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Occurrence and distribution of sesamoid bones in squamates: a comparative approach

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Abstract

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Sesamoids are defined as skeletal elements that develop within a continuous band of regular dense connective tissue (tendon or ligament) adjacent to an articulation or joint. In this work, we discuss developmental data on two squamate species, provide data on the onset of the squamate sesamoids and the muscles they are associated to. Our results show that *Mabuya mabouya* and *Liolaemus albiceps* exhibited rather similar ontogenetic patterns and that the first sesamoids appear in embryos. The ossifying sesamoid timing is different between *M. mabouya* and *L. albiceps*, being faster in the former. In adults, we found 41 sesamoids, considering both fore and hindlimbs. We did not find any intrataxonomic differences, or any differences between the right and left side of the specimens in relation to the sesamoid presence. We recognize four types of sesamoids: (1) embedded sesamoids; (2) interosseus sesamoids; (3) glide sesamoids; and (4) supporting sesamoids. A table is included with a preliminary survey of the sesamoid distribution pattern in 10 Squamate clades.

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Introduction

Sesamoids are currently defined as small osseous elements that are present within tendons at places where they wrap around bony prominences (Pearson and Davin 1921a; Romer 1956; Haines 1969; Sarin *et al.* 1999; Maisano 2002c). More recently, Vickaryous and Olson (2007) modified the term to 'skeletal elements that develop within a continuous band of regular dense connective tissue (tendon or ligament) adjacent to an articulation or joint'. Sesamoids are generally thought to arise through an interaction between mechanical and biological factors (Sarin *et al.* 1999). Most bones in the tetrapod body are connected to each other at joints. Generally, sesamoids are not connected to other bones, but instead are related to tendons and are thought to act like pulleys. They provide a smooth surface over which the tendons slide, thus increasing their ability to transmit muscle forces. Sesamoid bones are generally thought to be linked to mechanical stress (Haines 1969; Vickaryous and Olson 2007), although recently it has been stressed that many of them are sensitive to Hox gene knockout (Hall 2005). The fibrous tissue of the tendons typically surrounds sesamoid bones, except on the surfaces that are in contact with the parts over which they glide. Here, smooth articular facets are present (Gray 1918).

In spite of its intrinsic interest, sesamoid development, distribution and characteristics have seldom been considered outside of the hominid context. Data about these interesting structures are neglected in most of the survey about vertebrate skeleton structures, and are especially scarce when developmental processes are considered (Haines 1969; Maisano 2002c). This study provides additional developmental evidence to interpret the ontogenetic pattern of sesamoids in squamates. Our preliminary data will allow further comparisons among tetrapod taxa and provide basis to interpret the homologies of these components of the tetrapod limbs. To achieve these goals, we present developmental data of sesamoids in two squamate species and preliminary data on the distribution pattern of sesamoids among 10 Squamata clades. Our objectives were: to discuss data on the ontogeny of the squamate sesamoids, to establish their position and the muscles they are related to, and to present preliminary data about their distribution in the different squamate taxa surveyed.

Materials and Methods

Observations were made in cleared and stained skeletal whole-mounts, obtained following the protocol of Wassersug (1976). All observations and illustrations were made with a stereo dissection microscope Nikon SMZ1000 equipped with a camera lucida.

The ontogenetic pattern of sesamoids was analysed in a growth series of *Mabuya mabouya* (Scincidae) and *Liolaemus albiceps* (Liolaemidae). Lizards were dissected at embryonic stages 34, 39, and 40 and at post-embryonic stages. Embryos were staged according to the developmental table of Dufaure and Hubert (1961) for *Lacerta vivipara*. Post-embryonic stages were defined as neonate, juvenile, subadult and adult on the basis of body size and gonadal development. Table 1 was created based on two *L. albiceps* embryos at stage 39 and 40 according to Dufaure and Hubert (1961), two juveniles, two subadults and five adults (MCN 2582–2592), whose snout–vent length (SVL) is shown on the top of each column. Table 2 was created based on one *M. mabouya* embryo at stage 35 according Dufaure and Hubert (1961) (UIS_R 415); four embryos at stage 40 (Dufaure and Hubert 1961) (UIS_R 310, 314, 402, 404); two juveniles (UIS_R 348, 297); and five adults (UIS_R 290, 298, 349, 313, 319), whose SVL is shown on the top of each column. Only a brief description of the sequence of events in the development of sesamoids in *M. mabouya* is included.

The muscular association of sesamoids was determined by examining incompletely cleared specimens of *M. mabouya* (two specimens; UIS_R 321), *L. albiceps* (two specimens; MCN 2580) and *Cercosaura schreibersii* (one specimen; MCN 611).

Sesamoids were examined in the appendicular skeleton of adult specimens – all cleared and stained – of *Trachylepis maculilabris*: *N* = 1. DL uncatalogued. *Sphaerodactylus macrolepis*: *N* = 1. RT 13873; *Sphaerodactylus roosevelti*i: *N* = 1. RT 13848. *Leiosaurus catamarcensis*: *N* = 1. FML 00670-2. *Kentropix viridistriga*: *N* = 1. FML 01204. *Phymaturus punae*: *N* = 3. MCN 895, 965, 967. *Liolaemus albiceps*: *N* = 14. MCN 2580, 2582–2594. *Cercosaura schreibersii*: *N* = 1. MCN 611. *Mabuya mabouya*: *N* = 14. UIS_R 298, 297, 290, 310, 313, 314, 319, 320, 348, 349, 355, 404, 402, 415.

Data of all these specimens were used to create Table 3, which includes all the sesamoids present in them. In addition, we obtained data of particular sesamoids as the palmar sesamoid or the tibial lunula in the following specimens (not included in Table 3): *Polychrus acutirostris*: *N* = 9. FML 00140. MZUSP 08605, 08606, 08610, 08611, 48166, 48151, 48154, 48156. *Anolis sagrei*: *N* = 1. SDSU 2175; *Anolis lineatopus*: *N* = 1. SDSU 2157; *Anolis cristatellus*: *N* = 1. SDSU 2145; *Anolis coelestinus*: *N* = 1. SDSU 2148. *Anolis allogus*: *N* = 1. SDSU 2136; *Anolis carolinensis*: *N* = 2. FML no data. *Anolis macrolepis*: *N* = 1. SDSU 2183. *Anolis notopholis*: *N* = 1. SDSU 2188. *Anolis gundlachi*: *N* = 9. RT 14476–14484; *Anolis cuvieri*: *N* = 1. RT 59694. *Anolis evermani*: *N* = 5. RT 14485–88, 14491; *Anolis krugi*: *N* = 2. RT 14489-90; *Anolis stratulus*: *N* = 1. RT 14492. *Diplolaemus* sp.: *N* = 3. FBC 53–55, PT 4832; *Diplolaemus bibroni*: *N* = 1. MACN

Table 1 Sesamoid ontogenetic development in *Liolaemus albiceps*

	SVL	24.9–26.9	29.1–39	50.9	58.8	61.5	72.7	80.9	88.9	90.7
Sesamoids	Stage	Embryo stage 40	Juvenile	Subadult		Adult				
Distal phalangeal sesamoids in manus and pes		x	x	***x***	***x***	***x***	***x***	***x***	***x***	**X**
Palmar sesamoid		x	x	***x***	***x***	***x***	***x***	***x***	***x***	**X**
Tibial lunula		x	x	***x***	***x***	***x***	***x***	***x***	***x***	**X**
Pisiform		x	x	x	***x***	***x***	***x***	***x***	***x***	**X**
Ulnar patella		x	x	x	***x***	***x***	***x***	***x***	***x***	**X**
Dorsal pre-axial tibiofemoral lunula		x	x	x	***x***	***x***	***x***	***x***	***x***	***x***
Ventral pre-axial tibiofemoral lunula		x	x	x	***x***	***x***	***x***	***x***	***x***	***x***
Dorsal tarsal sesamoid			x	x	***x***	***x***	***x***	***x***	***x***	**X**
Sesamoid dorsal to the proximal radial epiphysis				x	x	x	***x***	***x***	***x***	**X**
Tibial patella					***x***	***x***	***x***	***x***	***x***	**X**
Ventral pre-axial tarsal sesamoid					***x***	***x***	***x***	***x***	***x***	**X**
Ventral metacarpophalangeal sesamoids (I–IV)					x	x	***x***	***x***	***x***	***x***
Ventral metatarsophalangeal sesamoids (I–IV)					x	x	x	x	x	***x***
Sesamoid ventral to the proximal fibular epiphysis							***x***	***x***	***x***	**X**
Sesamoid positioned on the cnemial crest							***x***	***x***	***x***	**X**
Sesamoid in the pre-axial niche of the proximal head of metatarsal V							***x***	***x***	***x***	**X**
Sesamoid dorsal to the radiale								***x***	***x***	**X**
Post-axial ligament sesamoid (fabella)									x	***x***
Sesamoid dorsal to the pisiform										**X**
Sesamoid lateral to the distal epiphyses of metacarpals and metatarsals							x	x	x	***x***

x, cartilaginous sesamoids; ***x***, cartilaginous sesamoids with evidence of ossifying; **X**, osseus sesamoids.

Table 2 Sesamoid ontogenetic development in *Mabuya mabouya*

Sesamoids	SVL Stage	27.4 Embryo stage 39	35 Embryo stage 40	38.92 Neonate	42.8 Juvenile	51.6 Adult	62.3	85.88	95.85	116.12
Pisiform		x	***x***	***x***	***x***	***x***	***x***	X	X	X
Parafibula		x	***x***	***x***	***x***	***x***	***x***	X	X	X
Tibial lunula		x	x	x	x	***x***	***x***	X	X	X
Dorsal pre-axial tubiofemoral lunula		x	x	x	x	x	x	x	x	***x***
Ventral pre-axial tibiofemoral lunula		x	x	x	x	x	x	X	X	X
Distal phalangeal sesamoids in manus and pes		x	x	x	x	x	***x***	X	X	X
Palmar sesamoid		x	x	x	x	x	x	X	X	X
Plantar sesamoid			x	x	x	x	x	X	X	X
Second palmar sesamoid				x	x	x	x	X	X	X
Post-axial ligament sesamoid (fabella)						x	x	X	X	X
Tibial patella							X	X	X	X
Third palmar sesamoid								x	X	X
Ventral metacarpophalangeal sesamoids (I–IV)								X	X	X
Ventral metatarsophalangeal sesamoids (I–IV)								X	X	X
Ventral pre-axial tarsal sesamoid								X	X	X
Sesamoid in the pre-axial niche of the proximal head of metatarsal V								***x***	X	X
Dorsal tarsal sesamoid								***x***	***x***	X
Sesamoid anterior to the pisiform								X	X	X
Sesamoid ventral to the distal femoral epiphysis									X	X

x, cartilaginous sesamoids; ***x***, cartilaginous sesamoids with evidence of ossifying; X, osseus sesamoids.

35850. *Diplolaemus sexcinctus*: *N* = 1. FML 16988. *Leiosaurus paronae*: *N* = 1. MACN 4386. *Leiosaurus belli*: *N* = 2. PT 3998–3999. *Leiosaurus catamarcensis*: *N* = 2. FBC 104–105.

Sesamoid data of adult specimens of *Calotes versicolor* (Agamidae), *Xantusia riversiana*, *Xantusia henshawi*, *Xantusia arizonae*, and *Lepidophyma gaigeae* (Xantusidae), *Callisaurus draconoides*, *Uta stansburiana* (Phrynosomatidae), and *Shinisaurus crocodilurus* (Shinisauridae) were obtained from the literature (Mathur and Goel 1976; Maisano 2002a,b; Conrad 2006).

We adopted the terminology and classification of muscles proposed by Russell (1988, 1993), Zaaf *et al.* (1999), Moro and Abdala (2004) and Abdala and Moro (2006). Histological sections of two sesamoids of the hand of a juvenile of *C. schreibersii* (FML w/data), the pisiform and the palmar sesamoid, are included to illustrate their relationships with the surrounding tendons. The formalin-fixed specimen was treated with 10% neutral buffered formalin, decalcified in 5% nitric acid, and dehydrated with graded alcohols. Serial sections were cut on an MSE sledge microtome, at 6 μm, along the long axis of the tendon, and at right and sagital angles to the bone. Six sections were mounted every 1 mm throughout each specimen and stained with Ehrlich's haematoxylin and eosin (McManus and Mowry 1968).

Abbreviations

RL, private collection of Raymond Laurent; MACN, Museo Argentino de Ciencias Naturales, Argentina; MCZ, Comparative Zoology Museum, Harvard University; MZUSP, Museo de Zoología de la Universidad de San Pablo, Brasil; FBC, Colección Félix B. Cruz, Argentina; FML, Colección Fundación Miguel Lillo; MCN, Colección del Museo de Ciencias Naturales, Universidad Nacional de Salta; SDSU, San Diego State University; PT, Proyecto Tupinambis; RT, private collection of Richard Thomas; UIS_R, Colección Herpetológica, Universidad Industrial de Santander.

Results

Developmental data in Mabuya mabouya *and* Liolaemus albiceps

As *M. mabouya* and *L. albiceps* exhibited rather similar ontogenetic patterns, we include a brief description of the main sesamoid developmental stages in *M. mabouya.*

Forelimb

Stage 34 (Dufaure and Hubert 1961): All the elements of the forelimb are cartilaginous. In the carpus, the ulnar, radial, central and the distal carpals are conspicuous. The first sesamoid appearing is the pisiform, which is located between the ulnar and the ulna distal epiphysis. In the manus, all the metacarpal bones are evident. The phalangeal formula is 1-2-3-4-2.

Stages 39–40 (Dufaure and Hubert 1961): the forelimb is complete. Digits are completely developed, with acute

Table 3 Distribution of the sesamoids based on ours and from the literature data (details in Materials and Methods section)

Sesamoids	% Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. Pisiform	100	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2. Tibial lunula	100	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
3. Dorsal pre-axial tibiofemoral lunula	100	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
4. Ventral pre-axial tibiofemoral lunula	100	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
5. Tibial patella	100	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
6. Dorsal tarsal sesamoid	85	x	x	x	x	x	x	x	x	x	x	x	x	?	x	x	x		x		?
7. Palmar sesamoid	85				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
8. Distal phalangeal sesamoids in manus and pes	85				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
9. Post-axial ligament sesamoid (fabella)	75	x	x	x	x	x	x	x	x	x	x	x	x	?	x	x			x		
10. Sesamoid ventral to the articulation of the proximal tarsal and metatarsal I	65	x	x	x			x	x					x	?	x	x	x	x	x	x	x
11. Parafibula	65	x	x	x	x	x	x	x	x	x	x	x	x								
12. Ulnar patella	60	x	x	x							x	x		x	x	x	x	x		x	x
13. Plantar sesamoid	60				x	x	x	x	x	x	x	x	x	?	x						x
14. Lateral post-axial tarsal sesamoid	40	x	x	x		x	x		x	x											x
15. Ventral pre-axial tarsal sesamoid	40	x	x	x	x		x								x	x			x		
16. Sesamoid dorsal to the proximal radial epiphysis	35	x	x	x			x		x						x	x					
17. Ventral metacarpophalangeal sesamoids (I–IV)	30				x	x	x		x							x	x				
18. Ventral metatarsophalangeal sesamoids (I–IV)	25				x	x	x		?							x	x				
19. Sesamoid in the pre-axial niche of the proximal head of metatarsal V	20				x				x							x					
20. Sesamoid anterior to the pisiform	20		x	x	x		x														
21. Sesamoid lateral and distal to the distal epiphysis of metatarsal V	20	x	x	x			x														
22. Sesamoid located between proximal radial and ulnar epiphysis	**20**	x	x	x			x														
23. Sesamoid positioned on the cnemial crest	15															x				x	x
24. Sesamoid in the glenoid cavity	15										x	x						x			
25. Sesamoid dorsal to the articulation of radiale with metacarpal I	15					x										x					x
26. Sesamoid ventral to the proximal fibular epiphysis	15						x	x								x					
27. Sesamoid in the trochanter	10				x			x													
28. Sesamoid dorsal to the articulation of ulnare with metacarpal IV	10							x							x						
29. Sesamoid dorsal to the radiale	10															x				x	
30. Second and third palmar sesamoid	10				x	x															
31. Ventral ligament sesamoid (in the ligaments ventral to the knee)	10											x	x								
32. Sesamoid dorsal located between humerus, radius and ulna	10			x					x												
33. Sesamoid ventral to the distal femoral epiphysis	5				x																
34. Sesamoid dorsal to the proximal fibular epiphysis	5						x														
35. Sesamoid ventral to the proximal radial epiphysis	5		x																		
36. Sesamoid ventral to the distal head of metatarsal I, II, III	5		x																		
37. Sesamoid dorsal located between distal tarsal IV and metatarsal IV	5					x															
38. Sesamoid dorsal to the metatarsal V	5					x															
39. Sesamoid dorsal to the pisiform	5															x					
40. Sesamoid lateral to the distal epiphyses of the metacarpals and metatarsals	5															x					
41. Sesamoid dorsal to the distal ulnar epiphysis	5						x														

Gekkonidae: 1. *Sphaerodactylus macrolepis,* 2. *Sphaerodactylus roosevelti,* 3. *Sphaerodactylus klauberi*. **Scincidae**: 4. *Mabuya mabouya*, 5. *Trachylepis maculilabris*. **Gymnophthalmidae**: 6. *Cercosaura schrebersi*. **Teiidae**: 7. *Kentropix viridistriga*. **Xantusidae**: 8. *Lepidophyma gaigeae,* 9. *Xantusia riversiana*, 10. *Xantusia henshawi,* 11. *Xantusia vigilis arizonae,* 12. *Xantusia vigilis vigilis*. **Shinisauridae**: 13. *Shinisaurus* sp. **Liolaemidae**: 14. *Phymaturus punae*, 15. *Liolaemus albiceps*. **Phrynosomatidae**: 16. *Uta stansburiana,* 17. *Callisaurus dracanoides*. **Polychrotidae**: 18. *Leiosaurus catamarcencis,* 19. *Anolis* sp. **Agamidae**: 20. *Calotes versicolor*.
x, present; ?, without data.

terminal phalanx, in ossification process. In the carpus, ulnar and radial are the biggest elements. Humerus's diaphysis, radio, ulna and metacarpals are also in the ossification process. The epiphysis and carpal elements are cartilaginous. New cartilaginous sesamoids appear: the palmar sesamoid, those in the ventral carpus surface, those proximal to the metacarpals II and III, and the supraarticular distal to the last phalanges. The ulnar patella is present only in *L. albiceps*. At stage 40 (Dufaure and Hubert 1961), the pisiform presents an ossification centre.

Neonate (38.92 mm): All the diaphyses are ossified; in the epiphyses of humerus and ulna there are secondary ossification centres. In the manus, only the ulnar and the distal carpals 4 and 5 present an ossification centre. The phalanges present only the proximal epiphysis; all epiphyses are cartilaginous, except that of the terminal phalanx that present an ossification centre. The pisiform presents an ossification centre; the palmar sesamoid and those supraarticular on the distal phalanges remain cartilaginous. In the pre-axial region, a new palmar sesamoid starts to develop, located between the radial, central and distal carpal 2.

Juvenile (42.8–51.6 mm): There are no changes in the sesamoid onset or structure.

Adult (>62.3 mm): The radius, the distal carpals 1 and 2, and the metacarpals have ossification centres in their proximal epiphyses. The supraarticular sesamoids on the penultimate phalanges show signals of ossification. In the adult of 85.88 mm, the carpus and sesamoids are ossified. In the palm of the hand, a new post-axial cartilaginous sesamoid appears. It is smaller than the other two palmar sesamoids. By 95.85 mm, the epiphyses of humerus, radius, ulna, metacarpals, and the three palmar sesamoids are already ossified. There is a new small sesamoid, anterior to the pisiform. By 116.12 mm, the distal epiphysis of the humerus, radius, and ulna is not fused to the diaphyses, and are separated by a suture. The bigger sesamoids are ossified, but the smaller one remains cartilaginous.

Hindlimb

Stages 39–40 (Dufaure and Hubert 1961): Embryo has the limbs fully developed, including metatarsals and phalanges. The fibular and the astragalus are fused forming the proximal tarsal. All the long bones present the diaphysis in ossification process; epiphysis and tarsal elements are cartilaginous. In both species appear cartilaginous sesamoids: the tibial lunula; the pre-axial dorsal and ventral tibial lunula in the femorotibial joint; the distal phalangeal sesamoids. The parafibula is present only in *M. mabouya*, and starts to ossify at the stage 40 (Dufaure and Hubert 1961). At the same stage, the embryo of *M. mabouya* presents the plantar sesamoid in the ventral surface of the tarsus; the proximal tarsal presents an ossification centre.

Neonate (38.92 mm): All bones of the posterior extremity have ossified diaphyses. The proximal tarsal shows two ossification centres. The tarsal distal 4, the distal epiphyses of metatarsals III and IV, and the epiphyses of the terminal phalanx have one ossification centre. There are several sesamoids with signals of ossification: the tibial lunulae, the tibial pre-axial dorsal and ventral lunulae on the tibio-femoral joint, the plantar sesamoid, the prearticular sesamoids on the last phalanx, and the parafibula.

Subadult juvenile (51.6 mm): Metatarsal III has an ossification centre on the proximal epiphysis. Digits II and III present ossification centres in all their phalanges. The tibial lunula presents one ossification centre. The sesamoid of the post-axial ligament, located laterally to the proximal epiphysis of the fibula, is cartilaginous.

Adult (>62.3 mm): The distal tarsal 3 has an ossification centre. The proximal epiphyses of metatarsals I, II and V and those of all phalanges present secondary ossification centres. The tibial patella is distinguishable on the distal dorsal epiphysis of the femur. The supraarticular sesamoids on the penultimate phalanx start to ossify. By 85.88 mm, all epiphyses are ossified but not yet fused to the diaphyses. The tarsus has all its elements ossified, with the astragalus and calcaneum fused. New small sesamoids appear: one dorsal between the proximal tarsal and the distal tarsal 4; two ossified sesamoids on the proximal tarsal; one completely ossified over each epiphysis distal and ventral of the metatarsals, and a smaller one, partially ossified, dorsal to the proximal epiphysis of the metatarsals IV and V. All sesamoids are ossified with the exception of the tibial lunula. By 95.85 mm, all epiphyses are ossified and fused to the diaphyses. A small and ossified sesamoid appears on the post-axial and ventral regions of the femur distal epiphyses. The sesamoid located between the proximal epiphyses of the metatarsals IV and V is ossified. In the adult of 116.12 mm, the femur distal epiphysis, the epiphysis of the tibia and of the fibula are ossified, but not fused. In the femur-tibia joint, the tibial pre-axial lunula is ossifying.

All these results are presented briefly in Tables 1 and 2. *Mabuya mabouya* and *L. albiceps* exhibited rather similar ontogenetic patterns and show that the first sesamoids appear in embryos. In *L. albiceps*, there were seven embryonic sesamoids, and 20 in the adult specimens (Table 1). In *M. mabouya*, there were eight embryonic sesamoids, and 19 in the adult specimens (Table 2). Most of them were cartilaginous in the embryo and ossify in adult stages between a size of 85.88, and 116.12 mm SVL. The number of sesamoids increased progressively throughout ontogeny in both species and consequently, larger specimens have more sesamoids. All sesamoids, which arose during embryonic stages, are found in all taxa surveyed (Table 3). During post-embryonic stages, other sesamoids appear that are also present in all taxa surveyed; but most of them are exhibited by only some of the taxa (Table 3).

In *M. mabouya*, the ossification process starts earlier than that in *L. albiceps* (Tables 1 and 2), showing the pisiform evidence of ossification at stage 40 of Dufaure and Hubert (1961). However, in *L. albiceps*, most sesamoids tend to have a longer ossification process than that in *M. mabouya.* In the latter, only the pisiform and the parafibula are ossifying during more than two developmental stages. Both species have almost all sesamoids ossified approximately at the same SVL (about 90 mm).

The overall distribution pattern of sesamoids in squamates, including their number and frequency, is summarized in Table 3. We found 41 sesamoids (Table 3), in the forelimbs (Fig. 1) and the hindlimbs (Fig. 2) of the specimens investigated in this study. We did not find any difference between the right and left sides of the specimens in relation to the sesamoid presence.

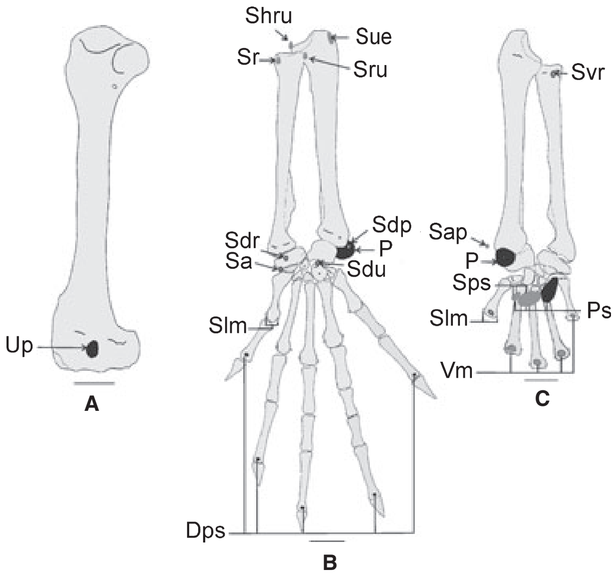


Fig. 1—Schematic drawings, based on multiple species, illustrating all the different possible sesamoids and their locations on the fore-limb—**A**. Dorsal view of humerus—**B**. Dorsal view of ulna, radius and hand—**C**. Ventral view of fibula, tibia and hand. Sesamoids present in most taxa are in black. Other sesamoids are in dark grey. dps: distal phalangeal sesamoid. p, pisiform; ps, palmar sesamoid; sa, sesamoid dorsal to the articulation of radiale with metacarpal I; sap, sesamoid anterior to the pisiform; sr, sesamoid dorsal to the proximal radial epiphysis; sdp, sesamoid dorsal to the pisiform; sdr, sesamoid dorsal to the radiale; sdu, sesamoid dorsal to the articulation of ulnare with metacarpal IV; shru, sesamoid dorsal located between humerus, radius and ulna; slm, sesamoid lateral to the distal epiphyses of the metacarpals and metatarsals; sps, second and third palmar sesamoid; sru, sesamoid located between proximal radial and ulnar epiphysis; svr, sesamoid ventral to the proximal radial epiphysis; sue, sesamoid dorsal to the distal ulnar epiphysis; up, ulnar patella; vm, ventral metacarpophalangeal sesamoids (I–IV). Scale bar = 1 mm.

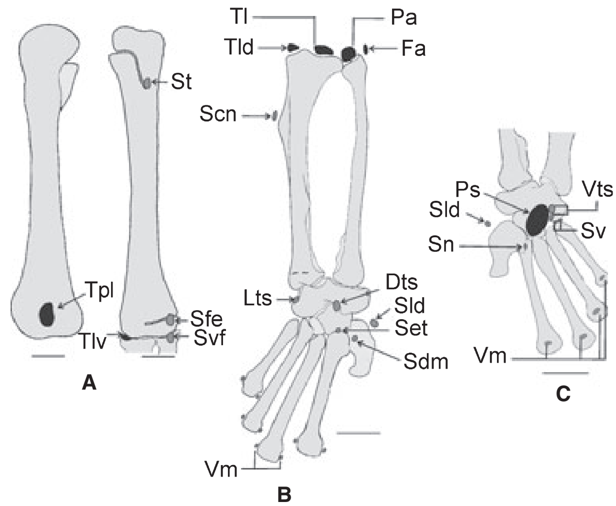


Fig. 2—Schematic drawings, based on multiple species, illustrating all the different possible sesamoids and their locations on the hind-limb—**A**. Dorsal and ventral view of femur—**B**. Dorsal view of fibula, tibia and pes—**C**. Ventral view of pes. 'Constat sesamoids' are in black. Other sesamoids are in dark grey. fa, post-axial ligament sesamoid (fabella). dts, dorsal tarsal sesamoid, located between proximal tarsal and distal tarsal 4; dps, distal phalangeal sesamoids in pes; lts, lateral post-axial tarsal sesamoid; pa, parafibula; ps, plantar sesamoid; scn, sesamoid located on the cnemial crest; sdf, sesamoid dorsal to the proximal fibular epiphysis; sdm, sesamoid dorsal to the metatarsal V; set, dorsal sesamoid located between distal tarsal IV and metatarsal IV; sfe, sesamoid ventral to the distal femoral epiphysis; sld, sesamoid lateral and distal to the distal epiphysis of metatarsal V; slm, sesamoids lateral to the distal epiphyses of the metacarpals and metatarsals; sn, sesamoid in the pre-axial niche of the proximal head of metatarsal V; st, sesamoid in the trochanter; sv, sesamoid ventral to the articulation of the proximal tarsal and metatarsal I; svf, sesamoid ventral to the proximal fibular epiphysis; tl, tibial lunulae; tld, dorsal pre-axial tubiofemoral lunula; tlv, ventral pre-axial tibiofemoral lunula; tp, tibial patella; vm, ventral metatarsophalangeal sesamoids (I–IV); vts, ventral pre-axial tarsal sesamoid. Scale bar = 1 mm.

Sesamoid categories and their relationships with the muscular system

Sesamoid categories and their location are shown in Table 4. We created four categories:

1 Embedded sesamoids: sesamoids included inside tendons; 22 of these could be found (Table 4) (Figs 3, 4). Most of the more powerful muscles of the limbs present embedded sesamoids (m. gastrocnemius femorotibialis; mm. flexor and extensor digitorum longus, etc.). These sesamoids are the closest related to the tendons, being surrounded in all their surfaces by tendinous tissue. In a histological section of the palmar sesamoid in *C. schreibersii*, a close relationship with the flexor tendon that surrounds it in all its limits is noticeable (Fig. 6A). This sesamoid presents a complex structure that includes its own muscle (Fig. 6A).

2 Interosseus sesamoids: located between bones and not directly related to tendons, but rather associated with ligaments. Six of these interosseus sesamoids were found (Table 4) (Figs 3, 4). In a first view, these sesamoids seemed to be not related with tendinous tissues; however, in all cases they are loosely attached to the closest ligaments.

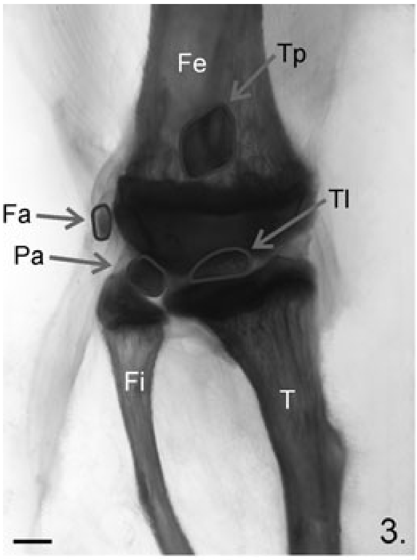
3 Glide sesamoids: providing smooth sliding surfaces for the flexor tendons in manus and pes. There was only one type of glide sesamoid, and only one in each digit (Table 4) (Fig. 5). This relationship of these bones to tendons is different from the other categories. In this case, all sesamoids serve as an appropriate surface for the over or underlying tendons sliding on.

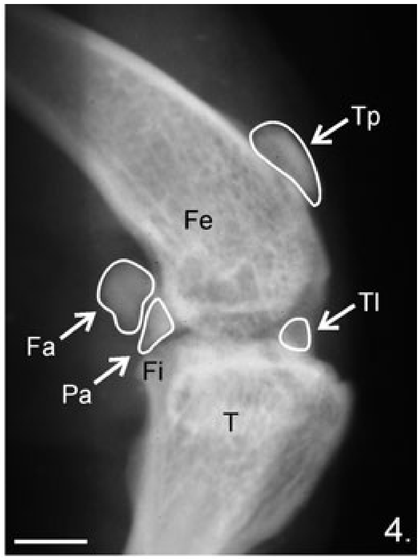
4 Supporter sesamoids: serving as muscle attachment areas to the corresponding bones. The pisiform (Fig. 1), attached to the m. abductor digitorum V, the extensor and flexor carpi ulnaris muscles, and the flexor retinaculum. Although the pisiform seems to be actually embedded in

Table 4 Sesamoid categories and their relation with the muscular system

	Muscle function	Tendon/tie of the muscle	Species *Cercosaura schreibersii*	*Liolaemus albiceps*	*Mabouya mabuya*
Embedded sesamoids					
Palmar sesamoid	Flexor	Flexor digitorum longus	x	x	x
Distal phalangeal sesamoids in manus and pes	Extensor	Extensor digitorum brevis	x	x	x
Tibial patella	Extensor	Femorotibialis (Quadriceps femoris)	x	x	x
Pisiform	Extensor	Extensor carpi ulnaris	x	x	x
	Flexor	Flexor carpi ulnaris	x	x	x
	Flexor	Flexor retinaculum		x	?
	Abductor	Abductor digitorum del dedo V	x	x	?
Ulnar patella	Extensor	Triceps	(–)	x	(–)
Dorsal tarsal sesamoid, positioned between proximal tarsal and distal tarsal 4	Extensor	Extensor digitorum brevis digit IV	x	x	?
Lateral post-axial tarsal sesamoid	Flexor	Peroneus longus	x	(–)	(–)
Post-axial ligament sesamoid (fabella)	Flexor	Gastrocnemius femoralis superficialis	x	x	x
Plantar sesamoid	Flexor	Flexor digitorum longus	x	(–)	x
Ventral pre-axial tarsal sesamoid	Pronator	Pronator profundus	x	x	x
Sesamoid ventral to the articulation of the proximal tarsal and metatarsal I		Tibial-metatarsal I tie	x	x	(–)
Sesamoid dorsal to the radiale	Abductor	Abductor polici longus	(–)	x	(–)
Sesamoid dorsal to the proximal radial epiphysis	Extensor	Extensor digitorum longus	x	x	(–)
Sesamoid positioned on the cnemial crest	Flexor	Flexor tibialis externus ?	(–)	x	(–)
Sesamoid ventral to the proximal fibular epiphysis	Flexor	F. digitorum longus	?	?	(–)
Sesamoid anterior to the pisiform	Extensor	Extensor carpi ulnaris	x	(–)	x
Sesamoid located between proximal radial and ulnar epiphysis	Extensor	Extensor carpi ulnaris	x	(–)	(–)
Sesamoid dorsal to the distal ulnar epiphysis	Extensor	Extensor carpi ulnaris	x	(–)	(–)
Sesamoid dorsal to the proximal fibular epiphysis	Extensor	Peroneus longus	x	(–)	(–)
Sesamoid ventral to the distal femoral epiphysis	Flexor	Iliofibularis?	(–)	(–)	x
Sesamoid dorsal to the pisiform	Extensor	Extensor carpi ulnaris	(–)	x	(–)
Sesamoids lateral to the distal epiphyses of the metacarpals and metatarsals	Flexor	Flexor digitorum longus?			
Interosseus sesamoids		**Bones**			
Tibial lunula		Femur-tibia	x	x	x
Dorsal pre-axial tubiofemoral lunula		Femur-tibia	x	x	x
Ventral pre-axial tibiofemoral lunula		Femur-tibia	x	x	x
Parafibula		Femur-fíbula	x	(–)	x
Sesamoid dorsal to the articulation of radiale with metacarpal I		Radial-Metacarpal I	(–)	x	(–)
Sesamoid in the pre-axial niche of the proximal head of metatarsal V		MtIV–MtV	(–)	x	x
Glide sesamoids					
Ventral metacarpophalangeal sesamoids (I–IV)	Flexor	Dorsal to the flexor digitorum longus tendon	x	x	x
Ventral metatarsophalangeal sesamoids (I–IV)	Flexor	Dorsal to the flexor digitorum longus tendon	x	x	x

x, present; (–), absent; ?, without data.





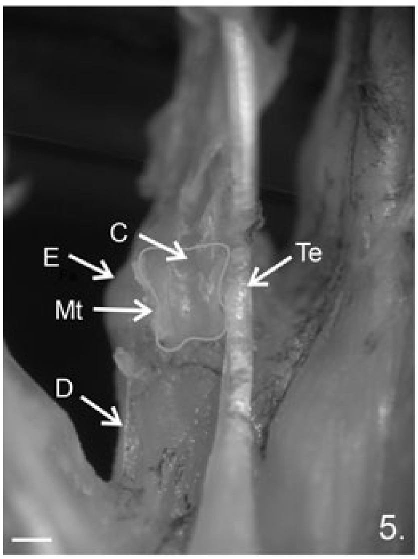


Fig. 3—*Tupinambis merianae* (PT w/d). Embedded and Interosseus sesamoids. Dorsal view of the hindlimb. fe, femur; fa, post-axial ligament sesamoid (fabella) (embedded); fi, fibula; tl, tibial lunula (interosseous); pa, parafibula (interosseous); tp, tibial patella (embedded); t, tibia. Scale bar = 1 cm.

Fig. 4—*Tupinambis merianae* (PT w/d). Embedded and Interosseus sesamoids. X-ray in lateral view of the hindlimb. fe, femur; fa, post-axial ligament sesamoid (fabella) (embedded); fi, fibula; tl, tibial lunula (interosseous); pa, parafibula (interosseous); tp, tibial patella (embedded); t, tibia. Scale bar = 1 cm.

Fig. 5—*Tupinambis meriane* (PT w/d). Glide sesamoids. Ventral view of metatarsus. The tendon glides in the concave surface of metatarsophalangeal sesamoid. c, concave surface; d, diaphyiseal metatarsal IV; e, distal epiphysis of metatarsal IV; mt, metatarsophalangeal sesamoid; te, tendon. Scale bar = 1 mm.

the tendons of the muscles it supports, the histological section shows a more light relationship with the surrounding tendon than that in the case of the embedded sesamoids. In fact, the relationships between the tendon and the pisiform have no difference from that showed by the ulna-tendon. Thus, the attachment of the pisiform to the tendons is more similar to that of a long bone than to an embedded sesamoid. In this histological section, the contact between pisiform and ulna is easily observed (Fig. 6B).

More sesamoids were observed in the hindlimb (13) than in the forelimb (7). There were a similar number of sesamoids associated with flexor muscles (10) and extensor muscles (11). Sesamoids were less frequent in relation to pronator (1) or abductor (2) muscles, and there was no adductor muscle with sesamoids. All of these sesamoids belong to the embedded category.

Discussion

Our developmental data show that sesamoid ontogeny trajectories tend to be very similar, even in two not closely related species, such as those analysed in this work. These similarities invite further exploration of potential common mechanisms underlying the origin and evolution of sesamoids in squamates. Differences are mainly related to the ossifying sesamoid timing, being faster in *M. mabouya* than in *L. albiceps*. Thus, most of the sesamoids of the former species are cartilaginous during most of their developmental time. On the contrary, most of the sesamoids of *L. albiceps* are in an ossification process during most of their developmental time. This developmental change in the timing of events – heterochrony, seems to lead to no appreciable change in size and shape, as the final result is basically the same, and all sesamoids are

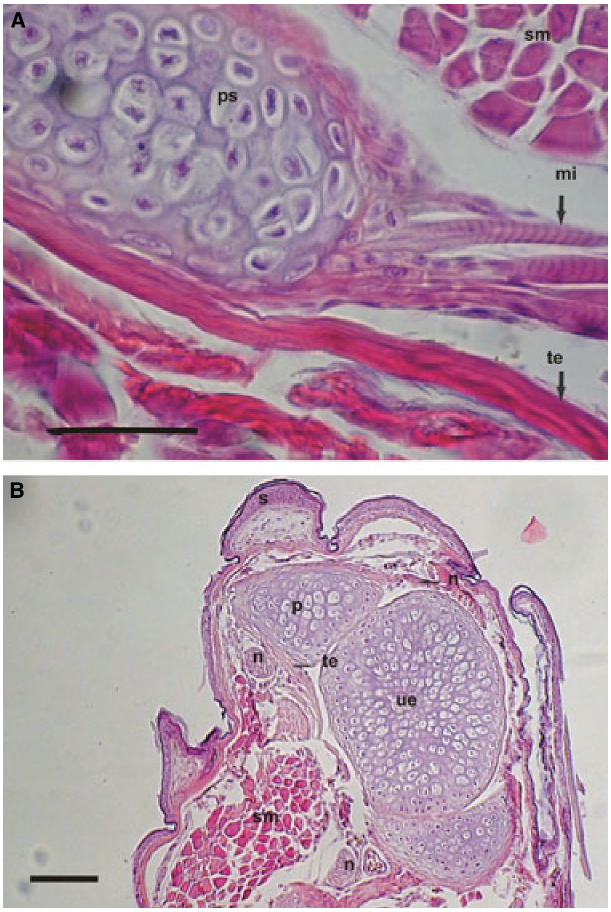


Fig. 6—**A**. *Cercosaura schreibersii* juvenile. A sagital section through the palm of the hand showing the cartilaginous palmar sesamoid surrounded by tendinous tissue. Scale bar = 35 μm—**B**. A transversal section of the proximal region of the hand showing the pisiform in contact with the epiphysis of the ulna, both cartilaginous, and the tendinous tissue surrounding them. Both sections were stained with haematoxylin and eosin (H & E). Scale bar = 100 μm. ap, aponeurosis; mi, muscular insertion; n, nerve; p, pisiform; ps, palmar sesamoid; sm, skeletal muscle; te, tendon; ue, ulna epiphysis.

osseous at approximately the same time (Tables 1 and 2). These heterocronic trends seem to be common among tetrapods. For instance, Vickaryous and Olson (2007) stressed that unlike most other sesamoids, ossification of the patella occurs relatively early in the ontogeny of mammals. However, this is not the case when our data are considered. In both *M. mabouya* and *L. albiceps*, the patella ossifies in subadult or adult stages (Tables 1 and 2). Whether these ossification patterns are phylogenetically fixed, or are intimately related to the functional demands of specific ecological contexts, remains currently unclear. Further broad comparative analyses of sesamoid development are needed to address this subject.

Our results indicate that most sesamoids seem to be indeed associated with areas experiencing mechanical stress, as many of them are only occasionally present, and when present, are embedded in tendons. In lizards, 69% of the total sesamoids are embedded in wrap-around tendons (Table 3). Wrap-around tendons are most characteristic of the limbs and are commonly wider at their point of bony contact so that the pressure is reduced (Benjamin and Ralphs 1998).

Interestingly, many sesamoids only appear during post-embryonic stages, and are found in different taxa, which suggest the importance of mechanical stress inducing their presence. These sesamoids could arise in response to the interaction between mechanical and biological factors (Sarin *et al.* 1999). If this is indeed the case, one could infer that, on one hand, mechanical stress created by flexion movements is approximately comparable with that provoked by extension movements, as flexor and extensor muscles have almost the same number of sesamoids. On the other hand, abduction, adduction, and pronation seem to induce less mechanical stress, as muscles implied in these movements only rarely show the presence of sesamoids. Our data show that in lizards the hindlimbs have more sesamoids than the forelimbs, thus supporting the current idea that the hindlimbs are under greater mechanical stress than the forelimbs during locomotion. Additionally, our data on those species with more than one specimen examined (*M. mabouya*, *L. albiceps* and *Phymaturus punae*) showed neither intrataxonomic variation nor variations related to the different sides of the specimens.

Thirty-one percent of the sesamoids are found in almost all taxa (Table 3). They appear at embryonic stages (Tables 1 and 2). Vickaryous and Olson (2007) established that an initial development and ossification in the early stages of the ontogeny are quite atypical characteristics of sesamoids. However, those are indeed intrinsic characteristics of the sesamoids that are commonly present in most limbed squamate taxa. The sesamoids are probably under strong genetic control as they are conserved in so many taxa. Moreover, experimental work on *Gallus* has demonstrated that the patella will develop under conditions of complete musculoskeletal paralysis (Vickaryous and Olson 2007), in essence thus without any biomechanical stimuli.

Vanden Berge and Storer (1995) consider three categories of intratendinous ossifications: periarticular ossifications that develop in a tendon of insertion; those that typically develop in tendons of short flexor and short adductor digital muscles attaching on the base of the thumb and hallux in the mammalian limb; and those skeletal elements that are incorporated into a joint capsule or joint cavity. Sesamoids of their two first categories are included in our embedded sesamoids, and their last one in our interosseus sesamoids. In the squamate knee-joint, there is a group of interosseus sesamoids not related to the tendon of any muscle, called periarticular elements by Vickaryous and Olson (2007). These periarticular elements are not the same as considered by Vanden Berge and Storer (1995), who used this term in a wider sense (see above). They are: the parafibula (lunula fibular *sensu* Maisano 2002a,b,c), in the femur-fibula joint, and the three tibial lunula in the femur-tibia joint: one in the post-axial region and two (one dorsal and the other ventral) in the pre-axial region. The parafibula is usually confused with the cyamella, which is in fact a mammalian sesamoid in the tendon of the m. popliteus (Pearson and Davin 1921a,b; Crum *et al.* 2003); this sesamoid is absent in squamates. The squamate parafibula is also confused with the fabela (pre-axial ligament sesamoid of Maisano 2002a,b,c), which is located near of the parafibula inside of the m. gastrocnemius tendon, and is present in Squamata. The only sesamoids that are less related to connective tissue are the glide sesamoids. In fact, these structures are under the flexor tendons and not embedded but only supporting them, presumably making their force transmission more efficient, and facilitating their sliding movement as the terminal phalanges are moved. Although these glide sesamoids are present in only about 45% of the squamate taxa surveyed, they are in fact the oldest sesamoids known. They were found in the palmar surface of the manus of the Permian lizard like captorhinids (Holmes 1977). Glide sesamoids, although on the dorsal surface of the manus, are also the oldest sesamoids reported in turtles (*Proganochelys quenstedi*, 230–200 Mya) (Vickaryous and Olson 2007). Although modern turtles are typically characterized as lacking appendicular ossicles (Haines 1969; Walker 1973), the bony projection covering the metacarpal–proximal phalanx joint of *Rhynoclemmys pulcherrima* hands (pers. obs.) could be considered a morphological integration of the glide sesamoid with the proximal phalanx, as defined by Vickaryous and Olson (2007).

The tendon-sesamoid relationship in all four categories described is variable. This fact is easily observed in the histological sections of the palmar sesamoid and pisiform. In the first case, the palmar sesamoid is completely embedded in the connective tissue (Fig. 6A), isolated of any other close structure. On the contrary, the pisiform is surrounded by the same type of tendon that surrounds the ulna, covering only the outer surfaces of both bones (Fig. 6B). These differences are probably related to the functions that each structure perform, and also suggest differences in the genesis of both pisiform and palmar sesamoids.

We believe that our more detailed classification of these structures, far for dealing only with semantic issues, allows delineating functionally relevant differences that should be investigated for a better understanding of the biological role of these structures. Moreover, it allows us to underline the very artificial nature of the sesamoid as a category. The differences described earlier strongly support Vickaryous and Olson's (2007) suggestion of the sesamoid as being a wastebasket for all manner of small and unusual skeletal elements.

Although our sample is far from enough, we think that this first survey of the complete onset of sesamoids in different clades of Squamata (Table 3) could give us a view of the general tendencies of sesamoid distribution within this clade. Further broad comparative analyses of the distribution patterns of sesamoids among tetrapods are needed to address this subject.

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