The oldest platypus and its bearing on divergence timing of the platypus and echidna clades

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Edited by David B. Wake, University of California, Berkeley, CA, and approved October 31, 2007 (received for review July 7, 2007)

Monotremes have left a poor fossil record, and paleontology has been virtually mute during two decades of discussion about molecular clock estimates of the timing of divergence between the platypus and echidna clades. We describe evidence from high-resolution x-ray computed tomography indicating that Tei­no­l­o­phos, an Early Cretaceous fossil from Australia’s Flat Rocks locality (121–112.5 Ma), lies within the crown clade Monotremata, as a basal platypus. Strict molecular clock estimates of the divergence between platypus and echidnas range from 17 to 80 Ma, but Tei­no­l­o­phos suggests that the two monotreme clades were already distinct in the Early Cretaceous, and that their divergence may predate even the oldest strict molecular estimates by at least 50%. We generated relaxed molecular clock models using three different data sets, but only one yielded a date overlapping with the age of Tei­no­l­o­phos. Morphology suggests that Tei­no­l­o­phos is a platypus in both phylogenetic and ecological aspects, and tends to contradict the popular view of rapid Cenozoic monotreme diversification. Whereas the monotreme fossil record is still sparse and open to interpretation, the new data are consistent with much slower ecological, morphological, and taxonomic diversification rates for monotremes than in their sister taxon, the therian mammals. This alternative view of a deep geological history for monotremes suggests that rate heterogeneities may have affected mammalian evolution in such a way as to defeat strict molecular clock models and to challenge even relaxed molecular clock models when applied to mammalian history at a deep temporal scale.

Mammalia | Monotremata | phylogeny | molecular clock

The timing of divergence of the two living monotreme clades is of general interest because it bears on basal events in mammalian history and provides independent calibration for understanding temporal aspects of the great radiation of therian mammals. Strict molecular clock estimates of the platypus-echidna divergence time range from 17 Ma to 80 Ma (1–11), with most opinions favoring the younger end of the spectrum (Table 1). Recent discoveries of Meso­zoic and earliest Cenozoic monotremes have also been challenged. Some analyses did not simultaneously sample both monotreme clades (9, 32), and molecular arguments favoring Marsupionta have also been challenged. Some analyses did not simultaneously sample both monotreme clades (9, 32), and neither species (Ornithorhynchus anatinus, Australia and surrounding islands) are as different from each other as they are from therian mammals, yet monotreme monophyly is supported by skeletal morphology (17–22), brain architecture (23, 24), facial electro-receptor arrays of unique structure (25), karyotype (26), and mitochondrial (27, 28) and nuclear genes (29). Monotremes are conventionally recognized as the sister clade to therian mammals, and to retain many plesiomorphic mammalian features that were transformed during therian evolution (30, 31).

Challenges to this conventional view were raised recently, when either an echidna or the platypus was found to be phylogenetically nested within, or as sister taxon to, marsupials. Evidence came from sequence analyses of 18s rRNA (32), and both mitochondrial (9, 32–37) and nuclear genes (7, 38, 39). These findings resurrected the obscure “Marsupionta” hypothesis, which contended that monotremes are derived marsupials who secondarily reacquired such ancient characters as ovipary. Formulated in 1947 by the great morphologist W. K. Gregory (17), this hypothesis has been largely disregarded ever since (29, 31). If true, it would profoundly alter the framework in which mammalian history is understood today.

The Marsupionta hypothesis has been unanimously rejected in favor of the conventional view of monotreme-therian relationships in all recent computed phylogenetic analyses that incorporated fossils and large samples of skeletal characters (18–22, 40–43), including our analysis. The molecular arguments favoring Marsupionta have also been challenged. Some analyses did not simultaneously sample both monotreme clades (9, 32), whereas those that sampled both platypus and echidna clades supported the conventional view of monotreme relationships (11, 27–29, 44). Analyses of large concatenated sets of nuclear

Author contributions: T.R., T.H.R., M.S., and M.O.W. designed research; T.R., T.H.R., P.V.-R., M.S., and M.O.W. performed research; M.S. contributed new reagents/analytic tools; T.R., M.S., and M.O.W. analyzed data; and T.R. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

Data deposition: Digital Morphology data have been deposited online at www.digimorph.org (http://digimorph.org/specimens/Tei­no­l­o­phos.trusleri/216575, http://digimorph.org/specimens/Tei­no­l­o­phos.trusleri/216570, and http://digimorph.org/specimens/Tei­no­l­o­phos.trusleri/212933)

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This article contains supporting information online at www.pnas.org/cgi/content/full/0706385105DC1.

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Table 1. Published estimates of echidna–platypus divergence times, including our relaxed molecular clock estimates (bottom three rows)

<table>
<thead>
<tr>
<th>Date</th>
<th>Data/method</th>
</tr>
</thead>
<tbody>
<tr>
<td>17–25 Ma (10)</td>
<td>Immunoglobulin IgM</td>
</tr>
<tr>
<td>18–27 Ma (4)</td>
<td>Molecular clock estimates based on Protamine P1 genes (290 bp for platypus, 311 bp for echidna)</td>
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<tr>
<td>20–45 Ma (6)</td>
<td>Molecular estimates based on mitochondrial ND1 protein sequences and assuming that the echidna-platypus split is 20–30% as old as the monotreme-marsupial split</td>
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<tr>
<td>25 Ma (7)</td>
<td>Molecular clock estimate based on single copy DNA–DNA hybridization data</td>
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<tr>
<td>25–30 Ma (5)</td>
<td>Molecular clock estimates based on partial mitochondrial 125 rRNA gene sequences</td>
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<tr>
<td>28–73 Ma (2)</td>
<td>Molecular clock estimates based on myoglobin, α-globin, and β-globin protein sequences</td>
</tr>
<tr>
<td>34 Ma (9)</td>
<td>Molecular clock estimate based on amino acid sequences for 12 mitochondrial proteins</td>
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<tr>
<td>54 Ma (1)</td>
<td>Molecular clock estimate based on α-and β-haemoglobin and myoglobin protein sequences</td>
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<tr>
<td>50–57 Ma (8)</td>
<td>Molecular clock estimate based on α-lactalbumin protein sequences</td>
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<tr>
<td>63.6 Ma (11)</td>
<td>Molecular clock estimate based on 66 nDNA, mDNA, tRNA genes</td>
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<tr>
<td>&gt;63.2 – 61.8 Ma (13)</td>
<td>Fossil (Monotrematum), paleomagnetic date</td>
</tr>
<tr>
<td>64–80 Ma (3)</td>
<td>Molecular clock estimates based on single copy DNA–DNA hybridization data</td>
</tr>
<tr>
<td>63.7 (95.0–39.7 Ma)</td>
<td>Relaxed molecular clock estimate based on Woodburne et al. IGF2 data (41)</td>
</tr>
<tr>
<td>79.5 (110.4–51.6 Ma)</td>
<td>Relaxed molecular clock estimate based on van Rheede et al. Dataset I DNA data (29)</td>
</tr>
<tr>
<td>88.9 (130.8–55.6 Ma)</td>
<td>Relaxed molecular clock estimate based on van Rheede et al. Dataset I amino acid data (29)</td>
</tr>
</tbody>
</table>

The monotreme stem was long thought to be represented by the extinct Mesozoic taxa Morganucodontidae (Late Triassic–Middle Jurassic) and Multituberculata (Late Jurassic–Paleogene) (52–53). Under that interpretation, the fossil record testified that monotremes and therians had separated by the Late Triassic. Howev'er, those relationships were unanimously overturned by computed phylogenetic analyses that incorporated large samples of skeletal characters. Morganucodontids are now thought to be the sister taxon to crown Mammalia, whereas multituberculates are stem therians (18–21, 40–43). The revised phylogeny also altered the evidentiary standing of the Triassic fossils, placing them on the mammalian stem rather than within the crown, and leaving us without any Triassic mammals or any stem monotremes.

Recent discoveries of Mesozoic fossils from the Southern Hemisphere have renewed hope of acquiring genuine clues to early monotreme history. One record is an isolated humerus from the Early Cretaceous (~106 Ma) Dinosaur Cove locality of Victoria, Australia, that resembles an echidna, but is sufficiently incomplete as to deny unequivocal attribution (54). All of the other fossils consist of isolated teeth and jaws, and the phylogenetic placement of each has been challenged on different grounds (40, 41, 55, 56, 60). The Early Cretaceous Australian fossil *Teinolophos* is among these.

A number of researchers have noted that dental morphology allies *Teinolophos* with monotremes (40, 41, 58–60), and specifically with the platypus (31, 56, 61). However, the complete absence of teeth in all known echidnas has left equivocal the nature of dental similarities that *Teinolophos* shares with *Oxurodon* and *Ornithorhynchus*. They could either be indications of true platypus affinities, or plesiomorphic characters present in monotremes ancestrally. Other researchers argued that *Teinolophos* retains a Meckelian canal that held postdental elements, another plesiomorphic feature placing *Teinolophos* outside of crown monotremes (22, 61). Previous phylogenetic analyses have all favored the hypothesis that *Teinolophos* branched from the monotreme stem (11, 21, 57, 59, 61, 62), in a relationship consistent with a comparatively recent divergence of the platypus and echidna clades.

Also assigned to the monotreme stem by several analyses are *Steropodon*, *Auskribosphenos*, *Bishops* (Early Cretaceous, Australia), *Ambondro* (Middle Jurassic, Madagascar), and *Asfaltomylos* (Middle–Late Jurassic, Argentina) (57, 58, 62). Believed to represent a clade that includes living monotremes, this
assemblage was named “Australosphenida,” and this hypothesis of relationships formed the basis for controversial arguments that the tribosphenic molar (57) and middle ear (61, 63) evolved independently in australosphenids and therians.

For our study, three specimens of *Teinolophos* (specimen numbers: NMV P216750, NMV P216575, and NMV P212933) were scanned at the University of Texas High Resolution x-ray Computed Tomography facility (Figs. 2 and 3). Two were edentulous jaws, and the third was a dentary with three teeth, all from the Flat Rocks locality, Wonthaggi Formation, Bunanrong Marine Park, Victoria, Australia, where the type specimen of *Teinolophos* was collected (64). These data were compared with scans of living monotremes including an adult *Ornithorhynchus* and a juvenile still in possession of its deciduous dentition, the fossil platypus *Ohsbudran*, the fossil mammaliaform *Morganucodon*, several eutriconodont fossils, several multituberculates, and a large collection of extant therian mammals scanned with HRXCT over the last decade (Fig. 4). The new morphological data were used to modify a large morphological data set published by Luo and Wible (21), which we analyzed using parsimony. Insofar as our results were inconsistent with all strict molecular clock estimates for the echidna–platypus split, we applied three different relaxed clock modes using different molecular clock estimates for the echidna–platypus split, we parsimony. Insofar as our results were inconsistent with all strict data were used to modify a large morphological data set and volumetric animations are available at http://digimorph.org/specimens/Teinolophos.trusleri/216575/.

Fig. 2. *Teinolophos trusleri* (NMV P216575). Volumetric reconstruction of left dentary, from HRXCT, in medial (A) and lateral (B) views, and in selected coronal cross sections (slice thickness = 0.019 mm). Medial tubercle (Mt) and mandibular canal (V3) are labeled. Complete CT serial section stacks and volumetric animations are available at http://digimorph.org/specimens/Teinolophos.trusleri/216750/.

sequences, to evaluate whether any molecular clock models is consistent with the interpretation of a deep geological age for the echidna–platypus divergence. Our matrix, modified character scores, sequence analyses, and methodology are described in supporting information (SI Text).

**Results**

Our analysis found that *Teinolophos* lies within the monotreme crown, as the most ancient member of the platypus clade, Ornithorhynchidae (Fig. 5). Its precise position among ornithorhynchids was sensitive to different taxon samples, but invariably *Teinolophos* clustered with other ornithorhynchids (see SI Text). The most compelling evidence to us of its platypus affinities was provided by the HRXCT scans, which revealed the presence of a hypertrophied mandibular canal coursing along the entire length of dentary in a position lateral to the molariform tooth roots, and which exits the ramus medially beneath a large medial tubercle (Figs. 1–3). Among extant mammals, only the platypus has such a hypertrophied canal and medial tubercle (Fig. 4).

Whereas diprotodont marsupials have an enlarged canal near the back of the jaw for insertion of the pterygoideus musculature (65), our comparative analysis indicates that only in ornithorhynchids is the mandibular canal hypertrophied along its entire length.

The ornithorhynchid mandibular canal transmits the mandibular artery and hypertrophied mandibular branch of the trigeminal nerve, in support of the electroreceptive bill that gives the duckbilled platypus its common name (66). The bill deploys an array of 60,000 mechanoreceptors along with 40,000 mucous gland electroreceptors. Electroreceptive nerve terminals lie in the ducts of glands that secrete mucous when immersed in water, and they measure electrical profiles of aquatic prey items (25, 67–72). Stimuli received by receptors in the bill are transmitted via comparatively huge mandibular and maxillary branches of the trigeminal nerve to an expansive population of neurons in the S1 somatosensory cortex that are bimodally responsive to both mechanical and electrical signals (25, 69, 70). Echidnas also have...
electroreceptive capabilities in their beaks (72), but their sensitivity is far less than in the platypus bill. The long-beaked echidnas have only ~2,000 electroreceptors, whereas the short-beaked echidna has only ~400 (25), which they use in probing moist substrate for food. Both have comparatively narrow mandibular canals, reflecting the plesiomorphic condition that is found in *Morganucodon* and all therians sampled (Fig. 4). Electroreception therefore appears to be an apomorphic characteristic of Monotremata, whereas the evolution of a specialized duckbill for high-resolution aquatic electroreception is unique to the platypus clade. *Teinolophos* preserves the oldest evidence of a duckbill in its hypertrophied mandibular canal.

Our analysis also affirms that the slightly younger (110 Ma) Australian fossil *Steropodon* (12, 73), known from a single broken jaw, is also a platypus. This unique specimen was preserved as an opal infilling of a natural mold, left after the actual bone and teeth dissolved. However, the dental resemblances to *Teinolophos* and *Obdurodon* are thoroughly documented (40, 41, 59, 60), and it preserves the edge of a large mandibular canal. The Paleocene fossil *Monotrematum* (74), based on three teeth from Argentina, is probably also a member of the platypus clade (*Ornithorhynchidae*) (31).

Optical data (61) has been interpreted as evidence for the presence of postdentary bones in *Teinolophos*. However, HRXCT data show no evidence of a postdentary trough or postdentary bones, suggesting that *Teinolophos* had a “standard” mammalian middle ear in which the auditory ossicles were separate from the lower jaw and hung suspended beneath the otic capsule, as in the platypus today (75, 76). This finding adds to the mounting evidence (60) that “Australosphenida” is a polyphyletic assemblage, with several of its members (*Teinolophos*, *Steropodon*) belonging to crown monotremes, whereas others (*Ausktribosphenos*, *Bishops*, *Ambondro*, *Asfaltomylos*) clustered consistently with therians. The precise positions of the latter taxa were also sensitive to taxon sampling in our analyses, and invarially they clustered with therian mammals (see SI Text).

Should the Australosphenida hypothesis fail, then so too would assertions based thereupon that the mammalian middle ear and tribosphenic molars evolved convergently (57, 58, 61, 62, 77).

The Flat Rocks locality was dated by using the fission track method at 121–112.5 Ma (64). The finding that *Teinolophos* is a platypus indicates that the platypus and echidna clades diverged during or before the Early Cretaceous. This date is more ancient by a factor of 7 than the youngest, and 50% older than the oldest strict molecular clock estimates (Table 1). The recent characterization of monotreme history as a “long-fuse” clade, whose diversification into platypus and echidna clades postdated the Cretaceous–Tertiary boundary (11), is difficult to reconcile with our more ancient divergence estimate, nor is there evidence of a diversity “explosion” at any time in monotreme history.

Although far older than any previous estimate, the accumulation of anatomical novelty in even the oldest monotreme fossils suggests that our estimate may underestimate the actual timing of the split between platypus and echidna. Viewing *Teinolophos* and *Steropodon* as platypuses in their ecological aspect suggests that, since the Early Cretaceous, rates of ecological exploitation and morphological diversification among monotremes may have been far slower than is the case in most or all therian clades. The 1,000-fold difference in species diversity found today (5 monotremes vs. 5,362 therian species; ref. 16) may be another indication that monotremes evolved at far slower rates than therians. Several molecular clock studies (6–9) have also suggested the possibility of a molecular evolution rate slowdown in monotremes. A rate slowdown in monotremes will result in estimates for the platypus–echidna divergence that are too young if calibrations are derived from therian taxa with faster rates of molecular evolution.

Given differences in rates of molecular evolution, we applied a relaxed molecular clock method (refs. 78–80; see SI Text) to reanalyze both the DNA and amino acid sequence versions of van Rheede et al.’s (29) data set I for 21 mammalian taxa. Each analysis gave a different estimate. The amino acid data of van Rheede et al.’s (29) data set I yielded a point estimate of 88.9 Ma for the platypus–echidna split, with 95% credibility intervals ranging from 130.8 to 55.6 Ma. This result overlaps broadly with the 121–112.5 Ma date for *Teinolophos*. However, the DNA data set of van Rheede et al. yielded a point estimate of 79.5 Ma (credibility interval 110.4 to 51.6 Ma). Previously, Woodburne et al. (41) reported a relaxed clock point estimate of 63.7 Ma (credibility interval 95.0–39.7 Ma) for the platypus–echidna split based on IGF2 amino acid sequences.

**Discussion**

Even considering *Teinolophos* as a crown monotreme, the monotreme fossil record remains dismally sparse and open to
温度更低（81）。在什么程度和到什么程度，卵生类的代谢率和呼吸速率远低于生活在现生有袋目中的哺乳动物。即使在早白垩世或更早之前，卵生类就已出现在世上并且通过直接的证据来支持这一结论。如果新的位置被接受，现生有袋类的起源和鸭嘴兽、针鼹的起源可能提供直接的证据来支持这一结论。但是，我们还没有在上三叠世的现生有袋类的化石（41）。