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Mitochondrial Genomics of Ostariophysan Fishes: Perspectives on Phylogeny and Biogeography

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Abstract. Ostariophysi is the second largest superorder within Teleostei. It contains five orders: Gonorynchiformes, Cypriniformes, Characiformes, Siluriformes, and Gymnotiformes. Resolving the higher-level relationships among ostariophysan and related fishes will aid in resolving basal teleostean divergence and provide basis to historical biogeographic analysis of major freshwater fish groups. In this study, we report the complete mitochondrial (mt) DNA sequences for eleven ostariophysan fishes and the results of phylogenetic analyses including these species plus four other ostariophysan and nine non-ostariophysan teleostean fishes. Maximum likelihood and maximum parsimony analyses reconfirmed clupeiforms as the closest relatives of ostariophysans. However, gonorynchiforms were closer to clupeiforms than to otophysans (ostariophysan groups excluding gonorynchiforms), thus raising a question over the current definition of Ostariophysi. The lack of clarity in otocephalan (ostariophysans + clupeiforms) basal relationships implies that such divergence took place over a short period of time. The monophyly of cypriniforms, characiphysans (characiforms, siluriforms, and gymnotiforms), and orders or superorders outside the ostariophysans examined here were conceivably reconstructed. The phylogenetic hypothesis suggests a Pangean origin of otophysans. Within characiphysans, gymnotiforms and siluriforms have independent evolutionary origins and evolutionary histories comparable to or older than that of characiforms. This helps to explain the present geographic distribution of characiphysans.

Key words: Teleost phylogeny — Regional bootstrap — Zoogeography — Freshwater fish diversity

Introduction

Teleostean fishes include about 23,500 species, amounting to nearly half of the extant vertebrate species (Nelson 1994). Within Teleostei, Ostariophysi is the second largest superorder, with five orders containing 63 families, nearly 1000 genera, and about 6500 species (Nelson 1994; Berra 2001). Except for Gonorynchiformes (e.g., milkfish), all the ostariophysan fishes (Cypriniformes, carps and loaches; Characiformes, tetras; Siluriformes, catfishes; Gymnotiformes, electric eels) have a specialized bony connection of transformed anterior vertebrae between the inner ear and swim-bladder. While gonorynchiforms (Anotophysi) live in mostly estuarine or marine environments, the bony swim-bladdered fishes (Otophysi) are almost exclusively primary freshwater inhabitants, which occur in all continents except Australia (Fig. 1). About 93% of primary freshwater fish species are Ostariophysi (Berra 2001). Because the volume of freshwater rivers and lakes worldwide collectively amount to less than $\frac{1}{2600}$ of that of oceanic

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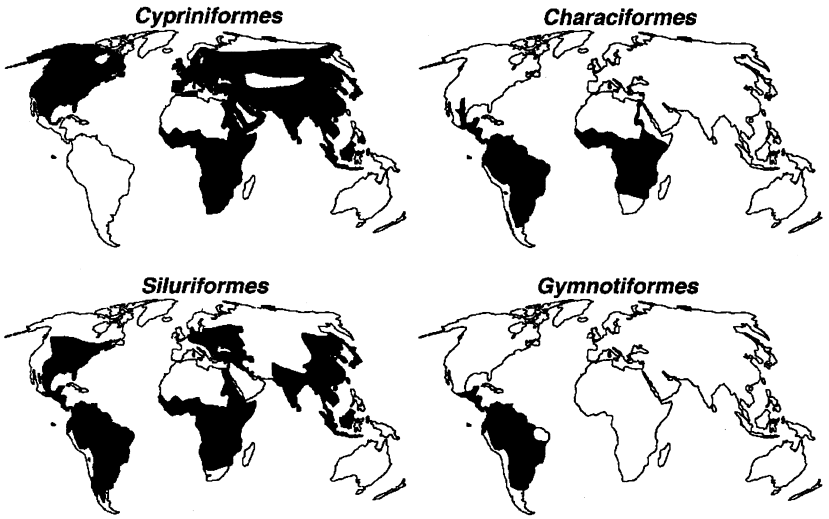

Fig. 1. Geographic distribution of the extant otophysan orders.

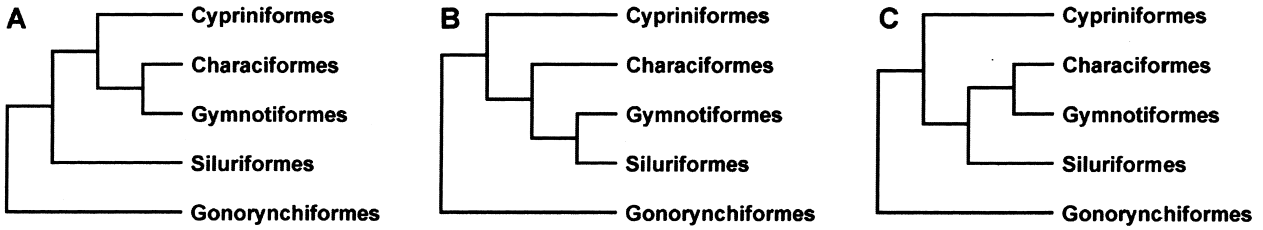

Fig. 2. Phylogenetic hypotheses of ostariophysan orders. **A** Greenwood and Rosen (1970) from morphological data. **B** Fink and Fink (1981) from morphological data, Dimmick and Larson (1996) from combined molecular and morphological data, and Ortí (1997) from molecular data from mtDNA and all codon-positions of the ependymin gene. Gonorynchiforms were not included in Ortí (1997). **C** Dimmick and Larson (1996) from molecular data only, and Ortí (1997) from molecular data from the first and second codon positions of the ependymin gene. Cypriniforms and gonorynchiforms were not included in Ortí (1997).

waters (Duxbury and Duxbury 1997), the biodiversity of freshwater fishes is therefore remarkably high, ostariophysan fishes being the major contributors.

The distribution patterns of primary freshwater fishes has provided a good evidence for the geological shaping of land and freshwater systems. As a representative freshwater fish group, the ostariophysans have been the focus of many biogeographical discussions (e.g., Novacek and Marshall 1976; Briggs 1979; Fink and Fink 1981; Howes 1991). Although Novacek and Marshall (1976) considered the present distribution of ostariophysan orders in relation to the movement of continents after Gondwanan land separation, Fink and Fink (1981) pointed out difficulties in biogeographic explanations for ostariophysan distribution. However, because different biogeographic opinions arose from different opinions on ostariophysan phylogeny, it is clear that a robust phylogenetic reconstruction is necessary.

With the Gonorynchiformes as an outgroup, two major morphological hypotheses have been put forward to explain otophysan phylogenetic relationships (Fig. 2A, B; Rosen and Greenwood 1970; Fink and Fink 1981). Although recent molecular studies resulted in the third hypothesis (Fig. 2C; Dimmick and Larson 1996; Ortí 1997), their suggestion was contradictory (Fig. 2B). The closest relative to the entire ostariophysan group (Otophysi + Anotophysi) has also been ambiguous (Greenwood et al. 1966; Rosen 1973; Gosline 1980; Lecointre and Nelson 1996; Johnson and Patterson 1996; Arratia 1999). Various hypotheses are shown diagrammatically in Fig. 3A and B. A recent molecular study (Lê et al. 1993) raised the possibility of a close ostariophysan-clupeiform relationship, whereby Johnson and Patterson (1996) proposed a new taxonomic unit, Otocephala (Ostariophysi + Clupeomorpha). Although this relationship was confirmed in a recent study (Inoue et al. 2001a), none of these molecular studies included anotophysans and hence there is currently no molecular information of the limit of ostariophysans (Fig. 3C).

For phylogenetic inferences among higher taxa, it is necessary to compare long DNA sequence data. Recent progress in molecular techniques has made it much easier to obtain complete nucleotide sequences of fish mitochondrial (mt) genomes (Miya and Nishida 1999). Such mt genomic data have been

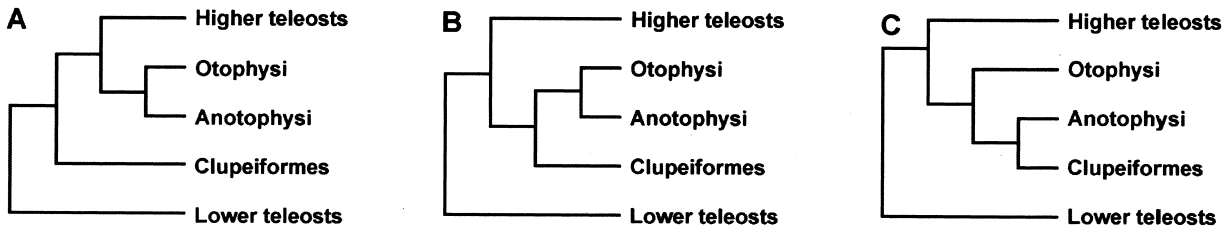

Fig. 3. Morphological hypotheses of basal teleostean phylogenies around ostariophysans. **A** collective representation of hypotheses by Greenwood et al. (1966) and Rosen (1973). The former presumed that protacanthopterygians were more basal to ostariophysans, and that clupeiforms and elopiforms formed a sister group. The latter assumed protacanthopterygians and other higher teleosts formed a sister group. Most morphologists have accepted the latter assumption (e.g., Lauder and Liem 1983; Nelson 1994). **B** collective representation of hypotheses by Gosline (1980), Johnson and Patterson (1996) and Arratia (1999). Gosline (1980) presumed the position of paracanthopterygians more basal to the ostariophysan + clupeiform cluster. Arratia (1999) did not include groups higher than protacanthopterygians in her analysis. **C** an alternative hypothesis based on mt genome data from this study. In this hypothesis, anotophysans and clupeiforms are closest relatives of each other. Recent molecular studies have indicated ostariophysan-clupeiform monophyly (Lê et al. 1993; Inoue et al. 2001b), but did not examine anotophysans so could not evaluate the plausibility of the two trees (B and C). Because of our hypothesis indicating the higher taxon including clupeiforms as a small group within otocephalans, we used Clupeiformes instead of Clupeomorpha throughout the text.

useful for resolving not only deep branches among teleostean fishes but also the percomorph 'upper-bush' (Miya and Nishida 2000; Inoue et al. 2001b; Miya et al. 2001). Also, advances in molecular statistical and phylogenetic inference theories and methodologies have enabled us to reconstruct the phylogenetic relationships among distantly-related taxa (Kishino and Hasegawa 1989; Yang 1996; Cao et al. 1999).

In this study, we determined the complete nucleotide sequences of mt genomes from eleven ostariophysan species and examined phylogenetic relationships based on data from these species plus 13 species sequenced previously, including four ostariophysans. The phylogenetic framework thus obtained, indicating ostariophysan limits and interrelationships, is helpful not only for phylogenetic explanations of freshwater fish diversity but also resolution of basal teleostean divergence.

Materials and Methods

Taxonomic Sampling

We sequenced ostariophysan mt genomes (Table 1) from three cypriniforms (*Sarcocheilichthys variegatus microoculus,* Cyprinidae; *Cobitis striata*, Cobitidae; *Lefua echigonia*, Balitoridae), two characiforms (*Phenacogrammus interruptus*, Alestidae; *Chalceus macrolepidotus*, Characidae), two gymnotiforms (*Eigenmannia* sp., Sternopygidae; *Apteronotus albifrons*, Apteronotidae), two siluriforms (*Pseudobagrus tokiensis*, Bagridae; *Corydoras rabauti*, Callichthyidae), and two gonorynchiforms (*Chanos chanos*, Chanidae; *Gonorynchus greyi*, Gonorynchidae). We selected these species together with four species previously sequenced so as to include at least two representatives of each major ostariophysan lineage, thereby subdividing possible long branches according to the strategy of Miya and Nishida (2000). Nine non-ostariophysan species selected for the outgroup included two clupeiforms, putative closest relatives to ostariophysans according to recent studies (Lê et al. 1993; Inoue et al. 2001b).

PCR and Sequencing

Procedures for the two-step PCR-direct sequencing method for mt genomes were as follows. Isolation of total genomic DNA from muscle tissue followed Asahida et al. (1996) or was done using a commercial kit (Qiagen). Seven fish-versatile long PCR primers (S-LA-16S-L, L2508-16S, L12321-Leu, H12293-Leu, H15149-CYB, H1065-12S, and S-LA-16S-H; for locations and sequences of these primers, see Miya and Nishida 2000; Inoue et al. 2000, 2001b; Ishiguro et al. 2001; Kawaguchi et al. 2001) worked in various combinations to amplify the entire mitochondrial genome in at least two reactions. Long PCR reaction conditions followed Miya and Nishida (1999). Short PCR with the long PCR products as template using 155 fish versatile primers followed the method of Miya and Nishida (1999) and Inoue et al. (2001b). We made a few species-specific primers when these versatile primers did not work. We designed short PCRs so as to overlap each other by 100–500 bp in turn. The short PCR products then worked as sequence templates with the same primers used in the PCR reactions. Commercial sequence kits (dye-labeled terminator sequence kits of Applied Biosystems and Amersham Pharmacia) and ABI model 373/377 automated DNA sequencers were used to obtain sequence data. Primer sequence data are available upon request.

Phylogenetic Analysis

The positions of structural genes and spacer regions were inferred on the basis of homology with previously reported sequences from several teleostean species (Table 1). First we determined the 5′ and 3′ ends of tRNA genes by manual alignment based on the structural model of Kumazawa and Nishida (1993). The termini of other structural genes were then determined so as to minimize any overlaps between genes coded on the same strand. Regarding protein-coding genes, the deduced amino-acid sequences were compared, first by automatic alignment using ClustalW (Thompson et al. 1994), followed by manual adjustment. For farther analyses of protein-coding genes, we made nucleotide sequence alignments following amino-acid level alignments as a guide.

The total number of nucleotide sites used in our phylogenetic inferences was 8196. We retained 7286 sites without gaps in the unambiguously aligned regions of 13 protein-coding genes, eliminating third codon-positions, and 910 sites of stem regions of 22 tRNA genes. These aligned sequences were compiled separately into 28 segments: 26 from the first and second codon positions for

Table 1. Fish species used in this study

Species	Locality[a]	Source (Voucher Specimen[b])	Accession No.
Ostariophysi			
Cypriniformes	(EA, AF, NA)		
Sarcocheilichthys variegatus microoculus	Lake Biwa, Japan (EA)	This study (CBM-ZF-10604)	AB054124
Cyprinus carpio	(EA)	Chang et al. (1994)	X61010
Carassius auratus langsdorfii	(EA)	Murakami et al. (1997)	AB006953
Danio rerio	(EA)	Broughton et al. (2001)	AC024175
Crossostoma lacustre	(EA)	Tzeng et al. (1992)	M91245
Cobitis striata	Lake Biwa, Japan (EA)	This study (CBM-ZF-10606)	AB054125
Lefua echigonia	Hino, Shiga, Japan (EA)	This study	AB054126
Characiformes	(AF, SA)		
Phenacogrammus interruptus	Unknown (AF)	This study (CBM-ZF-10607)	AB054129
Chalceus macrolepidotus	Unknown (SA)	This study (CBM-ZF-10608)	AB054130
Gymnotiformes	(SA)		
Eigenmannia sp.	Unknown (SA)	This study (CBM-ZF-10620)	AB054131
Apteronotus albifrons	Unknown (SA)	This study (CBM-ZF-10621)	AB054132
Siluriformes	(EA, AF, NA, SA)		
Pseudobagrus tokiensis	Japan (EA)	This study (CBM-ZF-10622)	AB054127
Corydoras rabauti	Unknown (SA)	This study (CBM-ZF-10623)	AB054128
Gonorynchiformes	(AF, MA)		
Chanos chanos	Slawesi, Indonesia (MA)	This study	AB054133
Gonorynchus greyi	Australia (MA)	This study (AM-I33768001)	AB054134
Outgroup			
Clupeiformes			
Sardinops melanostictus		Inoue et al. (2000)	AB032554
Engraulis japonicus		Inoue et al. (2001c)	AB040676
Anguilliformes			
Auguilla japonica		Inoue et al. (2001a)	AB038556
Osteoglossiformes			
Osteoglossum bicirrhosum		Inoue et al. (2001b)	AB043025
Pantodon buchholzi		Inoue et al. (2001b)	AB043068
Salmoniformes			
Oncorhynchus mykiss		Zardoya et al. (1995)	L29771
Salmo salar		Hurst et al. (1999)	U12143
Acanthomorpha			
Paralichthys olivaceus		Saitoh et al. (2000)	AB028664
Gadus morhua		Johansen and Bakke (1996)	X99772

[a] Letters in parentheses indicate the major distribution ranges. AF, Africa; EA, Eurasia; MA, marine; NA, North America; SA, South America.

[b] Abbreviations for specimen depository; AM, Australian Museum; CBM, Natural History Museum & Institute, Chiba.

13 protein-coding genes and two from concatenated stem regions of each of the H- and L-strand encoded tRNA genes. Alignment data are available upon request.

We employed maximum likelihood (ML) and maximum parsimony (MP) approaches among the 24 taxa for phylogenetic inferences (Table 1). Coding strands of these regions/genes were used when analyzed separately, and complementary strand sequences for L-strand-encoded genes when analyzed following concatenation.

We employed the nucleotide substitution model of TN93 (Tamura and Nei 1993) with rate optimization for ML analysis, since preliminary comparison of AIC (Akaike 1974) showed the model to fit the present dataset better than the HKY85 model (Hasegawa et al. 1985) adopted by many authors. Several candidate trees were obtained using the quick-add-OTU algorithm based on the approximate likelihood method (nucml program of MOLPHY version 2.3b3) (Adachi and Hasegawa 1996). We compared likelihood values of these initial candidate trees without gamma-corrections on concatenated sequences, so as to obtain a candidate tree with local bootstrap probabilities (BPs). Subsequently, we conducted further tree searches around the nodes with local BPs lower than 80%. Tree rearrangement regionally around two contiguous nodes enables likelihood comparison among 15 candidate trees, three nodes, 105, and so on, instead of three candidates for local rearrangement. In this analysis the calculation of likelihood values followed Cao et al. (1999) being performed separately on the 28 segments using PAML version 3.0c (Yang 1997) and totalml program of MOLPHY. Again, comparisons of AIC showed the rationale of such partitioning. The detailed comparison of candidate trees enabled the calculation of regional BPs helpful for assessing the reliability of the local BPs, summing the BPs of individual topologies with the Consense program of PHYLIP version 3.54c (Felsenstein 1993). All bootstrap resamplings followed the RELL method (Hasegawa and Kishino 1994) of 1000 times repetition for local or 10,000 for regional analyses.

For MP analysis, we took stepwise-addition and tree-bisection-reconnection search algorithms on concatenated sequences, jumbling the input order of OTUs 24 times with PAUP* version 4.0b (Swofford 1998). The tree search process was repeated 1000 times to obtain BPs.

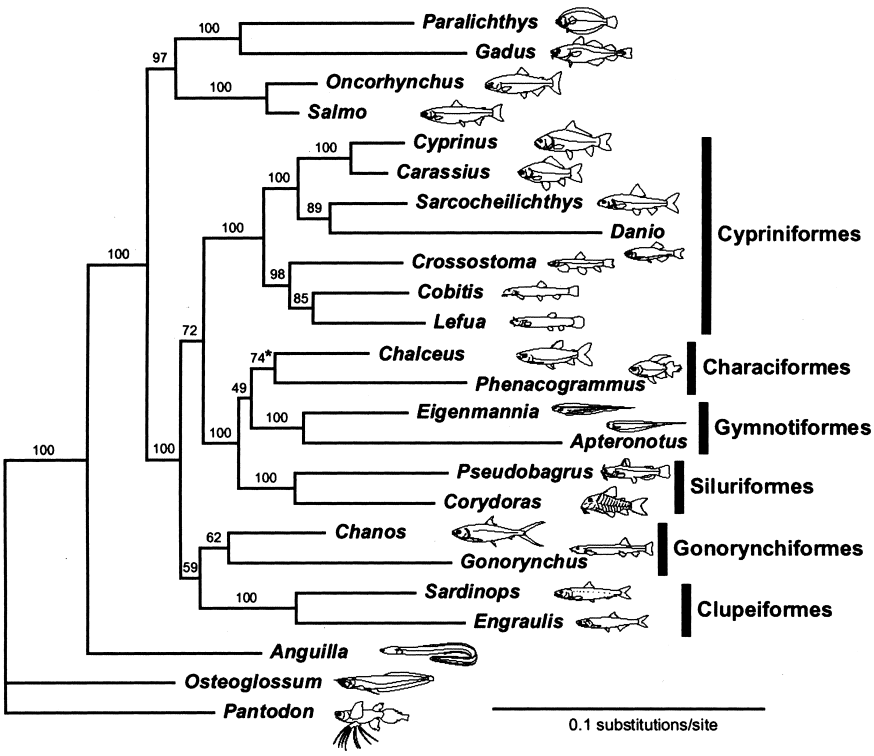

Fig. 4. ML tree based on gamma-corrected totalml topology. Numbers above branches are local RELL bootstrap probabilities (BPs) without gamma-correction. Regional RELL BPs of the following two combinations showed comparable values to local BPs in most cases (data not shown): (1) Fifteen possible topologies with *Phenacogrammus*, *Chalceus*, gymnotiforms, siluriforms, and outgroup, and (2) 105 topologies with cypriniforms, characiphysans, *Chanos*, *Gonorynchus*, clupeiforms, and outgroup. Regional BP at the characiform cluster (*) was considerably lower than local BP (36% versus 74%).

Results

Genome Organization

The organization of ostariophysan mt genomes followed that of generalized teleostean and vertebrate mt genomes. *Apteronotus*, *Eigenmannia*, *Chanos*, and *Gonorynchus*, however, showed unusual codon usages of the 5′ termini of a few protein-coding genes, while many of the start codons were "ATG" for most protein genes and "GTG" for the cytochrome oxidase subunit-I (COI) gene. We assumed non-canonical start codons in these genes. A 17-nt insertion sequence between the ATPase subunit-6 and COIII genes was found in *Corydoras*, while most vertebrate mt genomes sequenced to date have a head-to-tail junction between these genes. However, these specific features were phylogenetically uninformative in the present dataset, since none were shared by more than one of the species examined.

Phylogenetic Resolution

The totalml analysis of gamma-corrected, 28 partitioned ML analyses of 8196 nucleotide sites from the mt genomic data yielded a ML topology with resolution of the branching pattern among ostariophysan and related fishes (Fig. 4). MP analysis gave a similar result (Fig. 5). BP support for the nodes were strong in most cases (>80%) in both the ML and MP analyses. A few basal ostariophysan nodes were, however, marginal (50–80%) or weak (<50%). Local BP support for characiform monophyly in the ML tree was 74%. However, the gamma-corrected regional BP at the node of only 36% indicated that characiform monophyly is questionable. In other nodes with marginal BP support in the ML tree, regional BPs were comparable to local BPs based on non gamma-corrected concatenated analysis (data not shown). This upholds the reliability of the local BPs in ML analysis. Although the ML and MP tree topologies differed to some extent, they were statistically indistinguishable from each other (Tables 2, 3, trees #1, 2).

As for basal branching nodes, otocephalan monophyly and placement of lower (eel + osteoglossiforms) and higher (salmoniforms + acanthomorphs) teleosts were clear. Difference in likelihood values between the two alternatives in the ML and a conventional tree hypothesis (Table 2; tree #3) was significant.

ML analysis indicated that anotophysan-clupeiform monophyly (Fig. 4) was likely rather than the conventional hypothesis of ostariophysan monophyly, although the difference in likelihood was small (Table 2; tree #4). MP analysis showed a similar result, although indicating anotophysan paraphyly relative to clupeiforms.

Within otophysans, the oldest branching point leading to the extant groups was between cypriniforms and the other three orders, collectively referred to Characiphysi. On the other hand, the

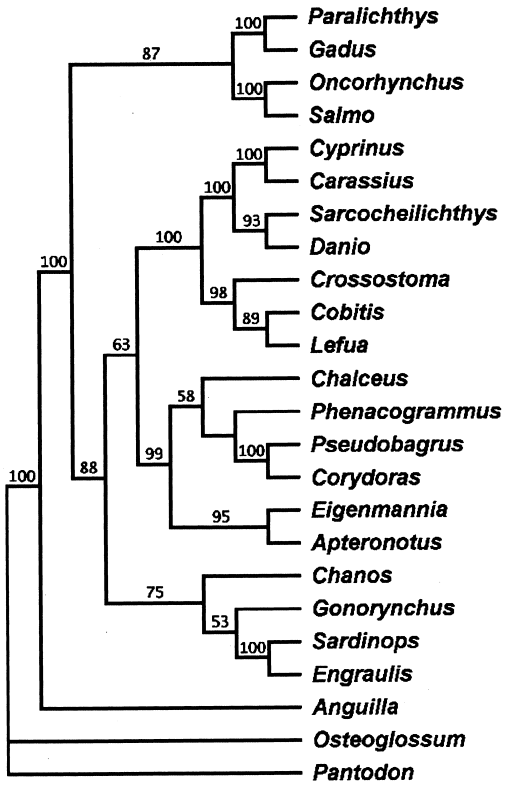

Fig. 5. MP tree based on the concatenated nucleotide sequence of 8196 sites (tree length = 12,111, consistency index = 0.4036, homoplasy index = 0.5964, retention index = 0.3534, and rescaled consistency index = 0.1426). Numbers above nodes are BPs (values above 50% are shown) derived from 1000 times iteration.

placement of siluriforms basal to other otophysans (hypothesis of Rosen and Greenwood 1970) gave a lower likelihood value and was also rejected by the Templeton test (Templeton 1983) of MP analysis (Table 3, tree #3).

Among characiphysans, the ML and MP trees showed different branching orders to some extent. While the monophyly of both gymnotiforms and siluriforms were evident, it was unclear which of the latter groups is the more basal. In contrast, gymnotiform-siluriform monophyly (Fink and Fink 1981) was only weakly supported. Although the hypothesis of Fink and Fink (1981) could not be rejected using the Kishino-Hasegawa criterion (Table 3; tree #4) (Kishino and Hasegawa 1989), it had a low BP (5.3% among 15 possible topologies). The overall regional BP support for the gymnotiform-siluriform monophyly including two other possible topologies was only 5.6%. The MP analysis gave a similar result, in which the gymnotiform-siluriform monophyly received only 7% of BP support. Our results largely agree with the topologies based on the ependymin sequence without 3rd codon positions (Ortí 1997) and the 28S rDNA sequence (Dimmick and Larson 1996) (Fig. 3C).

Discussion

Ostariophysan Monophyly Questioned

Our ML and MP analyses indicated that the recent views of ostariophysan monophyly are questionable. Anotophysan-clupeiform monophyly is rather possible. Because of a topological similarity between the ML and MP trees around basal otocephalans, we believe that the anotophysan affinity with clupeiforms is more likely than so far indicated by statistical tests. Anotophysan-clupeiform monophyly also implies that the independence of clupeomorphs is questionable even as a superorder, if we redefine monophyletic Ostariophysi on the other hand.

The lack of statistical clarity for basal otocephalan as well as basal otophysan relationships is not simply due to difficulties in resolving older branching patterns. Outside this cluster, the branching pattern of elopiforms, osteoglossiforms and salmoniforms was very clear, being in accord with a recent study (Inoue et al. 2001b). The divergence of these clusters is clearly older than the basal divergence of otocephalans. Then the splitting of basal otocephalan and otophysan clusters possibly occurred within a short period of time, resulting in the difficulties of resolution of their branching order.

Biogeographic Implications

Several authors have discussed historical otophysan biogeography, mainly on the basis of phylogenetic hypotheses and the distribution pattern of extant taxa (e.g., Novacek and Marshall 1976; Briggs 1979; Fink and Fink 1981; Howes 1991). There is no reliable otophysan fossil record before the Late Cretaceous (Fink et al. 1984). Nevertheless, Gondwanan (South America + Africa) or Pangean (all continents) distribution of extant taxa (Fig. 1) implies that the origin of otophysans is older than the Gondwanan separation in the Middle Cretaceous (Novacek and Marshall 1976; Howes 1991), possibly even older than the Pangean separation in the Jurassic, if all the lineages leading to extant taxa have been primary freshwater fishes.

Our analysis suggested that otophysan basal divergence took place at a time close to that of basal otocephalan divergence. The oldest otocephalan fossil record is the earliest Cretaceous anotophysan, *Aethalinopsis* (Patterson 1993). Recent molecular clock calibration indicated that the divergence time between cypriniforms and characiforms was about 250 MYA (Kumazawa et al. 1999). Accordingly, otophysan basal divergence took place no later than the Jurassic, possibly as early as the end of Permian Period on the Pangean Continent. Pangean separation in the Middle Jurassic may have been responsible for

Table 2. Comparisons between ML/MP trees and alternative hypotheses among basal otocephalan fishes

	ML		MP		
# Topology	ΔlnL ± S.E.	*p*	Length	z	*p*
1 (((Otop, ((*Chan*, *Gono*), Clup)), higher), lower);	−60228.0	(ML)	12113	−0.1857	0.4263
2 (((Otop, (*Chan*, (*Gono*, Clup))), higher), lower);	0.6 ± 19.1	0.4875	12111	(MP)	
3 ((((Otop, (*Chan*, *Gono*)), higher), Clup), lower);	39.8 ± 23.2	0.0435	12135	−1.6255	0.0520
4 ((((Otop, (*Chan*, *Gono*)), Clup), higher), lower);	1.6 ± 20.0	0.4681	12115	−0.3162	0.3759

Tree #3 is collectively derived from Greenwood et al. (1966) and Patterson and Rosen (1977) (Fig. 3A). Tree #4 is collectively derived from Gosline (1980) and Arratia (1999) (Fig. 3B). Abbreviations for five otocephalan lineages: *Chan*, *Chanos*; Clup, Clupeiformes; *Gono*, *Gonorynchus*; Otop, Otophysi. Topologies among characiphysan taxa are fixed as the ML and MP trees, respectively, for conparison. Probabilities (*p*) of the null hypothesis are one-tailed.

Table 3. Comparisons between ML/MP trees and alternative hypotheses among otophysan fishes

	ML		MP		
# Topology	ΔlnL ± S.E.	*p*	Length	z	*p*
1 (((((*Phen*, *Chal*), Gymn), Silu), Cypr), outgroup);	−60228.0	(ML)	12113	−0.1857	0.4263
2 ((((*Phen*, (*Chal*, Silu)), Gymn), Cypr), outgroup);	5.5 ± 19.1	0.3866	12111	MP	
3 (((((*Phen*, *Chal*), Gymn), Cypr), Silu), outgroup);	87.2 ± 25.8	0.0004	12153	−3.2796	0.0005
4 ((((*Phen*, *Chal*), (Gymn, Silu)), Cypr), outgroup);	9.6 ± 16.8	0.2839	12114	−0.2873	0.3869

Tree #3 is from Rosen and Greenwood (1970) (Fig. 2A). Tree #4 is from Fink and Fink (1981) (Fig. 2B). Abbreviations for five otophysan lineages: *Chal*, *Chalceus*; Cypr, Cypriniformes; Gymn, Gymnotiformes; *Phen*, *Phenacogrammus*; Silu, Siluriformes. Topologies among outgroup taxa are fixed as the ML and MP trees, respectively, for comparison. Probabilities (*p*) of the null hypothesis are one-tailed.

the present geographic pattern, in which cypriniforms show a largely Laurasian (North America + Eurasia) distribution and characiphysans (except for siluriforms), Gondwanan distribution (Figs. 1, 6A).

The analysis also indicated independent origins of gymnotiforms and siluriforms, rather than their monophyly as advocated by Fink and Fink (1981). Accordingly, the present characiphysan geographic pattern needs to be reconsidered. Our data imply that the divergence times of gymnotiforms and siluriforms are comparable to or older than characiform divergence. If so, and especially if siluriforms are the oldest among these three groups as indicated by the ML tree, siluriforms are likely to have originated in Gondwanaland leading to their present South American distribution on one hand, and African lineages subsequently dispersed into the Eurasian continent following land connections or accretion on the other (Fig. 6B) (e.g., Novacek and Marshall 1976). The existence of some related and even congeneric species across the African and Eurasian continents (Berra 2001) indicates Cenozoic faunal exchange between these two continents, thereby supporting the present scenario.

The restricted distribution of gymnotiforms to South America is also explainable, if one of the following ad hoc assumptions is invoked; Gymnotiforms comprised a small localized lineage in the South American portion of Gondwanaland before its separation, or the African counterpart of the gymnotiform lineage became extinct following competi-

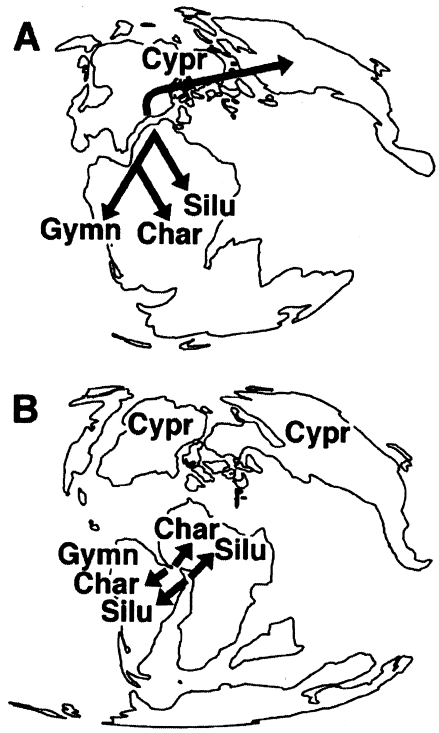

Fig. 6. Historical biogeography of otophysan fishes based on the ML tree and molecular clock calibration (Kumazawa et al. 1999). **A** ancestral otophysan stock divergence into cypriniforms (Cypr) and characiphysans (siluriforms; Silu + characiforms; Char + gymnotiforms; Gymn) on the Pangean land mass at about 250MYA, and subsequent characiphysan radiation on Gondwanaland. **B** Gondwanan separation in the Middle Cretaceous resulting in present day characiform and gymnotiform geographic ranges. Coastline patterns followed Smith et al. (1994).

tion with mormyrids, which have a similar ecology of electro-location in muddy waters.

Problems for Future Study

We were unable to obtain the clear phylogenetic resolution around basal otocephalan and otophysan relationships. Also, the branching order of characiphysan orders and characiform monophyly were relatively unclear. Although the two characiform species analyzed in this study were from South America and Africa (Table 1), they did not necessarily represent the closest relatives across the Atlantic (Ortí 1997). Furthermore, we have not discussed biogeography of lineages related to Laurasian-derived land masses. Finally, we have some reservations regarding the possibility of marine dispersal of siluriforms owing to the existence of recent marine families (Berra 2001) and Late Cretaceous fossil records from estuarine sediments (Nolf and Stringer 1996; Poyato-Ariza et al. 1999). The addition of several taxa with biogeography-oriented taxon sampling in the future may be helpful for further discussions.

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