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# Monotremes (Prototheria)

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## Abstract

**Monotremes are the sole living representatives of Prototheria and include the duckbilled platypus and four species of echidnas. Monotremes are restricted to Australia and New Guinea and exhibit a mosaic of primitive features that are similar to reptiles. Molecular time estimates for the platypus–echidna split, based on diverse methods and data, average 49 million years ago (Ma), but have a wide range (89–17 Ma). All of these estimates are younger than the oldest putative platypus fossil (121–112.5 Ma). Better constraints on the platypus–echidna divergence time will require improved sampling of the fossil record and of the monotreme genome.**

Monotremes are the sole living representatives of the mammalian Subclass Prototheria (*1*). Living monotremes have traditionally been viewed as the descendants of pre-tribosphenic mammalian ancestors that lacked the distinctive tribosphenic molar that is capable of both shearing and grinding functions. Following the recent discoveries of several tribosphenic mammals from the Mesozoic of Gondwana, Luo *et al.* (*2, 3*) proposed that tribosphemy evolved independently in the Laurasian clade Boreosphenida, which includes marsupials and placentals, and the Gondwanan clade Australosphenida, which includes monotremes. Other authors argue in favor of a single origin for tribosphenic mammals that excludes living and fossil monotremes (*4, 5*).

Extant Monotremata are the Duckbilled Platypus and four species of echidnas (Tachyglossidae; Fig. 1), which are confined to Australia and New Guinea (*6*). Platypuses and echidnas exhibit a mosaic of reptile-like primitive features that were inherited from early therapsids, more derived features such as hair and three middle ear ossicles that are shared with other living mammals, and evolutionary specializations that are unique to Monotremata and to each monotreme family. Primitive features include a cloaca and the retention of certain bones in the skull and shoulder girdle. Monotremes also lay shell-covered eggs that are hatched outside of the body of the mother. Here, we review relationships and divergence times of the monotremes.

Ornithorhynchidae contains one living species (*Ornithorhynchus anatinus*) that is restricted to Australia. The platypus is semiaquatic. Adult platypuses have hornlike plates that replace functional teeth and an electroreceptive bill. The electroreceptive bill is supported by a hypertrophied mandibular branch of the trigeminal nerve that courses through an enlarged mandibular canal. Tachyglossidae includes two extant genera (*Tachyglossus, Zaglossus*). Echidnas, also known as spiny anteaters, are edentate insectivores that are variably covered with spines. That ornithorhynchids and tachyglossids are each other's closest relatives among extant mammals is supported by both morphological and molecular data (*7–10*). Putative anatomical shared-derived characters uniting the two families include pincer-like anterior extensions of the premaxillae, electroreception, and enlargement of the trigeminal complex (*9, 10*). The most recent analyses of complete mitochondrial genomes (*11*) and concatenated nuclear genes (*12*) firmly resolve platypus and echidnas on a long branch separated from other living mammals.



**Fig. 1** The Short-beaked Echidna (*Tachyglossus aculeatus*), Family Tachyglossidae, which occurs in Australia and New Guinea. Credit: M. Westerman.
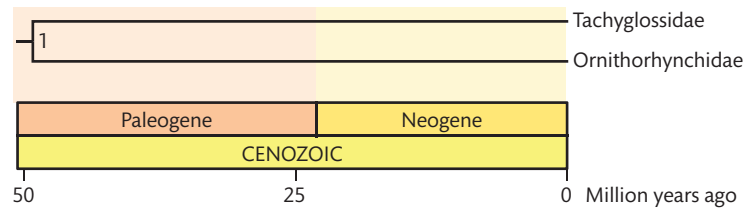
**Fig. 2** A timetree of monotremes (Prototheria). Divergence times are from Table 1.

The oldest undisputed platypus fossils belonging to crown-group Monotremata are from the Oligocene of Australia, ~25 Ma, and belong to *Obdurodon* (13–15). The earliest fossil echidna is *Zaglossus robustus* from the middle Miocene of Australia, ~15 Ma (15). The phylogenetic affinities of older monotreme fossils, including the early Paleocene *Monotrematum* from South America (16) and the early Cretaceous Australian fossils *Steropodon* (110 Ma) (16) and *Teinolophos* (121–113 Ma) (5, 17), are less clear. *Steropodon* was originally classified as a monotreme and possible ornithorhynchid (18) based on distinctive features of the lower molars that are shared with the Miocene *Obdurodon insignis*. *Steropodon* was later excluded from a monophyletic Ornithorhynchidae by Flannery *et al.* (19) because its age was incompatible with 80–64 Ma molecular clock dates for the ornithorhynchid–tachyglossid split based on DNA hybridization (20). Other previous molecular clock dates for the ornithorhynchid–tachyglossid divergence range from 64 to 17 Ma (13) and would seem to preclude the inclusion of *Teinolophos*, *Steropodon*, and possibly even *Monotrematum*, from a monophyletic Ornithorhynchidae. Rather, molecular clock dates suggest that platypus-like fossils from the early Cretaceous represent lineages before the echidna–platypus divergence. Pascual *et al.* (16) suggested the possibility of a paraphyletic Ornithorhynchidae with tachyglossids as specialized platypuses of uncertain relationship to known ornithorhynchids. However, a recent cladistic analysis of morphological characters supports the monophyly of an ornithorhynchid clade that includes *Steropodon* and *Teinolophos* to the exclusion of tachyglossids (5). Further, x-ray-computed tomography shows that *Teinolophos* had a hypertrophied mandibular canal along the entire length of the dentary and that the canal exits the ramus of the jaw medially below a large medial tubercle (5). Among living mammals these derived features are only found in the platypus (5). The mandibular canal of the platypus transmits the mandibular artery and hypertrophied mandibular branch of the trigeminal nerve. These structures support the electroreceptive bill of the Duckbilled Platypus. The finding that the hypertrophied mandibular canal also occurs in *Teinolophos* suggests that ornithorhynchids with electroreceptive bills had evolved no later than 113 Ma. There is also an isolated humerus from the early Cretaceous (106 Ma) of Australia that is echidna-like but unfortunately too incomplete to allow for unambiguous taxonomic assignment (21). Nevertheless, the weight of evidence from the fossil record and from x-ray-computed tomography make a strong case for platypus monophyly, inclusive of *Teinolophos*. This finding necessitates a minimum date of 113 Ma for the ornithorhynchid–tachyglossid split.

The large difference in time between the early Cretaceous separation of ornithorhynchids and tachyglossids suggested by paleontology and the late Cretaceous, Paleogene, or even Neogene separation suggested by molecular clocks is now reduced by a recent molecular clock date of 89 Ma for the ornithorhynchid–tachyglossid split (5). This date is based on an amino acid sequence data set that includes segments from five different proteins (12) and has a 95% credibility interval (131–56 Ma) which overlaps with the early Cretaceous date for *Teinolophos* (5). In contrast, yet another recent molecular clock date based on *IGF2* sequences is in better agreement with earlier molecular clock dates and suggests an early Paleogene (64 Ma) split between ornithorhynchids and tachyglossids (4). The timetree date for the split between platypus and echidnas (Fig. 2), which is based on molecular clock estimates that are summarized in Table 1 (also see reference 5), is 49.1 Ma. We note that several studies (5, 22, 23) have suggested the possibility of a molecular evolution rate slowdown in monotremes, which will result in estimates for the platypus–echidna split that are too young if clock calibrations are derived from other mammalian taxa with faster rates of molecular evolution. Determination of the relationships and divergence times among living and fossil monotremes awaits the discovery of additional fossil material and robust molecular

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

| Timetree | | Estimates | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Node | Time | Ref. (*4*) | | Ref. (*5*)(a) | | Ref. (*5*)(b) | | Ref. (*20*) | | Ref. (*22*) | | Ref. (*23*) | Ref. (*24*) | |
| | | Time | CI | Time | CI | Time | CI | Time | CI | Time | CI | Time | Time | CI |
| 1 | 45.9 | 63.7 | 95–40 | 88.9 | 131–56 | 79.5 | 110–52 | 72 | 80–64 | 53.5 | 57–50 | 25 | 63.6 | 75–52 |

| Timetree | | Estimates (*Continued*) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Node | Time | Ref. (*25*) | Ref. (*26*) | | Ref. (*27*) | | Ref. (*28*) | | Ref. (*29*) | | Ref. (*30*) | | Ref. (*31*) |
| | | Time | Time | CI | Time | CI | Time | CI | Time | CI | Time | CI | Time |
| 1 | 45.9 | 34 | 27.5 | 30–25 | 50.5 | 73–28 | 32.5 | 45–20 | 22.3 | 27–18 | 21 | 25–17 | 54 |

*Note*: Node times in the timetree represent the mean of time estimates from different studies. NP, not provided. Confidence intervals are Bayesian 95% credibility intervals for refs. (*4, 5*) and 95% confidence intervals for ref. (*24*). For other studies, times are midpoints and confidence intervals are ranges (*20, 22, 26–28, 30*) or a "20% margin of error" (*29*, p. 460).

dates that encompass more extensive sampling from the monotreme genome.

## Acknowledgment

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