the TIMETREE of LIFE

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Lungfishes (Dipnoi)

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Abstract

Lungfishes (Subclass Dipnoi) number only six species in three families, but are an important group of vertebrates because of their close relationship to tetrapods. Phylogenetic analyses of morphological and molecular data agree that African lungfishes (Protopteridae) and South American Lungfish (Lepidosirenidae) are closest relatives. Molecular clock analyses suggest that the divergence of these families from the Ceratodontidae (e.g., Australian Lungfish) occurred in the Permian 277 (321–234) million years ago (Ma). The divergence of South American and African lungfishes was in the early Cretaceous, 120 (165–94) Ma, and was probably related to the breakup of Gondwanaland.

The six species of lungfish are the living representatives of the Subclass Dipnoi. These species are divided into two suborders (Lepidosirenoidei and Ceratodontoidei) and three families (Lepidosirenidae, Protopteridae, Ceratodontidae) (*1*). Several additional families are known from fossils extending back to the Devonian (416–359 Ma). Lungfishes comprise one of three extant groups of Sarcopterygii, along with tetrapods and coelacanths (*1*). Living species are characterized by stocky, eellike bodies and fleshy fins without spines or rays. The paired pectoral and pelvic fins are paddle-like in the Australian Lungfish (Ceratodontidae) and whiplike in the African and South American lungfishes (Protopteridae and Lepidosirenidae). All extant species of lungfish are obligate air-breathers, and the African and South American lungfishes have the ability to aestivate during periods of drought (for months at a time in the case of African lungfishes) (*1*). The Australian Lungfish, *Neoceratodus* (Fig. 1), is a riverine species able to tolerate water with low oxygen content, not unlike many ray-finned fishes with the ability to breathe air, but does not aestivate. Extant lungfishes are intolerant of marine conditions, and are restricted to freshwater habitats, as were most Mesozoic lungfishes (*2*). Paleozoic lungfishes included numerous marine representatives, however, and the group may have originally been marine (*3*). Here, the relationships of the three living families of lungfishes are reviewed and the first estimates of divergence times are presented based on analyses of published sequence data.

The fossil record of lungfishes is moderately complete. Tooth plates and scales are well represented, but skeletal material is relatively rare (*2*, *4*). In addition to these remains, fossilized burrows are known (*5*), some harboring skeletal remains. The earliest fossils of sarcopterygians on the lungfish lineage (rather than tetrapod or coelacanth lineage) are from the Devonian (*6*). True members of the Subclass Dipnoi also appear in the Devonian, and the peaks of diversity of Dipnoi were in the Devonian and Triassic (251–200 Ma) (*6*). These early lungfishes represent extinct groups, and the living families appear later in the fossil record. The three extant families are all known from the Cretaceous (146–66 Ma) (as fossils

Fig. 1 An Australian Lungfish (*Neoceratodus forsteri*). Credit: J. Joss.
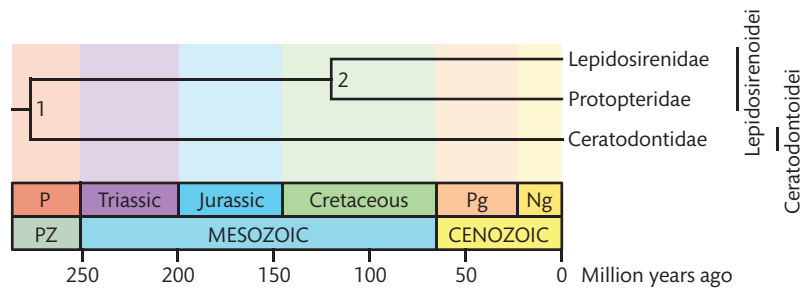
Fig. 2 A timetree of lungfishes. Divergence times are from Table 1. *Abbreviations*: Ng (Neogene), P (Permian), Pg (Paleogene), and PZ (Paleozoic).

of the three modern genera) (*7–9*). Ceratodontidae has been suggested to extend back to the Triassic (251–200 Ma), depending on how fossil taxa are allocated (*6, 7*). Species diversity declined in the Cenozoic, although several extinct species, including some in extinct genera, occur as late as the Pleistocene (1.81–0.01 Ma) (*7*).

The phylogenetic relationships of the living lungfish families are not controversial. It is universally accepted that Dipnoi is monophyletic, and that within Dipnoi the African and South American lungfishes (Protopteridae and Lepidosirenidae) are closest relatives. They share numerous anatomical characters, including external larval gills, fin shape, and two-lobed lungs (*1*), and are in fact often grouped together in the Family Lepidosirenidae. These relationships are strongly supported by both morphological cladistic (*10, 11*) and molecular studies, including molecular studies employing nuclear or mitochondrial data (*12–16*). Molecular data also support the monophyly of Protopteridae, the only family that includes more than one living species (*17*).

There have been no published molecular timing analyses among the three families of lungfish. Therefore, we conducted two molecular clock analyses using published sequence data and the Bayesian program Multidivtime (*18*). One analysis includes all families and uses published *RAG1* and *RAG2* nucleotide sequences from another study (*12*). The other analysis includes only Lepidosirenidae and Protopteridae, but uses amino acid data for six genes (*RAG1*, *RAG2*, *TPI*, *GAG*, *ALDc*, and *GAD65*) from three studies (*12, 19, 20*). Methodology is as described elsewhere (*21*). Several vertebrate outgroups are included in both analyses for calibration purposes (*Mus*, *Oryctolagus*, *Homo*, *Gallus*, *Xenopus*, *Danio*, and *Carcharhinus*/*Triakis* in the two-gene set; *Mus*, *Homo*, *Gallus*, *Danio* in the six-gene set), although these do not appear in the timetree.

Seven minimum and three maximum constraints were used in the two-gene data set, based on fossil data obtained from the literature (*6, 7, 9, 22, 23*). These include the divergence of Lepidosirenidae and Protopteridae (minimum, 92.7 Ma); the divergence of Lepidosirenoidei and Ceratodontoidei (minimum, 199 Ma); the divergence of primates and rodents (minimum, 62 Ma); the divergence of mammals and birds (minimum, 312 Ma, maximum, 370 Ma); the divergence of amniotes and amphibians (minimum, 330 Ma, maximum, 370 Ma); the divergence of tetrapods and lungfish (minimum, 404 Ma); and the divergence of ray-finned and lobe-finned fishes (minimum, 416 Ma, maximum, 495 Ma). For the six-gene data set, only the primate/rodent and mammal/bird divergences were used. The following Multidivtime parameters were employed in both analyses: rttm (450), rttmsd (100), bigtime (3000). For the two-gene analysis, rtrate was set at 0.001, rtratesd at 0.0005, brownmean at 0.0025, and brownsd at 0.0025. For the six-gene analysis, these values were 0.04, 0.04, 0.001, and 0.001, respectively.

The results of both analyses for the Protopteridae/Lepidosirenidae divergence are similar, with confidence intervals that broadly overlap (Table 1). The timetree (Fig. 2) shows that the African and South American lungfishes diverged in the early Cretaceous, 120 (165–94) Ma. This date agrees well with the fossil evidence, as it is not substantially earlier than the earliest fossils of African lungfish that appear beginning in the Cenomanian stage of the Cretaceous (100–93 Ma) (*9*). The divergence between these two families and Ceratodontidae occurs much earlier, in the Permian, 277 (321–234) Ma. This date also agrees well with the fossil record, as putative ceratodontids are known from the Triassic, and several other Triassic genera of dipnoans are thought to be more closely related to lepidosirenoid lungfishes (*2, 6, 7*).

Table 1. Divergence times (Ma) among lungfishes and their confidence/credibility intervals (CI).

Timetree		Estimates			
		(a)		(b)	
Node	Time	Time	CI	Time	CI
1	277.0	277	321–234	–	–
2	120.0	114	154–94	126	165–95

Note: Node times in the timetree are averages from two data sets: two-gene analysis (a) and six-gene analysis (b).

The divergence of protopterid and lepidosirenid lungfishes has long been suggested to be related to Gondwanan breakup, because these families are restricted to freshwater and have fossil records extending back to the Cretaceous, but restricted to Africa and South America, respectively (*2, 24, 25*). The timetree supports this hypothesis, as the South Atlantic Ocean opened largely during the Aptian and Albian stages of the Cretaceous, 125–100 Ma (*26*), the time period during which these families diverged according to the molecular time estimate. The ceratodontids are a much older, and formerly more widespread, group. The divergence of ceratodontid and lepidosirenoid lungfishes was too early (277 Ma) to be explained by continental vicariance, as the continents were joined into the supercontinent Pangaea at this time (*2*). Further, although now restricted to Australia, fossils referable to ceratodontids have been described from Mesozoic deposits in Africa and South America, indicating a much wider distribution (*27, 28*). In the future, it is likely that additional fossils, rather than molecular data, will contribute more to elucidating the biogeographic history of the lungfishes, a relict group.

Acknowledgment

References

1. J. S. Nelson, *Fishes of the World*, 4th ed. (John Wiley, San Francisco, 2006).
2. L. Cavin, V. Suteethorn, E. Buffetaut, H. Tong, *Zool. J. Linn. Soc.* **149**, 141 (2007).
3. K. S. W. Campbell, R. E. Barwick, *J. Morph. Supp.* **1**, 99 (1986).
4. C. R. Marshall, *J. Morph. Supp.* **1**, 151 (1986).
5. D. S. Berman, *J. Paleont.* **50**, 1034 (1976).
6. R. Cloutier, P. E. Ahlberg in *Interrelationships of Fishes*, M. L. J. Stiassny, L. R. Parenti, G. D. Johnson, Eds. (Academic Press, San Diego, 1996), pp. 445–479.
7. A. Kemp, *J. Paleont.* **71**, 713 (1997).
8. H. P. Schultze, in *Fosiles y Facies de Bolivia. Volumen I—Vertebrados*, R. Suarez-Soruco, Ed. (Revista Tecnica de Yacimientos Petroliferous Fiscales Bolivianos 12, Santa Cruz, 1991), pp. 441–448.
9. A. M. Murray, *Fish Fisheries* **1**, 111 (2000).
10. H. P. Schultze, C. R. Marshall, in *Palaeontological Studies in Honour of Ken Campbell*, P. A. Jell, Ed. (Association of Australasian Palaeontologists, Brisbane, 1993), pp. 211–224.
11. H. P. Schultze, in *Mesozoic Fishes 3—Systematics, Paleoenvironments and Biodiversity*, G. Arriata, A. Tintori, Eds. (Verlag Dr. Friedrich Pfeil, Munchen, 2004), pp. 463–492.
12. H. Brinkmann, B. Venkatesh, S. Brenner, A. Meyer, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4900 (2004).
13. H. Brinkmann, A. Denk, J. Zitzler, J. J. Joss, A. Meyer, *J. Mol. Evol.* **59**, 834 (2004).
14. S. B. Hedges, C. A. Hass, L. R. Maxson, *Nature* **363**, 502 (1993).
15. A. Meyer, S. I. Dolven, *J. Mol. Evol.* **35**, 102 (1992).
16. R. Zardoya, A. Meyer, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5449 (1996).
17. M. Tokita, T. Okamoto, T. Hikida, *Mol. Phylogenet. Evol.* **35**, 281 (2005).
18. J. L. Thorne, H. Kishino, *Syst. Biol.* **51**, 689 (2002).
19. K. Kikugawa *et al.*, *BMC Biol.* **2**, 3 (2004).
20. K. Lariviere *et al.*, *Mol. Biol. Evol.* **19**, 2325 (2002).
21. M. P. Heinicke, W. E. Duellman, S. B. Hedges, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10092 (2007).
22. M. J. Benton, P. C. J. Donoghue, R. J. Asher, in *The Timetree of Life*, S. B. Hedges, S. Kumar, Eds. (Oxford University Press, New York, 2008), pp. 35–86.
23. J. E. Blair, S. B. Hedges, *Mol. Biol. Evol.* **22**, 2275 (2005).
24. J. G. Lundberg, in *Biological Relationships between Africa and South America*, P. Goldblatt, Ed. (Yale University Press, New Haven, 1993), pp. 156–199.
25. M. J. Novacek, L. G. Marshall, *Copeia* **1976**, 1 (1976).
26. A. G. Smith, D. G. Smith, B. M. Funnell, *Atlas of Mesozoic and Cenozoic Coastlines* (Cambridge University Press, Cambridge, 1994).
27. M. Martin, *Neus Jahrb. Geol. Palaontol., Abhandlung* **169**, 225 (1984).
28. M. Martin, *C. R. Acad. Sci. Paris, Sci. Ter. Planets* **325**, 635 (1997).