L. (1993). Filogenia dos generos de gymnotiformes (Actinopterygii, Ostariophysi), com base em caracteres esqueleticos. *Comun. Mus. Cienc. PUCRS, Serie Zool*. **6**, 85–130.

Triques, M. L. (1996a). *Eigenmannia vicentespelaea*, a new species of cave dwelling electrogenic neotropical fish (Ostariophysi: Gymnotiformes: Sternopygidae). *Rev. Fr. Aquariol.* **23**, 1–4.

Triques, M. L. (1996b). *Iracema caiana*, new genus and species of electrogenic neotropical freshwater fish (Rhamphichthyidae: Gymnotiformes: Ostariophysi: Actinopterygii). *Rev. Fr. Aquariol.* **23**, 91–92.

Triques, M. L. (1998). *Tembeassu marauna*, new genus and species of electrogenic neotropical fish (Ostariophysi: Gymnotiformes: Apteronotidae). *Rev. Fr. Aquariol.* **25**, 5–10.

Van Der Bank, F. H. and Kramer, B. (1996). Phylogenetic relationships between eight African species of Mormyriform fish (Teleostei, Osteichthyes): Resolution of a cryptic species and reinstatement of Cyphomyrus Myers, 1960. *Biochem. Systematics Ecol*. **24**, 275–290.

Vawter, L. and Brown, W. M. (1993). Rates and patterns of base change in the small subunit ribosomal RNA gene. *Genetics* **134**, 597–608.

Werner, C. (1994). Die kontinentale Wirbeltierfauna aus der unteren Oberkreide de Sudan (Waidi Milk Formation). *Berliner Geowiss. Abh. E* **13**, 221–249.

Wheeler, W. C. and Honeycutt, R. L. (1988). Paired sequence difference in ribosomal RNAs: evolutionary and phylogenetic implications. *Mol. Biol. Evol.* **5**, 90–96.

Zakon, H. H. (1986). The electroreceptive periphery. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 103–156. New York: John Wiley & Sons.

Zuckerkandl, E. and Pauling, L. (1962). Molecular disease, evolution and genic heterogeneity. In *Horizons in Biochemistry* (ed. M. Kasha and B. Pullman), pp. 189–225. New York: Academic Press.

Zuckerkandl, E. and Pauling, L. (1965). Evolutionary divergence and convergence in proteins. In *Evolving Genes and Proteins* (ed. V. Bryson and H. J. Vogel), pp. 97–166. New York: Academic Press.

View publication stats