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Evolution of the patellar sesamoid bone in mammals

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The patella is a sesamoid bone located in the major extensor tendon of the knee joint, in the hindlimb of many tetrapods. Although numerous aspects of knee morphology are ancient and conserved among most tetrapods, the evolutionary occurrence of the patella is highly variable. Among extant (crown clade) groups it is found in most birds, most lizards, the monotreme mammals and almost all placental mammals, but it is absent in most marsupial mammals as well as many reptiles. Here we integrate data from the literature and first-hand studies of fossil and recent skeletal remains to reconstruct the evolution of the mammalian patella. We infer that patellae most likely evolved between four to six times in crown group Mammalia: in monotremes, in the extinct multituberculates, in one or more stem-mammal genera outside of therian or eutherian mammals, and up to three times in therian mammals. Furthermore, a patella was lost several times in mammals, not including those with absent hindlimbs: once or more in marsupials (with some re-acquisition), and at least once in bats. Our inferences about patellar evolution in mammals are reciprocally informed by the existence of several human genetic conditions in which the patella is either absent or severely reduced. Clearly, development of the patella is under close genomic control, although its responsiveness to its mechanical environment is also important (and perhaps variable among taxa). Where a patella is present it plays an important role in hindlimb function; especially in resisting gravity by providing an enhanced lever system for the knee joint. Yet the evolutionary origins, persistence and modifications of a patella in diverse groups with widely varying habits and habitats -- from digging to running to aquatic, small or large body sizes, bipeds or quadrupeds -- remain complex and perplexing, impeding a conclusive synthesis of form, function, development and genetics across mammalian evolution. This meta-analysis takes an initial step toward such a synthesis by collating available data and elucidating areas of promising future inquiry.

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Running head: Patellar evolution in mammals

INTRODUCTION

This meta-analysis addresses the evolution of the ossified patella (tibial sesamoid or “kneecap” bone) in mammals. We compiled voluminous literature and firsthand observational data on the presence or absence of the patella in extinct and extant mammals, then conducted phylogenetic analysis of patellar evolution by mapping these data onto a composite phylogeny of mammals (Kielan-Jaworowska et al. 2004; Luo 2007a; Luo 2007b) using multiple phylogenetic optimization methods. We used the results to address patterns of acquisition and loss of this structure within Mammaliaformes. In particular, we investigated whether an ossified patella was ancestrally present in all crown group Mammalia, and lost in particular groups especially marsupials (Metatheria), or whether it evolved multiple times in separate crown clades. Furthermore, if the patella had multiple origins, how many times was it gained or lost, and what did it become if it was lost (such as a vestigial fibrocartilage versus complete loss)? These were our study’s key questions. We provide some broader context here first.

Some aspects of the morphology of the knee in tetrapods (four-legged vertebrates bearing limbs with digits) are evolutionarily ancient. Tetrapods had their ancestry amongst lobe-finned sarcopterygian fish, in which jointed, muscular fins transitioned into limbs. Early stages of distinct bony articulations between the femur and tibia-fibula are evident in the hind fins/limbs of Devonian (~370 million years ago; Mya) animals such as *Eusthenopteron*, *Panderichthys*, and *Ichthyostega* (Ahlberg et al. 2005; Andrews & Westoll 1970; Boisvert 2005; Dye 1987; Dye 2003; Haines 1942). These fossil sarcopterygians also have subtle differences between the homologous joints in the pectoral fin/forelimb and the pelvic fin/hindlimb, indicating that specification of forelimb/hindlimb identity was already in place (Boisvert 2005; Daeschler et al. 2006; Shubin et al. 2006). Furthermore, the morphology of the forelimb and hindlimb joints indicates divergent functions of these limbs, with the forelimb evolving into a more “terrestrialized” capacity earlier than the hindlimb (Pierce et al. 2012). Developmental and morphological modifications to the hindlimb and particularly the mid-limb joint between the stylopod and zeugopod continued, until a recognizable knee articulation of almost modern, derived aspect arose in tetrapods of the Carboniferous period, ~350 Mya (Dye 2003).

Sesamoids are best defined as “skeletal elements that develop within a continuous band of regular dense connective tissue (tendon or ligament) adjacent to an articulation or joint” (Vickaryous & Olson 2007). The tibial patella is a sesamoid bone that arises during development within the main extensor tendon of the knee, subsequently ‘dividing’ it (though there remains some continuity) into the quadriceps and patellar tendons (the latter is sometimes inappropriately called the patellar ligament) (Bland & Ashhurst 1997; Fox et al. 2012; Pearson & Davin 1921a; Tecklenburg et al. 2006; Tria & Alicea 1995; Vickaryous & Olson 2007). These tendons span from the quadriceps muscle group to the tibia (Fig. 1). The patella itself tends to be incorporated mainly into the vastus muscles of the quadriceps in mammals, with the tendon of *M. rectus femoris* lying more superficial to them (Tria & Alicea 1995), with variable degrees of attachment to it (Jungers et al. 1980). Hereafter, the term patella implies ossification and hindlimb localization unless otherwise specified (some literature inconsistently and confusingly refers to non-ossified cartilaginous structures in this location as patellae), and implicitly refers to either a single patella or the left and right patellae normally present in an individual. There is an “ulnar patella” in the forelimbs of some taxa (notably lizards, but also some frogs, birds and mammals (Barnett & Lewis 1958; Haines 1940; Maisano 2002a; Maisano 2002b; Pearson & Davin 1921a; Pearson & Davin 1921b; Romer 1976; Vanden Berge & Storer 1995; Vickaryous & Olson 2007) but a full discussion of this enigmatic structure is beyond the scope of this study. Figure 2 depicts the anatomical orientations used throughout this study to refer to tetrapod limbs.

The patella appears broadly similar amongst mammals possessing it, as far as has been studied, although it varies greatly in size, generally in accordance with body size. It ossifies endochondrally; from a cartilaginous precursor (*i.e.* anlage (Vickaryous & Olson 2007)); relatively late in gestation (*e.g.* sheep, goats (Harris 1937; Parmar et al. 2009)) or sometime after birth (*e.g.* rabbits, rats, mice, humans (Bland & Ashhurst 1997; Clark & Stechschulte 1998; Patton & Kaufman 1995; Spark & Dawson 1928; Tria & Alicea 1995; Walmsley 1940)). Very recently, the development of the patella in mouse embryos was re-examined and the claim made that the patella develops as a process that branches off the femur, strongly influenced by mechanical loading in that region (Eyal et al. 2015). Whether this truly happens as described in mice, let alone other mammals, and whether it can be accepted as unexpected support for the “traction epiphysis” origin of patellar sesamoids (*e.g.* Pearson & Davin, 1921a; Pearson &

Davin, 1921b), remains to be determined, but the surprising results deserve attention. The general form of the mature osseous patella in mammals is a hemispherical structure, with a superficial surface (covered by fibrocartilage (Clark & Stechschulte 1998) and quadriceps tendon fibres (Bland & Ashhurst 1997)) and a deep surface which articulates with the femur, gliding along the patellar sulcus or groove in this bone. In maturity, the patella is composed of an outer lamellar cortex enclosing an inner cancellous bone structure with marrow spaces, and has an articular hyaline cartilage lining on the deep surface for articulation with the patellar sulcus (groove) of the femur (Benjamin et al. 2006; Clark & Stechschulte 1998; Vickaryous & Olson 2007).

The vastus muscles' tendons (especially *M. vastus intermedius*) may have a fibrocartilaginous region at the approximate position of the patella, called the "suprapatella" or "patelloid" (Fig. 1). the latter two terms are sometimes used synonymously, though "suprapatella" is more usual when an osseous patella is also present, and "patelloid" when it is not. The suprapatella is described as proximal to the patella, occasionally with a fat pad interposed between it and the ossified patella (Fig. 1), whilst the patelloid is described as occupying the same approximate region that a bony patella would (though absence of a patella makes this difficult to objectively assess) (Bland & Ashhurst 1997; Jungers et al. 1980; Ralphs et al. 1991; Ralphs et al. 1998; Ralphs et al. 1992; Reese et al. 2001; Walji & Fasana 1983). It is not clear whether the fibrous patelloid in some marsupials (and perhaps some bats (Smith et al. 1995)) is homologous to the suprapatella, equivalent to an evolutionarily reduced patella, or an independently occurring structure.

The human patellar anlage is first visible at O'Rahilly stage 19, and chondrifies at stage 22. Ossification begins 14 weeks after birth (Merida-Velasco et al. 1997a; Merida-Velasco et al. 1997b; Tria & Alicea 1995), but is not grossly visible until 4-6 years of age (when multiple, eventually-coalescing centres of ossification can be seen radiographically (Ogden 1984)) and sometimes not in its fully ossified form until adolescence. The patella is the only sesamoid bone counted regularly among the major bones of the human body (Vickaryous & Olson 2007), although there are other, much smaller sesamoids in the hands and feet (and in some cases even the spine (Scapinelli 1963)). Other small sesamoids, such as the lunula, fabella, cyamella and

parafibula, also occur in the knee joint in many tetrapod species including some mammals (Fig. 1); these occur sporadically in humans (Pearson & Davin 1921a; Sarin et al. 1999).

The patella is covered by the thickest layer of articular cartilage in the human body (Palastanga et al. 2006). The patella may thus also play a protective role for the underlying joint architecture (Haines 1974), in addition to protecting the patellar tendon from excessive compressive stresses (Giori et al. 1993; Sarin & Carter 2000a; Wren et al. 2000). The patellar tendon itself, to the extent that its properties are known for some species (e.g. humans), is stiff and strong, able to withstand about twice as much stress as typical knee joint ligaments and enduring strains (i.e. lengthening) of up to 11-14% (Butler et al. 1986). Regional variations in the microscopic anatomy of the human patella have also been recognised, for example in tissue thickness and nerve arrangement), which may reflect load distribution (Barton et al. 2007; Eckstein et al. 1992; Toumi et al. 2006; Toumi et al. 2012). There is convincing evidence from numerous species that excessive loads on the patella can lead to degeneration of the articular cartilages and damage to the underlying bone, leading to osteoarthritis (Aglietti & Menchetti 1995; Hargrave-Thomas et al. 2013; Tria & Alicea 1995), so those regional variations of patellar structure are likely important. Similarly, the tissues involved in anchoring the patellar tendon to the proximal and distal surfaces of the patella as well as to the proximal tibia (tuberosity/tubercle) vary in their composition and presumably are adapted, and exhibit phenotypic plasticity, to reduce the risk of tendon avulsion from the bone (Evans et al. 1991).

Functions of the patella notwithstanding, there was once some enthusiasm for its outright removal for treatment of certain joint problems. Patellectomy was first performed in 1860 and for some time was an established treatment option for several conditions (Pailthorpe et al. 1991; Sweetnam 1964). However, partial and complete patellectomies are now considered as last resort salvage procedures; this is also the mainstream view of the veterinary profession (Langley-Hobbs 2009). The historical lack of clarity on the pros and cons of patellectomy was summarised eloquently by *The Lancet*, stating, “Sadly, most of our interventions on the patella are empirical, and are supported more by the enthusiasm of proponents than by a very deep knowledge of the biology or biomechanics of this unusual joint. The knee cap could do with more scientific attention” (Editors 1992).

The latter complaint regarding lack of scientific attention to form, development, function and clinical treatment of the patella applies even more so to non-human tetrapods. One exception is a study that measured the inter- and intra-specific variability of the patellae and other bones (Raymond & Prothero 2012). They found generally greater variation in patellae (and other sesamoids) vs. “normal” long bones. Their inference was that this greater variability might pertain to the “intermembranous” [*sic*- intramembranous] development of sesamoids, vs. an endochondral location in long bones. However, the patella and most other major limb sesamoids of mammals are pre-formed in cartilage and thus clearly are endochondral bones (Farnum 2007). Yet the latter study reinforces that sesamoids are more variable than most other bones, in part due to their mechanical environment, in part due to their embedding in soft tissues (themselves quite variable) such as tendons and ligaments (Bland & Ashhurst 1997; Clark & Stechschulte 1998), and perhaps due to other factors not yet understood. This uncertainty about the causes of variability in the patella may also relate to incomplete understanding of its mechanical loading and function *in vivo*, as follows.

Where a patella is present in its typical form, its primary function is to modify the mechanical advantage (ratio of output force to muscle force) at the knee joint, by increasing the moment arm of the tendon in which it is embedded and thereby altering the amount of force needed from the quadriceps muscles in order to generate a particular moment (torque; rotational force) about the knee joint (Alexander & Dimery 1985; Fox et al. 2012; Haines 1974; Heegaard et al. 1995; Herzmark 1938; Howale & Patel 2013; Tecklenburg et al. 2006). In humans, the patella causes the quadriceps muscle group’s moment arm about the knee to increase as the knee becomes more extended, causing the amount of quadriceps muscle force required per unit of patellar tendon force (i.e. at the insertion onto the tibial tubercle) to vary significantly across knee joint flexion-extension (Aglietti & Menchetti 1995; Fellows et al. 2005). By articulating with the femur, the patella also transmits some forces of the quadriceps muscle group directly onto the femur (the patellofemoral joint reaction force); forces which can reach a maximum of 20-25 times body weight (Aglietti & Menchetti 1995).

The mobility of the patella is an important aspect of its function. While, in humans, the patella mostly flexes and extends relative to the femur as the knee is flexed and extended, it also translates and pitches (tilts) and rolls (Aglietti & Menchetti 1995; Fellows et al. 2005), leading to variable contact between the patella and femur that is reflected in the angled facets of the human patella (Lovejoy 2007). In contrast to the situation in humans (as well as in early hominins such as *Australopithecus*), in chimpanzees and presumably many other primates (as well as other taxa such as sheep (Bertollo et al. 2012; Bertollo et al. 2013)), the patella remains in tight articulation with the femur throughout the knee's range of motion, reducing patellofemoral stresses especially when the knee is strongly flexed, as it habitually is in those non-human primates (Lovejoy 2007). Other primates show varying degrees of specialization of patellar morphology that alter the moment arm of the patellar tendon, with great apes apparently having a patella most specialized for widely varying knee joint postures (Pina et al. 2014). It has been claimed that in hominids and ursids (bears) alike, there is an association between plantigrady (flat-footedness), increased knee range of motion, and patellar mechanics (Lovejoy 2007); this is an interesting hypothesis that deserves further consideration.

In the elbow of humans and other mammals, there is an extension of the ulnar bone called the olecranon (process), which serves a lever-like function analogous to that of the patella (Herzmark 1938). However, a mobile sesamoid bone like the patella has a more flexible ("dynamic gearing") function in improving mechanical advantage compared with an immobile retroarticular process like the olecranon (Alexander & Dimery 1985). There tends to be an inverse relationship between mechanical advantage and speed of joint motion (Hildebrand 1998), thus a high mechanical advantage is not necessarily useful in all cases, which may in part explain the variable occurrence, size and shape of the patella in animals with different lifestyles and modes of locomotion. Biomechanical studies of primates (Lovejoy 2007; Pina et al. 2014) and domesticated mammalian species (*e.g.* dogs (Griffith et al. 2007; Kaiser et al. 2001), sheep (Bertollo et al. 2012; Bertollo et al. 2013), horses (Schuurman et al. 2003; Wentink 1978)) have contributed some knowledge of how the patella functions in these groups, or in individual species, but a general "functional synthesis" for the patella is still lacking.

De Vriese performed pioneering comparative analyses and attempted syntheses of patellar size and morphology in comparison to other leg bones, between species and among multiple individuals in some species (De Vriese 1909). No clear correlations were observed between the size of the patella and other major hindlimb bones (femur, tibia, and fibula). A correlation was claimed between the sizes of the patella and the talus (or intermedium) in the ankle, although no clear, plausible mechanistic/functional justification was suggested and no statistical analyses were performed. Somewhat oddly, no relationship was evident between the size and shape of the patella and the femoral patellar groove (De Vriese 1909). The more restricted but quantitative analysis of Valois (Valois 1917) focused mainly on primates and challenged many of De Vriese's claims that mechanical or physiological explanations of patellar morphology have "no scientific merit". Haxton also criticised De Vriese for focusing on relative length of bones; his own "patellar index" based on relative width found no correlation with animal speed or size, but he inferred that the patella confers functional advantages in knee extension (Haxton 1944). There has been little examination of these questions in a modern comparative, rigorously statistical context since these studies. A notable exception is a study of the distal femur and patellar groove in bovid mammals, indicating increased mechanical advantage of the knee in larger species (Kappelman 1988).

The occurrence of an ossified patella in the knee joint is not universal among tetrapods (Fig. 3). It is absent in extinct early Tetrapoda and crown clade Lissamphibia (Dye 1987; Haines 1942; Herzmark 1938; Vickaryous & Olson 2007), all non-avian dinosaurs, Crocodylia, and Testudines (turtles), and all other extinct tetrapods. In contrast, an ossified patella is present in many or most Squamata (lizards and kin) with limbs (Camp 1923; Carrano 2000; De Vriese 1909; Dye 1987; Dye 2003; Gauthier et al. 2012; Haines 1940; Haines 1942; Hutchinson 2002; Hutchinson 2004; Jerez & Tarazona 2009; Maisano 2002a; Regnault et al. 2016; Vickaryous & Olson 2007). Patellar status (used throughout our study to refer to presence/absence) is unknown for the (mostly extinct) Rhynchocephalia (sister group to Squamata), although a patella is at least sometimes present in the tuatara *Sphenodon* – the only extant rhynchocephalian (Regnault et al. 2016). An apparent sesamoid bone was noted in the knee joint region of a specimen of *Macrocnemus*, a mid-Triassic (~235 Mya) reptile, which may be the earliest identified occurrence of a patella in any animal group (Rieppel 1989), although this structure may have

been a different sesamoid bone or ossicle. There have been anecdotal accounts of fibrocartilaginous or “fibrovesicular” patelloids in some reptiles such as turtles and crocodiles (Haines 1940; Haines 1942; Pearson & Davin 1921a; Pearson & Davin 1921b)), but these are not well-explored. Thus, although such fibrous tissues seem to be excellent candidates for intermediate character states between “absence of ossified patella (normal extensor tendon)” and “presence of ossified patella”, empirical grounding for this transformational sequence within Sauropsida is weak.

No patella has been observed in early, stem-group birds throughout the Jurassic and Cretaceous periods, except in the well-documented Cretaceous Hesperornithes, diving birds with vestigial wings and an extremely large and unusually shaped patella, resembling that in some extant diving birds (Lucas 1903; Marsh 1875; Martin 1984; Martin & Tate 1976; Shufeldt 1884; Thompson 1890). A patella is found in some Cenozoic fossil bird specimens, most notably archaic penguins, and commonly among many crown clade birds (Dye 1987; Dye 2003; Hutchinson 2001; Hutchinson 2002; Ksepka et al. 2012; Shufeldt 1884; Vickaryous & Olson 2007; Walsh & Suarez 2006). A recent study by Regnault *et al.* inferred that a patella was probably ancestrally present in the common ancestor of Hesperornithes and living birds over 70 Mya (Regnault et al. 2014). However, the patella was lost (and in some cases replaced by fatty cartilaginous tissue) in some large flightless birds such as emus, cassowaries and the extinct moa, yet unexpectedly is present as a double ossification in the knee joints of ostriches (Chadwick et al. 2014).

A patella is generally found in two of the three crown groups of Mammalia: Eutheria (Fig. 3) and Monotremata (see Fig. 4A-D), but not in most Metatheria (see Fig. 4E, F) (Dye 1987; Dye 2003; Vickaryous & Olson 2007). This raises the question whether this patella represents independent, convergent evolutionary origins in the Eutheria and Monotremata, or an ancestral origin for all three groups, with loss of the ossified patella amongst most Metatheria. To address this question, we conducted phylogenetic character mapping with Mesquite software (Maddison & Maddison 2014) that reconstructed patellar evolution in Mammalia. Using likelihood methods, we also traced the most likely pattern of evolution over existing phylogenies, and considered alternate proposed topologies to test how they affected our reconstructions. Based on the predicted

evolutionary patterns and individual morphologies, we propose suggestions as to the lifestyle of particular taxa, and consider where general correlations between lifestyle and patellar presence/absence might exist (or not).

Mottershead called the patella “that prince among sesamoids” but questioned whether it is “not typical of its kind” (Mottershead 1988). But is there even a “typical” patella? Our synthesis of key data from morphology and function to phylogeny, development and genetics allows us to evaluate just how “typical” any patella is, even for a mammalian patella.

MATERIALS AND METHODS

We surveyed the literature and additional specimens (see Supplementary Table S1) and coded the patella as absent (score = 0), fibrocartilaginous (i.e., “patelloid”; score = 1), or ossified (score = 2) for each taxon in our analysis, with “?” denoting an ambiguous character coding. We used two phylogenetic optimization methods in Mesquite software to reconstruct possible evolutionary polarity of the patella in the clade Mammaliaforma (with a focus on Mammaliaformes), as follows. First, for broad reconstruction across Tetrapoda, we used a phylogeny based on Shedlock and Edwards (2009) and Gauthier et al. (1988), with average branch lengths they derived from several studies. Some aspects of the phylogeny remain controversial, such as the position of Testudines (turtles; Hedges 2012). Reconstruction was performed using Mesquite’s parsimony algorithm and unordered character states and results are illustrated in Figure 3. As this analysis only involved major clades and not any stem lineages, it was intended as purely illustrative of general patterns and the current state of knowledge, given that patellar evolution across Tetrapoda had not been analyzed phylogenetically before.

We adopted composite phylogenetic trees for our study taxa (Archibald 1998; Beck 2012; Bi et al. 2014; Cardillo et al. 2004; Forasiepi et al. 2006; Gatesy et al. 2013; Goloboff et al. 2009; Kielan-Jaworowska et al. 2004; Luo et al. 2003; Luo 2007a; Luo et al. 2002; Luo 2007b; May-Collado et al. 2015; Meredith et al. 2009; Meredith et al. 2011; Mitchell et al. 2014; O’Leary et al. 2013; O’Leary & Gatesy 2008; dos Reis et al. 2012; Rose 2006; Sánchez-Villagra et al. 2007; Song et al. 2012; Spaulding et al. 2009; Springer et al. 2003; Springer et al. 2007; Springer et al.

2009; Thewissen 1990; Thewissen et al. 2007; Wible et al. 2007; Zack et al. 2005). As defined by several authors, the clade Mammaliaformes includes crown group Mammalia plus closely related extinct stem-mammals such as the iconic *Morganucodon* and the more recently discovered *Sinoconodon*, and is characterised by diagnostic features involving the teeth, jaw and inner ear (Kielan-Jaworowska et al. 2004; Rose 2006). Extant mammals (crown group Mammalia) include three main clades: Placentalia, Marsupialia and Monotremata. Placentalia lie within the Eutheria; Marsupialia lie within the Metatheria, and Monotremata lie within the Australosphenida, all of which diverged during the Mesozoic, pre-dating the K-Pg extinction event ~66 Mya.

We reconstructed patellar evolution amongst Mesozoic Mammaliaformes, including key fossils with hindlimb material. The main topology used was based on Bi et al. (2014), congruent with other sources (e.g. Rose 2006; Luo 2007b; O'Leary et al. 2013). We used parsimony optimisation, with both unordered and ordered states, and maximum likelihood optimisation. We also reconstructed ancestral states over an alternative topology with †*Eomaia* placed on the therian stem (after O'Leary et al., 2013) using parsimony optimisation and unordered states. Results are illustrated in Figures 5 and S4.

To examine these evolutionary patterns in more detail, we performed further reconstructions of ancestral states within both Metatheria and Eutheria. For Metatheria, we used topology and approximate divergence times based on (Cardillo et al. 2004, Forasiepi et al., 2006, Forasiepi, 2006, Sánchez-Villagra et al., 2007, Luo et al., 2003, Meredith et al., 2009, Springer et al., 2009, Beck, 2012, Mitchell et al., 2014), and ran reconstructions with parsimony optimisation for unordered and ordered states and maximum likelihood. An alternative topology with Microbiotheriidae nested slightly further within Marsupialia (after May-Collado et al., 2015) was also explored using parsimony optimisation and unordered character states. Results for Metatheria are illustrated in Figures 6 and S5.

For Eutheria, our topology is based on O'Leary et al. (2013) with minor modifications and reconstructed with parsimony optimisation and unordered states. Results are illustrated in Figure 7. For the four major crown placental groups, *per* O'Leary *et al.* (2013), the first divergence was

323 Xenarthra, followed by Afrotheria, and finally the branching of Boreoeutheria into
 324 Laurasiatheria and Euarchontoglires. Other studies have suggested Afrotheria as the first group
 325 to diverge, followed either by Xenarthra (Springer et al. 2003; Springer et al. 2007) or
 326 Laurasiatheria (Goloboff et al. 2009) or alternatively a basal branching between Boreoeutheria
 327 and Atlantogenata (comprised of Afrotheria+Xenarthra; dos Reis et al. 2012; Meredith et al.
 328 2011; Song et al. 2012). These alternative topologies did not change our fundamental results.
 329 Divergence order within Laurasiatheria was (see Figure 7 for subgroupings): Eulipotyphla
 330 (called Lipotyphla by O’Leary *et al.* (2013); Pholidota+Palaenodonta;
 331 Carnivora+Miacidae+Creodonta (Spaulding et al. 2009); Chiroptera; Condylarthra (see below);
 332 Mesonychia; Perissodactyla; Cetartiodactyla. Within Euarchontoglires, the order of divergence
 333 was (see figure for subgroupings): (Scandentia+Dermoptera)+Primates; (Glires+Rodentia).
 334 Regarding individual species, *Leptictidium* was placed with Macroscelidea based on its relation
 335 to the mapped *Leptictis*; *Carodnia* was placed within Afrotheria; Mesonychia (*Mesonyx et al.*)
 336 were placed between Euungulata (Perissodactyla and Cerartiodactyla) and Chiroptera;
 337 *Protungulatum* was placed between Mesonychia and Chiroptera. Among species broadly
 338 described as “condylarths”, *Phenacodus* (albeit a likely perissodactyl), *Hyopsodus*, *Tetraclaenus*
 339 and *Copecion* were grouped together (Thewissen 1990); *Mioclaenus* was grouped with
 340 *Hyopsodus* (Zack et al. 2005); *Meniscotherium* was grouped with *Phenacodus* (Wible et al.
 341 2007); *Arctocyon* and *Chriacus* were grouped together and with other condylarths (Archibald
 342 1998). O’Leary *et al.* (2013) placed *Notharctus* just outside Primates, but noted that this was
 343 possibly incorrect as they did not include other notharctids, therefore Notharctidae were here
 344 placed within Primates. *Plesiadapis* was placed as the sister taxon to Primates. O’Leary *et al.*
 345 (2013) placed *Rodhocetus* basal to Cetartiodactyla, but *Artiocetus* and *Basilosaurus* as basal
 346 Cetacea. For consistency with other studies including additional archaic Cetacea (O’Leary &
 347 Gatesy 2008; Thewissen et al. 2007), *Rodhocetus* was retained within the stem cetaceans (Gatesy
 348 et al. 2013). Additional archaic Perissodactyla and Artiodactyla not included in O’Leary *et al.*
 349 (2013) were here grouped with included group members. Additional stem placentals, including
 350 Taeniodonta, Pantodonta, Dinocerata and selected individual species, were not included in the
 351 study of O’Leary *et al.* (2013) and here were placed equally as stem groups. O’Leary *et al.*
 352 (2013) placed *Protolipterna* basal to Euungulata, but do not incorporate other archaic South
 353 American ungulates, whereas this study did not include Litopterna. Pending more analysis,

Notoungulata were here placed as stem placentals. Tillodontia, another stem placental group, was not included in this study due to lack of identified specimens with sufficient hindlimb material.

Our analysis involved numerous challenges and caveats. Many anatomical studies of extant or extinct species omit any mention of the patella, leaving its provenance in these taxa as uncertain. Interpretation of patellar status is especially challenging in fossils due to the rarity of finds with extensive, articulated postcranial material, the potential occurrence of other small non-patellar bones in the knee joint, and the uncertain age of the animal at time of death versus the developmental timing of sesamoid ossification (usually unknown; often relatively late in ontogeny). For the present analysis, statements in the primary literature regarding patellar status were generally accepted at face value except when superseded by more recent observations. From some publications with high quality photographs, patellar status was tentatively interpreted even if not discussed in the original text. In some cases, patellar status was confirmed by direct observation (e.g. Fig. 4; Figs. S1-S3; Table S1). Drawings found in secondary citations were mostly not been taken as definitive evidence, as we noticed examples of discrepancies between primary references and such drawings found in review articles or even textbooks, which may simply assume patellar status in mammals. Also, many mammalian groups are found over long temporal and geological spans, thus we were cautious about using the presence of a patella in one or a few individual extant or fossil specimens to infer presence throughout the group, although in some cases there was clearly enough conservatism within a clade to score it for all members.

An important knee structure related to the patella is the femoral patellar or intercondylar sulcus (groove) (Norell & Clarke 2001; Polly 2007). This sulcus is anatomically associated with a true patella (Figs. 1,4) in terms of its direct role in guiding the patellar sesamoid and tendon's path of movement during leg flexion/extension, and in mediolaterally confining the patellar tendon, which may enhance osteogenic stresses favouring the formation of a patella (Sarin & Carter 2000b; Wren et al. 2000). In the absence of an observed patella in fossil specimens, this sulcus at the distal end of the femur is sometimes treated as evidence of a patella even in the absence of the observed bone itself. We deemed this conclusion to be unwarranted. For example, the

evolution of a patellar sulcus in early pygostylian birds substantially predated the evolution of an ossified patella in later ornithurine birds; moreover the sulcus was retained in some avian taxa that lost the patella (Clarke & Norell 2002; Hutchinson 2002; Livezey & Zusi 2006; Regnault et al. 2014). In contrast, a prominent sulcus is absent in many Squamata despite the presence of a patella (S.R. and J.R.H., pers. obs.). Together these observations indicate that these two anatomical features are not obligatorily coupled, so reliance on the observed presence of an ossified patella in fossil specimens was warranted. Nonetheless, at least among mammals the complete absence of a femoral patellar sulcus might be indicative of the absence of an ossified patella (Chester et al. 2012).

RESULTS AND DISCUSSION

Our overall evolutionary reconstruction of the patella for Mesozoic mammals is shown in Fig. 5, for Metatheria/Marsupialia in Fig. 6, and for Cenozoic Eutheria/Placentalia in Fig. 7, with details for specific taxa in Table S1 and alternative phylogenetic analyses in Figs. S4 and S5. Here we sequentially summarize and discuss our findings for five subgroups of Mammaliaforma (especially Mammaliaformes): (1) Mesozoic pre-therians and stem-therians; (2) Mesozoic Metatheria and Eutheria; (3) Cenozoic Monotremata; (4) Cenozoic Metatheria, and (5) Cenozoic Eutheria. We then conclude with a general synthesis of our study's insights (as well as uncertainties) and a consideration of how available and emerging data on developmental genetics of the patella might help shed light on the "evo-devo" of the patella, augmenting the phylogenetic and anatomical insights that this study focuses on.

1. Mesozoic pre-therian and stem-therian mammals

The earliest mammals as widely construed include *Sinoconodon*, the Morganucodonta and Docodonta. These were mostly small, probably insectivorous animals, that appear to have lacked a patella, although it is unclear whether the known specimens contain sufficient postcranial material or are from verified adults, to allow for definitive conclusions. The absence of a clear patella in two stunningly preserved docodonts (the scansorial [climbing-adapted] *Agilodocodon* and fossorial [digging-adapted] *Docofossor*) lends credence to the conclusion that it was

generally absent in early mammals (Luo et al. 2015b; Meng et al. 2015). There is convincingly strong evidence of absence of a patella in earlier pre-mammals in lineages dating from the divergence of Synapsida and Sauropsida/Reptilia (~320 Mya), including the ancestral “pelycosaurs”, therapsids and cynodonts (Kemp 2005).

Australosphenida, the clade containing and thus ancestral to extant Monotremata, diverged from other mammals extremely early, possibly in the mid-Jurassic (Kielan-Jaworowska et al. 2004). There is little postcranial material for any extinct members of this lineage however, and no hindlimbs (Kemp 2005). The patella in crown clade monotremes will be discussed below.

Fruitafossor, from the late Jurassic (150 Mya), diverged after the Australosphenida (Luo & Wible 2005). Its relationship to other early mammals is complicated by its mixture of characters in the molar teeth, middle ear and elsewhere. *Fruitafossor* is described as lacking a patella, and it is proposed to have had a fossorial lifestyle.

The Eutriconodonta were found abundantly across the world from the middle Jurassic to early Cretaceous periods (Kielan-Jaworowska et al. 2004). Among eutriconodonts, a poorly developed distal femoral patellar groove is found but a patella is absent.

The Allotheria were an extremely successful and widely dispersed group of mammals, among which the best understood are the multituberculates (Kielan-Jaworowska et al. 2004; Wilson et al. 2012). Generally Allotheria are found from the late Triassic to the Eocene periods, thus this group spans much of the heyday of the (classic) dinosaurs, and survived the K-Pg extinction (Kielan-Jaworowska et al. 2004). Multituberculates were predominantly small animals, either herbivorous or omnivorous (Kielan-Jaworowska et al. 2004). A patella is noted for the nearly complete multituberculate *Ptilodus*, a proposed scansorial animal from the early Cenozoic. A patella is also present in the Cretaceous multituberculate *Chulsanbaatar*. It is unclear whether a patella is typical of all members of the multituberculate group and is under-reported due to lack of hindlimb material for most group members, or whether it occurs only among selected species, although the former seems more plausible. A patella is not reported, however, for the early Jurassic basal *Rugosodon*, a proposed multituberculate specimen with one relatively intact knee

joint (Yuan et al. 2013), so it is conceivable that an ossified patella evolved later within the Allotheria (Fig. 5).

Specimens of the diverse group “Haramiyida” are mostly restricted to cranial material, and the relationship of this ancient group to other Allotheria and Mammaliaformes has been controversial (Butler 2000; Kielan-Jaworowska et al. 2004; Rose 2006). However, several recently described more complete haramiyid specimens from the Jurassic with at least one preserved knee joint lack a patella (Bi et al. 2014; Zheng et al. 2013; Zhou et al. 2013). These new specimens have been interpreted to support an Allotheria clade including a paraphyletic “Haramiyida” (but a valid clade Euharamiyida including many “haramiyid” taxa) and Multituberculata (Fig. 5), although new analyses of a key specimen of *Haramiyavia* concluded that the haramiyids and multituberculates are not closely related (Luo et al. 2015a). The inclusion of the “Euharamiyida” in Allotheria pushes the divergence date of the group significantly earlier into the late Triassic, whereas multituberculates themselves appear only in the middle to late Jurassic. Final resolution of this controversy will undoubtedly require additional fossil material.

Symmetrodonta were a group of diverse, small mammals widely distributed in time from the late Triassic to the late Cretaceous (Kielan-Jaworowska et al. 2004). In the subgroup of spalacotheroids, a patella is reported for one fairly complete specimen (*Zhangheotherium*) but not for another (*Akidolestes*) (Chen & Luo 2012; Luo & Ji 2005) (these two specimens are coded oppositely in character matrices in some subsequent publications, probably in error (Bi et al. 2014; Zhou et al. 2013)); a patella was absent in *Maotherium*.

Eupantotheria was a diverse group found commonly from the mid-Jurassic to the early Cretaceous (Kielan-Jaworowska et al. 2004). The patella is reported as absent in both an early European specimen (*Henkelotherium*, late Jurassic) and a later South American specimen (*Vincelestes*, early Cretaceous) (Fig. 5). Of the large group of dryolestid Eupantotheria, which possibly survived past the K-Pg boundary, patellar status is unknown.

The tribotherians were the earliest-diverging group to share key molar features with the therians. However, no postcranial specimens have been reported; thus nothing is known of their patellar morphology (Kielan-Jaworowska et al. 2004).

The single specimen of *Juramaia* from the Jurassic (~160 Mya) unfortunately lacks hindlimb material; therefore its patellar status is unknown. Based on its forelimb, *Juramaia* is proposed to have been scansorial or possibly arboreal (Luo et al. 2011). The later specimen of *Eomaia* from the early Cretaceous includes all limb elements, and is described with a patella (Ji et al. 2002). Based on limb and foot features, *Eomaia* was probably scansorial or arboreal. In the original publication, *Eomaia* was described as the earliest eutherian mammal (Fig. 5), however a more recent and much more extensive analysis confidently placed *Eomaia* prior to the eutherian/metatherian divergence (O'Leary et al. 2013) and thus at least as a stem member of the clade Theria (see Figure S4). *Eomaia* (and presumably *Juramaia*) postdate the divergence of the Symmetrodonta, but their positions relative to the Eupantotheria remain to be determined, as does any close relationship between these two key taxa. Lacking a better alternative, here we refer to these taxa as “Theria”, and in Fig. 5 vs. Fig. S4, consider the consequences of *Eomaia*'s phylogenetic position on our conclusions.

In surveying the available data mapped onto our composite phylogeny (Figs. 5, S4), it becomes evident that an ossified patella evolved multiple times (at least four) along the mammalian stem lineages during the Mesozoic era, whether using parsimony or maximum likelihood optimisation methods: at some highly uncertain time in the long mammalian lineage that led to Monotremata, in multituberculates/Allotheria, in *Zhangheotherium* or a direct ancestor, and likely twice (or between one to three times, depending on the placement of *Eomaia*; See Figs. 5 and S4) in the clade containing *Eomaia* and Theria (Metatheria and Eutheria). This remains the same if the Euharamiyida are not included with multituberculates but pre-dated crown Mammalia, as suggested by some recent studies (e.g. Luo et al. 2015a).

2. Mesozoic Metatheria and Eutheria

The two major extant mammalian groups, the Metatheria and Eutheria (together forming the clade Theria), diverged as early as the Jurassic (Fig. 5). The earliest fossil defined as

metatherian, *Sinodelphys*, dates from the early Cretaceous of China (125 Mya, approximately contemporary to *Eomaia*), and lacks a patella (Luo et al. 2003). A patella also seems absent in the less complete Cretaceous metatherian *Asiatherium* (Szalay & Trofimov 1996).

The earliest occurrences of the patella in definitive eutherians (Figs. 5,7) are in the late Cretaceous *Ukhaatherium* (Horovitz 2003), a relatively unspecialized form, and in *Zalambdalestes* (Wible et al. 2005), a more specialized taxon sometimes described as resembling later lagomorphs (Rose 2006). Patellar status at the crown group node for Theria (plus *Eomaia*) remains ambiguous (Figs. 5,6,S4), as we consider below.

3. Cenozoic Monotremata

The origins of the Monotremata (egg-laying mammals) are poorly understood. They are considered extant members of the clade Australosphenida (the alternative term Prototheria has been superseded), and hence with early roots in the Mesozoic. Molecular studies based on the sequenced genome of the platypus corroborate the long held interpretation that the monotremes diverged prior to the metatherian/eutherian split, consistent with proposed fossil-based phylogenies (Warren et al. 2008). Unfortunately, there are almost no reported hindlimb specimens of any extinct monotreme (including probable early monotreme fossils found in South America; (Musser 2003)), with the exception of the Pleistocene *Zaglossus* (echidna) from Australia and New Guinea (which may be the same as the extant species of that name). Unfortunately, although fossil *Zaglossus* hindlimb elements exist, including an articulated knee, neither presence nor absence of the patella has been reported (Murray 1984). The extant monotremes, the platypus (*Ornithorhynchus anatinus*) and the echidnas (Tachyglossidae, two genera *Zaglossus* and *Tachyglossus*; four known species) all have substantial patellae (see Fig. 4A, B, C, D) (Herzmark 1938; Rowe 1988). It is unclear when the two extant monotreme genera diverged although a date early in the Cretaceous has been proposed (Rowe et al. 2008), and it is impossible for now to date the appearance of the patella in the monotreme lineage. Regardless, an ossified patella is homologous for this crown clade (Fig. 5), and alternative phylogenetic topologies do not change the general pattern of patellar evolution (Fig. S4).

4. Cenozoic Metatheria

All extant Metatheria are within the subgroup of Marsupialia, however non-marsupials did exist earlier during the Cenozoic. As documented in the pioneering study of sesamoids in Marsupialia by Reese et al. (2001), the patella is absent in the great majority of extant marsupial species, both from Australia and the Americas (Flores 2009; Herzmark 1938; Holladay et al. 1990; Reese et al. 2001; Rose 2006; Rowe 1988), including the sole surviving North American marsupial, the North American opossum (*Didelphis virginiana*, see Fig. 4E, F showing tendon lacking a true patella. Many marsupials have other sesamoids in the knee region (e.g. the parafibula, lateral sesamoid, or “sesamoid bone of Vesalli”), as well as a fibrocartilaginous “patelloid”, which may to some degree serve the mechanical function of a bony patella (Reese et al. 2001). However, the mechanics of a fibrous or bony patella remain essentially unstudied (to our knowledge) in non-placental mammals, so this is simply speculation. Studies have claimed some association between reduction of the patella in many marsupials and locomotor style or ecology (Holladay et al. 1990; Reese et al. 2001), but these deserve testing with more detailed sampling across phylogeny and ontogeny.

Nonetheless, a true, ossified patella is found in a small number of extant marsupial species among otherwise divergent clades, both from Australia: at least several Peramelidae or bandicoots, and the two marsupial mole species of *Notoryctes*; and from South America: *Tarsipes*, an opossum; and several, and possibly all, Caenolestidae or shrew opossums (see Fig. 6: note collapse of several large clades in terms of total number of species, in which no species have been shown to possess a patella; Table S1).

Possibly uniquely among crown clade marsupials, bandicoots also possess a chorioallantois fused to the uterine epithelium (i.e. a true placenta) (Freyer et al. 2003; Padykula & Taylor 1976), which combined with a patella led to the initial suggestion that they might actually be eutherians (Reese et al. 2001). However, more recent molecular and fossil-based phylogenetic studies provide no support for that hypothesis of eutherian bandicoots (Asher et al. 2004; Meredith et al. 2008b; Sanchez-Villagra et al. 2007; Westerman et al. 2012). Bandicoots clearly are metatherians, and their chorioallantois is thus a convergently evolved trait rather than plesiomorphic. It remains to be determined whether a patella is present in all or only some bandicoots, as so far it is only reported in the Peramelinae of dry or temperate forests of

Australia, not yet in the Peroryctinae of tropical rainforests of New Guinea, or the more distantly related bilbies (Groves & Flannery 1990; Meredith et al. 2008a; Westerman et al. 2012).

Similarly, a comprehensive study of the Caenolestidae remains to be performed, much as a more thorough study of the major marsupial clade Diprotodontia (wombats, kangaroos and kin) is needed.

Not surprisingly given the absence of a patella in most extant marsupials, the patella is absent in the early Cenozoic Metatheria *Pucadelphys*, *Mayulestes*, and the later *Herpetotherium*.

Unexpectedly, a patella is reliably reported in the Borhyaenoidea, an unusual group of dog-like carnivorous South American marsupials found from the Paleocene through the Miocene (Argot 2002; Argot 2003a; Argot 2003b; Argot 2003c; Argot 2004; Argot & Babot 2011; de Muizon et al. 1997). Patellar status in some members of Borhyaenoidea (*e.g.* *Borhyaena* itself and *Lycopsis* (Argot 2004)), and in the more inclusive group Sparassodonta, is uncertain due to the incomplete state of specimens (C. Argot, pers. comm.) Szalay and Sargis (2001) noted other enigmatic fossils from the Palaeocene of Brazil that they assigned to Metatheria, but the phylogenetic relationships of those fragmentary remains are unclear and no patellae were shown (Szalay & Sargis 2001). However, no patella is reported in extant or recent carnivorous marsupials such as *Thylacinus*, thus it is not obligatorily correlated with that particular ecological adaptation.

Two related, pernicious problems remain for interpreting the evolution of the patella in Metatheria that may have ramifications for all of Mammalia/Mammaliaformes. First, Szalay and Sargis (2001:pp.164-5) reported the presence of an ossified patella in older individuals of *Didelphis virginiana* in their study of an ontogenetic series from this species. They stated (p.165) “In older individuals there is occasionally an elongated and small sesamoid ossification within the tendon of the quadriceps femoris where it crosses the knee joint when the knee is flexed.” However, this observation was not documented with illustrations or photographs (especially tissue histology or x-rays) and hence remains simply tantalizing. Similarly, Owen (Owen 1866) commented that some marsupials had no ossifications in their patellar tendon but others had “only a few irregular specks of ossification” and a “distinct but small bony patella in the *Macropus Bennettii*.” In contrast, Reese et al. (2001) and Holladay et al. (1990) respectively sampled 61 specimens (~39 adults) from 30 species of marsupials and 3 macropodid specimens

(of unknown maturity), documenting no ossified patellae except as noted in bandicoots (Holladay et al. 1990; Reese et al. 2001). It remains possible that patellar ossification occurs variably in older individuals among Metatheria, which would help explain its patchy description in known taxa.

If the latter situation is the case (i.e., the literature is unclear about patellar ossification in marsupials because they have more inherent variability), then it relates to a second problem, a cladistic one of character coding and transformational homology (*sensu* (Brower & Schawaroch 1996; Pinna 1991). Should character states of the patella in metatherians, or even all mammals and their kin, be coded as an ordered transformational series such as absent (0), fibrocartilaginous (1) or ossified (2), or as an unordered series (i.e. should evolutionary steps be required to go from 0-1-2 as 2 steps, or unordered allowing 0-2 transformations as 1 step)? We chose the unordered character option by default for all crown group mammals, but the legends for Figs. 5-7 explain how an ordered option changes (or does not change) our results. An endochondral ossification of the patella is certain, but a fibrocartilaginous or otherwise soft tissue composition of the patella (coded as state 1) is not unambiguously the necessary (i.e. ordered) precursor character state to state 2 (ossified patella in adults). The solution to both of these problems lies in more developmental data for the patella in diverse mammalian species, in addition to more scrutiny of the adult morphology in extant and fossil Mammalia (especially Metatheria).

As noted briefly in the Introduction, many marsupials have a primarily fibrocartilaginous patelloid in place of an ossified patella and some other mammals may have a “suprapatella”. The developmental and evolutionary relationships of these structures remain somewhat unclear, particularly as some marsupials with an ossified patella (*e.g.* bandicoots) also possess a patelloid (Reese et al., 2001), suggesting that the patelloid is not developmentally equivalent to the patella in marsupials (Vickaryous & Olson 2007). If so, this would suggest independent evolutionary histories of these two structures. Further work is required to clarify the relationships of the patelloid and suprapatella at least in extant taxa, before definitive evolutionary trajectories can be inferred.

A non-osseous patelloid/suprapatella is also found in several closely related modern placental clades that lie far from the base of Eutheria (Fig. 7), suggesting that these represent independent acquisitions. We have not attempted to explicitly reconstruct the evolution of the patelloid in Eutheria. Lewis (1958) and Broome and Houghton (1989) speculated that the mammalian patelloid might be a precursor to the tibial epiphysis (Broome & Houghton 1989; Lewis 1958) -- a so-called “traction epiphysis” (Vickaryous & Olson 2007) -- but considering that the patelloid evolved after the tibial tuberosity (and proximal epiphysis) of mammals, not before it, and lies proximal rather than distal to the patella, we reject this hypothesis. Furthermore, this same phylogenetic evidence indicates that the patelloid in Euarchontoglires, some Carnivora and bandicoots is not ancestrally associated with leaping or other behaviours (e.g. Jungers et al., 1980)-- as Walji & Fasana (1983) caution, its ancestral mechanical environment and roles in different behaviours remain unclear, although it does seem to be associated with knee hyperflexion like a typical fibrocartilaginous “wrap-around” tendon (e.g. Ralphs et al. 1991; Alexander & Dimery, 1985).

Our unordered parsimony reconstruction indicated that an ossified patella was absent in the ancestor of Metatheria, then evolved in the ancestor of Borhyaenoidea and Marsupialia. The most likely pattern shows loss of the patella in the basal lineages of Marsupialia, with subsequent re-acquisition in certain groups (Notoryctidae, possibly with Thylacomyidae + Peramelidae, and Tarsipedidae) (Fig. 6). Ordered parsimony reconstruction resulted in subtle differences; making some nodes less ambiguous (i.e. state 1 within basal Marsupialia) and others more ambiguous (such as the ancestor of Borhyaenoidea and Marsupialia, which becomes equally parsimonious between states 1 and 2). In contrast, Maximum likelihood reconstruction suggested multiple evolutions of the osseous patella in Metatheria, perhaps preceded in some taxa by a patelloid, but this is unclear. Because presence of a patelloid has not been clearly excluded in some extant marsupials (e.g. Petauridae, Acrobatidae) and is unlikely to be fossilised, its reconstruction must be treated carefully. Finally, alternative placement of Microbiotheriidae did not drastically alter the reconstructions (Fig. S5).

5. Cenozoic Eutheria

The Placentalia include all extant Eutheria as well as some fossil stem taxa (Fig. 7). Although there is some fossil evidence for placentals pre-dating the K-Pg event (Archibald et al. 2011), as well as substantial molecular dating consistent with an older placental radiation, the timing of the placental radiation remains highly controversial. However, our major conclusions about patellar evolution in placentals are not dependent on how this controversy is ultimately resolved, as a recent large-scale phylogenetic analysis convincingly established the presence of a patella as a derived character state in the ancestral placental irrespective of its true date of divergence (O'Leary et al. 2013).

Fossil evidence supports the presence of the patella in essentially all Cenozoic placental groups (Fig. 7; also see Table S1 and Figs. S1-S3, with citations therein). Specimens with sufficient hindlimb material to make a determination of the patella are rare in the early Cenozoic Paleogene period (~66-23 Mya), but Paleocene groups in which a patella has been reported include the Taeniodonta (small to medium sized fossorial animals), Pantodonta (early herbivores), Palaeonodonta (small, possible insectivores; perhaps related to pangolins), “Condylarthra” (a diverse assemblage of putatively related taxa, probably polyphyletic, including both herbivores and carnivores, many of which may be stem members of subclades within the placental crown group), and the important Plesiadapiformes, a sister group to crown clade primates (and possibly members of the clade Primates as well) (Bloch & Boyer 2007; Silcox 2007). In general, the evolutionary relationships between Paleocene taxa and more recent placentals remain enigmatic.

Eocene placentals include examples whose close relationships to modern groups are well accepted. Among Eocene groups (Fig. 7, Table S1), a patella has been reported in older, extinct groups such as “Condylarthra”, Creodonta (carnivores), Mesonychia (carnivorous/omnivorous artiodactyls or cetartiodactyls), Dinocerata (large rhinoceros-like herbivores), Brontotheriidae (large equid-like herbivores), and Notoungulata (fairly diverse South American hoofed herbivores; probably related to Afrotheria) (O'Leary et al. 2013), as well as in extinct species (in parentheses, see Table S1 for citations) recognized as stem members of several extant groups: Glires (*Rhombomylus*), Perissodactyla (*Propalaotherium*), early Sirenia retaining hindlimbs (*Pesoziren*, *Protosiren*), Proboscidea (*Numidotherium*, *Moeritherium*, *Barytherium*), Rodentia (the horse-sized *Pseudotomus*, *Paramys*), Pholidota (*Eomanis*), Artiodactyla (*Gervachoerus*),

early Cetacea retaining hindlimbs (*Maiacetus*) and Chiroptera (*Icaronycteris*, *Tachypterion*). A patella is also reported for several Eocene primates, including the lemur-like Notharctidae (*Northarctus*) and the tarsier-like *Omomys* and *Archicebus*, in addition to the enigmatic primate *Darwinius*.

Despite an extensive literature search, we found no reports attesting to the presence of the patella in certain important and widely cited Paleocene and Eocene species, including: *Protungulatum*, frequently cited as the earliest true placental; *Miacis*, *Vulpavus*, *Viverravus* and *Didymictis*, which are stem Carnivora (Gregory 1920; Heinrich & Houde 2006; Heinrich & Rose 1995; Heinrich & Rose 1997; Samuels et al. 2013); *Pakicetus*, a fully quadrupedal early cetacean (though sometimes reconstructed with a patella as in Fig. 7 and Fig. S1 M, N) (Thewissen et al. 2001); *Leptictis*, possibly related to crown clade lagomorphs (Rose 1999); *Sinopa*, a creodont (Matthew 1906); and the early primates *Adapis*, *Leptadapis*, *Teilhardina*, and *Cantius* (Dagosto 1983; Gebo et al. 2012a; Gebo et al. 2012b; Rose & Walker 1985; Schlosser 1887; Szalay et al. 1975). There is no reason to expect that the patella is missing in these species. These absences are more likely due to incompleteness of the fossil record and/or literature descriptions and images. Moreover, the massive collections of Eocene specimens from the Messel and Green River lagerstätten in Germany and Wyoming have not yet been fully described (Grande 1984; Schaal & Ziegler 1992). There are many examples of the patella in specimens from extant placental groups across the more recent Miocene, Oligocene, Pliocene and Pleistocene, but a comprehensive search of the literature for those geologic epochs was deemed redundant for our major conclusions.

Based on fossil evidence plus extensive genomic DNA sequencing, there is a consensus that crown clade placentals can be historically and geographically defined by four major groups: Xenarthra, Afrotheria, Euarchontoglires (further divided into Euarchonta; featuring Primates; and Glires) and Laurasiatheria (Rose 2006). These in turn may be resolved, with somewhat less consensus, into 19 crown clade “orders” (Fig. 7) (O’Leary et al. 2013). In two of these orders, the afrotherian clade Sirenia and the cetacean branch of (Cet)artiodactyla (laurasiatherian clade), extant members have extensively reduced or absent hindlimbs and thus lack skeletal knee structures, including a patella. In contrast, the patella is retained among the aquatic seals and sea

lions in Carnivora, although unlike Sirenia and Cetacea these animals still display some terrestrial habits and thus presumably still employ the antigravity mechanism that the patella is involved in at the knee. A patella is documented as present in at least some members of all other 17 placental “orders” (e.g. Figs. 7, S1-S3, 4G,H; Table S1) (de Panafieu & Gries 2007; De Vriese 1909; Dye 1987; Herzmark 1938; Lessertisseur & Saban 1867; Rose 2006).

The evolution of the Cetacea presents an interesting scenario regarding patellar evolution (Fig. 7). Cetaceans evolved from a common ancestor with other (cet)artiodactyls (Spaulding et al. 2009; Thewissen et al. 2007). Early members, *Diacodexis* and *Indohyus*, shared morphological similarities with both extant groups of Cetacea (toothed and baleen whales) and yet retained a patella (Rose 1982; Thewissen et al. 2007), much as stem Sirenia did (Domning 2001; Zalmout 2008). Patellar status in *Pakicetus*, a presumptive early cetacean with full hindlimbs, remains uncertain based on the primary literature, but presence is likely considering the presence of a patella in its closest relatives. *Rodhocetus* and *Ambulocetus*, probably semi-aquatic early cetaceans, still had large hindlimbs and patellae (Madar et al. 2002). The pelvis and hindlimbs are greatly reduced in the later cetaceans *Dorudon* and *Basilosaurus*, but a patella is still present in these animals (Gingerich et al. 1990; Uhen 2004). It is not clear exactly when the patella was lost in later cetaceans with increasingly reduced hindlimbs.

Bats present another interesting case of patellar evolution (Fig. 7; Table S1). The patella is generally present in bats (Pearson & Davin 1921b). A patella is also reported in a well-preserved hindlimb of an early Eocene bat, *Icaronycteris*, of intermediate form but proposed to be a microchiropteran (Jepsen 1966). However, in studies of multiple genera of modern bats including members from both of the major subgroups Megachiroptera and Microchiroptera (which is possibly paraphyletic), a patella was noted as absent in four species of the megachiropteran *Pteropus* (flying foxes of various sizes), and a few individual species of *Cephalotes*, *Epomophorus* and *Vespertilio* (De Vriese 1909; Lessertisseur & Saban 1867; Smith et al. 1995). No obvious lifestyle distinction was noted for the *Pteropus* genus as compared to many other bats, hence the loss of the patella in members of this particular subgroup (and others) remains mysterious. In general, bat hindlimbs are highly derived, adapted to hanging and pulling rather than pushing. A few bats such as the vampire bats are actively quadrupedal (Adams &

Thibault 2000; Riskin & Hermanson 2005). Bat hindlimbs are articulated in abduction, so that the knee faces dorsally; as in the original ancestral orientation for Tetrapoda (Fig. 2) (Neuweiler 2000; Schutt & Simmons 2006). There remains a need for a comprehensive study of the patella in bats (Smith *et al.* only studied 31 specimens of 13 species), but this is challenging due to the existence of >900 extant bat species (Jones et al. 2002). The microstructure of the “patelloid” in *Pteropus* is generally similar to that in many marsupials (e.g. deep layer of fibrocartilage; superficial layer of dense connective tissue contiguous with the quadriceps/patellar tendon) (Smith et al. 1995). This also raises the question of whether the patella only ossifies later in adulthood in *Pteropus*, rather than not ossifying at all.

General evolutionary patterns and ambiguities

Considering the above distributions of patellar presence/absence in Mammalia (Figs. 5-7; Figs. S4,S5) and our data matrix (Table S1), the simplest interpretation of the evolutionary record of the patella in mammals (by parsimony-based mapping of presence/absence) is that this structure arose independently at least four times (but possibly up to six), mostly during the Mesozoic era: 1, in Australosphenida ancestral to modern monotremes; 2, in Multituberculata (later than *Rugosodon*); 3, in Symmetrodonta (specifically in Spalacotheroidea that were ancestral to *Zhangheotherium* but not *Akidolestes*); 4-6, in early Theria (including Eutheria, Metatheria, *Eomaia* and related stem groups; depending on topology between one and three times this clade); Conceivably, a single common patelloid precursor may pre-date the evolutions of the bony patellae, or the patella may have arisen fewer times and undergone loss (and re-gain) in some lineages, similarly to the pattern in Metatheria. Each of these scenarios remain difficult to test purely with fossil evidence, however, due to the typical lack of preservation of cartilaginous structures.

Once the bony patella evolved in Eutheria, it was highly conservative in its presence. There are very few examples of fossil or extant Eutheria in which the hindlimb remains intact but the patella is absent. A caveat is that many fossil specimens are not sufficiently complete for a definitive rejection of patellar occurrence in those taxa. Still, the evolutionary stability of the patella in Eutheria stands in contrast to its general variability across mammals, and suggests

some conserved functional requirement and/or ontogenetic mechanism that remains to be determined.

Although an ossified patella is absent in the majority of Metatheria, it is reported in several groups (Figs. 6, S5). This likely represents loss and regain(s) of the early metatherian patella. Importantly, in this case the presence of a fibrocartilaginous “patelloid” in most marsupials shows a clear evolutionary polarity from an ossified patella to a non-ossified patelloid, and back again in the case of the secondary gain of ossification, in each case within Metatheria (Reese et al. 2001). This “patella to patelloid” transition suggests the reverse may also be possible – that a soft tissue patelloid may represent the evolutionary precursor to an ossified patella – but it has yet to be clearly documented. There is no obvious lifestyle or biomechanical correlate among all four groups of patella-bearing Metatheria: the notoryctid moles are underground burrowers, and bandicoots may dig for insects, but *Tarsipes* is a nectar feeder and the borhyaenoids were largely terrestrial carnivores. In contrast, other Australasian carnivorous marsupials including the recently extinct thylacine, and the extant quoll, numbat and Tasmanian devil are not reported to have a patella.

The large size of the patella in the monotreme platypus might be related to its aquatic (and partly fossorial; burrowing of dens) lifestyle. Reduction of the patella in the Cetacea and Sirenia is not intrinsically correlated with their aquatic lifestyle, but with the reduction of the hindlimbs as part of their particular adaptations. Elsewhere in groups with aquatic adaptations, for example in various diving birds, an unusually large patella is found. The other monotremes, the echidnas, also burrow and the long-beaked species (*Zaglossus*) lives in underground dens-- further suggesting an association between fossorial habits and the presence or enlargement of a patella in Monotremata, as well as in some fossil Mammaliaformes (multituberculates?) but curiously not in other fossorial stem taxa (*e.g.* the docodont *Docofossor*). It seems premature to weave detailed scenarios around the high degree of convergent evolution of the patella in mammals until the function and genomic control of the patella are better understood, and improved phylogenetic sampling improves resolution of when it evolved in particular lineages.

Patellar developmental genetics

Molecular phylogenomics provides a potential independent or synergistic approach to resolving issues of patellar evolution. If specific genomic sequence signatures could be associated with patellar status, then comparison of the genomes of the various extant but widely separated groups with a patella might indicate whether these represent convergence events or a common ancestral event (*i.e.* identified via shared evolutionarily transmitted genetic markers required for patellar development). For example, it has recently been shown that the ability to taste sweet carbohydrates in hummingbirds represents a trait convergence. Hummingbirds diverged from the insectivorous swifts, in which the sweet taste receptor is inactivated by mutations in the receptor coding gene. In hummingbirds, the ability to taste sweet has been re-acquired, apparently through molecular adaptation of the umami receptor to detect sweet molecules (Baldwin et al. 2014). It would be helpful to understand the (developmental) genetics of the patella as a step toward the identification of such sequence signatures. Developmental genetic studies in two mammals, humans and mice, have identified genes required for correct patellar specification. The known functions of some of these genes are informative regarding their requirements.

There are currently approximately 12 human genetic disorders with identified molecular bases that regularly include abnormal, reduced or absent patellae (hypoplasia or aplasia) as an important aspect of the phenotype (reviewed by Bongers *et al.* (2005), see also Warman *et al.* (2011) and Supplementary Table S2 for details). There are also several genes whose genetics in mice indicates relevance to patellar development at least in rodents. A detailed discussion of all these syndromes and genes is beyond the scope of this study. However, the known patellar genes can be broadly organized according to three major developmental processes: limb specification and pattern formation (transcription factors such as *LMX1B*, *TBX4*, *PITX1* and mouse *Hoxaaccdd-11*, *OSR1*, *OSR2*, *SOX11*, and signalling factor *WNT7A*); bone development, biochemistry and regulation (*GDF5*, *CHRNA*, *SLC26A2*, *COL9A2*, *AKT1*); and genes involved in DNA replication and chromatin (*ORC1*, *ORC4*, *ORC6*, *CDT1*, *CDC6*, *GMNN*, *CDC45*, *RECQL4*, *KAT6B*, *ESCO2*). Of these, the genes of replication and chromatin are the most unexpected, and potentially of the most interest for evolutionary studies. Patellar ossification may be dependent on the timing of DNA replication in particular cells, or else may be affected by aberrant gene regulation resulting from mutations in replication and chromatin factors. In

either case, the target genes mis-regulated in these syndromes, if they can be identified, may provide useful evolutionary markers to distinguish convergent from homologous patellar status.

Developmental studies in mouse or chick embryos, sometimes with induced paralysis, document the additional importance of local environmental factors in patellar ontogenesis (Hosseini & Hogg 1991; Mikic et al. 2000; Nowlan et al. 2010a; Nowlan et al. 2010b; Osborne et al. 2002; Rot-Nikcevic et al. 2006). Similarly, embryonic development and hindlimb activity in the case of particular marsupials may be important in understanding the diversity of patellar states in this group. A better understanding of these environmental processes will also be helpful to disentangle genomic versus epigenomic regulation of patellar development, and hence evolution.

CONCLUSIONS

How “the mammalian patella” evolved

The widespread, repeated evolution of the patella across evolution argues for an important role in locomotor biomechanics. In animals lacking a patella (*e.g.* Lissamphibia, Testudines, Crocodylia; as well as many extinct lineages of tetrapods), the consequences of this ancestral absence for hindlimb function remain mostly unstudied. This mystery is striking, in particular, within Mammalia where most marsupials lack an ossified patella, as did numerous fossil stem-mammals, despite seeming to share common ecological niches and the associated locomotor requirements. This sporadic occurrence in marsupials and stem mammals contrasts with its near universality and evolutionary stability in the Eutheria as noted above.

The exact number of independent origins of a patella among mammals remains unclear, but we have estimated at least four convergent episodes inside Mammaliaformes, and several instances of patellar loss (with apparent re-gain in some marsupials). The pattern of acquisition and loss will require revisiting as new fossil material is discovered, as our evolutionary reconstructions are dependent on single specimens for many ancient taxa. Moreover, patellar status has not been verified for all >5,000 eutherian and >330 metatherian species (Wilson & Reeder 2005), so it is possible that additional placental species (other than the fully aquatic forms) may be found

lacking, or marsupials having, a patella. A recent evolutionary study documented many apparently independent evolutionary origins of the caecal appendix in mammals; thus the convergent evolution of complex anatomical structures like the patella has precedent (Smith et al. 2013). Similarly, blue coloration among tarantula spiders apparently involves at least eight independent evolutionary acquisitions, among different microscopic anatomical structures affecting spectral reflectance hence general external color (Hsiung et al. 2015). A better understanding of the genomic signatures required for development of such novel structures should be very helpful to deconstruct the observed complex patterns of evolution, distinguishing between convergent evolution (homoplasy) and shared inheritance (synapomorphy/homology).

Given that the patella evolved, and was also lost, multiple times in mammals and other Tetrapoda (Fig. 3), one thing is clear. Much as we have referred to “the patella” throughout this study, there is no such thing—perhaps not even a single “mammalian patella”. The story of patellar evolution is one of many patellae; a story of diverse evolutionary origins as well as forms, functions, ontogenies and perhaps even diverse underlying genetics. Mottershead (1988) wondered if the patella is “not typical of its kind” for a sesamoid bone (Mottershead 1988). Yet even patellae are not necessarily typical for patellae, let alone other sesamoids-- there are double or fatty patellae in some birds (Regnault et al. 2014), proximal suprapatellae and/or fibrocartilaginous patelloids in many marsupials, no patellae at all in many species, and even amongst those animals that have patellae, there are numerous shapes and sizes of patellae (Figs. 4, S1-S3), suggesting still-unappreciated lifestyle constraints in patellar (and knee joint) mechanics.

Future prospects

Our discussion of patellar evolution in Mammalia has identified several areas where key questions remain unresolved, in addition to uncertainties about the amount of convergence/parallel evolution in origins of the patella and about specific roles of (and interactions between) genetic/developmental factors in patellar formation/loss. Considering that mechanical loads are known to play an important role in the development of sesamoid bones (in particular in early ontogeny), studies linking these loads to genetic/developmental control as well as broad evolutionary patterns could prove very insightful, especially in explaining the

907 seemingly large amount of patellar homoplasy in mammalian evolution. Mammals may be less
 908 sensitive (*i.e.* more genetically assimilated (Vickaryous & Olson 2007)) than birds in terms of
 909 the relative influence of mechanical loads on bone (including sesamoid) ontogeny (Nowlan et al.
 910 2010b) - this idea deserves better testing as insight into load-based influences improves.
 911 Furthermore, indications that some bones within an organism may be more responsive to their
 912 loading regime (Nowlan et al. 2010a) may be of great relevance to interpreting patellar biology
 913 and evolution, but at present strong inferences cannot be drawn about how variable the patella's
 914 responsiveness to mechanics is within or among organisms. There is clearly much room for
 915 further study of the patellae of mammals and other tetrapods, and here we have noted directions
 916 in which these might most beneficially be directed.

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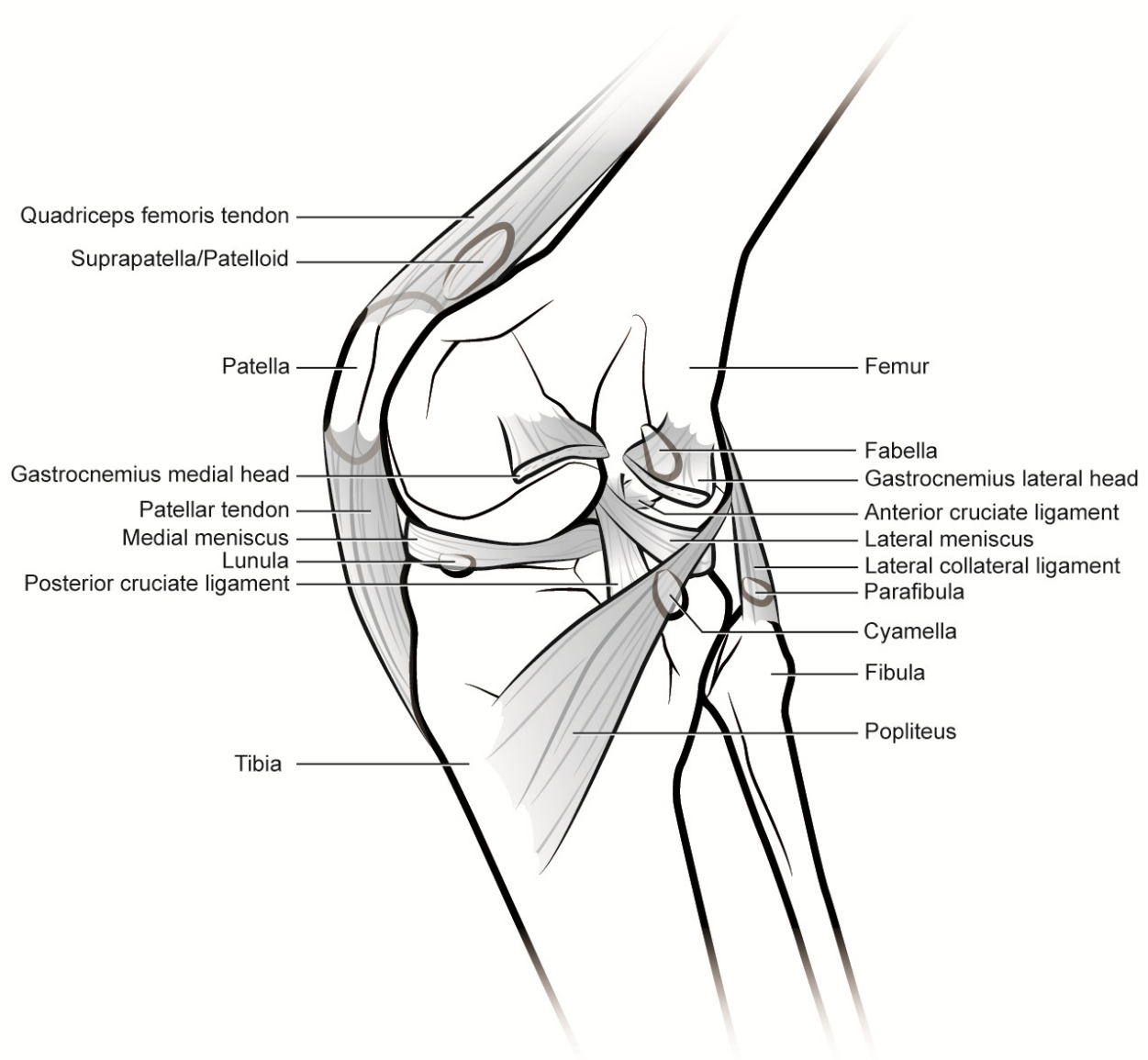
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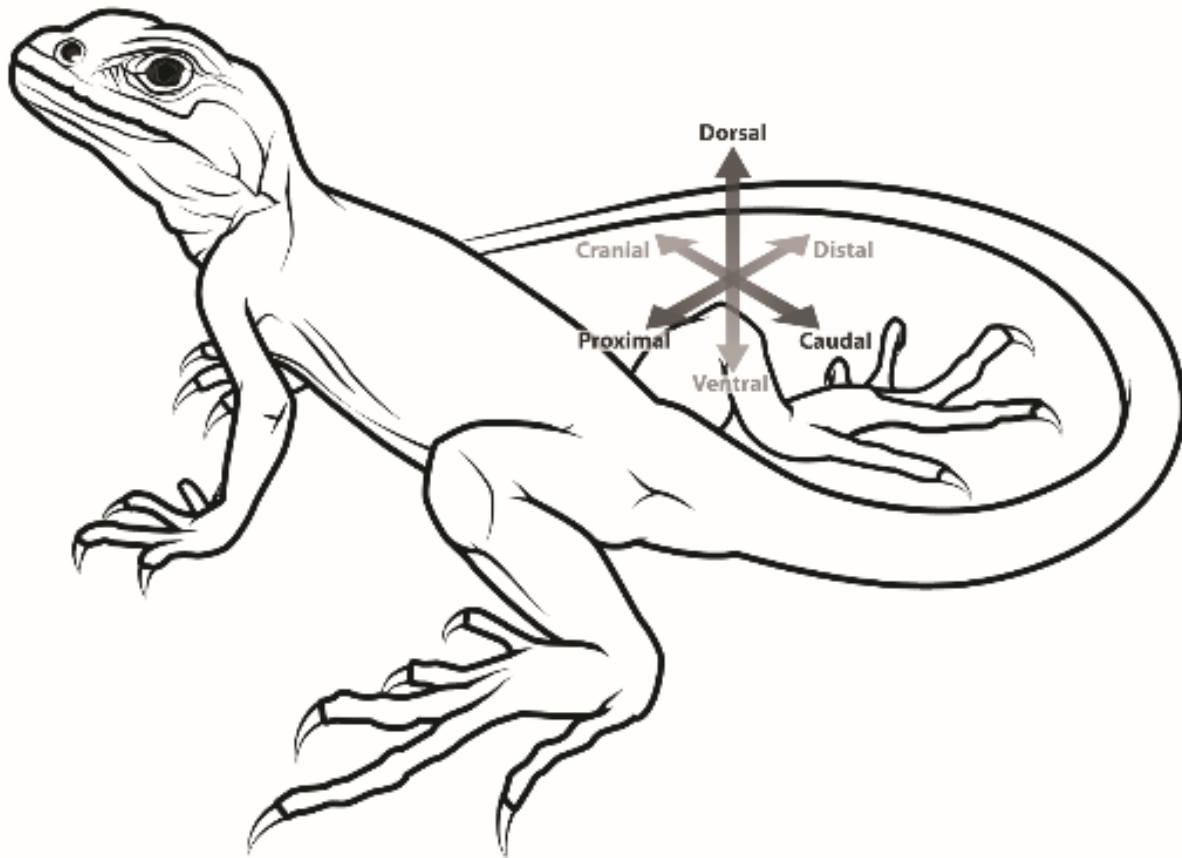
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FIGURES

Figure 1. Generalized knee showing sesamoid bones found in various mammals, although possibly no species includes all of these (patella, lunula, cyamella, fabella and parafibula). Also shown are relevant muscles, ligaments and other anatomical elements that lie close to the sesamoids of the knee joint. The knee is in medial view and the medial collateral ligament has been removed.

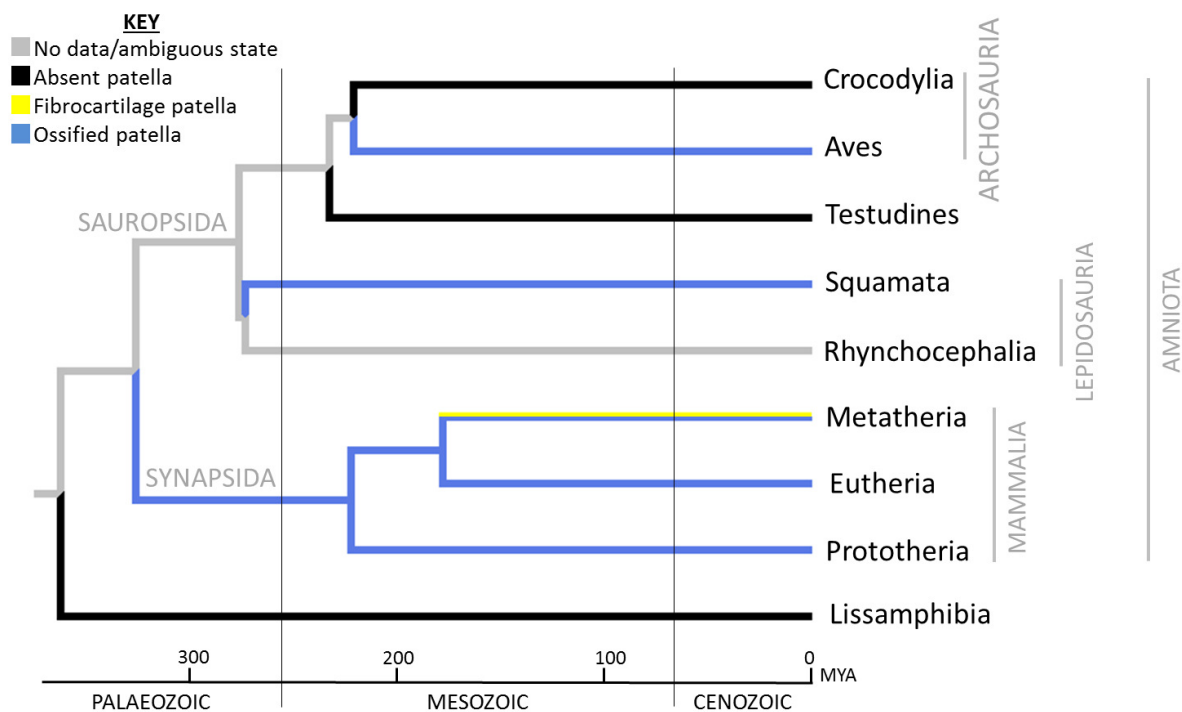


1460 Figure 2. Generalized tetrapod with anatomical/developmental axes defined for the hindlimb:
 1461 cranial/caudal (towards the head/tail respectively), proximal/distal (toward/further from the trunk
 1462 respectively), dorsal/ventral (towards the back/belly respectively).
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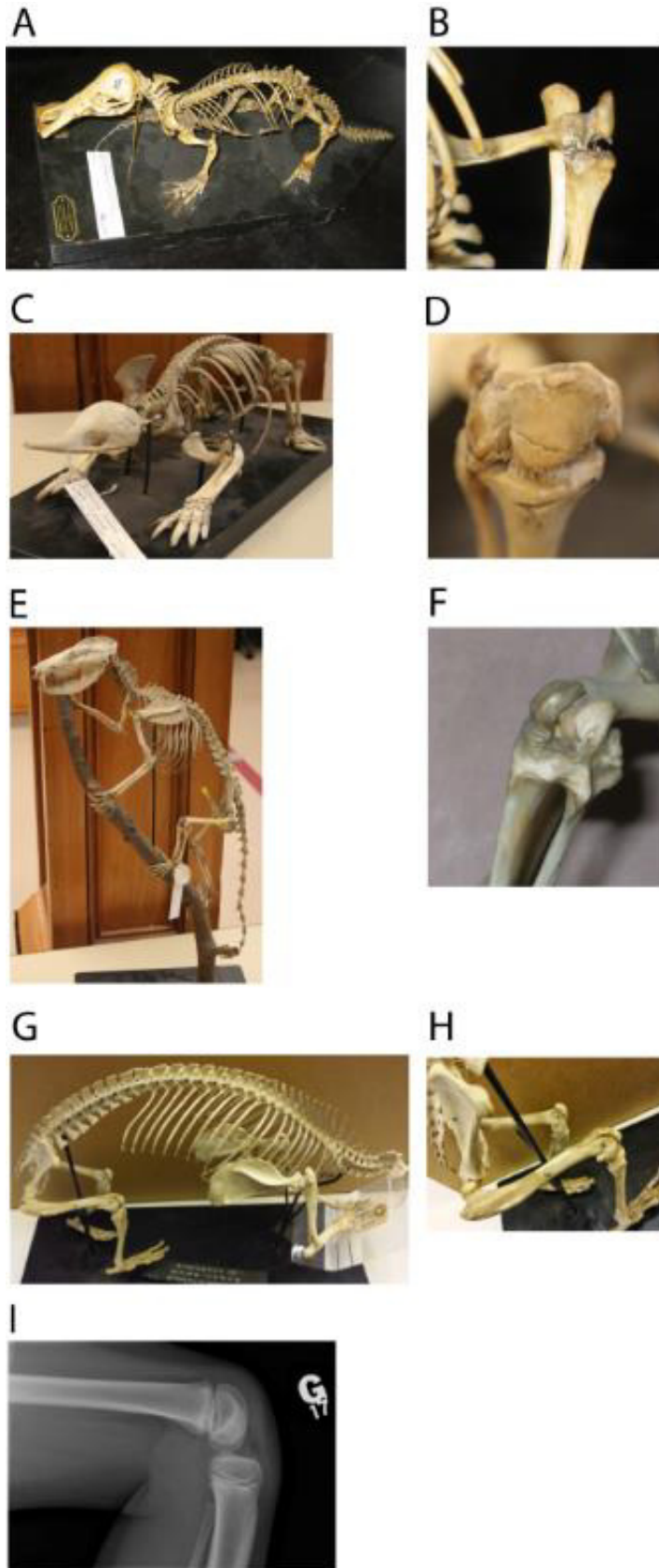


1464

Figure 3. Reconstruction of ancestral patellar states in Tetrapoda, showing the major extant clades. Reconstruction was performed using Mesquite's parsimony algorithm and unordered character states, where 0 (black) = absent patella, 1 (yellow) = soft tissue patella/patelloid, and 2 (blue) = ossified patella; see Methods for further details. The distribution of the ossified patella among extant clades has been interpreted as three occasions of independent evolution (in Aves, Squamata, and Mammalia) (Dye, 1987, Haines, 1940), a conclusion strongly reinforced by specific fossil evidence (absence or equivocality of a patella in all outgroups). Reconstruction within Mammalia is explored in more depth in Figures 5-7. MYA= millions of years from present.



1476 Figure 4. Examples of tetrapods with or without patellae. A, B. *Ornithorhynchus anatinus*
1477 (Monotremata: duck-billed platypus, Redpath Museum specimen 2458). C, D. *Tachyglossus*
1478 *aculeatus* (Monotremata: echidna, Redpath Museum specimen 2463). E, F. *Didelphis virginiana*
1479 (Metatheria: North American opossum, Redpath Museum specimen 5019). G, H. *Procavia*
1480 *capensis* (Eutheria: Afrotheria: Cape hyrax, Uncatalogued Horniman Museum specimen,
1481 London, UK). I, knee of patient with Meier-Gorlin Syndrome (Guernsey et al. 2010). For more
1482 images of mammalian patellae (or lack thereof in some marsupials), see Figures S1-S3.
1483



1484

Figure 5. Reconstruction of ancestral patellar state in Mesozoic mammals, using Mesquite's parsimony algorithm and unordered character states, where 0 (black) = absent patella, 1 (yellow) = soft tissue (i.e. fibrocartilaginous in adults) patella/pateloid, and 2 (blue) = ossified patella. Uncertain patellar state is indicated by grey branches and question mark (?) following taxon name, and key fossils with hindlimb material are denoted by †. This reconstruction suggests that a bony patellar sesamoid has evolved at least five times within Mammaliaformes. Reconstruction using ordered parsimony and maximum likelihood methods similarly resulted in five instances of ossified patellar evolution. An alternative topology with †*Eomaia* placed on the therian stem (after O'Leary et al., 2013), shown in Figure S4, similarly found 4-6 instances of bony patella evolution. Evolution within Metatheria is explored in more detail in Figure 6, and within Eutheria in Figure 7. MYA= millions of years from present.

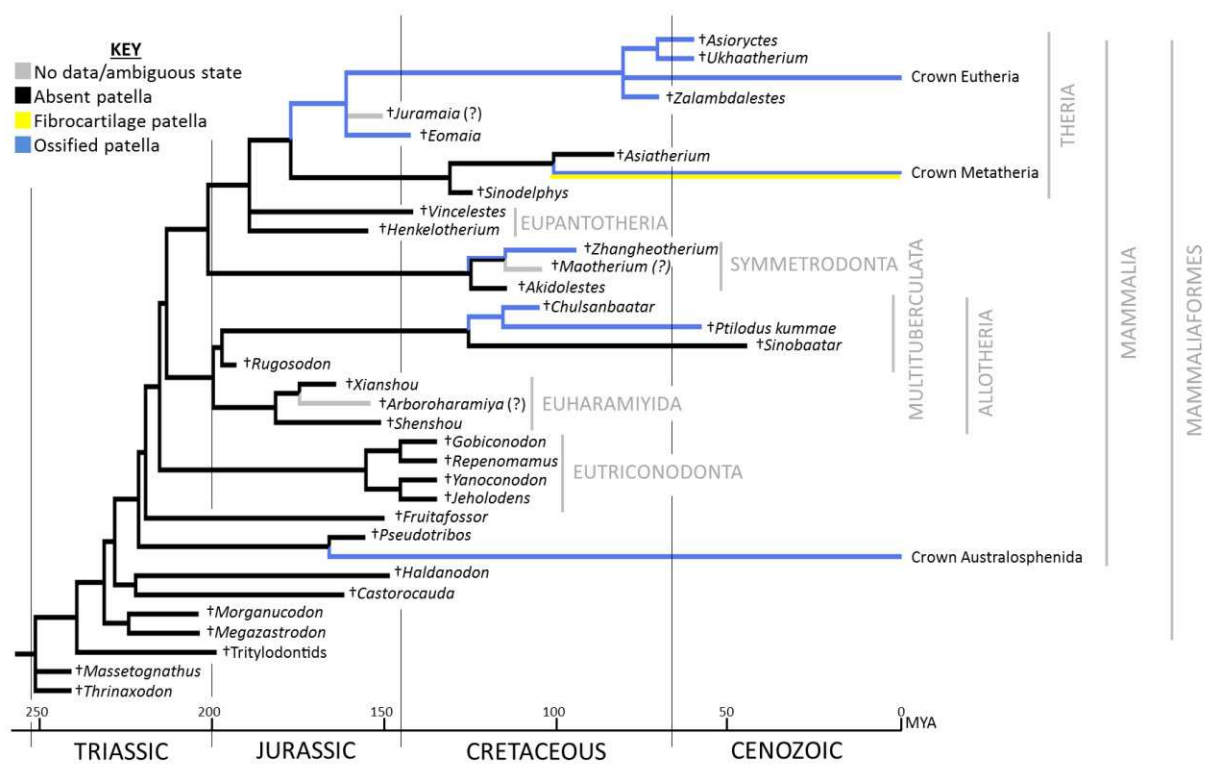
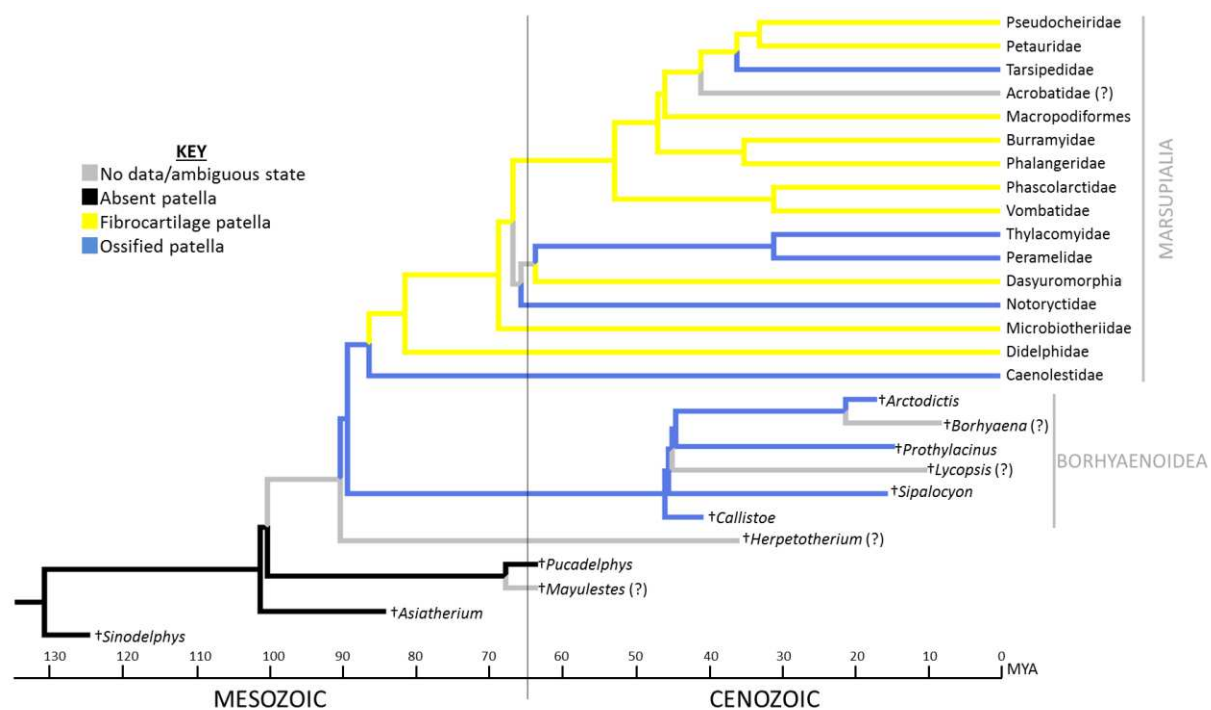


Figure 6. Reconstruction of ancestral patellar state in Metatheria, using Mesquite's parsimony algorithm and unordered character states, where 0 (black) = absent patella, 1 (yellow) = soft tissue (i.e. fibrocartilaginous in adults) patella/patelloid, and 2 (blue) = ossified patella. Uncertain patellar state is indicated by grey branches and question mark (?) following taxon name, and key fossils with hindlimb material are denoted by †. The reconstruction suggests that the ossified patella evolved between 1-5 times in Metatheria, with loss and reversion (to a fibrocartilaginous patella/patelloid and back) or as independent instances. States 0 (no patella) and 1 (fibrocartilage patella in adults) are equally parsimonious at the base of the tree. Ordered parsimony reconstruction yields similar results but state 1 is most parsimonious ancestrally. Maximum Likelihood analysis results in five independent instances of patellar evolution (in Borhyaenoidea, Caenolestidae, Notoryctidae, Thylacomyidae + Peramelidae, and Tarsipedidae), with an ancestral state of either 0 or 1 depending on the states assigned to the earliest fossils such as *Sinodelphys*. An alternative topology with Microbiotheriidae nested slightly further within Marsupialia (after May-Collado et al., 2015), shown in Figure S5, similarly shows between 1-5 instances of bony patella evolution but with more internodes of ambiguous character state (i.e. equivocal between states 1 and 2). MYA= millions of years from present.



1517 Figure 7. Reconstruction of ancestral patellar state in Eutheria, using Mesquite's parsimony
 1518 algorithm and unordered character states, where 0 (black) = absent patella, 1 (yellow) = soft
 1519 tissue (i.e. fibrocartilaginous in adults) patella/patelloid (state not present in Eutheria other than
 1520 some bats; see main text), and 2 (blue) = ossified patella. Uncertain patellar state is indicated by
 1521 grey branches and question mark (?) following taxon name, and key fossils with hindlimb
 1522 material are denoted by †. Our reconstruction suggests that the ossified patella evolved only once
 1523 within Eutheria and (as far as is currently known) has only been lost by the bat genus *Pteropus*
 1524 (not counting groups which have lost hindlimbs; e.g. *Trichechus manatus*/crown Sirenia,
 1525 *Tursiops truncatus*/crown Cetacea). Taxa within Euarchontoglires (e.g. rats, mice, guinea pigs,
 1526 rabbits, most primates), some Carnivora, and possibly some Chiroptera have been found to
 1527 possess a suprapatellar structure similar to the patelloid, shown by vertical black bars at the
 1528 appropriate branches. MYA= millions of years from present, P ϵ = Paleocene, Eo = Eocene, OG
 1529 = Oligocene, MI = Miocene (most recent Cenozoic epochs not labelled).
 1530

