Biochemical and Biophysical Research Communications 421 (2012) 228–231

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Evolutionary landscape of amphibians emerging from ancient freshwater fish inferred from complete mitochondrial genomes

Xiao-tong Wang [a,b,1], Yan-feng Zhang [c,1], Qian Wu [d], Hao Zhang [a,*]

[a] College of Animal Science and Technology, China Agricultural University, Beijing 100193, China
[b] Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China
[c] State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China
[d] School of Biological Science and Medical Engineering, Beijing University of Aeronautics & Astronautics, Beijing 100191, China

ARTICLE INFO

Article history:
Received 14 March 2012
Available online 4 April 2012

Keywords:
Amphibian
Marine amphibian
Freshwater fish
Saltwater fish
Complete mitochondrial genome

ABSTRACT

It is very interesting that the only extant marine amphibian is the marine frog, *Fejervarya cancrivora*. This study investigated the reasons for this apparent rarity by conducting a phylogenetic tree analysis of the complete mitochondrial genomes from 14 amphibians, 67 freshwater fishes, four migratory fishes, 35 saltwater fishes, and one hemichordate. The results showed that amphibians, living fossil fishes, and the common ancestors of modern fishes are phylogenetically separated. In general, amphibians, living fossil fishes, saltwater fishes, and freshwater fishes are clustered in different clades. This suggests that the ancestor of living amphibians arose from a type of primordial freshwater fish, rather than the coelacanth, lungfish, or modern saltwater fish. Modern freshwater fish and modern saltwater fish were probably separated from a common ancestor by a single event, caused by crustal movement.

1. Introduction

The different species of fish that inhabit lakes, rivers, streams, and oceans have evolved over millions of years and are adapted to their preferred environments. Fish are categorized into two groups based on their tolerance for salinity: freshwater fish and saltwater fish.

Freshwater areas are fewer, more isolated, and more unstable compared with the oceans, which makes them more prone to rapid changes, including, drying out, sudden and sharp rises in levels, and internal ecosystem deterioration. Thus, organisms found in freshwater, including freshwater fish, are more likely to have experienced severe ordeals and stronger selective pressure.

To date, the only known marine amphibian is the marine frog (*Fejervarya cancrivora*). It is possible that amphibians lost their ability to adapt to the salinity of ocean water after they originated from saltwater fish. However, the rarity of marine amphibians suggests it is more likely that all amphibians evolved directly from freshwater fish.

A large number of complete mitochondrial genomes from fish species have been released in recent years, which provides adequate genetic markers for delineating the genetic relationship among freshwater fish, saltwater fish, and amphibians. This facilitates the elucidation of whether amphibians share more common ancestors with freshwater or saltwater fish.

2. Materials and methods

2.1. Data collection

Complete mitochondrial genomes were acquired from GenBank database and used for phylogenetic tree analyses. Freshwater fish, migratory fish, and saltwater fish were classified according to the Fishbase (http://www.fishbase.gr/search.php). Classification abbreviations were prefixed to Latin names of each species used in the study, where "*F*-" denoted freshwater fish, "*S*-" denoted saltwater fish, "*A*-" denoted amphibians, and "*FS*-" denoted migratory fishes. In addition, the abbreviation "*L*-", indicated species regarded as living fossils. Complete mitochondrial genomes were acquired for 14 amphibians (*A*), as follows: *A-Ambystoma mexicanum*, *A-Andrias japonicus*, *A-Buergeria buergeri*, *A-Bufo japonicus*, *A-Fejervarya cancrivora*, *A-Hyla japonica*, *A-Mantella madagascariensis*, *A-Microhyla okinavensis*, *A-Rana nigromaculata*, *A-Rana plancyi*, *A-Ranodon sibiricus*, *A-Rhacophorus schlegelii*, *A-Xenopus laevis*, *A-Xenopus tropicalis*. Mitochondrial genomes of 67 freshwater fishes (*F*) were as follows: *F-Acantopsis choirorhynchos*, *F-Acheilognathus typus*, *F-Acipenser dabryanus*, *F-Alburnus alburnus*, *F-Aphyocypris chinensis*, *F-Barbatula toni*, *F-Barbonymus gonionotus*, *F-Barbus barbus*, *F-Barbus trimaculatus*, *F-Botia macracantha*, *F-Catostomus commersonii*, *F-Chanodichthys mongolicus*, *F-Cobitis choii*, *F-Cranoglanis bouderius*, *F-Cycleptus elongatus*, *F-Cyprinella lutrensis*, *F-Distoechodon tumirostris*, *F-Eigenmannia* sp., *F-Esomus metallicus*, *F-Galaxiella nigrostriata*, *F-Gnathopogon elongatus*, *F-Gobio gobio*, *F-Gymnocypris przewalskii*, *F-Gyrinocheilus aymonieri*, *F-Hemibarbus barbus*, *F-Hiodon alosoides*,

---

* Corresponding author. Fax: +86 10 62731265.
E-mail address: zhanghao827@163.com (H. Zhang).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.03.141

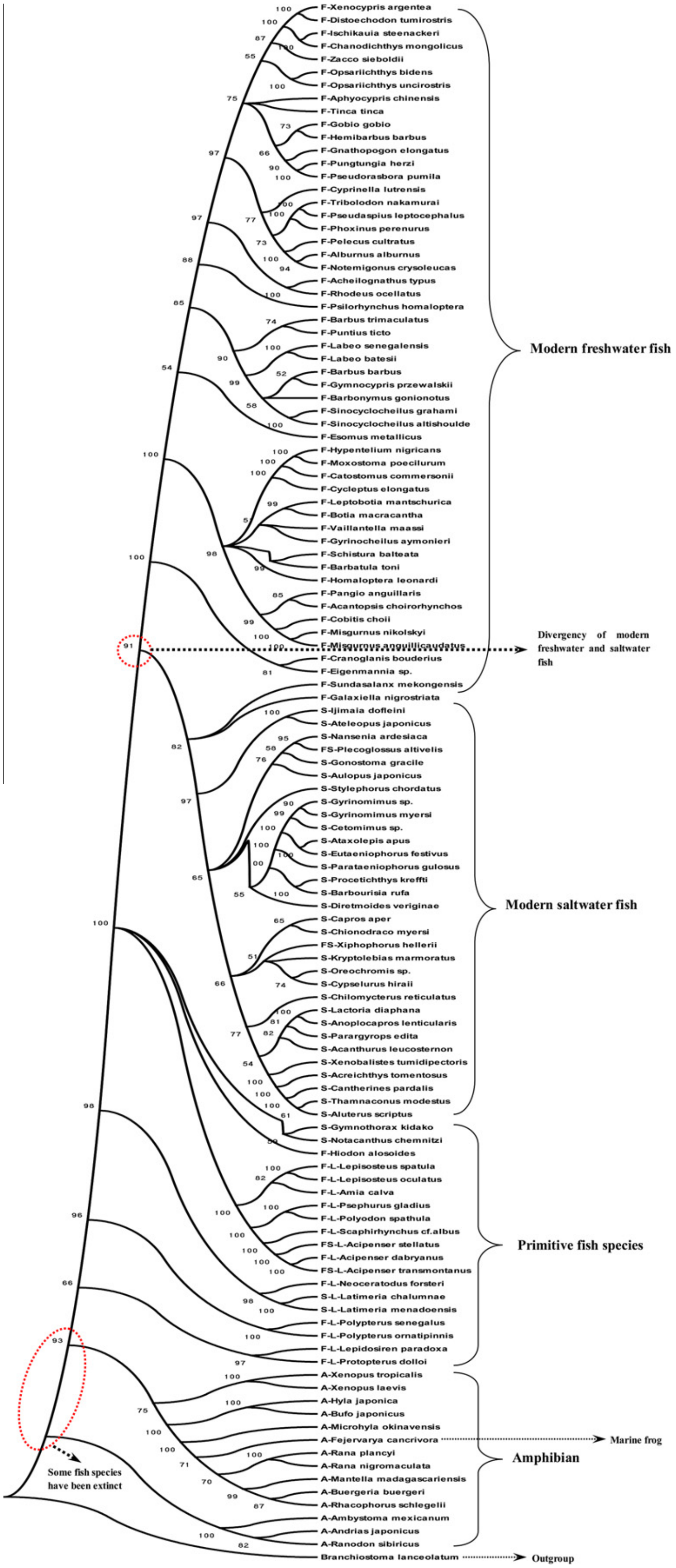

Fig. 1. The NJ tree based on a Tamura–Nei model constructed using the complete mitochondrial genomes of all the study species.

F-Homaloptera leonardi, *F-Hypentelium nigricans*, *F-Ischikauia steenackeri*, *F-L-Amia calva*, *F-L-Lepidosiren paradoxa*, *F-L-Lepisosteus oculatus*, *F-L-Lepisosteus spatula*, *F-L-Neoceratodus forsteri*, *F-L-Polypterus ornatipinnis*, *F-L-Polypterus senegalus*, *F-L-Protopterus dolloi*, *F-Labeo batesii*, *F-Labeo senegalensis*, *F-Leptobotia mantschurica*, *F-Misgurnus anguillicaudatus*, *F-Misgurnus nikolskyi*, *F-Moxostoma poecilurum*, *F-Notemigonus crysoleucas*, *F-Opsariichthys bidens*, *F-Opsariichthys uncirostris*, *F-Pangio anguillaris*, *F-Pelecus cultratus*, *F-Phoxinus perenurus*, *F-Polyodon spathula*, *F-Psephurus gladius*, *F-Pseudaspius leptocephalus*, *F-Pseudorasbora pumila*, *F-Psilorhynchus homaloptera*, *F-Pungtungia herzi*, *F-Puntius ticto*, *F-Rhodeus ocellatus*, *F-Scaphirhynchus cf. albus*, *F-Schistura balteata*, *F-Sinocyclocheilus altishoulderus*, *F-Sinocyclocheilus grahami*, *F-Sundasalanx mekongensis*, *F-Tinca tinca*, *F-Tribolodon nakamurai*, *F-Vaillantella maassi*, *F-Xenocypris argentea*, *F-Zacco sieboldii*. Mitochondrial genomes of four migratory fishes (*FS*) were as follows: *FS-Acipenser stellatus*, *FS-Acipenser transmontanus*, *FS-Plecoglossus altivelis*, *FS-Xiphophorus hellerii*. Mitochondrial genomes of 35 saltwater fishes (*S*) were as follows: *S-Acanthurus leucosternon*, *S-Acreichthys tomentosus*, *S-Aluterus scriptus*, *S-Anoplocapros lenticularis*, *S-Ataxolepis apus*, *S-Ateleopus japonicus*, *S-Aulopus japonicus*, *S-Barbourisia rufa*, *S-Cantherines pardalis*, *S-Capros aper*, *S-Cetomimus* sp., *S-Chilomycterus reticulatus*, *S-Chionodraco myersi*, *S-Cypselurus hiraii*, *S-Diretmoides veriginae*, *S-Eutaeniophorus festivus*, *S-Gonostoma gracile*, *S-Gymnothorax kidako*, *S-Gyrinomimus myersi*, *S-Gyrinomimus* sp., *S-Ijimaia dofleini*, *S-Kryptolebias marmoratus*, *S-L-Latimeria chalumnae*, *S-L-Latimeria menadoensis*, *S-Lactoria diaphana*, *S-Nansenia ardesiaca*, *S-Notacanthus chemnitzi*, *S-Oreochromis* sp., *S-Parargyrops edita*, *S-Parataeniophorus gulosus*, *S-Porichthys myriaster*, *S-Procetichthys kreffti*, *S-Stylephorus chordatus*, *S-Thamnaconus modestus*, *S-Xenobalistes tumidipectoris*. The single hemichordate mitochondrial genome was that of *Branchiostoma lanceolatum*.

2.2. Phylogenetic tree analyses

Sequences with or without control regions were multiple aligned using the ClustalW program [1] with default parameters and manually correction. Aligned sequences with gaps were treated as missing data. Alignments were used to construct phylogenetic trees with the MEGA4 program using each method and each model with 1000 bootstrap replicates, where *B. lanceolatum* was designated as the outgroup. The initial phylogeny placed *S-Porichthys myriaster* in a distinct clade, because the three putative control regions found in its mitochondrial genome were not present in other mitochondrial genomes, where only one control region was present. Thus, this species was excluded from subsequent phylogenetic tree construction.

3. Results

The results showed that bootstrap values of phylogenetic trees constructed using sequences with control regions were higher than those without the control regions (Supplementary data 1: Figs S1–1 and S1–2), so subsequent phylogenetic trees were constructed using only sequences with control regions.

The topologies of all phylogenetic trees constructed using each method and each model provided in MEGA4 were very similar (Supplementary data 2: Figs S2–1 to S2–27). Therefore, only the phylogenetic tree based on the Neighbor-joining (NJ) method with the Tamura–Nei model is shown (Fig. 1). The results indicated that amphibians, living fossil fishes, saltwater fishes, and freshwater fishes are clustered in different lineages. There are some exceptions, i.e., *Sundasalanx mekongensis*, *Galaxiella nigrostriata*, and saltwater fish are clustered together, while some fishes not accepted as living fossils are clustered together with accepted living fossil fishes. Likely reasons for these exceptions are discussed in the following section.

4. Discussion

This study found that the only extant saltwater amphibian, *F. cancrivora*, was clustered together with other amphibians (Fig. 1), rather than being placed in a separate clade. This suggests that *F. cancrivora* originally lived in freshwater and subsequently migrated to the seashore alone, indicating that all living amphibians share a common ancestor with freshwater fish. *Ichthyostega* is the oldest known terrestrial tetrapod and its fossils are found in freshwater deposits [2], which was consistent with our study. Fig. 1 shows that only two types of living fossil fishes, *Latimeria chalumnae* and *Latimeria menadoensis*, live in saltwater, but it is known that *Latimeria* can live in freshwater and later move to saltwater [3]. This suggests that the ancestor of all living fossil fishes lived in freshwater. Living fossil fishes are closely related to amphibians and this further suggests that amphibians are derived from freshwater fish.

Three types of fish that were not previously regarded as living fossils were clustered with living fossil fish, i.e., *Gymnothorax kidako*, *Notacanthus chemnitzi*, and *Hiodon alosoides*. However, these three species were classified as ancient fish in a previous study [4]. In particular, *G. kidako* is classified in the genus *Gymnothorax*, where several species exhibit some degree of cutaneous respiration [5]. Cutaneous respiration is an ancestral characteristic, so these three types of fish should probably be regarded as living fossils. Thus, it was reasonable that *G. kidako*, *N. chemnitzi*, and *H. alosoides* were clustered together with known living fossil fish.

According to the separation order shown in Fig. 1, the first amphibian was derived earlier than all living fossil fishes, which argues against the prevailing view that the coelacanth (i.e., *L. chalumnae* and *L. menadoensis*) or lungfish (*Neoceratodus forsteri*, *Lepidosiren paradoxa*, and *Protopterus dolloi*) were the ancestors of all living amphibians [6,7]. These results also suggested that *L. paradoxa* and *P. dolloi* were the closest living relatives to the ancestor of living amphibians, rather than *Latimeria*. This analysis also predicted that saltwater fishes and freshwater fishes were probably separated from a common ancestor, which might have occurred with the crustal movement in the late Triassic (about 200 Mya) [8]. Furthermore, *Cranoglanis bouderius* was separated first in the freshwater clade, which agreed with the discovery that *Siluriformes* were found in the late Jurassic (157 Mya) [9].

S. mekongensis [10] belongs to the Sundasalangidae family, where some members are diadromous, i.e., they can move between salt and fresh water. Like *S. mekongensis*, the black-stripe minnow (*G. nigrostriata*) is a species of diadromous fish in the Family Galaxiidae, where most members are diadromous [11]. The most closely related species to these two types of fish are diadromous. Thus, *S. mekongensis* and *G. nigrostriata* are clustered into the saltwater fish clade, although they are freshwater fish. It can be deduced that the ancestors of *S. mekongensis* and *G. nigrostriata* may have been saltwater fish.

In conclusion, phylogenetic tree analyses based on complete mitochondrial sequences suggested that the ancestor of living amphibians arose from a single type of primordial freshwater fish, which was not a coelacanth or a lungfish. It was also suggested that the ancestors of modern saltwater fish and modern freshwater fish separated from a common ancestor in a single event, possibly during a period of crustal movement.

Acknowledgment

This work was funded by National Natural Science Foundation of China (Grant Nos. 30871770 and U1036604).

Appendix A. Supplementary data

Supplementary figures S1–1, S1–2, S2–1~S2–27. The GenBank ID of the sequence used in this paper are showed in Supplemental information Table S1. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2012.03.141.

References

[1] J.D. Thompson, T.J. Gibson, D.G. Higgins, Multiple sequence alignment using ClustalW and ClustalX, Current Protocols in Bioinformatics (2002).

[2] G.R. Zug, L.J. Vitt, J.P. Caldwell, Herpetology: An Introductory Biology of Amphibians and Reptiles, Academic Press, 2001.

[3] L. Cavin, P.L. Forey, E. Buffetaut, H. Tong, Latest European coelacanth shows Gondwanan affinities, Biology Letters 1 (2005) 176–177.

[4] J.G. Inoue, M. Miya, K. Tsukamoto, M. Nishida, Basal actinopterygian relationships: a mitogenomic perspective on the phylogeny of the "ancient fish", Molecular Phylogenetics and Evolution 26 (2003) 110–120.

[5] H. Heatwole, J. Powell, Resistance of eels (Gymnothorax) to the venom of sea kraits (*Laticauda colubrina*): a test of coevolution, Toxicon 36 (1998) 619–625.

[6] B. Fritzsch, Inner ear of the coelacanth fish *Latimeria* has tetrapod affinities, Nature 327 (1987) 153–154.

[7] J.M. P Joss, Lungfish evolution and development, General and Comparative Endocrinology 148 (2006) 285–289.

[8] W. Wong, Crustal movements and igneous activities in Eastern China since Mesozoic time. 1, Bulletin of the Geological Society of China 6 (1927) 9–37.

[9] Z. Peng, Phylogenetic Relationships of Eurasian Catfishes (Otocephala: *Siluriformes*) and Divergence Time Estimates for Major Otocephalan Clades, Chinese Academy of Sciences, 2005.

[10] M. Kottelat, *Sundasalanx mekongensis*, a new species of clupeiform fish from the Mekong basin (Teleostei: Sundasalangidae), Ichthyological Exploration of Freshwaters 10 (1999) 337–344.

[11] http://fishbase.org/Summary/FamilySummary.cfm?id=79&lang=English. 5/3/2012.