

On the Presence of the Patella in Frogs

VIRGINIA ABDALA ^{1*}, MIRIAM C. VERA,² AND MARÍA LAURA PONSSA²

¹IBN CONICET-UNT, Horco Molle s/n. Cátedra de Biología General, Facultad de Ciencias Naturales e IML, Instituto de Biodiversidad Neotropical, Miguel Lillo 205,

San Miguel de Tucumán, 4000, Argentina

²UEL CONICET-FML, Unidad Ejecutora Lillo, Miguel Lillo 251, San Miguel de Tucumán, 4000, Argentina

ABSTRACT

The patella is one of the most studied sesamoids. Historically, the patella is described as a big sesamoid embedded in the tendon of the quadriceps femoris muscle. This sesamoid is studied from developmental, functional, clinical, and anatomical perspectives. The presence of a patella is reported in squamates, birds, and mammals. Lissamphibians are identified as the major lineage that fail to develop a patella. However, this sesamoid is reported at least once in anurans, but without detailed anatomical discussions. Through anatomical and histological studies we examined the topography and tissue composition of two structures that we identify as the proximal and distal patellae in several anuran species. We explored the evolution of these sesamoids through ancestral state reconstruction, finding that they are ancestral for amphibians and possibly tetrapods as a whole. The presence of these patellae in anurans would roll back their origin to the last common ancestor of tetrapods. From a functional perspective, the overwhelming evidence of fibrocartilage as a clear response to compression suggests that the fibrocartilaginous patellae could also withstand the mechanical stress generated on the knee undergoing compression during limb extension. *Anat Rec*, 00:000–000, 2017.

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Key words: sesamoids; patellar tendon; fibrocartilage

INTRODUCTION

Sesamoids have been defined as osseous structures usually embedded within a tendon (Barnett and Lewis, 1958; Burton, 1973; Le Minor, 1988; Hoyos, 2003; Vickaryous and Olson, 2007; Doherty et al., 2010; Ponssa et al., 2010). The study of sesamoids in non-clinical contexts undergone a renaissance, since their role in the interface of genetic/epigenetic factors, already noted by Pearson and Davin (1921a,b) has been recently re-evaluated (Newman and Müller, 2005; Vickaryous and Olson, 2007; Eyal et al., 2015). One of the most studied sesamoids is the patella, which is among the largest sesamoids described in tetrapods and probably the one more regularly counted among the osseous elements of the human body (Vickaryous and Olson, 2007; Regnault et al., 2014, 2016; Chadwick et al. 2017; Samuels et al., 2017). The patella is typically a big bone embedded in the tendon of the extensor femoris muscle, which has

been studied from multiple perspectives: developmental (Walsmley, 1940; Bland and Ashhurst, 1997; Reese et al., 2001; Vickaryous and Olson, 2007; Eyal et al., 2015), functional (Oxnard, 1971; Clarck and Stechschulte, 1998; Sarin et al., 1999), clinical (Blazina et al., 1973), and anatomical (Chadwick et al., 2014; Regnault et al., 2016; Samuels et al., 2017; Chadwick et al., 2017) among many others. The presence of the patella has

*Correspondence to: Miss Virginia Abdala, Instituto de Biodiversidad Neotropical, IBN CONICET-UNT, Horco Molle s/n. Cátedra de Biología General, Facultad de Ciencias Naturales e IML, Miguel Lillo 205, 4000 San Miguel de Tucumán, Argentina. E-mail: virginia@webmail.unt.edu.ar

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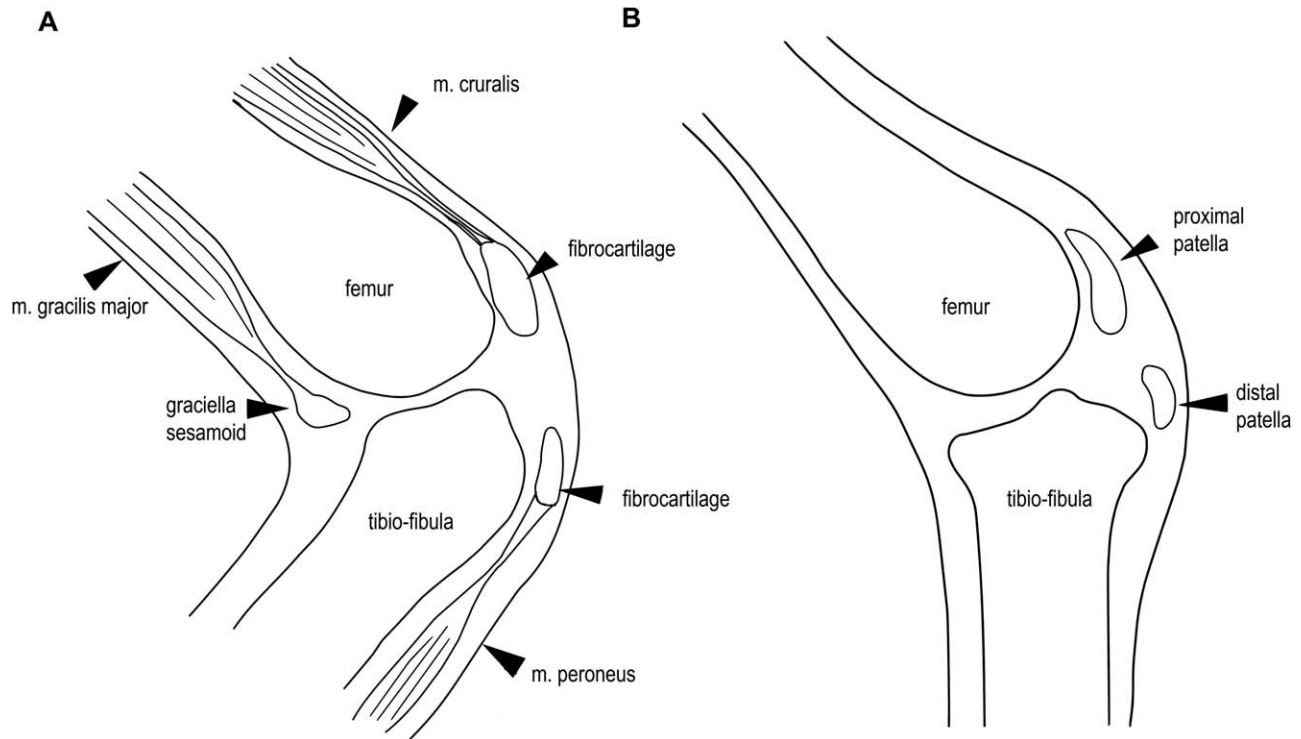


Fig. 1. Schematic representation of the knee. (A) Knee joint of a frog. (B) Knee joint of *Struthio camelus*, redrawn from Chadwick et al. (2014).

been reported in most tetrapod taxa: squamates (Jerez et al., 2010; Regnault et al., 2016), birds (Hudson et al., 1965; Vanden Berge and Storer, 1995; Chadwick et al., 2014; Regnault et al., 2014; Hutchinson et al., 2015), and mammals (Wiedersheim, 1907; Vaughan, 1959; Reese et al., 2001; Scott and Springer, 2016; among many others). Vickaryous and Olson (2007) stressed that lissamphibians are one of the major lineages which fail to develop the patella. However, there is at least one report of what seems a cartilaginous patella in amphibians (Hebling et al. 2014, their Fig. 3A). These authors correctly identify this sesamoid in one of their histological sections, but without discussing this finding.

The presence of a patelloid, defined as an additional sesamoid embedded in the quadriceps tendon and composed of fibrocartilage, has been reported in certain taxa, such as peramelid marsupials (Reese et al., 2001) and placental mammals (Vickaryous and Olson, 2007). In the definition of this sesamoid as a patelloid lies the idea that “true” sesamoids are osseous, as defined above. However, the concept of sesamoid should be revisited once again. As Vickaryous and Olson (2007) pointed out, sesamoids can be composed of many different tissues: bone, fibrocartilage, and even fat, but three aspects are shared in all these cases: (a) an embedding tendon, which became heterogeneous due to a genetic stimulus and the epigenetic signals from the surrounding environment, (b) the proximity to a joint, and (c) the mechanical effort that this tendon must support. The definition given by Regnault et al. (2016) can also be taken into consideration: “any organized, intratendinous/intraligamentous structure including those composed of fibrocartilage (e.g., the cartilago transiliens; see Tsai and Holliday, 2011).”

In this study, we investigate the presence, topography, and tissue composition of two structures that we identify as the proximal and distal patella in certain frog species. We also present histological data which allows us to identify the tissue structure of these sesamoids. The presence of patellae in anurans would roll back the origin of these structures to the last common ancestor of tetrapods, indicating that they should be regularly considered among the skeletal elements of this big clade. Thus, it would defy the notion of extreme variability associated to most sesamoids.

MATERIALS AND METHODS

A total of 20 species of seven families were analyzed. All specimens are from a collection in LGE: Laboratorio de Genética Evolutiva, FML: Fundación Miguel Lillo, Tucumán, Argentina (Appendix), and therefore an animal-use protocol was not required for this study. Observations were made in cleared and stained skeletal whole-mounts prepared following the protocol of Wassersug (1976). All the observations and illustrations were made with a stereo dissection microscope Carl Zeiss Discovery V8.

We also analyzed histological samples of femur and tibia-fibula epiphyses of post-metamorphic and adult specimens of *Leptodactylus latinasus*, *L. mystacinus*, *L. bufonius*, *Melanophryniscus rubriventris*, *Phyllomedusa sauvagii*, *Rhinella arenarum*, *Scinax fuscovarius*, *Telmatobius oxycephalus*, and *Xenopus leavis*. Specimens were fixed in a 10% formaldehyde solution for 24 hr. The extracted hind limbs were decalcified with 5% formic acid (5 mL of formic acid, 5 mL of formaldehyde and 100 mL of distilled water) for 1–6 weeks, depending on the sample size. The material was then dehydrated in

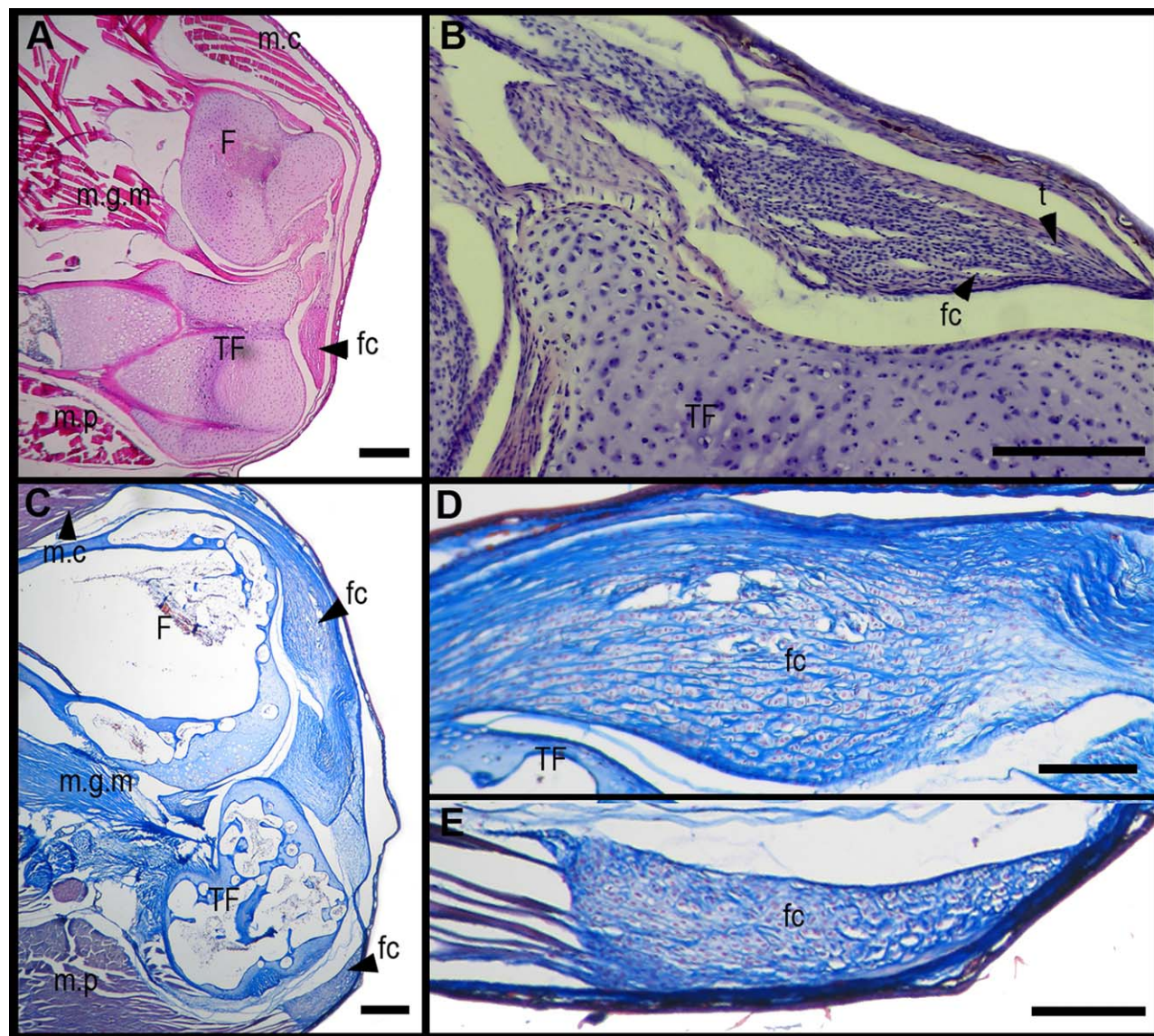


Fig. 2. Fibrocartilage over the epiphyses of *Leptodactylus latinasus*. (A, B) Knee of a juvenile specimen (SVL 22.94 mm, scale bar 200 μ m) with details of the fibrocartilage-like distal patella (scale bar 200 μ m). (C–E) Adult specimen (SVL 30.86 mm, scale bar 200 μ m) with details of the fibrocartilaginous proximal patella over the femur (D), and the distal patella over the tibio-fibula (E) (scale bar 100 μ m). F, femur; fc, fibrocartilage; m.g.m, muscle gracilis major; m.c, muscle cruralis; m.p, muscle peroneus; t, tendon; TF, tibio-fibula.

graded ethanol series and in n-butyl ethanol, and embedded in Histoplast embedding medium. Serial sagittal sections 7-mm thick were cut with a rotary microtome (Microm HM 325), and stained with Masson trichrome and Hematoxylin-Eosin. With the Masson trichrome technique, both the collagen fibers present in the extracellular matrix (ECM) and the fibrocartilage are stained blue, while the cellular nucleus is stained purple. Histological samples were observed under an optical microscope (Leica ICC 50 HD) and photographed with Nikon Coolpix P6000 digital camera. Terminology of sesamoids follows Ponssa et al. (2010).

For ancestral state reconstruction, we coded the presence of patella in tetrapod taxa based on our own data and the literature. An absence/presence matrix considering the proximal and distal patella was constructed. The

characters were assigned a state of "0/1" (proximal and distal patella absent and present) to reflect their polymorphism. However, alternate coding (i.e., 0 or 1) was also used, based on two reasons: on the one hand, the distribution pattern of these elements in the tetrapod tree allows inferring that both were present in the last common ancestor of the clade; and on the other hand, we tried to avoid the complete ambiguity generated by the 0/1 coding in almost all clades. Although we are aware that this decision might be conflictive, interesting patterns might emerge, despite the limitations of our data. Additionally, a coding taking into account the adult patellar tissue was performed. In the later, states were coded as: (0) absent sesamoid; (1) fibrocartilage; (2) cartilage; (3) bone. We coded additive characters using parsimony, on the phylogeny proposed by Hugall et al. (2007),

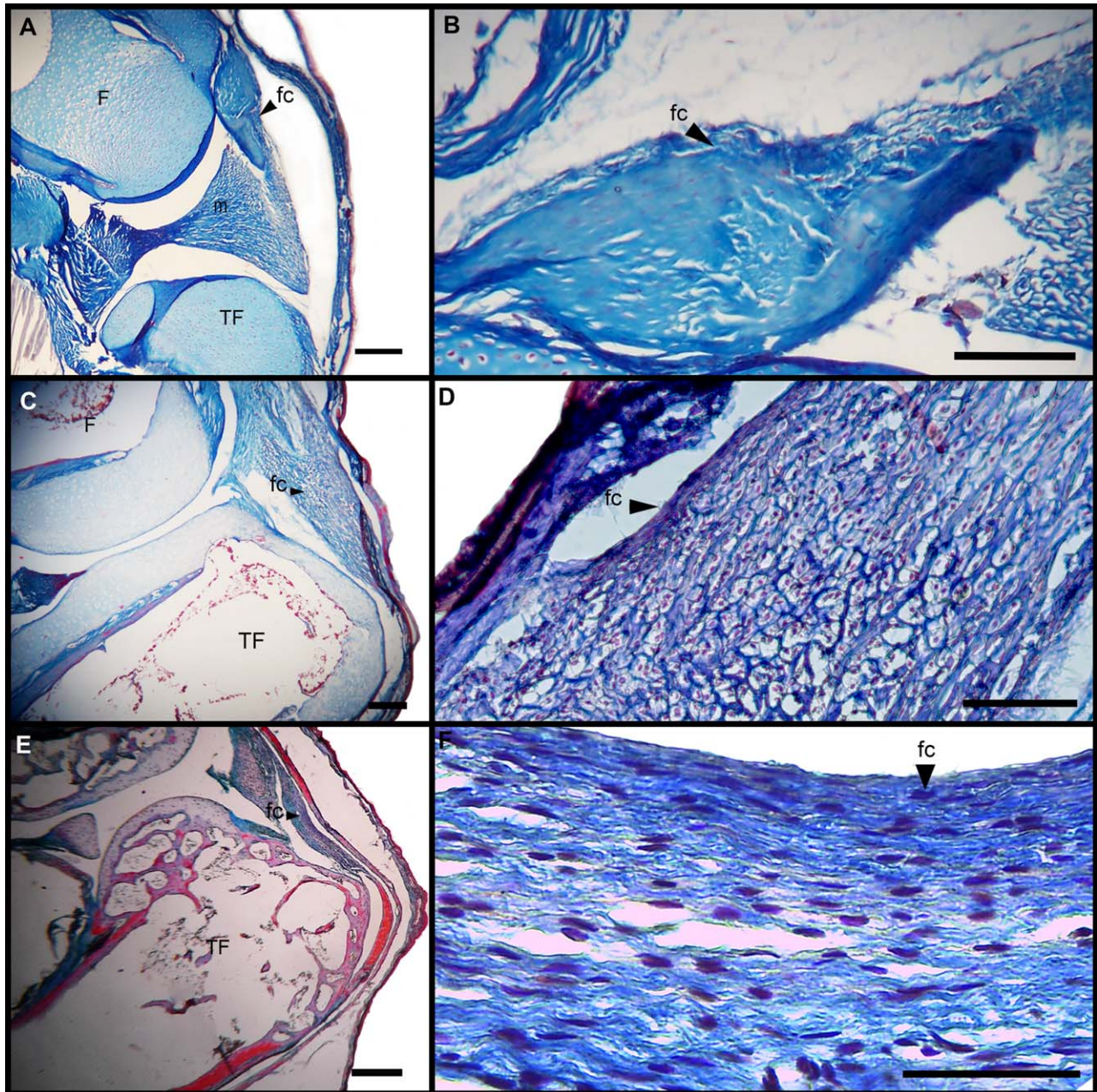


Fig. 3. (A, B) Juvenil specimen of *Xenopus leavis* (SVL 28.74 mm, scale bar 200 μ m) with a detail of the fibrocartilagenous proximal patella over the femur (scale bar 100 μ m). (C, D) Knee joint of an adult of *Phyllomedusa sauvagii* (SVL 60 mm, scale bar 400 μ m) with a detail of the fibrocartilagenous distal patella over the tibiofibula (scale bar 200 μ m). (E, F) Knee joint of an adult *Melanophryniscus rubriventris* (SVL 42 mm, scale bar 400 μ m) with a detail of the fibrocartilagenous distal patella over the tibio-fibula (scale bar 50 μ m). F, femur; fc, fibrocartilage; m, meniscus; TF, tibio-fibula.

with TNT software (Goloboff et al., 2003), and considering only unambiguous changes. The resulting cladogram was edited with Winclada 1.00.08 software (Nixon, 2002).

RESULTS

The examination of the diaphanized specimens showed no visible structure that could be identified as a patella in their knees. However, the histology of the

knee of *L. latinasus* showed a fibrocartilagenous structures located on the laterodorsal epiphysis of the femur and the tibio-fibula. We identified the one embedded in the insertion tendon of the cruralis muscle as the proximal patella; while the other fibrocartilagenous structure was embedded in the origin tendon of the m. peroneus (Fig. 1A,B), and corresponds to the distal patella. The detail of the fibrocartilagenous structures reveals a tissue with more cells than those in the associated tendon,

TABLE 1. Anuran species that underwent histological examination

Specie	Proximal patella	Distal patella	Tissue	Juvenil/adult	Technique
Hylidae					
<i>Dendropsophus nannus</i>	-	-	-	J/A	Stained skeletal
<i>Hypsiboas riojanus</i>	-	-	-	J/A	Stained skeletal
<i>Lysapsus limellum</i>	-	-	-	J/A	Stained skeletal
<i>Scinax fuscovarius</i>	-	X	Fibrocartilaginous	J/A	Histology/stained skeletal
<i>Phyllomedusa sauvagii</i>	-	X	Fibrocartilaginous	J/A	Histology/stained skeletal
Bufonidae					
<i>Melanophryniscus rubriventris</i>	-	X	Fibrocartilaginous	A	Histology
<i>Rhinella arenarum</i>	-	-	-	J	Histology/stained skeletal
<i>Rhinella major</i>	-	-	-	J/A	Stained skeletal
Ceratophryidae					
<i>Ceratophrys cranwelli</i>	-	-	-	J/A	Stained skeletal
<i>Lepidobatrachus llanensis</i>	-	-	-	J/A	Stained skeletal
<i>Lepidobatrachus leavis</i>	-	-	-	J/A	Stained skeletal
Leptodactylidae					
<i>Leptodactylus bufonius</i>	-	X	Appearance of a tissue intermediate between fibrocartilage and tendon	J/A	Histology/stained skeletal
<i>Leptodactylus fuscus</i>	-	-	-	-	Stained skeletal
<i>Leptodactylus latinasus</i>	X	X	Fibrocartilaginous	J/A	Histology/stained skeletal
<i>Leptodactylus mystacinus</i>	-	X	Fibrocartilaginous	J/A	Histology
<i>Leptodactylus chaquensis</i>	-	-	-	J/A	Stained skeletal
<i>Pleurodema borellii</i>	-	-	-	J/A	Stained skeletal
Microhylidae					
<i>Elachistocleis bicolor</i>	-	-	-	J/A	Stained skeletal
Pipidae					
<i>Xenopus laevis</i>	X	-	Fibrocartilaginous	J	Histology/stained skeletal
Telmatobiidae					
<i>Telmatobius oxycephalus</i>	-	X	Fibrocartilaginous	J/A	Histology/stained skeletal

Presence of the Proximal and Distal Patella. **X**, presence; -, absence.

which are slightly ordered and surrounded by two or three layers of parallel and slightly wavy collagen bundles running along the axis of the tendon (Fig. 2). In juvenile specimens, the fibrocartilage over the tibio-fibula presents a thickened ribbon shape (Fig. 2A,B), which changes with aging, becoming spindle shaped in older specimens (Fig. 2C–E). The fibrocartilage presents rounded nuclei, slightly ordered in rows, with parallel and slightly wavy collagen fibers. These fibers are continuous with those of the menisci, where they adopt a more disordered pattern. In adult specimens, there is a higher proportion of fibrous tissue interspersed with the cartilaginous cells. In the periphery of the structure, the nuclei tend to be flatter (Fig. 2D,E). The collagen bundles constitute the tendon, peripherally blended with the cellular structure. The tendon exhibits abundant cells with flattened nuclei which tends to be aligned. A region with intermediate characteristics can be distinguished in the area blended between the fibrocartilaginous structure and the tendon. Cells are typical chondrocytes surrounded by an areola (Fig. 2B,D,E).

A fibrocartilaginous proximal patella located over the femur was observed in juveniles of *Xenopus laevis* (Fig. 3A,B) (Table 1). The proximal patella in *X. laevis* shows few chondrocytes at the edges of the structure, closely

packed with wavy collagen fibers (Fig. 3B). The proximal patella of *Leptodactylus latinasus* shows chondrocytes on the entire surface, with parallel collagen fibers (Fig. 2D). A big distal patella over the tibio-fibula was observed in adults of *Phyllomedusa sauvagii*, with its chondrocytes disposed in rows or in groups, exhibiting small and rounded nuclei with collagen fibers highly interwoven between them (Fig. 3C,D). A thinner, distal patella was observed in adults of *Melanophryniscus rubriventris* (Fig. 3E); with fibroblast-like cells disposed in rows that become flatter toward the edges of the structure (Fig. 3F). The collagen fibers exhibit a parallel or wavy disposition (Fig. 3F). *Leptodactylus latinasus* was the only species showing both proximal and distal patellae composed by fibrocartilage (i.e., over the femur and the tibio-fibula) (Fig. 2C,D,F). Other anuran species with evidence of patellar elements are listed in the Table 1.

The parsimony ancestral state reconstruction supports the hypothesis that the proximal patella appears at the base of the tetrapod clade (Fig. 4A), with independent lost in certain amphibians and Rhyncocephalia. This patella is lost in the turtles + birds + crocodylians clade, but is reacquired in certain birds (Fig. 4A). The absence of a distal patella is a synapomorphy of tetrapods, with instances of convergent acquisitions in amphibians and

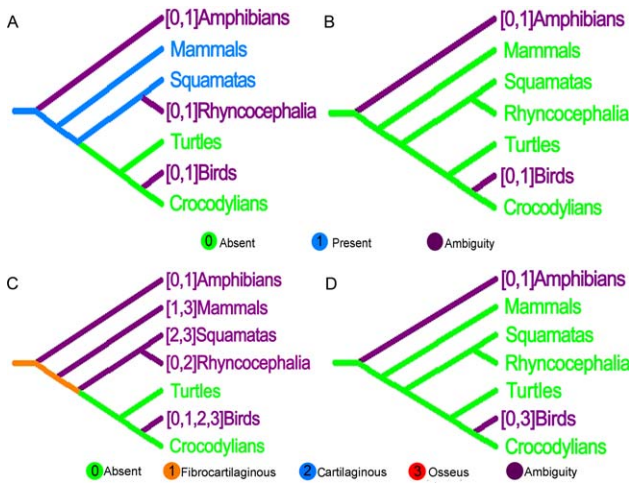


Fig. 4. Parsimony ancestral state reconstruction over the cladogram proposed by Hugall et al (2007). (A, B) Optimization considering the distribution pattern of the proximal and distal patella, respectively. (C, D) Optimization considering the patellar tissue of both sesamoids, proximal and distal, respectively. (0) absent (1) fibrocartilage (2) cartilage (3) bone.

birds (Figs. 4B and 5B). The ancestral reconstruction of the patellar tissue shows that a fibrocartilaginous proximal patella is the ancestral state in tetrapods clade (Fig. 4C). The fibrocartilage-like distal patella appears as an independent acquisition of amphibians, while the osseous condition is an independent acquisition in birds (Fig. 4D).

The optimization considering the presence/absence polymorphism as “presence,” supports the presence of a proximal patella as a synapomorphy of amniotes (Fig. 5A). The optimization in the turtles + birds + crocodylians clade is ambiguous, which brings out two possibilities: either its absence in turtles and crocodylians represents independent losses, or the losses occurred at the base of this clade and the presence in birds is thus a reacquisition (Fig. 5A).

The ancestral reconstruction of patellar tissue shows that a fibrocartilaginous proximal patella is the ancestral state in tetrapods clade. The cartilage-like patella appears as a putative synapomorphy of the Squamata + Rhyncocephalia clade (Fig. 5C). The cartilaginous and osseous states appear as independent acquisitions in mammals and birds (cartilaginous), and mammals, squamata, and birds (osseous). The fibrocartilage-like distal patella is present in amphibians, and it is ossified in birds (Fig. 5D).

DISCUSSION

For a long time, the patella has been considered a structure exclusively present in placental mammals (Reese et al., 2001 and references therein). However, as more studies were performed, data showed that it is broadly present in several tetrapod taxa (Jerez et al., 2010; Otero and Hoyos, 2013; Chadwick et al., 2014; Regnault et al., 2016; Samuels et al., 2017; Chadwick et al., 2017). Our results allow us to identify the fibrocartilaginous structure embedded in the tendon of the m. cruralis of several frog species as the proximal patella.

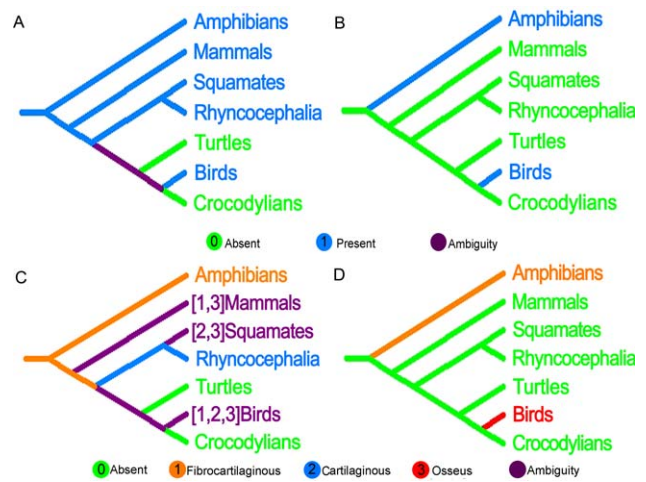


Fig. 5. Parsimony ancestral state reconstruction over the cladogram proposed by Hugall et al (2007). The polymorphism of presence/absence is considered as “presence.” (A, B) optimization considering the distribution pattern of the proximal and distal patella, respectively. (C, D) optimization considering the patellar tissue of both sesamoids, proximal and distal, respectively.

We also identify the fibrocartilage embedded in the peroneus longus tendon as the distal patella (Chadwick et al., 2014).

Reese et al. (2001) described a fibrocartilaginous structure in several marsupial families. Since this structure is not composed by bone, they decided to name it patelloid, instead of patella. They consider the patelloid as phylogenetically different to the placental patella, because the latter is osseous. The authors also differentiate five patelloid types according to their histologic structure, and consider them adaptations to the mechanical stress exerted on the joint (Reese et al., 2001, p. 294). We adopt the proposal of Vickaryous and Olson (2007) and Regnault et al. (2016) detailed above. They do not take into consideration the tissue composition of the structures to defining them as sesamoids. Thus, according to our homology criterium, marsupials have a fibrocartilaginous patella. Pearson and Davin (1921a,b) proposed naming “orthosesamoids” and “hemisesamoids” those sesamoids composed by bone and thickened cartilage, respectively, and “pseudosesamoids” to the pathological ossifications, exostoses, etc. As far as we know, all these terms have not been used again, despite subtly describing the sesamoids. Thus, we propose that any structure embedded in the tendon of the extensor muscles of the knee and located adjacent to the femur distal epiphysis should be considered a proximal patella, independently of its tissue composition. The same applies to any structure embedded in the origin tendon of the peroneus longus muscle, which should be considered a distal patella (Chadwick et al., 2014). Thus, we propose that the suprapatella embedded in the quadriceps tendon of rabbits and rats (Ralphs et al., 1991; Tischer et al., 2002) should also be defined as a proximal patella.

The proposal of using the same name for the structures that we found in frogs than that of other tetrapods, is to avoid the proliferation of terms for clearly homologous structures. In fact, sesamoid names are

usually given considering the tendon in which they are embedded; e.g., os peroneum for the sesamoid related to the *m. peroneus longus* insertion tendon (Vickaryous and Olson, 2007); fabella for the sesamoid embedded in the origin tendon of the *m. gastrocnemius* (Pearson and Davin, 1921a,b); cyamella for the sesamoid embedded in the *m. popliteus* (Pearson and Davin, 1921a,b), graciella for the sesamoid embedded in the insertion tendon of the *m. gracilis major* (Ponssa et al., 2010); patella within the main extensor insertion tendons of the knee (Regnault et al., 2014, 2016), etc. The fibrocartilaginous structure that we detected in frogs is embedded in the tendon associated to the *m. cruralis*, inserted distally in the tibiofibula through the patellar ligament. When present, it runs over the knee joint in all tetrapod taxa. The *m. cruralis* muscle is part of the *triceps femoris* (Příkril et al., 2009), whose tendon houses the patella in many tetrapod taxa, such as lizards (Regnault et al., 2014, 2016). The distal patella is less frequent among tetrapods, but more frequent and bigger than the proximal one among anurans, thus its distribution pattern is puzzling (see Chadwick et al., 2017).

The presence of the proximal and distal patellae in frogs allows inferring that they were present in the development program of the last common ancestor of the tetrapod clade. In fact, our optimization shows that the proximal patella was present as the ancestral state for all the tetrapod clade, being thus homologue in all tetrapod groups. The patella could be related to locomotion on land, which could have required the reinforcement of precise limb points to assure efficient displacement in terrestrial landscapes. Locomotion on land involves extension and flexion movements in addition to the abduction and adduction movements already present in fishes. The extension and flexion movements require of particular sets of muscles, such as the extensors *femoris*, whose tendon the proximal patella is always associated to. Interestingly, only small sesamoids, such as the fabella and cyamella, are associated to the ventral surface of the knee joint, although flexor muscles tend to be more complex and to perform more complex movements than the extensors (Pereira et al., 2015). The most evident difference between the extensor and flexor surface of the knee is that the convexity of the former generates an intense compression of the tendon of the extensor muscles over the epiphyses of the joint bones. In addition, the resting position in frogs is analogous to the jumping position in humans; thus, the patellae of frogs are under constant compressional stress. It has been already proposed that the proximal patella might protect the lower end of the femur from the friction of the quadriceps tendon (Walsmley, 1940). However, the alternative idea that the function of this patella is to protect the tendon from the compression of the femur lower end (see Benjamin and Ralphs, 1998; Reese et al., 2001; Regnault et al., 2014) is also valid. A pathological condition in humans, the “jumper’s knee,” indicates that a bony patella might not be the best solution to protect the tendon from overuse in jumping animals. The jumper’s knee is a relatively common inflammatory condition that causes pain in the anterior aspect of the knee, caused by a patellar tendinitis which starts as an inflammation of the patellar tendon, where it attaches to the proximal patella. This condition can also progress by tearing or degeneration of the tendon, to become a tendinopathy

(Engebretsen and Bahr, 2007). It could be thus inferred that a bony tissue is unable to respond to the strength demanded by jumping, causing the tendon to suffer varying degrees of injuries. It is the entheses between the patellar tendon and the proximal patella, subjected to stress concentration at the hard–soft tissue interface, the one which is vulnerable to overuse injuries in sports (Benjamin et al., 2006). Tendon cells are capable of altering the composition of the ECM in response to changes in the mechanical load, through the formation of a fibrocartilaginous matrix at the areas of the tendons and ligaments under mechanical stress (Benjamin and Ralphs, 1998). The presence of fibrocartilaginous patellae might alleviate this stress, since this tissue is found in areas of the body undergoing high mechanical load (Benjamin et al. 1991), but it is lost in the absence of muscles loads (Zelzer et al., 2014). The structure is also more fibrous, and hence more flexible to respond to this stimulus, thus avoiding tendon injuries, additionally it has been showed that tendons with more fibrocartilaginous entheses exhibit more compliance (Benjamin and Ralphs, 1998). In addition to all these aspects, the type of mechanical stress generated through intense jumping should also be addressed as a potential cause of the lack of ossification. Sarin and Carter (2000) have proposed the possibility that hydrostatic stress on sesamoids could inhibit their ossification process. This could explain why the anuran patellae tend to be fibrocartilaginous, despite the fact that the anuran bauplan is basically adapted to jump (Reilly and Jorgensen, 2011), and that jumping is one of the most mechanically challenging locomotion modes in tetrapods. Interestingly, rabbits and kangaroos also present a fibrocartilaginous proximal patella (Reese et al., 2001; Tischer et al., 2002). Reese et al. (2001) concluded that differences in the histological structures (e.g., degree of differentiation of the cells, density and orientation of collagen fibers, etc.) of patelloids (non-ossseous patella) in different families of marsupials are adaptations to the mechanical stresses acting on the stifle joint, while its presence could be attributed to phylogeny (p. 294). Following the same rationale, it could be inferred that the origin of many sesamoids can be mostly explained by a process of metaplasia, generated on a very versatile tissue, such as a presumptive tendon (Ponssa et al., 2010; Eyal et al., 2015), with the genetic potential inherited from the ancestors (Doherty, 2007; Doherty et al., 2010; Ponssa et al., 2010; Chadwick et al., 2014; Eyal et al., 2015).

The parallel gradation observed between the tissue development of the sesamoid and the spatial configuration of the entheses, appears to be aligned with the evolution of the patellar tissue. The entheses is the complex attachment between a flexible tendon, and a rigid and stiff bone (Conizzo et al., 2013). To adjust to the conditions which arise at the blending of two different materials, the entheses comprises a transitional tissue with graded morphology and mechanobiology (Conizzo et al., 2013). In a study on the structure of the human patella and its entheses, Toumi et al. (2012) showed that the central and lateral parts of the quadriceps–patella entheses are more fibrocartilaginous than the medial part, suggesting that in these regions the patella is more compressed during knee extension. The overwhelming evidence of fibrocartilage as a clear response to compressive forces (Benjamin and Ralphs, 1998; Tischer et al.,

2002; Toumi et al., 2012; Conizzo et al., 2013) indicates that it could also withstand the mechanical stress generated during jumping.

The ancestral reconstruction character shows that the patellar fibrocartilage tissue is the basal state for tetrapods. This reconstruction is in accordance with that of Summers and Koob (2002), who proposed that the evolutionary transition of tendons able to respond to compressive loading with a fibrocartilaginous pad can be traced sometime in or before the Silurian (440 to 410 mya). After that, by the Devonian, sesamoids would have formed in bony fishes (Summers and Koob, 2002). It could be hypothesized that during the evolution of the knee framework, a presumptive tendon wrapping around the epiphysis of the knee bones might have been under a strong mechanical stress, which generated a metaplastic fibrocartilage that become incorporated to the tetrapod genetic program. In ancestral anurans, the demanding movements implied in the ability to jump arrested the development conducting to a bony patella, maintaining fibrocartilage as the reinforcement structures of the knee. Thus, a fibrocartilaginous patella would result from a neotenic phenomenon at a tissue level. The same process could be proposed to explain the fibrocartilaginous patella of any tetrapod.

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APPENDIX: SPECIES AND MATERIAL EXAMINED OF DIAPHANIZED DATA

LGE: Laboratorio de Genética Evolutiva, FML: Fundación Miguel Lillo.

Ceratophrys cranwelli: FML 29848–29849; LGE 00175, 00176, 07413, 09111, 09113, 10505, 10506, 10511. *Dendropsophus nanus*: FML 29802–29811. *Elachistocleis bicolor*: FML 29792–29801. *Hypsiboas riojanus*: FML 2766/7, 2766/16, 01294/45, 01294/18, 01294//81, 01529/23, 2766/10, 01294/42, 01294/5, 01297/24, 01529–51, 01297/70, 01297/44, 01529/101. *Lepidobatrachus laevis*: FML 29842–29845; LGE 12069, 12079. *Lepidobatrachus llanensis*: FML 29846–29847; LGE, 12029, 12030, 12068, 12073, 12075. *Leptodactylus bufonius*: FML 00256/10, 00256/76, 00256/126, 00256/148, 00256/160, 00256/202, 00256/235, 00256/246, 00256/249, 00256/266, 00587/5, 00587/6, 00589, 00672 (A), 00672 (B), 03568 (A), 03568 (B), 04908 (A), 04908(B), 04908(C), 08010–08011, 09779–09780, 12129–12139, 12144. *Leptodactylus chaquensis*: FML29751–29768. *Leptodactylus fuscus*: FML29777–29791. *Leptodactylus latinasus*: FML 29734–29748. *Lysapsus limellus*: FML 29812–29824. *Phyllomedusa sauvagii*: FML 2430/5, 4899/6, 4899/13, 29832. *Pleurodema borellii*: FML 01434–7, 15298, 02235–64, 25152, 25153, 25154, 25155, 25156, 25157. *Rhinella arenarum*: FML 29858–29865. *Rhinella major*: FML 29833–29841. *Scinax fuscovarius*: FML 29825–29831. *Telmatobius oxycephalus*: FML02867/7–02867/9. *Xenopus laevis*: FML 29850–29853.