the TIMETREE of LIFE

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Labyrinth fishes (Anabantoidei)

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Abstract

Labyrinth fishes (Anabantoidei) are grouped into three families (Osphronemidae, Helostomatidae, and Anabantidae) within the teleost Order Perciformes. Recent phylogenetic analyses have resulted in major changes in their classification. The Family Belontiidae is no longer recognized. The pike-head *Luciocephalus*, previously considered a separate family (Luciocephalidae) and closest relative of the remaining anabantoids, is now placed in a derived position within the Osphronemidae. The anabantoid timetree shows that the three families diverged either in the Middle Eocene (~40 million years ago, Ma) or in the late Cretaceous (~90 Ma) depending on the assignment of the only available anabantoid fossil.

Labyrinth fishes form a natural (monophyletic) group of teleost fishes, the Suborder Anabantoidei. They are arranged into three families: Osphronemidae (~120 species; gouramies, paradise fishes, and fighting fishes; Fig. 1), Helostomatidae (one species, Kissing Gourami), and Anabantidae (28 species; climbing gouramies and bushfishes). Although a comparatively small group, anabantoids exhibit a striking variation in size, ranging from dwarfed forms such as *Parosphromenus ornaticauda*, with 19 mm standard length, to large forms such as the giant gouramies of the genus *Osphronemus*, with up to 70 cm standard length (*1, 2*). A number of species play an important role as food fishes and are widely used in aquaculture, whereas others are important and highly colorful aquarium fishes. Labyrinth fishes show an astonishing diversity in breeding behavior that is rarely found in any other fish group (*3, 4*). Parental care is dominant and occurs in 16 of the 19 anabantoid genera.

Reproductive modes range from free-spawning without parental care to substrate spawning, submerged plant nest building, bubble nesting, and mouthbrooding with parental care. Labyrinth fishes are diagnosed by the presence of a peculiar organ above the gills (suprabranchial), consisting of a greatly modified upper element of the gill arches (epibranchial 1), which is housed in a cavity above the gills. Both, the wall of the cavity and the modified epibranchial are covered with respiratory epithelium, and assist in accessory air-breathing. The subrabranchial organ is also called labyrinth organ because of its complex folding that greatly increases respiratory surface. Labyrinth fishes are typically grouped together with the snakeheads (Channoidei, Channidae) in the Labyrinthici (*5*). Here, I review the relationships and divergence times of labyrinth fishes that include the three families: Osphronemidae, Helostomidae, and Anabantidae.

Although anabantoids were already recognized as a natural assemblage in the early nineteenth century by Cuvier and Valenciennes (*6*) their phylogenetic intrarelationships have been highly contentious. Most of the controversy is focused on the relative phylogenetic position of the enigmatic pike-head *Luciocephalus pulcher*. This highly morphologically derived teleost (*7*) was originally included in the family Esocidae (Esociformes) by Gray (*8*), but subsequently considered a member of the Anabantoidei by Bleeker (*9, 10*). Later on, Berg (*11*) and Liem (*12*) rejected a close relationship of the two taxa.

Fig. 1 A fighting fish (*Betta channoides*) from Borneo. Credit: Z. Hang.
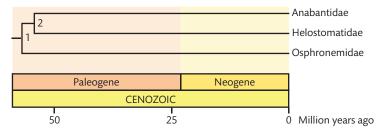
Fig. 2 A timetree of labyrinth fishes (Anabantoidei). Divergence times are from Table 1.

However, it is now generally accepted that *Luciocephalus* belongs to the anabantoids based on several derived morphological characters (*13–15*).

The first phylogenetic hypothesis of anabantoid relationships was proposed by Lauder and Liem (*15*), who divided the suborder into five families: Luciocephalidae, Anabantidae, Helostomatidae, Osphronemidae, and Belontiidae. They identified Luciocephalidae as the most basal anabantoid family, Anabantidae as the closest relative of Helostomatidae, and Osphronemidae as the closest relative of Belontiidae. However, Britz (*13, 14, 16*) and Britz *et al.* (*17*) revised Lauder and Liem's hypothesis in essential aspects. Britz (*13*) showed that *Luciocephalus* belongs to a monophyletic group, called Osphronemidae (*18*), that includes *Osphronemus*, and Liem's belontiids. The monophyly of Osphronemidae and Anabantidae is well supported by morphological studies (*5, 15*). The only molecular phylogenetic study addressing anabantoid relationships was based on four mitochondrial genes (*cyt b, 12S rRNA, tRNA Val,* and *16S rRNA*) and one nuclear gene (*RAG1*) and an extensive taxonomic coverage (*4*). The monophyly of both Osphronemidae and Anabantidae are well supported (Fig. 2).

Based on morphological evidence, Lauder and Liem (*15*) considered *Helostoma temminkii*, the only representative of the Helostomatidae, to be the closest relative of the Family Anabantidae. The molecular phylogenetic analyses on the other hand were unable to resolve the relative position of the Helostomatidae with respect to the other two anabantoid families. While the mitochondrial genes indicated that Anabantidae is the closest relative of the group containing Osphronemidae and Helostomidae, *RAG1* and a combined nuclear and mitochondrial DNA data set showed that Osphronemidae is the closest relative of the group containing Anabantidae and Helostomidae. Short internal branches connecting the Osphronemidae, Anabantidae, and Helostomatidae along with a wide posterior probability distribution of the root location may account for this lack of resolution (*4*).

Clearly, further morphological and molecular data studies are needed to rigorously test basal anabantoid relationships.

Rüber *et al.* (*4*) also estimated divergence times among the anabantoid families based on two separate data sets, using either the four mitochondrial genes or a combined data set consisting of the mitochondrial genes plus the nuclear gene *RAG1* and applying a Bayesian approach. The anabantoid fossil record that can be utilized for the calibration of the lineage divergence times is scarce. The only known articulated anabantoid fossil is from the genus *Osphronemus* (*19, 20*). It was found in the Sangkarewang Formation (Central Sumatra) dated with palynological data as late Eocene to early Oligocene (37–28.5 Ma) by Barber *et al.* (*21*). It is not possible to assign the fossil *Osphronemus* to any extant species in that genus with certainty, nor is it possible to assign it without doubt to *Osphronemus*.

Therefore, two different age estimates based on different assignments of the fossil *Osphronemus* were conducted: (a) assignment of the fossil to the most recent common ancestor of *Osphronemus* and its closest relative *Belontia* (first calibration) and (b) assignment of the fossil to the most recent common ancestor of *Osphronemus* (second calibration). Calibration with fossils from early-diverging lineages, based on both, mitochondrial genes and the combined mitochondrial and nuclear gene data set, indicate a Middle Eocene (~40 Ma) origin of anabantoids as well as a Middle Eocene Helostomatidae–Anabantidae split (Table 1). Divergence times based on the combined mitochondrial plus nuclear data resulted in estimates that were on average a few million years younger than those derived from the four mitochondrial genes using the first calibration (Table 1). The second calibration, based on the four mitochondrial genes, on the other hand, resulted in a late Cretaceous age, ~90 Ma, for the origin of anabantoids and the Helostomatidae–Anabantidae split (Table 1).

Table 1. Divergence times (Ma) and confidence/credibility intervals (CI) among labyrinth fishes (Anabantoidei).

Timetree		Estimates					
		Ref. (*4*)(a)		Ref. (*4*)(b)		Ref. (*4*)(c)	
Node	Time	Time	CI	Time	CI	Time	CI
1	56.9	41.7	50–35	37.7	44–32	91.3	127–64
2	54.3	39.7	48–33	36.2	42–31	87.0	122–61

Note: Node times in the timetree represent the mean of time estimates from different columns. Times from the analysis of four mitochondrial genes assigning the fossil *Osphronemus* as a basal branch (plus *Belontia*) are shown in (a), from the analysis of four mitochondrial genes plus the nuclear gene *RAG1* assigning the fossil *Osphronemus* to a basal branch (plus *Belontia*) are shown in (b), and from an analysis of four mitochondrial genes assigning the fossil *Osphronemus* to a living lineage of *Osphronemus* are shown in (c).

Anabantoids show a disjunct South and Southeast Asian–African distribution that might be indicative of a restricted Gondwana distribution. With both the *RAG1* and a combined nuclear and mitochondrial DNA data set used by Rüber *et al.* (*4*), a basal split of labyrinth fishes into osphronemids vs. helostomids plus anabantids was favored. The first two families are exclusively found in Asia, whereas the third family is found on both continents, with *Anabas* (Anabantinae) from Southeast Asia as the closest relative of the remaining anabantids (Ctenopominae), which occur in Africa. Divergence time estimates of an Asian–African separation of these subfamilies ranged from (a) 35.1 (CI 43–28) based on mitochondrial DNA and 30.8 Ma (CI 37–25 Ma) based on a combined mitochondrial DNA plus nuclear *RAG1* data set for the first calibration to (b) 77.0 Ma (CI 109–53 Ma) based on mitochondrial DNA for the second calibration. The African–Asian biogeographic split within labyrinth fishes indicates either a divergence at the Eocene–Oligocene (first calibration) or an Upper Cretaceous–Paleocene divergence (second calibration). Thus, the oldest divergence time estimate based on the second calibration is close to the suggested divergence of the Madagascar and Indian continent from Africa at 165–121 Ma. On the other hand, late Mesozoic dispersal from Africa to Asia, or vice versa, via land bridges between Africa, India, and Eurasia cannot be ruled out based on the first calibrations. It has been pointed out repeatedly that the late Mesozoic–early Cenozoic history of the Gondwana breakup is still poorly known and thus allows for wide speculations regarding African–Asian biotic exchanges via land bridges (*22, 23*). Given the poor fossil record of anabantoids, as well as the uncertainty in the phylogenetic placement of the fossil *Osphronemus*, it seems premature to draw any firm conclusion regarding the relative role that drift vicariance and dispersal have played in shaping anabantoid African–Asian distribution (*4*).

Acknowledgments

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