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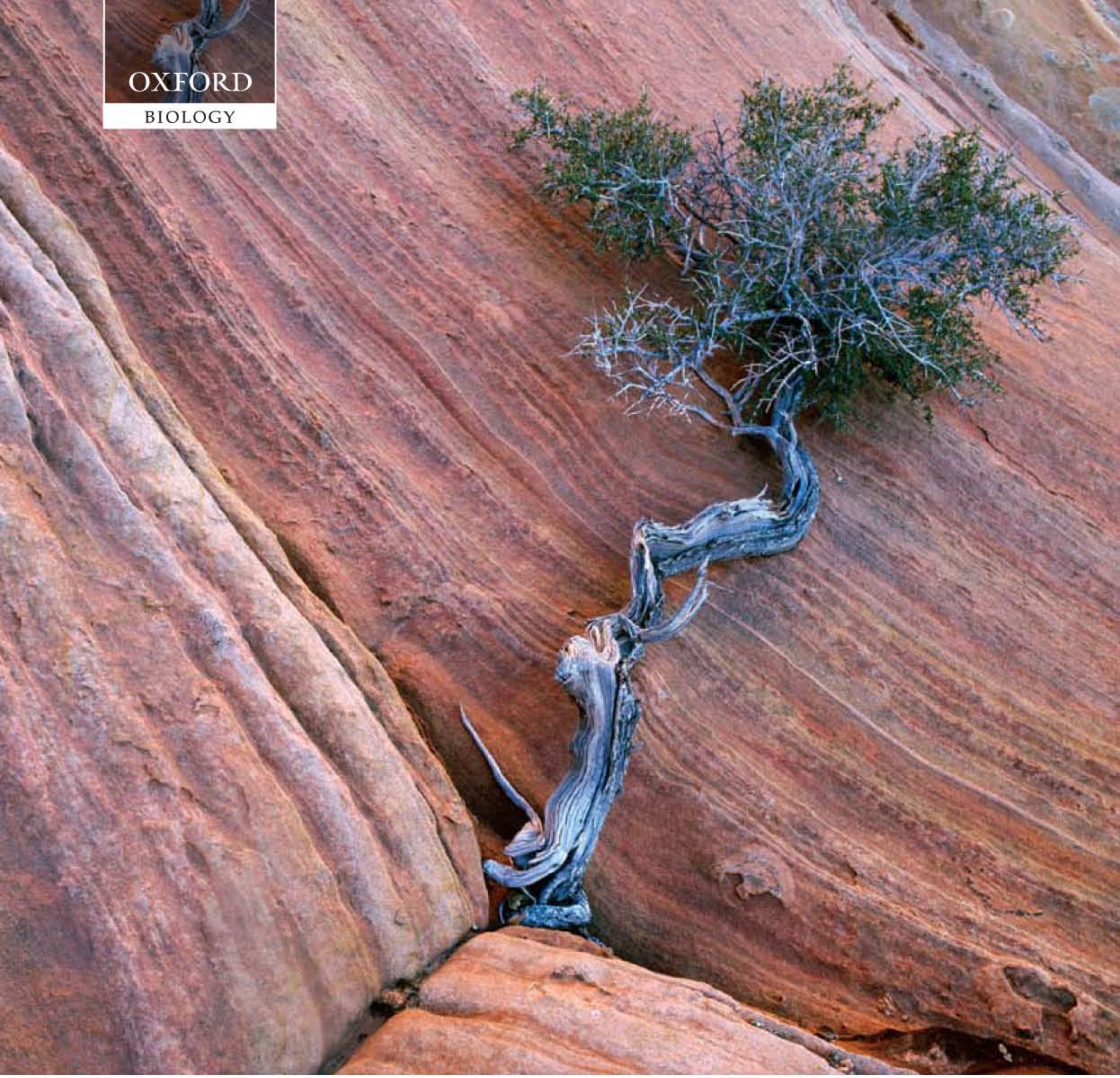

the TIMETREE of LIFE

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Marsupials (Metatheria)

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Abstract

Opossums, kangaroos, koalas and their kin (marsupials) are grouped into ~20 families and seven orders in the mammalian infraclass Metatheria. Recent phylogenetic analyses of mitochondrial, nuclear, and mixed molecular/morphological data sets support a basal split between didelphimorph opossums and all other marsupials. These analyses also support Australidelphia as a natural group, consisting of all Australasian marsupials plus the South American Order Microbiotheria. The timetree suggests that marsupial interordinal diversification began in the late Cretaceous, ~78 million years ago (Ma), and continued into the Paleocene (66–56 Ma). Most interfamilial diversification occurred during the Eocene (56–34 Ma) and Oligocene (34–23 Ma).

Marsupialia is a diverse group comprising ~330 living species that occur in North America, South America, and Australasia (*1*). Metatheria includes marsupials as well as stem fossils that are more closely related to Marsupialia than to Placentialia. Marsupials, like placentals, are viviparous mammals with mammary nipples. Marsupials generally lack the chorioallantoic placenta that occurs in placental mammals, have a very short gestation period, and give birth to young that are at an early stage of development. Neonates make their way from the mother's urogential aperture to a nipple, usually enclosed within an abdominal marsupium, where they begin suckling and continue development through an extended period of pouch life. Living marsupial species are placed in seven orders and 16–22 families (*1–4*). Here we review the phylogenetic relationships and divergence times among the living families of marsupials.

Division of living marsupials into seven monophyletic orders is supported by numerous studies of anatomical and molecular information (*2*). The orders are Didelphimorphia (New World opossums), Paucituberculata (South American caenolestids), Microbiotheria (the Chilean *Dromiciops gliroides*), Peramelemorphia (Australasian bandicoots), Dasyuromorphia (Australasian dasyurids and their relatives; Fig. 1), Notoryctemorphia (the Australian marsupial moles), and Diprotodontia (Australasian vombatiforms and phalangeridans). Relationships among these groups have been highly contentious, but the seminal work of Szalay (*5*) linked microbiotheres to Australasian forms in a monophyletic Australidelphia based on a shared, derived ankle-joint morphology. Subsequent studies produced inconsistent results on the placement of *Dromiciops* within this clade, but the most recent and comprehensive molecular analyses (*6*, *7*) resolve *Dromiciops* as the closest relative to a monophyletic group of all living Australasian marsupials. The relationship among ameridelphian orders remains an open question, but nuclear DNA sequences (*6*) and complete mitochondrial genomes (*8*) favor paucituberculates as the closest relative of Australidelphia.

Fig. 1 The Australian numbat (*Myrmecobius fasciatus*), which is the sole living representative of the dasyuromorphian Family Myrmecobiidae. Credit: M. Westerman.

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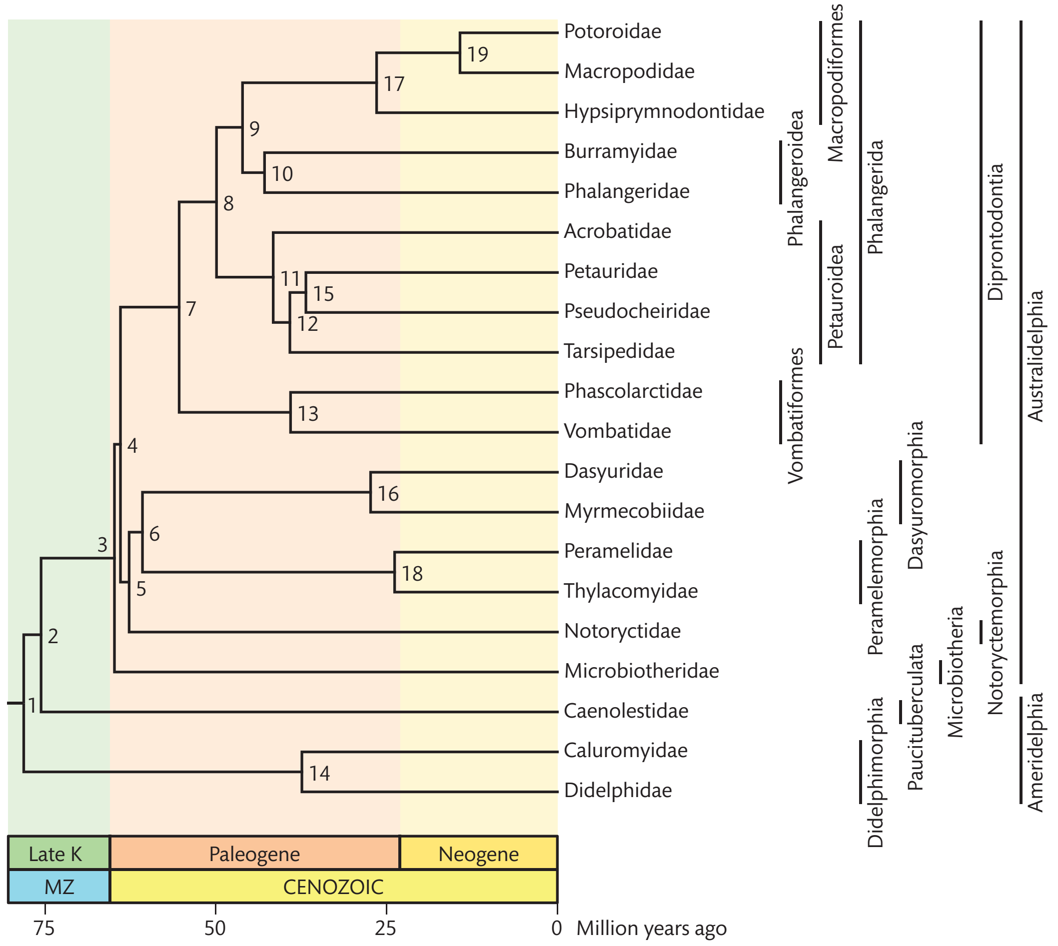

Fig. 2 A timetree of marsupials (Metatheria). Divergence times are shown in Table 1. *Abbreviations*: MZ (Mesozoic) and K (Cretaceous).

Disagreement about the phylogeny of Australasian orders has involved the positions of peramelemorphians and notoryctemorphians, specifically whether either or both of these groups are closer to dasyuromorphians than to diprotodontians. *Notoryctes* is specialized for fossorial life and has been since the early to middle Miocene (*9*) and there has been no morphological consensus about its phylogenetic position. Notoryctids and dasyurormorphians share a duplication in their protamine P1 introns (*10*). Bandicoots have syndactyl hind feet, a derived trait in which they resemble diprotodontians (*11*), but comparative serology (*12*) and DNA sequences (*6–8, 13*) resolve them as part of a clade with dasyuromorphians and *Notoryctes*.

Support for the reciprocal monophyly of didelphimorphian Families Caluromyidae and Didelphidae is equivocal at best. Familial distinction of these groups is based on DNA hybridization (*4*) and supported by Reig *et al.*'s (*14*) morphological data. Patton *et al.* (*15*) and Jansa and Voss (*16*) failed to obtain caluromyid monophyly with mitochondrial and nuclear DNA sequences, respectively, and the latter authors expressed concerns about the character analyses of Reig *et al.* (*14*). Family-level relationships among dasyuromorphians are less controversial. Krajewski *et al.* (*17*) found moderate support for a close relationship between dasyurids and thylacinids, apart from myrmecobiids, in a data set of mitochondrial *cytochrome b* and *12S rRNA* and nuclear protamine sequences. Wroe (*18*) cited two dental characters supporting the same arrangement. Reconstructing the affinities of *Macrotis* (bilbies) and *Chaeropus* (pig-footed bandicoots) has been central to understanding the phylogeny of peramelemorphians. Isolation of *Macrotis* in a distinct family (Thylacomyidae) was suggested by the serological

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

Timetree		Estimates							
Node	Time	Ref. (*8*)		Ref. (*12*)		Ref. *(13)*		Ref. (*30*)	
		Time	CI	Time	CI	Time	CI	Time	CI
1	78.1	69	76–62	80.7	–	78.1	87–68	83	100–69
2	75.6	60	68–55	–	–	75.6	84–33	78	–
3	64.8	50	55–43	–	–	64.8	70–57	65	74–57
4	63.9	–	–	–	–	63.9	70–56	–	–
5	62.7	41	45–36	–	–	62.7	68–55	61	70–53
6	60.7	–	–	–	–	60.7	67–54	58	67–49
7	55.3	46	51–41	58.6	–	55.3	61–48	47	59–36
8	49.9	–	–	55.1	–	49.9	56–44	40	53–29
9	46.1	–	–	–	–	46.1	52–40	–	–
10	42.8	–	–	44.5	–	42.8	49–37	–	–
11	41.6	–	–	–	–	41.6	48–36	–	–
12	39.1	–	–	NP	–	39.1	45–33	–	–
13	39.0	–	–	41.5	–	39.0	45–33	32	46–20
14	37.4	51	57–44	49.8	–	37.4	45–30	37	51–26
15	36.8	–	–	41.8	–	36.8	43–31	–	–
16	27.3	–	–	–	–	27.3	31–25	–	–
17	26.4	–	–	–	–	26.4	31–22	–	–
18	23.8	25	29–21	36.4	–	23.8	28–20	–	–
19	14.2	20	25–16	24.1	–	14.2	18–12	–	–

Note: Estimates for the timetree are based on a study of five nuclear-protein-coding genes (*13*). Estimates from other studies were excluded if they were for nodes that were topologically inconsistent with that in Fig. 2. Confidence intervals are Bayesian 95% credibility intervals (*13*), one standard deviation (*8*), and 95% highest posterior densities (*30*). Point estimates and confidence intervals from refs. (*8* and *30*) were measured directly from Figure 3 (p. 194) and Figure 3 (p. 704), respectively.

studies of Kirsch (*12*), but Westerman *et al.* (*19*) used 12S rRNA sequences to show that the extinct *Chaeropus* (Chaeropodidae) is even more distantly related to the typical bandicoots (Peramelidae) than is *Macrotis*. By contrast, sequences from the nuclear *Rag1* gene suggest that *Macrotis* is the closest relative of Peramelidae plus *Chaeropus* (*20*).

Diprotodontia is the most diverse marsupial order, though its family-level classification has been relatively stable in recent decades. The union of wombats (Vombatidae) and koalas (Phascoloartctidae) in a clade apart from other diprotodontians was first suggested by sperm morphology (*21*) and serological data (*12*), but has since been verified by numerous morphological and molecular studies. This basal split led Aplin and Archer (*2*) to divide living diprotodontians into Suborders Vombatiformes and Phalangerida, the latter comprising a diverse set of possum- and kangaroo-like species. Monophyly of the kangaroos and their relatives (Macropodiformes) has also been strongly supported, with morphological studies (*3*) suggesting a separation between kangaroos (Macropodidae) and potoroos (Potoroidae). However, ankle-joint morphology (*22*) and mitochondrial rRNA genes (*23, 24*) resolve the Musky Rat-Kangaroo (*Hypsiprymnodon moschatus*) as closest to all other living macropodiforms. Although DNA hybridization analyses (*4*) supported the monophyly of possum-like ("phalangeriform") diprotodontians, morphological and DNA sequence studies have been equivocal on this point (*24*). In particular, sequence data have suggested that a clade of phalangers (Phalangeridae) and pygmy-possums (Burramyidae) is more closely related to macropodiforms than to other phalangeriforms (*13*). DNA hybridization (*4*) data united Petauridae, Pseudocheiridae, and Acrobatidae into a single clade (Petauroidea), within which the morphologically enigmatic Honey Possum (*Tarsipes rostratus*, Tarsipedidae) has been placed using albumin microcomplement fixation (*25*), DNA hybridization (*26*), mitochondrial DNA (*24*), and nuclear DNA (*13*) data.

The earliest fossil record of Metatheria is the stem taxon *Sinodelphys szalayi* from China at 125 million years (Ma) (*27*). Recent fossil discoveries suggest that the earliest split among living Metatheria has a minimum age of Lancian (69–66 Ma) based on the oldest didelphimorph (*28*) or possibly Judithian (79–73 Ma) depending on the phylogenetic position of polydolopimorphs (*28*, *29*).

Numerous studies have reported molecular dates for one or a few cladogenic splits between major marsupial lineages. However, only a few molecular-dating studies have included representatives of all seven marsupial orders (Table 1). Kirsch *et al.* (*4*) estimated divergence times among all marsupial orders and 17 marsupial families using a molecular clock for single-copy DNA hybridization data. Nilsson *et al.* (*8*) used a relaxed clock method with complete mitochondrial genome sequences to estimate dates for all extant orders and 13 families. Drummond *et al.* (*30*) used a relaxed clock method with sequences for five nuclear genes to estimate divergence times among all marsupial orders and 12 families. The timetree of marsupials shown in Fig. 1 is based on Meredith *et al.*'s (*13*) relaxed clock analysis of five nuclear gene sequences and includes all Holocene marsupial orders and families except for the recently extinct Thylacinidae (Tasmanian Wolf) and Chaeropodidae (Pig-Footed Bandicoot).

The timetree shows the earliest divergence among living marsupials, between Didelphimorphia and other taxa, as 78 Ma (Fig. 2). Other molecular estimates for the last common ancestor of Marsupialia range from 100 to 64 Ma (*8, 13, 31–34*). All of these dates are younger than *S. szalayi* and are consistent with the hypothesis that *S. szalayi* is a stem fossil. Other interordinal splits in the timetree are in the late Cretaceous/Paleocene and range from 75–61 Ma. The date for the last common ancestor of the Australasian clade is 64 Ma. This date supports the hypothesis that marsupials dispersed from South America to Australia, via Antarctica, before the submergence of the South Tasman Rise at ~64 Ma (*35*).

Didelphimorphs had a most recent common ancestor 37 Ma in the middle Eocene. Steiner *et al.* (*36*) also obtained a middle Eocene date, albeit slightly older (~40 Ma), for this split. Steiner *et al.* noted that the middle Eocene origin for extant didelphids coincides with one of the first unequivocal phases of Andean uplift, which precipitated a shift from warm woodland habitats to cooler, drier climates.

Within the Australasian clade, basal splits in Peramelemorphia and Dasyuromorphia are at 24 and 27 Ma, respectively, in the Oligocene (Fig. 2). Nilsson *et al.* (*8*) obtained a similar estimate for the base of Peramelemorphia (25 Ma) based on mitogenomic sequences. The basal split within Diprotodontia is at 55 Ma in Meredith *et al.* (*13*), but Nilsson *et al.* (*8*) and Drummond *et al.* (*30*) both obtained a younger date (~47–46 Ma) for the base of Diprotodontia. Other interfamilial divergences within Diprotodontia range from 50 to 14 Ma on the timetree. Molecular clock dates based on DNA hybridization place interfamilial divergences within Diprotodontia in a similar range of 59–24 Ma. Divergence dates for diprotodontian families based on DNA hybridization, complete mitochondrial genome sequences, and nuclear gene sequences are generally consistent with Case (*37*), who suggested that all of the major diprotodontian lineages were present in the Eocene.

During the middle to late Eocene, podocarp dominated forests in Australia were replaced by more diverse Nothofagus dominated forests (*37*). This floristic change may have promoted the diversification of arboreal possum families in the mid- to late Eocene. Radiation of the terrestrial forms probably did not occur until the forests began to open up with an herbaceous angiosperm under story in the Oligocene. Fossil material from the Australasian Paleogene is needed to test Case's (*37*) hypothesis and molecular dates for the early diprotodontian radiation.

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